ASPECTS OF CONDITION IN CAPTIVE AND FREE-RANGING DOLPHINS.

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ABSTRACT

Aspects of morphological and physiological condition of captive and net-caught dolphins were studied with respect to age, diet, reproduction and seasonal changes in environmental factors. These investigations aimed to provide base-line data on marine mammal physiological and morphological condition indices and to expand the knowledge of condition assessment of small cetaceans. By examining the interactive nature of animal condition and growth, diet and the environment, the study investigated the potential usefulness of animal condition indices to assess and monitor habitat quality.

Base-line values for haematological and blood chemistry parameters were established for five captive bottlenose dolphins (*Tursiops truncatus*), using monthly blood samples, collected between 1992 and 1995, and corresponding measures of body weight and blubber thickness; feeding, dietary energy intake, water temperature and behavioural and medical records. Curves of best fit were used to interpret trends between leukocytic, haematological and blood chemistry parameters and morphology, diet and water temperature. WBC's and WBC differential cell types were related more distinctly to clinical changes in health, than to condition. RBC, HB and PCV were useful as short-term indicators of nutritional condition, with enhanced haematological status associated with greater food intake and improved diet quality. Weight was a more effective long-term indicator of nutritional status. The importance of blubber thickness measurements as a morphological index of condition was reinforced, with relationships between blubber thickness and haematology highlighting interactions between morphological and physiological condition. The complex interactions between haematology, diet, temperature and weight emphasised the need to account for all influential factors when considering haematological condition.

The blood chemistry parameters of serum creatinine, BUN, BUN:creatinine and serum albumin provided the best indication of nutritional condition and diet quality in captive dolphins. Creatinine and BUN:creatinine also reflected changes in morphological condition. Reduced ALP was associated with nutritional stress but was more effectively used as an indication of metabolism and growth activity. LDH and CPK were highly variable and fluctuated with changing activity levels, limiting their use as condition indices. Serum sodium was an important indication of diet quality and hydration status. Many of these parameters effectively illustrated that physiological condition is compromised outside of the thermoneutral zone of the species.

Bottlenose and common (*Delphinus delphis*) dolphins, incidentally caught in shark nets, were used to investigate the condition of free-ranging dolphins. Blood samples, taken as soon as possible after capture from drowned animals, were used to analyse physiological parameters. Dissections provided morphological data. Age and dietary status were assessed using growth layer groups in teeth and stomach content analyses, respectively. Morphological measures of net-caught dolphins reflected important interactions with diet, water temperature and reproductive status. Weight, girth and blubber thickness were valuable indicators of condition, although blubber thickness and proportional blubber weight were the best indicators of nutritional status. Condition of net-caught dolphins was difficult to assess in terms of leukocytic and haematological parameters. Stress- and *post-mortem*-associated changes, and variable data, reduced the usefulness of some parameters. WBC counts reflected on immunological status and served to emphasise changes in health, which may lead to deterioration in condition. WBC's showed potential as indicators of habitat quality, in terms of parasite load and/or pollution. Interactions between RBC, HB, PCV, MCV and dietary, morphological and environmental factors proved valuable in the short-term. Several haematological parameters were related to morphological condition, with weight, girth and blubber thickness, showing potential as non-invasive predictors of haematological status. Relationships between haematology and water temperature implied that dolphins have physiological and environmental "zones of comfort", within which condition is optimised.

LDH and CPK were elevated by capture stress and *post-mortem* changes. Serum sodium and plasma glucose were good indicators of short-term nutritional status, but were altered by stress, which reduced their usefulness as condition indicators. BUN, albumin, globulin, albumin:globulin, total cholesterol and triglycerides appeared to be little affected by these changes and are potentially valuable indicators of condition in net-caught and/or free-ranging dolphins, particularly with respect to nutritional condition, and associated changes in morphology. Although data were limited, these parameters show the greatest potential as physiological indicators of animal condition and environmental health.

The limitations of this study are recognised, however, results highlight the feasibility of using net-caught specimens to monitor the condition of free-ranging marine mammals, and the marine habitat as a whole. Relationships established in this study will be used to elucidate further on the interactive nature of marine mammal condition and aid in the development of a non-invasive condition assessment technique.

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CHAPTER 1:

GENERAL INTRODUCTION: NATURE, USE AND IMPLICATIONS OF CONDITION INDICES.

The relationship between "good growth" and "good food" has been appreciated since long before the present century. However, only in the early 1920's was it recognised that something more than "good food" was required and that satisfactory growth was only possible on a "balanced" diet (Hopkins 1920, 1925 in McCance and Widdowson 1962). Subsequent research has vindicated this foresight, and a great many further discoveries have been made about the bearing of nutrition on growth. All studies have emphasised the co-ordinated nature of the process and its important influence on successful development (McCance and Widdowson 1962).

There are four major "forces" that determine how an animal will develop, and the control and synchronisation of these ultimately regulate the development process (McCance and Widdowson 1962). These forces include firstly, the plane of nutrition, which can be affected by external temperature and the activity of the animal; secondly, the incentive to grow, the intensity of which regulates food requirements; thirdly, the size of the animal, which controls the metabolic rate and through it, to a large extent, the intake of food and other aspects of psychological and physiological development; and lastly, the age of the animal. Given a satisfactory plane of nutrition, each stage of development is passed at a finite chronological age (McCance and Widdowson 1962). These four forces were identified through early studies on human nutrition and growth and the changes which occur during malnutrition. These forces, and the factors that influence them, play an essential role in the growth, development and eventual successful reproductive and breeding processes that ensure propagation of all animal species.

The term "condition" has been used by various authors in several, slightly different, contexts. Condition is described in the Concise Oxford Dictionary of Current English (Allen 1991) as a "particular state of being" or "a state of physical fitness, especially good health". The McGraw-Hill dictionary of scientific and technical terms (Lapedes 1978) does not define condition but describes "health" as a "state of dynamic equilibrium between an organism and its environment in which all functions of mind and body are normal". For the purposes of this study, "condition" was broadly defined as:- "the interactive status of an animal's physiology and morphology relative to influencing factors, such as age, sex, reproduction, diet and nutrition, and the environment".

Condition studies, early in the 20th century, were used initially in the assessment of nutrition and malnutrition in children, as well as in the monitoring of optimal growth and form in domestic livestock (Butcher and Harris 1957, Lewis 1957, Winchester *et al.* 1957, McCance and Widdowson 1962). The majority of these studies involved the identification of morphological and/or tissue changes - considered to be related to nutrient deficiencies (Krehl and Hodges 1965). But, results showed that tissue changes were usually the end result of prolonged nutrient deprivation and although some physical measures (height, weight, muscular and skeletal development, skinfold thickness) (Shenkin and Steele 1978) were of great value, the long-term nature of these types of assessment reduced their usefulness as indicators of overall condition. In the search for parameters that could be used to provide a more effective short-term indication of condition, it was found that traditional morphological assessments were associated with physiological changes (Standard *et al.* 1959, Bleiler and Schedl 1962, Hegsted 1962, Baum *et al.* 1975, Krehl and Hodges 1965, Shenkin and Steele 1978). This was the forerunner of present-day physiological investigations in which it is possible to assess nutritional status and condition almost instantaneously using blood and urine.

The establishment of physiological indices of condition was invaluable in the assessment and monitoring of nutritional condition in humans, and its bearing on growth and development. However, wildlife biologists also sought indices that would relate well with the recent nutritional status of wild animals and thus would be useful for evaluating trends in habitat conditions. This was particularly vital for the management of commercially important wildlife species, which were vulnerable to extreme environmental changes. The assessment of nutritional status in populations of wild animals historically included measures of age-specific body size, skeletal size, bone marrow and kidney fat, deposited fat reserves, antler development (in cervids), adrenocortical hypertrophy, rumen content analysis and abdominal parasite counts (Bailey 1968, Smith 1970, Kirkpatrick *et al.* 1975, Brooks 1978, Hanks 1981, Lochmiller *et al.* 1985a, 1989, Fowler and Sniff 1992, Krebs and Singleton 1993, Fournier and Hines 1994). However, the majority of these particular measures of condition are destructive and can only be used on dead animals.

The invasive nature of most of these indices of condition proved that none were entirely satisfactory, particularly in terms of wildlife management. As wildlife management became more intensive, the need for good indices of animal and habitat conditions increased. In the late 1960's and early 1970's, biochemical analyses of blood and urine were already holding promise as reliable nutritional indices for non-domestic terrestrial mammals (Youatt *et al.* 1965, McCullagh 1969, Anderson *et al.* 1970, Franzmann 1972, LeResche *et al.* 1974, Seal *et al.* 1975). However, the influence of geographical, seasonal and annual changes in the palatability of different forages, coupled with the fluctuating contributions of particular forages to the total intake, made it difficult to evaluate terrestrial habitat in terms of its ability to support animals in healthy condition (Seal *et al.* 1978a, 1978b). Metabolic tuning of white-tailed deer (*Odocoileus virginianus*), for example, to habitat quality is reflected in growth, coat moult, antlerogenesis, bone marrow fat content, bodily fat distribution, resistance to disease and many aspects of reproduction (puberty, fecundity and natal fawn survival) (Riney 1955, Verme 1965). Each of these characteristics may be influenced by specific nutrients and by variation in protein and energy content of the available diet (Seal *et al.* 1978b). Quantitative and qualitative adjustments of metabolic state in ruminants were known to occur in response to changes in the composition of the diet with respect to protein and energy. As a result, it became vital to assess the influence of dietary composition and adequacy on morphological and physiological condition (Anderson *et al.* 1965, Silver *et al.* 1969, Robinette *et al.* 1973, LeResche *et al.* 1974, Torell *et al.* 1974, deCalesta *et al.* 1975, Hyvarinen *et al.* 1975, Short 1975, Seal *et al.* 1978a, 1978b, Warren *et al.* 1981, 1982, DelGiudice *et al.* 1987b, Lochmiller *et al.* 1985b, 1985d, 1986, 1987, 1988, 1989). Physiological indices have also been used in the assessment of animal survival (Ullrey *et al.* 1967, Thompson *et al.* 1973, Smith *et al.* 1975, Warren *et al.* 1982) and the monitoring of growth (Verme 1965, Youatt *et al.* 1965, Holter and Hayes 1977). In addition, several authors have investigated the interaction between reproduction and condition in both terrestrial mammals (Verme 1965, Youatt *et al.* 1965, Robinette *et al.* 1973, Lochmiller *et al.* 1988, Allaye Chan-McLeod *et al.* 1994, Hellgren et al. 1997, Oullet *et al.* 1997, White *et al.* 1997) and birds (Fairbrother *et al.* 1990, Chastel *et al.* 1995). The importance of body condition has been emphasised by recent studies on white-tailed and roe (*Capreolus capreolus*) deer, which have shown that maternal condition has a direct effect on the sex ratio of offspring (Burke and Birch 1995, Wauters *et al.* 1995).

The use of physiological indices of condition has grown exponentially. From being limited to use only in human and agricultural nutrition research, the same indices have become indispensable in wildlife science. Blood plasma is a dynamic medium; it is readily available, changes in relation to the physiological status of an animal, and is a sensitive index of condition. Terrestrial mammal studies have generally concluded that combinations of physiological and morphological condition assessments provide a wider perspective of metabolic function than using only a single index. Although body size, weight and fatness remain important condition indices (Altmann *et al.* 1993, Virgl and Messier 1993, Neumann and Willis 1994, Cavallini 1996, Oullet *et al.* 1997), blood parameters respond acutely to dietary changes, and thus, offer information on short term or recent metabolic function, as opposed to morphological or tissue traits, which offer an historical assessment of long-term metabolic function (Lochmiller *et al.* 1986). The collaborative use of physiological assessments, together with the more "traditional" morphological measurements of condition has made both invaluable tools in wildlife conservation and management.

Results from these types of studies have emphasised the fact that monitoring the body condition of wild animals can provide valuable information concerning the potential survival, reproductive success and "well-being" of their populations (Kirkpatrick 1980, Hanks 1981, Arnould 1995). Additionally, studies are able to reflect the nutritional state of animals and can provide insight into factors influencing the interactions between animal and habitat (Virgl and Messier 1993). Changes in animal condition may manifest themselves in changes in population structures, sizes and reproductive capabilities (Robinette *et al.* 1973, Eberhardt and Sniff 1977, Hanks 1981, Fowler and Sniff 1992, Allaye Chan-McLeod *et al.* 1995, White *et al.* 1997). In this way, animal condition can be a vital link to the assessment, monitoring and management of habitat quality. In terrestrial mammals, several physiological indices of condition have been found to provide insight into "habitat health" (Seal and Hoskinson 1978, Seal *et al.* 1978a, Warren *et al.* 1981, 1982, Lochmiller *et al.* 1985b, 1985c, 1988, Dinkines *et al.* 1991, Sams *et al.* 1998). Authors of recent terrestrial studies have also found certain physiological condition indices to be sensitive to population density (DelGiudice *et al.* 1991a, Soveri *et al.* 1992, Sams et al. 1996, 1998, Oullet *et al.* 1997, Blumstein and Foggin 1997, Sams *et al.* 1998). One of the main problems as far as terrestrial game management is concerned, is the inability to determine whether the quality and quantity of the available grazing is sufficient to maintain the current game populations. Animals suffering from nutritional deficiencies, are stressed and prone to outbreaks of disease, and often show poor reproduction and survival rates. Through the identification of nutritionally stressed animal populations, it will be possible to determine, more scientifically, whether specific game populations should be controlled, and to what extent (Grant *et al.* 1992). The establishment of baseline physiological values has now become a vital component of management and conservation policies of other animal species. Particular emphasis is being placed on endangered species and/or animals at risk from disease, pollution and habitat degradation. Some of these species include the Hawaiian monk seal (Banish and Gilmartin 1988), seabirds (Work 1996), turtles (Bolten and Bjorndal 1992), foxes (Mainka 1988, McCue and O'Farrell 1992) sand gazelles (Vassart *et al.* 1994) and mink (Weiss *et al.* 1994).

Studies on marine mammal condition are understandably less extensive than those on terrestrial mammals, due mainly to the animals' restricted availability and less accessible habitat. Similar to early terrestrial studies, basic morphological measurements formed the basis of condition assessment in the first investigations of marine mammals (Ridgway and Fenner 1982). These types of investigations were carried out on specimens obtained during whaling (Lockyer *et al.* 1984, 1985, Lockyer 1986) and similar types of operations (Lockyer 1993a). However, morphological measures have remained an important component in the condition assessment of marine mammals, although investigations are now less invasive (Fowler and Sniff 1992, Rosen and Renouf 1997). Improved technology has extended the nature and use of these measures, particularly in terms of their prediction capabilities (Wells *et al.* 1992, Costa *et al.* 1993). The importance of body fat for marine mammals is well documented (Whittow 1987); measures of blubber/fat proving invaluable as indicators of morphological and nutritional condition in both captive and free-ranging marine mammals (Ridgway and Fenner 1982, Lockyer *et al.* 1984, 1985, Lockyer 1986, 1993, Ognetov 1990, Slip 1992, Wells *et al.* 1992, Worthy *et al.* 1992, Renouf *et al.* 1993, Costa *et al.* 1993, *et al.* 1996). This variability not withstanding, data obtained from captive animals have provided a valuable insight into many aspects of marine mammal physiology, metabolism, nutrition, health and survival (Elsner and Wickham 1988).

Haematology has been used extensively in the investigation and interpretation of physiological adaptations to the marine environment (Medway and Geraci 1964, Medway and Moldovan 1966, Horvath *et al.* 1968, De Monte and Pilleri 1968a, 1968b, 1972, 1977, 1983, 1988, Bryden and Lim 1969, Harkness and Grayson 1969, Geraci 1971, Lane *et al.* 1972, Korzhuev and Galzova 1973, Bogdanova and Lebedev 1973, White *et al.* 1976, Ridgway *et al.* 1984, 1986, Horning and Trillmich 1997). These studies have provided valuable insights into how marine mammals are physiologically adapted to living in the marine environment. Haematological differences have also been used to examine and distinguish distinctive taxonomic groups of marine mammals (Hersh and Duffield 1990).

For wild dolphins, haematology and blood chemistry values determined for stranded animals cannot be assumed to be normal, since such animals generally have at least one pathological condition. However, these data do provide valuable insight into the physiology of otherwise inaccessible dolphin species (Rhinehart *et al.* 1995). Normal values for wild dolphins were characteristically estimated from studies on captive dolphins. But some investigators became concerned that these "normal" values reflected adaptations to a specialised environment and thus, were not normal for healthy, free-ranging dolphins. Several authors have investigated the influence of captivity on marine mammal haematology and blood chemistry (Medway and Geraci 1978, Geraci and St. Aubin 1979, Irvine *et al.* 1980, Cornell 1983, Asper *et al.* 1990, Bossart and Dierauf 1990, Nielsen 1995). Physiological differences between captive and free-ranging marine mammals have been ascribed to a number of different factors, including higher immune activity in wild animals, reflecting that wild cetaceans are exposed to a greater antigenic challenge resulting from parasites and marine pollution. Other physiological parameters, which differ between captive and wild dolphins, have been related to diet (Kaneko 1980 in Asper *et al.* 1990). Fish vary considerably in fat and protein composition, and it was therefore postulated that these differences were, in part, related to the types and quantities of food eaten by the dolphins. Varying degrees of activity have also been found to contribute to differences in the physiology of captive and free-ranging marine mammals (Asper *et al.* 1990).

Enzymes conventionally used to assess liver damage in man and domestic animals have been applied to clinical evaluation of whales and dolphins (Ridgway 1965, Geraci and St. Aubin 1979, Bossart and Dierauf 1990). Alkaline phosphotase, for example, can reportedly be used as an indicator of pending disease (Fothergill *et al.* 1991). As with terrestrial mammals, certain aspects of marine mammal blood chemistry have been found to be valuable indicators of physical maturity

(Fothergill *et al.* 1991).

Normal blood values, established for captive animals, have provided a baseline for diagnosing pathology in captive and wild dolphins. Sampling blood from captive and wild marine mammals has also highlighted the effects that different types of "indeterminable natural environmental pressures i.e. parasitism, competition.... almost always arbitrarily referred to as "stress" " (Geraci and Medway 1973) can have on blood parameters (Medway and Geraci 1964, Colgrove 1978, Asper *et al.* 1990, Dierauf 1990). Artificial changes in blood chemistry due to stress-related procedures on marine mammals generally concur with those described for terrestrial mammals (Seal *et al.* 1972, Hofmeyer *et al.* 1973, Seal and Hoskinson 1978, Kock et al. 1987a, 1987b, Joubert and Stander 1990, Chapple et al. 1991, Dabbert and Powell 1993, De Villiers *et al.* 1995, Whittington and Grant 1995).

Although the important interaction between short-term physiological indices and longer-term morphological indices of condition has been discussed for marine mammals (Ridgway and Fenner 1982, Ridgway and Kohin 1995), in-depth investigations into these relationships, and the influence of environmental variables on them, are lacking. The main reasons for this are directly associated with the reduced accessibility of marine mammals and the difficulties associated with conducting controlled experimental studies, similar to those conducted on terrestrial mammals.

Traditional morphological condition indices maintain their usefulness in the marine environment and have recently been implemented in the investigation of changes in population growth rates of endangered Stellar sea lions (*Eumetopias jubatus*) (Merrick *et al.* 1995) and southern elephant seals (*Mirounga leonina*) (Hindell *et al.* 1994). Total body weight, particularly, continues to be an important indicator of overall body condition (Baker *et al.* 1994a, 1994b) and has been used, in conjunction with indices of nutritional status, has been used to assess differences in the duration of pregnancy, reproductive success; and growth and development of offspring in several species of seal (Boyd 1984, 1996, Arnbom *et al.* 1993, Iverson *et al.* 1993, Lunn and Boyd 1993, Muelbert and Bowen 1993, Walker and Bowen 1993, Lunn *et al.* 1994)), and polar bears (Derocher and Stirling 1995).

With developing technology, condition assessments of free-ranging marine mammals using physiological parameters are becoming increasingly more feasible. These types of condition assessments include in-depth investigations of blood physiology, particularly in the establishment of base-line values for endangered species (Banish and Gilmartin 1988), as well as non-invasive investigations of blubber thickness, condition and body composition (Gales and Burton 1987, Slip 1992, Wells *et al.* 1992, Worthy et al. 1992, Muelbert et al. 1993, Renouf et al. 1993, Wells, 1993, Gales *et al.* 1994, Arnould 1995, Rosen and Renouf 1997). The accessibility of seals on land during

Rosen and Renouf 1997).

Physiological studies of the blood of marine mammals were facilitated in the 1960's with the advent of marine mammal capture for dolphinariums. Although some earlier studies made use of opportunistically available marine mammals through harpooned/incidentally caught and stranded specimens, the increased accessibility of dolphins in captivity made physiological studies of marine mammal blood easier (Elsner and Wickham 1988). The majority of the early studies involved the establishment of basic, baseline values for bottlenose dolphins (*Tursiops truncatus*) as this was the easiest species to maintain in captivity (Medway and Geraci 1964, 1965, Ridgway *et al.* 1970, Geraci and Medway 1973, 1974, Geraci and St. Aubin 1979). However, a number of other cetacean species, from a variety of sources, have been opportunistically investigated. These include Risso's dolphin (*Grampus griseus*) (De Monte and Pilleri 1968b); Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) (Ridgway 1965); striped (*Stenella coeruleoalba*) and common (*Delphinus delphis*) dolphins (De Monte and Pilleri 1977); harbour (*Phocoena phocoena*) (Anderson 1968) and Dall's (*Phocoena dalli*) porpoises (Ridgway 1965); killer whales (*Orcinus orca*) (Newman and McGeer 1966) and long-finned pilot whale (*Globicephala melaena*) (Medway and Moldovan 1966).

With the growth in popularity of oceanariums it was soon recognised that the maintenance of marine mammals in captivity was extremely difficult. Any stresses imposed by captivity itself, were compounded by the composition and quality of food and water, disease and training or experimental regimes (Ridgway 1965, Engelhardt 1979, Elsner and Wickham 1988). In view of the value of the individual animal it became more important to confirm and maintain the health of these animals in captivity (Ridgway 1965, Ridgway *et al.* 1970, Medway and Geraci 1978, Engelhardt 1979, Van Foreest 1980, McConnell and Vaughan 1983, Cornell 1983, Clarke 1990, Asper *et al.* 1990, Thurman and van der Elst 1995). Regular haematological and plasma chemistry tests became the preferred method for the monitoring of health and condition of captive animals. As a result, routine clinical tests associated with the care and handling of dolphins in captivity have permitted workers to accumulate considerable data on haematology and serum chemistry, particularly for the bottlenose dolphin (Asper *et al.* 1990, Thurman and van der Elst 1995). Regular samples have provided information on "normal" clinical values, which are used to monitor changes in health status. Long-term records provide an unprecedented opportunity for examining age- and sex-related differences in normal haematology and serum chemistry values (Asper *et al.*, 1990). Although the volume of data on captive marine mammal haematology is large, particularly for the more common species, there is often great variation among results from different authors and it is at times difficult to accept reported values as normal or base-line values. Some of this variation has been attributed to individual and population differences and varying sampling techniques, storage conditions and/or laboratory methodologies (Geraci and Medway 1974, Engelhardt 1979, Castellini seasonal haul out periods has resulted in the majority of studies on marine mammal body condition and/or composition concentrating on otarids and pinnipeds (for example Slip 1992, Worthy *et al.* 1992, Gales *et al.* 1994, Arnould 1995, Rosen and Renouf 1997). Similar studies have been carried out on polar bears (Atkinson and Ramsay 1995, Atkinson *et al.* 1996). Cetaceans are less accessible and as a result, condition assessments of healthy, wild cetaceans are severely limited (Rhinehart *et al.* 1991, 1992, Wells 1991, 1993).

Seabirds have been used successfully as biomonitors of fish stocks (Crawford *et al.* 1983, Batchelor and Ross 1984, Berruti and Colclough 1987, Montevecchi and Berruti 1991, Adams *et al.* 1992, Bost and Le Maho 1993, Montevecchi 1993a, 1993b, Weimerskirch *et al.* 1994, Montevecchi and Myers 1995, 1996, Montevecchi *et al.* 1995). In addition, the physiology of seabirds has recently been investigated with respect to seasonal changes in food resources (Work 1996). Until recently, similar studies using other marine species were lacking. Recently, the presence and numbers of dolphins and/or turtles in an area have been used as an indication of environmental status, especially in areas where these species are under threat from human-related activities (Kendall 1993, Garber and Burger 1995).

Marine mammals are top predators in the marine environment and, as a result, their condition is a direct reflection of the food resources and overall condition of the habitat in which they live. As discussed above, biomonitoring of the marine environment is becoming increasingly important in light of expanding coastal development and associated population, pollution and industrial expansion, placing pressure on marine based resources and habitats (Brown 1986, Lundin and Lindén 1993, Ngoile and Horrill 1993, Agardy 1994, Lubke and Britz, 1994, Simmonds 1994). Condition assessment of marine mammals is growing in importance as the techniques become more advanced and the results prove their effectiveness. However, it is still unclear exactly how physiological and morphological indices of condition interact, not only with one another, but also with changes in age, diet, reproduction and environmental parameters such as water temperature and pollution.

In this regard, the main aim of this study was to expand the knowledge of the physiological and morphological parameters useful in the condition assessment of dolphins; and provide additional insight into the interactions between these parameters and influential factors such as diet and water temperature. Objectives of the study included the identification and characterisation of parameters which could be used most effectively in the assessment and monitoring of free-ranging animal condition, and could thus be used as biological indices of habitat condition. To achieve these aims, a number of different aspects of condition assessment were investigated.

Chapter 3 outlines the establishment of "normal" base-line haematological values for five captive bottlenose dolphins. These base-line values were established through the use of regular analyses of blood samples from captive dolphins of known age and sex, which were assessed in association with routine monitoring of medical and behavioural data, carefully monitored feeding and dietary composition records, and records of the water temperature. In addition to these data, morphological parameters (weight and blubber thickness) were monitored in order to provide base-line information pertaining to the interactions between these variables in the controlled captive environment. These data provide "normal" haematological values for captive bottlenose dolphins and describe the influence of age, sex, morphology, diet and water temperature on haematological parameters.

Chapter 4 similarly establishes base-line values for blood chemistry parameters of captive bottlenose dolphins and investigates the interactions between captive dolphin blood chemistry and aspects of morphology, age, diet and water temperature. The values and relationships discussed in Chapters 3 and 4 are important for a number of reasons. Both chapters identify those haematological and blood chemistry parameters that best reflect condition in captive dolphins. The base-line values are useful for comparison with similar results obtained from other captive and/or free-ranging specimens. The relationships established in these chapters reflect base-line trends between blood parameters and influential factors such as diet and temperature, which can be used to investigate and/or compare similar trends in other groups and/or populations of dolphins.

In this regard, the base-line results and trends outlined in these two chapters serve as comparative data for condition measures obtained from incidentally caught dolphins, which are discussed in Chapters 5, 6 and 7. Chapter 5 outlines the results of morphological measurements obtained from net-caught bottlenose and common dolphins. These measurements include external measures of total body weight, length and girth, as well as internal measures of blubber thickness, blubber weight, muscle weight and proportional blubber and muscle weights. These data are used to assess the influence of age, sex, diet and water temperature on the morphological parameters of free-ranging dolphins; and identify those parameters which best reflect morphological condition in net-caught dolphins.

Chapters 6 and 7 deal with the haematology and blood chemistry, respectively, of the net-caught dolphins for which the morphology has been described in Chapter 5. Results of blood analyses from incidentally caught animals provide base-line data for a sub-sample of a population of free-ranging, presumably, healthy, coastal dolphins from the south-east coast of southern Africa. Values measured for haematological and blood chemistry parameters in net-caught dolphins are interpreted with respect to species, age, morphology, diet and water temperature. These analyses are used to identify those blood parameters which best allude to physiological condition of free-ranging dolphins with respect to factors such as diet and temperature.

Comparative analyses between data obtained from captive and net-caught animals are outlined in Chapter 8. Differences and similarities between the two study groups are interpreted with respect to aspects of the captive and free-ranging environment, including activity levels, diet and water temperature. The potential effects of other influential variables, which play a role in maintaining optimal condition in dolphins, are also discussed. The value of certain parameters as condition indices is highlighted and the potential of their use in the assessment of habitat quality and condition is reviewed. Options for future studies, provided by the results of this study, are considered.

CHAPTER 2:

STUDY SITES

CAPTIVE DOLPHINS

The five captive bottlenose dolphins (*Tursiops truncatus*) used in this study were housed in the dolphin lake of the Port Elizabeth Museum and Oceanarium Complex in Humewood, Port Elizabeth, South Africa. Built in 1967, this pool measures 61.5 m in length and 55.4 m at its widest point. The depth of the pool ranges between 0.6 m and 4.6 m. The total capacity of the pool is 4 742 400 litres. The water in the pool is seawater, pumped from the ocean, at a rate of 20 000 l/hour. Seawater passes through a sedimentation tank before being chlorinated (0.3 parts per million) and filtering into the main pool. A sand filtration system filters water at 1 million l/hour and five per cent of the total water volume is replaced daily. Sodium hypochlorite and copper sulphate are added to prevent algal growth (Clokie 1993).

FREE-RANGING DOLPHINS

The free-ranging dolphins from which morphological and physiological data were obtained during the study were common (*Delphinus delphis*) and bottlenose dolphins, which are resident along the south-east coast of southern Africa (Fig. 2.1). The common dolphins in this area show a combination of the morphological factors used to distinguish two separate species of common dolphin (*D. delphis* and *D. capensis*) by Heyning and Perrin (1994). Therefore, for the purposes of this study, the nomenclature of common dolphins will remain as classified by Linnaeus.

Oceanographic features

The south-east coast of southern Africa is defined as the length of coastline between Mossel Bay, Western Cape and Cape Vidal, Kwazulu-Natal (Fig. 2.1). This portion of coast borders on the south west Indian Ocean, within which large-scale, long-term circulation patterns are set up by the dominant winds (Heydorn *et al.* 1978, Schumann 1987). The predominant feature of this area is the strong, well-defined Agulhas boundary current, which flows southwards along the coast. The Agulhas current has an average velocity of approximately 1 m/sec and frequently exceeds 2 m/sec at its core. It flows closer inshore between Durban and Port Elizabeth, with parts of it over the continental shelf and the core often over the continental slope. Between East London and Port Elizabeth the Agulhas Current begins to diverge from the coastline, veering south before encountering the edge of the Agulhas Bank; and finally eastwards to form the Agulhas Return Current (Heydorn *et al.* 1978, Ross 1984).

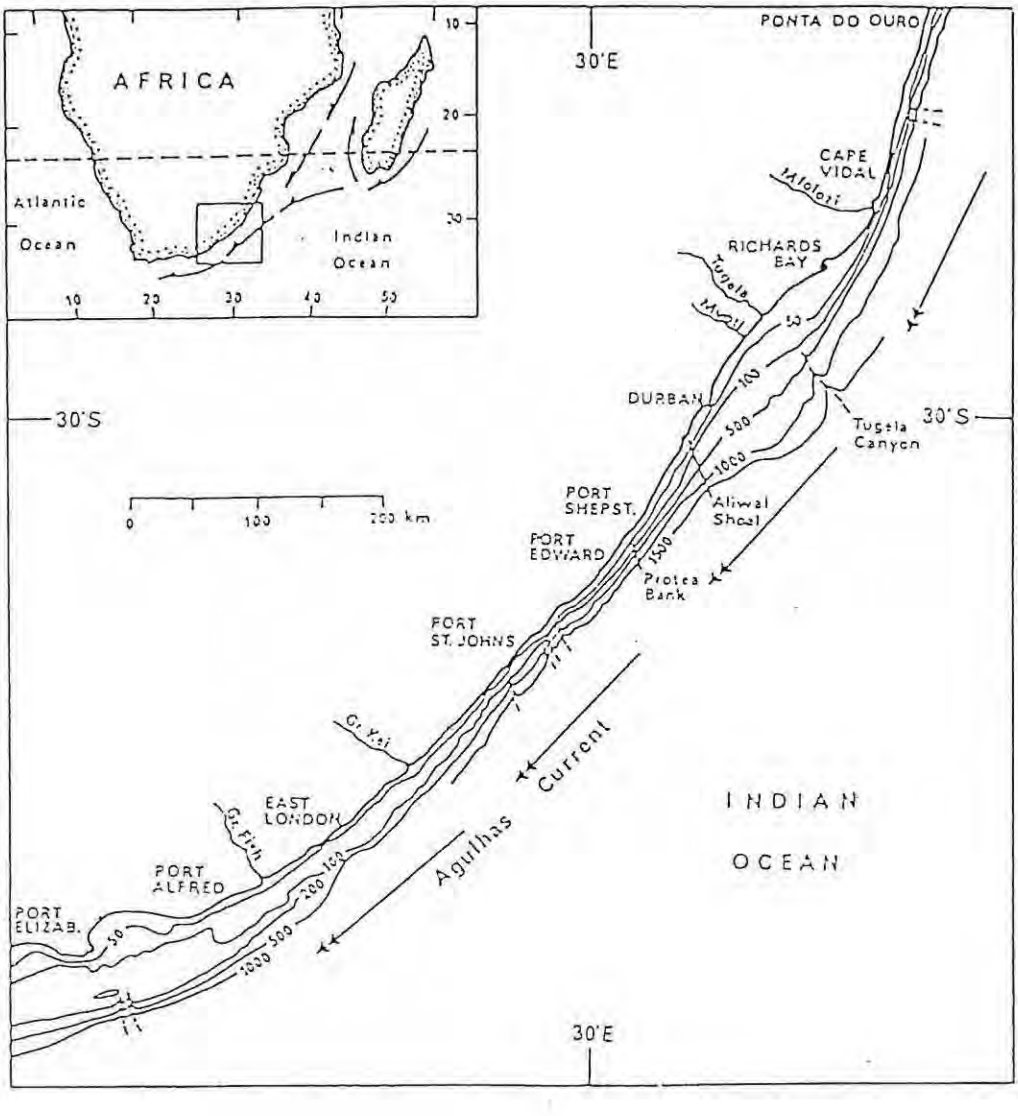


Figure 2.1 Map illustrating the oceanographic and physical features of the south-east coast of southern Africa (from Heydorn *et al.* 1978).

The hydrography of coastal waters between the inshore edge of the Current and the coastline is complex and not very well understood. The surface waters of the Agulhas Bank are well mixed in the upper 50 m and are subject to broad seasonal fluctuations in temperature and salinity. The surface temperature of the Agulhas Current varies seasonally from 22°C in August to 27°C in March, decreasing with depth. Under the surface mass lies "Central Water", from about 200 m to 800 m depth.

The average temperature in the upper 50 m varies between 15°C (August) and approximately 20°C (February). Central Water spreads up the continental slope and over the shelf, occasionally reaching the surface and the coastline (Heydorn *et al.* 1978, Schumann 1987).

Physical features

The south-east coast of southern Africa can be divided into two distinct systems. South of Port Elizabeth the coast is characterised by a series of warm, sheltered bays, connected by stretches of open, rocky shore. Water depth, in these bays, is generally less than 50 m, and always less than 100 m. At Port Elizabeth, the coastline angles north-eastwards until East London, after which it runs north to south. To the north-east, the coast is exposed and almost devoid of sheltered bays (Ross 1984).

The continental shelf along the south-east coast of southern Africa varies considerably in width. It is at its widest in the south, on the Agulhas Bank, where it reaches a width of 240 km. Further east, between Port St. Johns and Durban, it narrows to 8-10 km. Between Durban and Cape St. Lucia, in northern Kwazulu-Natal, the shelf widens again to approximately 40 km, at the submerged Tugela River delta. Further northwards, the shelf is reduced again, to less than 3 km in some parts. North of Durban, the continental shelf is broad and slopes gently, formed from sediments spilt over from the delta. Further south, between Port Shepstone and Port St. Johns, the continental slope is extremely steep, with gradients up to 12°. West of Algoa Bay the slope becomes progressively more gradual as far as Cape Agulhas (Heydorn *et al.* 1978, Ross 1984).

Biological features

The diversity in oceanographic and environmental conditions along the south-east coast has promoted a wide variety of flora and fauna. It is likely that the seasonal temperature variations in the region may be important in that animals may proliferate when conditions are optimal. Larger animals may respond through feeding migrations or by congregating in pockets of suitable water (Heydorn *et al.* 1978). The annual appearance of pilchard shoals in Kwazulu-Natal waters during winter is a regular phenomenon. Their northward movement can be followed from East London, where the pilchard shoals appear for the first time, until they disappear north of Durban. This influx of pilchards into Natal usually occurs in June of July, but may extend into August and September (Baird 1971). The pilchard shoals are accompanied by, and form an important food resource for, seabirds such as Cape Gannets (*Sula capensis*), a number of predatory fish and large schools of bottlenose and common dolphins (Baird 1971, Ross 1984, Cockcroft and Peddemors 1990).

Studies based on this annual migration have hypothesised as to the driving force behind this extensive movement of marine biota along the south-east coast of southern Africa (Davies 1956, Baird 1971, Crawford 1981, Armstrong *et al.* 1987). Davies (1956) speculated that the "sardine run" was a northward movement of juveniles, from their nursery grounds between Lamberts Bay and False Bay, comparable with that up the west coast of South Africa. However, further evidence has led to a revised proposal regarding a more detailed age-specific distribution pattern along the coastline (Crawford 1981, Armstrong *et al.* 1987). Crawford (1981) provides evidence that the clupeoid shoals first tend to move eastwards, away from the south coast fishing grounds, during late summer and autumn. It has been suggested that an inshore counter-current and eastward extension of cool inshore water is the mechanism for transporting the shoals eastward for the winter run along the Natal coast (Heydorn *et al.* 1978).

On the east coast of southern Africa, numbers of small cetaceans are caught incidentally in non-commercial gillnets set off Kwazulu-Natal beaches frequented by tourists (Cockcroft 1990, Cockcroft and Ross 1991). These nets are set to catch and reduce the population of sharks, reducing the probability of contact between bathers and sharks. However, they are also responsible for the incidental mortality of a number of dolphin and small whale species. The most frequently caught species are bottlenose, common and humpback (*Sousa chinensis*) dolphins (Best and Ross 1977, Cockcroft 1990, Cockcroft and Ross 1991). The regional and seasonal distribution, as well as the age and sex composition, of these catches has been thoroughly investigated (Cockcroft 1990, Cockcroft and Ross 1991). The dolphins caught in these nets have provided a valuable sub-sample of the local dolphin population, as well as those pelagic species, which are caught during the annual fish migration. Studies of these animals have provided vital information concerning various aspects of the biology of dolphin species that reside and/or feed in the coastal waters of Kwazulu-Natal. These studies include investigations into taxonomy and general biology (Ross 1984, Ross *et al.* 1994), age, growth and reproductive biology (Cockcroft and Ross 1989, Mendolia 1990). The incidentally caught dolphins also supply important information on distribution (Findlay *et al.* 1992), diet and feeding (Cockcroft and Ross 1983, 1990b, Barros and Cockcroft 1991, Young and Cockcroft 1994) and pollutant loads (Cockcroft *et al.* 1989, 1990). Recently, samples from these animals examined the genetic relationships between regional stocks of bottlenose dolphins (Smith-Goodwin 1997). No physiological studies have been carried out on these animals.

CHAPTER 3:

MORPHOLOGICAL AND PHYSIOLOGICAL INDICATORS OF CONDITION IN CAPTIVE BOTTLENOSE DOLPHINS (*Tursiops truncatus*):

I. HAEMATOLOGY.

INTRODUCTION

Studies of marine mammal condition have, until recently, relied on morphological parameters, particularly weight (Eberhardt and Sniff 1977, Fowler and Sniff 1992). Although weight has been regarded as a more traditional measure of body condition, it remains a valuable indicator of condition for marine mammals (Baker *et al.* 1994a, 1994b, Hindell *et al.* 1994, Merrick *et al.* 1995). Total body weight has been extensively used in the assessment of condition, with respect to nutrition, duration of pregnancy, reproductive success, and growth and development in a number of different seal species (Boyd 1984, 1996, Arnbom *et al.* 1993, Iverson *et al.* 1993, Lunn and Boyd 1993, Muelbert and Bowen 1993, Walker and Bowen 1993, Lunn *et al.* 1994), as well as polar bears (Derocher and Stirling 1995).

Measurements of blubber thickness, as indices of morphological condition, were, in the past, only available from dead animals such as stranded and whaling specimens (Ridgway and Fenner 1982, Lockyer *et al.* 1984, 1985, Lockyer 1986). The importance of body fat or "blubber" in marine mammals is well documented (Whittow 1987) and measures of blubber have already proved to be vital in the assessment of morphological and nutritional condition of marine mammals. Recently, ultrasound technology has allowed this valuable aspect of morphology to be incorporated into the condition assessment of both captive and free-ranging marine mammals (Kastelein and van Battum 1990, Slip 1992, Castellini and Calkins 1993, Wells *et al.* 1992, Costa *et al.* 1993).

These morphological measures of condition offer a historical assessment of long-term metabolic function and condition (Lochmiller *et al.* 1986). Blood parameters, in contrast, respond acutely to nutritional and/or environmental changes and are therefore able to provide an almost instantaneous indication of short-term physiological condition and metabolism. Consequently, terrestrial mammal studies have generally concluded that combinations of physiological and morphological condition indices provide a wider perspective than using only a single index. The importance of interactions between morphological and physiological condition has also been emphasised for marine mammals (Ridgway and Fenner 1982, Ridgway and Kohin 1995).

Haematological parameters of marine mammals were investigated as early as 1902 (Joliet 1902 in De Monte and Pilleri 1988). Initial haematological studies were opportunistic, but with the advent of dolphinariums and oceanariums, marine mammals were made more accessible and the extent and nature of marine mammal physiological studies has steadily grown. As a consequence, haematology has played an important role in the assessment and monitoring of captive marine mammal health (Ridgway 1965, Ridgway *et al.* 1970, Medway and Geraci 1978, Engelhardt 1979, Van Foreest 1980, McConnell and Vaughan 1983, Cornell 1983, Clarke 1990, Asper *et al.* 1990, Thurman and van der Elst 1995, Nielsen 1995). Haematology has also been used extensively in the investigation and interpretation of physiological adaptations to the marine environment (Medway and Geraci 1964, Medway and Moldovan 1966, Horvath *et al.* 1968, De Monte and Pilleri 1968a, b, 1972, 1977, 1983, 1988, Bryden and Lim 1969, Harkness and Grayson 1969, Geraci 1971, Lane *et al.* 1972, Korzhuev and Galzova 1973, Bogdanova and Lebedev 1973, White *et al.* 1976, Ridgway *et al.* 1984, Ridgway 1986).

The majority of the early studies provided baseline values for species, listing results with limited additional data on age, sex and other potentially influential factors. Animals in captivity provided the base from which haematology could be examined in terms of age and sex and monitored over long periods of time. With the help of these studies, captive animals have provided valuable haematological data, in conjunction with an insight into how certain factors affect haematological components. Although the effect of factors such as age and sex on blood parameters has been investigated for several species, few have taken into account the interactions between blood parameters and morphology, temperature and diet (Medway and Cornell 1989, Asper *et al.* 1990).

Studies on the haematology of terrestrial mammals and the influence of environmental, social and seasonal factors have been highly successful, with many authors identifying particular parameters that are useful in assessing nutritional condition and general physiological health. A number of these parameters have subsequently been related to the health of the environment as a whole. Strictly controlled studies on captive terrestrial mammals such as collared peccaries (*Tayassu tajacu*) (Lochmiller *et al.* 1985b, 1985d, 1988, Sams *et al.* 1998) and white-tailed deer (*Odocoileus virgianus*) (Seal *et al.* 1978a, 1978b, Warren *et al.* 1981, 1982, Dinkines *et al.* 1991) have shown that haematological parameters are valuable indices of animal condition, nutritional status and habitat quality. Studies of terrestrial animal condition using haematology encompass a number of different species and results emphasise the value of these types of studies (Bailey 1968, Smith 1970, Franzmann 1972, Hyvarinen *et al.* 1975, Seal *et al.* 1975, Hanks *et al.* 1976, Brooks 1978, Robinson 1979, Melton and Melton 1982, DelGuidice *et al.* 1987a, 1987b, Henke and Demarais

1990, Grant *et al.* 1992, Altmann *et al.* 1993, Boren *et al.* 1993, Choquenot 1993, Crete *et al.* 1993, Hellgren *et al.* 1993, Knick *et al.* 1993, Van Rooyen 1993, Rietkerk *et al.* 1994, Wolkers *et al.* 1994a, Clode *et al.* 1995, Gallivan *et al.* 1995, Huot *et al.* 1995). Terrestrial studies have also successfully illustrated the interaction between body condition, nutrition, fertility, reproductive success (Robinette *et al.* 1973, Hanks 1981, Allaye Chan-McLeod *et al.* 1994, 1997, White *et al.* 1997) and population density (DelGiudice *et al.* 1991a, Soveri *et al.* 1992, Sams *et al.* 1996, 1998, Oullet *et al.* 1997).

Similar studies, examining the relationship between animal condition and habitat condition, in the marine mammal field are lacking. In light of their success in the terrestrial environment, this limitation is unfortunate. Captive animals, because of their controlled environment, provide an ideal sample in which to examine and monitor changes in blood parameters according to known fluctuations in age, morphology, social status and dietary and/or environmental changes. Baseline haematological values, obtained from captive dolphins, have proved invaluable in the study of marine mammal physiology (Elsner and Wickham 1988). In this respect, examination of the relationships between haematology and morphological and nutritional condition may play a similarly important role. In addition, these base-line relationships, established using captive animals, will provide vital data for comparative studies for use in the field.

The aims of this study were as follows:

- To produce base-line haematological values for healthy captive bottlenose (*Tursiops truncatus*) dolphins,
- to identify and investigate the effects of age, weight, water temperature and diet on haematological parameters in captive bottlenose dolphins and
- to produce an index of condition for captive bottlenose dolphins using haematological parameters.

MATERIALS AND METHODS

Five captive bottlenose dolphins, from the Port Elizabeth Museum, Port Elizabeth, South Africa (see Chapter 2), were used during this study. All five animals were the small, inshore form of *Tursiops* (Ross and Cockcroft 1990). The life histories of the animals concerned are as follows:

A. Dimple

Sex: Female
Origin: Wild caught, Algoa Bay, South Africa - 12 November 1963

Date of death: 16 August 1995
Estimated age at death: >38 years

B. Dolly
Sex: Female
Origin: Captive born (dam: Dimple / sire: Daan, wild caught, Algoa Bay)
Date of birth: 12 December 1968

C. Thandi
Sex: Female
Origin: Wild caught, Algoa Bay, South Africa - 29 December 1981
Date of death: 30 October 1995
Estimated age at death: >25 years

D. Simo
Sex: Male
Origin: Captive born (dam: Thandi / sire: free-ranging male)
Date of birth: 2 March 1982
Date of death: 6 June 1995
Age at death: 13 years, 3 months

E. Domino
Sex: Male
Origin: Captive born (dam: Dolly / sire: Simo)
Date of birth: 12 October 1990

ENVIRONMENT - WATER TEMPERATURE

All animals were housed in a single dolphin "lake" measuring approximately 45 m by 60 m and containing 4.72 million litres of chlorinated seawater (see Chapter 2). Water temperature was measured three times daily at an inlet pipe. Daily, weekly and monthly mean water temperatures were calculated from these records.

BEHAVIOURAL MONITORING

Daily comments on behaviour, recorded by training staff, were examined for periods of aberrant feeding, behaviour and social interactions. Special note was made of periods of illness and the administration of medication and other treatment.

FOOD CONSUMPTION

The diet of the five animals used in this study was composed of a variety of locally available fish and squid species. Diet and feeding levels for each animal were recorded daily. These records included the proportional species composition of the diet; the total feed weight offered and the portion of total feed consumed. Daily, weekly and monthly food intake was calculated using these records. The energy content of the diet was calculated for each animal, using data available in the literature (Table 3.1).

In order to assess the short- and long-term effects of food consumption on physiological parameters, food consumption and feeding levels were assessed using several different categories. Food consumption was assessed by monitoring the total amount of food consumed in the week prior to blood sampling. Dietary energy intake was estimated using a number of different categories, including the energy intake of the day prior to blood sampling and the mean daily energy intake of the week in which blood was sampled. Additional measures of energy intake were the mean daily energy intake of both the week and month prior to blood sampling.

MORPHOLOGICAL PARAMETERS

All five animals were weighed (to the nearest kilogram) regularly - at least once a month, mostly coinciding with blood sampling procedures. During this procedure animals were confined in stretchers, out of the water, and weighed using a winch scale.

Blubber thickness was measured (to the nearest millimetre) monthly using a portable ultrasound probe (Renco Lean Meter SN 6232, U. S. Pat. 4359055 (Renco Corporation, Minneapolis, U. S. A.)). Initially blubber thickness was measured at three locations: mid-way between the blowhole and the dorsal fin - dorsally, laterally and ventrally. Subsequently, additional measures of blubber thickness were taken at three different girth locations: axilla, maximum and anus. Dorsal, lateral and ventral blubber thickness measurements were taken at all three girth locations. From these, a mean blubber thickness was calculated for each animal.

PHYSIOLOGICAL - BLOOD SAMPLING AND ANALYSES

Blood was sampled monthly from all five animals. Blood samples were taken more frequently if illness was suspected and during the monitoring of treatment. During sampling, the animal was confined in a stretcher and blood was sampled from a blood vessel on the dorsal or ventral surface of the tail fluke. Between six and 10 ml of blood was drawn using an 18-gauge needle and vacutainer, directly into specialised vac-u-test ® tubes. Blood samples for haematological analyses were sampled into EDTA vac-u-tubes. Samples were sent (at room temperature) for analysis directly to the haematology laboratory at the Port Elizabeth branch of the South African Institute for Medical Research. All analyses were completed within 4 hours of sampling.

Table 3.1: Calorific values for prey species typically consumed by captive and net-caught dolphins. All values refer t wet weight. Some conversions from dry weight to wet weight have been made using ratio values recorded Balmelli and Wickens (1994).

Prey family/spp.	Cal. value (kJ/g)	Mean cal. value (kJ/g)	Ref.*
LOLIGONIDAE			
Loligo reynaudii	4.55	**4.04**	9
	4.50		5
L.vulgaris	3.32		3
	3.62		2
	3.21		4
Squid	4.55		6
	3.08		15
Chokka squid	4.74		11
	4.81		13
SEPIIDAE			
Sepia officinalis	3.18		3
CLUPEIDAE		**6.06**	
Etrumeus spp.	4.72	**5.51**	14
Round herring	5.90		13
	5.91		10
Sardinops sagax	7.66	**6.87**	12
(previously S. ocellatus)	8.60		15
	4.86		14
Pilchard	6.42		10
	5.49		11
	5.87		13
	6.82		8
	7.54		15
	8.59		8
ENGRAULIDAE			
Engraulid spp.	5.10	**5.79**	14
Engraulis capensis	5.56		9
Anchovy	6.74		8
	5.17		13
	6.28		11
	5.91		10
T. vitrirostris	5.06		14
MYCTOPHIDAE			
Myctophidae	5.6-8	**4.90**	7
Lanternfish	4.33		13
	5.46		10
MERLUCCIDAE			
Hake	3.13	**4.02**	15
Cape hake	4.87		13
Stockfish	4.07		8
SCORPAENIDAE			
Jacopever	4.44	**3.76**	13
Helicolenus dactylopterus	3.07		15
TRIGLIDAE			
Red Gurnard	3.19		15
POMATOMIDAE			
Pomatomus saltatrix	6.20	**6.36**	1
	6.51		14
HAEMULIDAE			
Pomadasys commersonii	3.44	**3.92**	14
P.olivaceum	4.40		14
SPARIDAE			
Acanthopagrus berda	5.48	**5.20**	14
C. puniceus	6.14		14
Diplodus cervinus	4.90		14
D.sargus	6.03		14
Pagellus bellotti	3.91		14
	5.29		12
Rhabdosargus sarba	4.62		14
R. thorpei	4.39		14
Sarpa salpa	5.95		14
Cheimerus nufar	5.32		14
SCIAENIDAE			
Argyrosomus hololepidotus	5.22		1
LEIOGNATHIDAE			
Secutor insidiator	4.43		14
CARANGIDAE			
Horse mackerel	4.87	**5.89**	13
	5.63		8
	6.46		10
Trachurus delagoa	6.85		14
T. trachurus	5.63		12
MUGILIDAE			
Liza dumerii	4.83	**4.65**	14
L.richardsoni	4.25		9
Mugil cephalus	4.86		14
SCOMBRIDAE			
Scombridae	10.30	**8.94**	7
Chub mackerel	7.73		13
Scomber japonicus	7.64		14
	10.10		15

contd...

Table 3.1: contd.

References* (pertaining to Table 3.1)

1. Van Wyk 1944
2. Gouveia and Gouveia 1951 in Croxall and Prince 1982
3. Coppini 1972 in Croxall and Prince 1982
4. Pandit and Magar 1972
5. Cooper 1979
6. Heath 1980
7. Simmonds and Tanner 1980
8. Batchelor and Ross 1984
9. Heath and Randall 1985
10. Prosch 1986
11. Jackson and Place 1990
12. Ross and Cockcroft 1990
13. Balmelli and Wickens 1994
14. Sink 1994
15. Unpublished data, Port Elizabeth Oceanarium

Leukocytic and haematological analyses were done using a Coulter Counter T890. Automated counts of white blood cells (WBC), red blood cells (RBC), platelets and reticulocytes, as well as measurements of haemoglobin (HB) concentration and packed cell volume (PCV) were made in the Coulter counter. The indices of mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were calculated automatically by the Coulter counter. A corrected index of the reticulocyte count was calculated manually (taking into account PCV and the fact that reticulocytes take two days to mature). The proportional contribution of neutrophils, monocytes, lymphocytes and eosinophils to the total WBC count was investigated manually using a light microscope. The different cell types were counted and expressed as a percentage of the total WBC count.

DATA ANALYSES

Data from 1992 to 1995 were used in analyses. Although blood had been sampled from 1978 for some animals, records from previous years were not as extensive. In addition, I did not have intimate knowledge of feeding, behaviour and illness for the years prior to 1992. For each animal, overall mean values were calculated for each blood parameter, using all recorded data points. Minimum-maximum ranges and 90 % confidence limits were also calculated. Outlying data points were identified using box-whisker plots and Wilk-Shapiro Rankit plots (Zar 1984). Outliers were examined in relation to known periods of illness and/or medication and the subjective records of behaviour and removed from the data. Mean values, minimum-maximum ranges and 90 % confidence limits were calculated for the remaining "normal" data (excluding outliers). The two data sets, inclusive and exclusive of outliers, were examined for significant differences using the Mann-Whitney U-test (Zar 1984).

Overall significant differences between mean morphological, dietary and physiological values for the group of five animals were examined using One-way Analysis of Variance. Follow-up analysis of significant differences between specific animals was carried out using Two-sample t-tests. Levels of significance for the latter were adjusted by (alpha/k) (Bonferroni adjustment) where k is the number of two-sample t-tests done for each variable.

In order to investigate the relationships between seasonal, environmental, dietary, morphological and physiological parameters, "normal" data (exclusive of outliers) were examined using multiple correlation analysis, done separately for each of the five captive dolphins. In an effort to streamline the analyses and identify those parameters, which would best reflect dolphin condition, the number of significant relationships ($p \leq 0.05$) between blood parameters and independent variables such as diet, water temperature, etc. were counted. Only those blood parameters having more than 15 significant relationships with independent variables were used in further analyses.

Multivariate statistics were used in an attempt to better interpret interactions between haematological and independent parameters. These included Cluster analysis and Stepwise Linear Regression (Statistica ver. 5). However, results were highly complex and not consistent between animals. The value of these types of statistics is acknowledged, but a larger sample size, incorporating a wider variety of age groups, was needed to validate interactions between haematology and influential factors using these particular statistical analyses.

In order to better identify and interpret significant correlations between blood parameters and independent variables, curves of best fit (Curve Expert 1.1) were plotted, using all "normal" data. From a choice of eight model families, encompassing 32 different regression models, computed curves of best fit were assessed in terms of their biological importance and significance. In order to reduce the complexity of the best-fit curves, the model types were maintained at the lowest degree, for example, only third degree polynomial curves were used.

RESULTS

Only those blood parameters with more than 15 significant relationships with independent variables were included in further analyses. Parameters excluded from analyses, with less than 15 correlations, included MCH, MCHC, lymphocytes and monocytes. Although these parameters are potentially important, their usefulness was limited, in relation to the other parameters used during this study.

ENVIRONMENT – WATER TEMPERATURE

Mean water temperature of the dolphin pool during the course of the study was 21.1°C. Minimum water temperature was 15.5°C in July 1992; maximum water temperature was 27.1°C in February 1992. In October 1994 the recording of water temperature in the dolphin pool was terminated due to logistical problems. Therefore mean weekly and monthly temperatures from the previous years were used for the period between October 1994 and the end of the study.

DETERMINATION OF STATISTICAL OUTLIERS

Box-whisker plots for captive dolphin morphological, dietary and associated haematology data obtained during the current study are presented in Appendix 1. Although, this appendix does not present outliers obtained by Wilk-Shapiro runs plots, it does illustrate the overall range of data, outside of which outlying and/or extreme data were identified.

The removal of statistical outliers was supported by investigation of associated medical, behavioural and feeding records. A total of 84 outlying data points (5.65 % of the total) were removed from Dimple's data. Of these, 84.5 % could be attributed to periods of aberrant behaviour, feeding, illness and/or medication. Fifty outliers (2.6 % of total) were removed from the data of Dolly. Seventy four percent of these could be related to errant periods during the study. Only 4 % of the data were identified as outliers for Thandi, 88.3 % of which could be associated with periods of illness, medication and/or a change in behaviour or feeding. Approximately 83 % of the 5.5 % of data removed from Simo's data could also be related to similar events. Sixty-one data points (3.7 %) were removed from Domino's analyses, but only 70.5 % of these could be related to deviant feeding or behaviour and/or illness and medication.

Central values of the independent variables (water temperature, age, weight, blubber thickness, diet) (before and after the outliers were excluded) were not significantly different (Mann-Whitney U-test, $p>0.05$). For this reason, mean values discussed below relate to "normal" data (exclusive of outliers), unless stated otherwise. However, it is important to note that in several cases, the exclusion of outliers had a marked effect on the standard deviations of parameter means. Mean values for particular morphological and physiological parameters changed significantly after the exclusion of outlying data points. These will be discussed separately in the relevant sections.

MORPHOLOGICAL PARAMETERS

a. Weight

The mean weights of both Dimple (154.30 ± 4.50 kg) (mean ± standard deviation) and Domino (132.90 ± 10.71) were significantly lower than other animals (Two sample t-test; $p<0.001$) (Fig. 3.1). Dolly (181.00 ± 8.56 kg), Thandi (176.80 ± 9.03 kg) and Simo (176.80 ± 7.97 kg) all had similar mean weights (Two sample t-test, $p>0.005$).

Dimple's mean body weight during the study (1992-1995) was only slightly lower than that recorded prior to 1992. However, the mean body weights of Dolly, Thandi and Simo between 1992 and 1995 were all greater than that of prior to 1992 (Fig. 3.1). Domino showed a gradual gain in weight during the study (Fig. 3.1E). Dramatic increases in weight were associated with pregnancy for both Dolly (immediately prior to the study) and Thandi (in the latter half of 1993) (Fig. 3.1B & C). Dramatic reductions in total body weight were associated with illness and were particularly evident for Dimple, Thandi and Simo (Fig. 3.1). During the study, both Thandi and Domino had

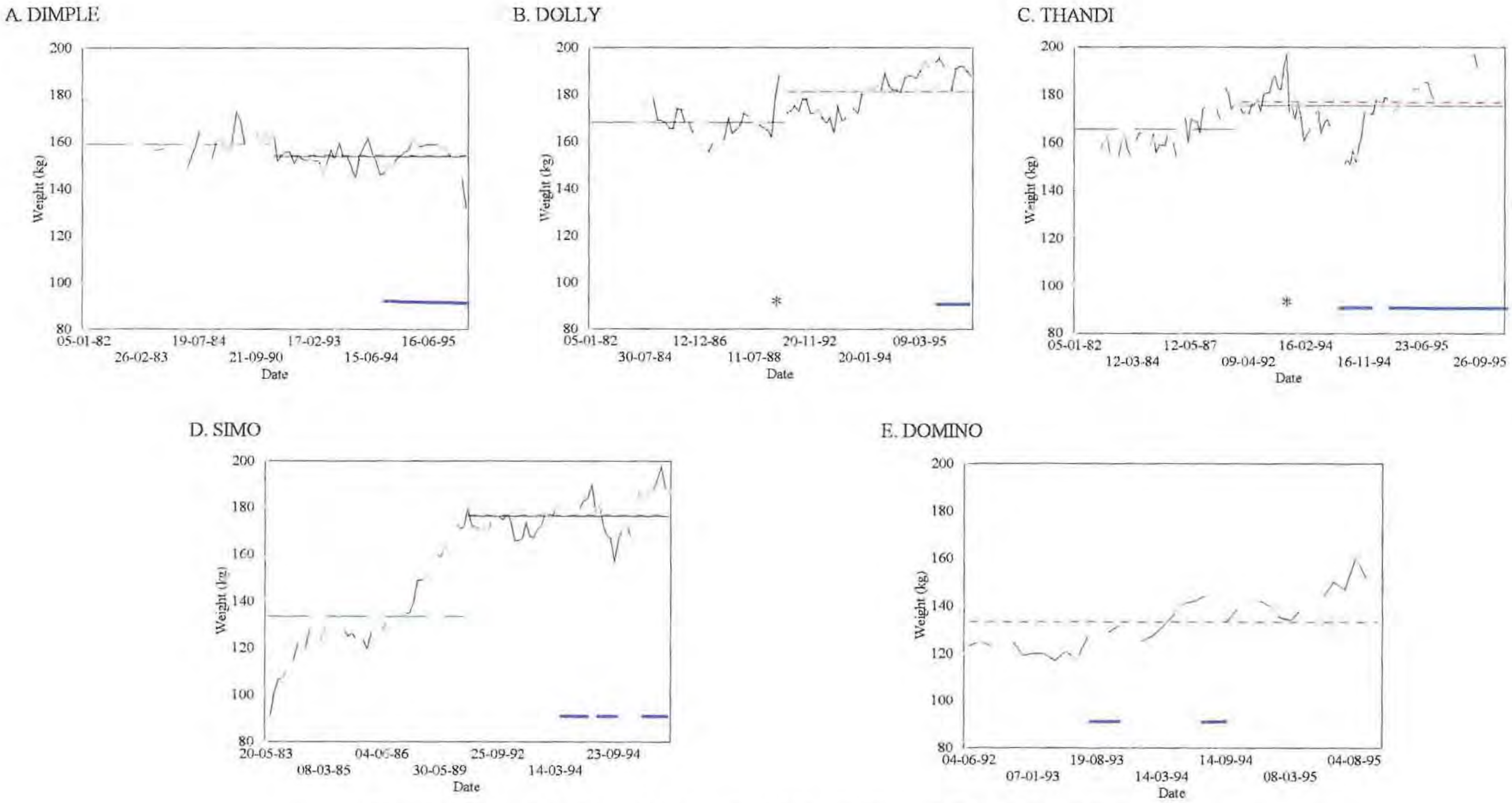


Figure 3.1: All recorded weights of the five captive dolphins (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the mean weight of all records prior to the study (– –); an overall mean weight for the study between 1992 and 1995 (—) and a "normal" (see text) mean weight for the study (- - -) (*: parturition; ▬ : extended period of illness and/or treatment or period of aberrant feeding and/or behaviour).

the most widely ranging total body weights, although significantly low weights were recorded for only Dimple and Thandi (Appendix 1.1).

There were several positive correlations between total body weight and diet for captive dolphins (Table 3.2). This was expected as food intake of the captive dolphins in this study was calculated as a proportion of body weight.

Table 3.2: Correlation coefficients (r), levels of significance (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$) and sample sizes (n) for significant relationships between total body weight and other independent variables for the five captive dolphins (Data are presented as r*(n)) (NS: non-significant ($p>0.05$)).

Parameter	Dimple	Dolly	Thandi	Simo	Domino
Age	NS	0.84***(44)	NS	0.55***(46)	0.88***(37)
D1	NS	0.32*(38)	0.47***(52)	NS	0.56***(34)
E1	NS	0.47**(38)	0.32*(54)	NS	0.63***(34)
E2	NS	NS	0.33*(54)	NS	0.61***(34)
E3	NS	NS	NS	NS	0.55***(33)

D1: Total food consumption of week prior to blood sample, E1: Dietary energy intake of day prior to blood sample, E2: Mean daily energy intake of week in which blood was sampled, E3: Mean energy intake of week prior to the blood sample.

b. Blubber thickness

Due to logistical problems, blubber thickness was measured irregularly and for four animals only between 1993 and 1995 (Fig. 3.2). Overall, mean blubber thicknesses were significantly different between animals (1 way ANOVA: $F=6.12$; $p\leq0.001$; $n=48$). However, the ranges of blubber thickness measurements overlapped considerably (Appendix 1.1B). Two sample t-tests showed that Dimple's mean blubber thickness (21.49 ± 1.52 mm) was significantly greater than both Thandi (18.25 ± 2.56 mm) ($t=3.81$; $p<0.001$; $df=23$) and Dolly (20.12 ± 1.10 mm) ($t=2.45$; $p=0.02$; $df=21$). Both Dolly's ($t=2.26$; $p=0.03$; $df=22$) and Domino's (20.80 ± 2.34 mm) ($t=-2.66$; $p=0.01$; $df=24$) blubber was also significantly thicker than that of Thandi. However, when levels of significance were adjusted (Bonferroni correction) to $p=0.003$, only Dimple's and Thandi's mean blubber thickness remained significantly different. A significantly high blubber thickness of 25.00 mm was recorded for Dolly, but it was suspected that this was an incorrect reading, as the range for this animal was fairly restricted (Appendix 1.1). The irregularity of blubber thickness measurements made it impossible to investigate any potential relationship between blubber thickness and illness, treatment or periods of aberrant behaviour and/or feeding (Fig. 3.2). Blubber thickness was not significantly correlated with weight or diet ($p>0.05$). However, Domino's blubber thickness was negatively correlated with the mean water temperature of the week ($r=-0.74$, $p<0.05$, $n=13$) and month ($r=-0.83$, $p<0.01$, $n=13$) prior to the blood sample.

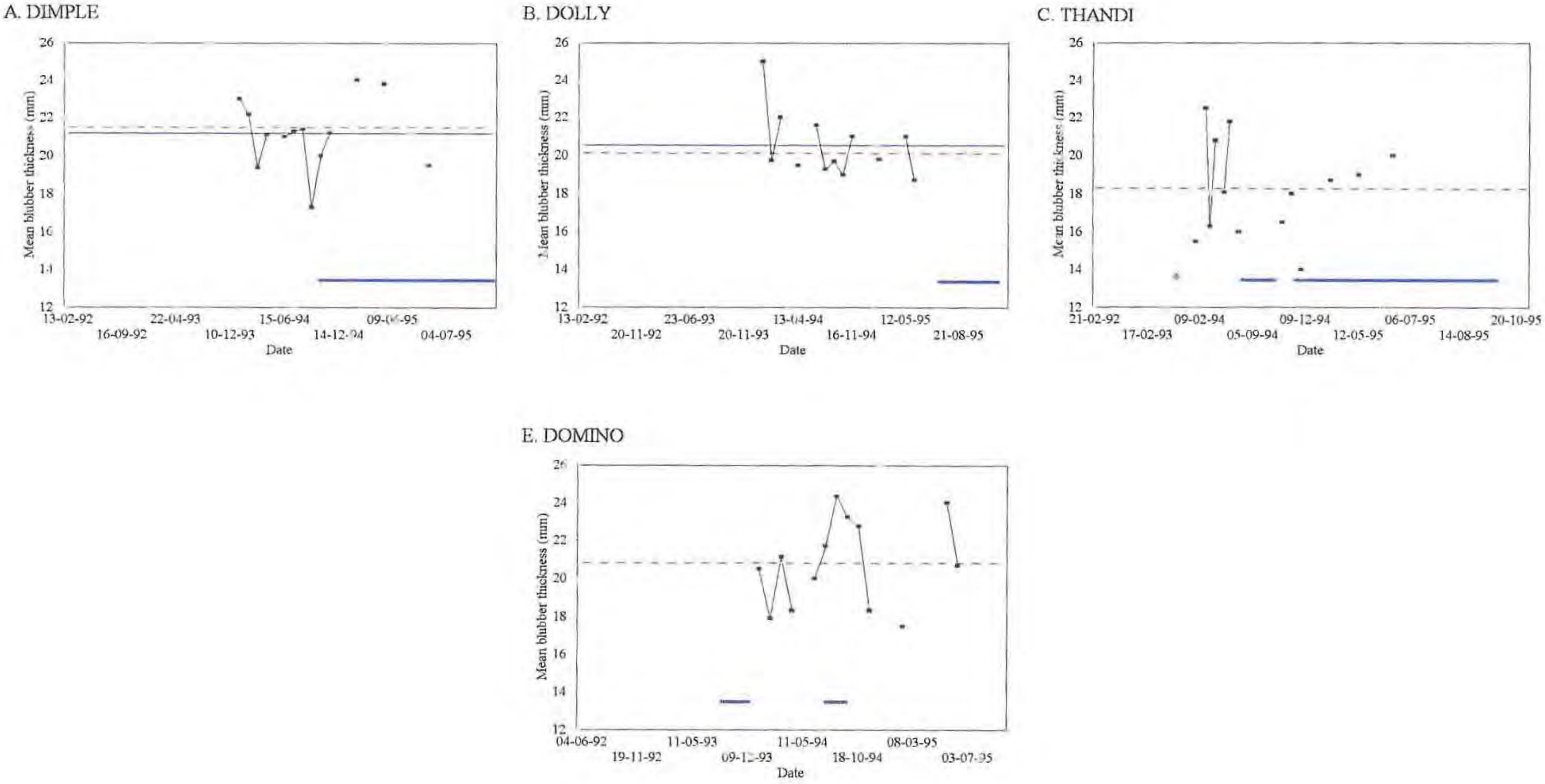


Figure 3.2: Measurements of blubber thickness (mm)(—) for all five captive dolphins (A. Dimple; B. Dolly; C. Thandi; D. Domino). Also shown, for each animal, is the overall mean blubber thickness (—) and the "normal" (see text) mean blubber thickness (- - -) for the study between 1992 and 1995 (*: parturition; —: extended period of illness and/or treatment or period of aberrant feeding and/or behaviour).

FOOD CONSUMPTION

Total food consumption in the week prior to blood sampling fluctuated irregularly, with deviations being most severe for Thandi (63.28 ± 12.55 kg/week) (Fig. 3.3C). The majority of food intake fluctuations and associated outlying data could be associated with known changes in the health status of the animals (Fig. 3.3). In this context, long-term illness and antibiotic treatment of Thandi resulted in highly deviant feeding levels and no outlying data being identified for this animal (Appendix 1.2).

Mean levels of food consumption were significantly different between all five animals (1 way ANOVA: F=207.65; p<0.001; n=229; Two sample t-test; p<0.001), except Thandi and Simo (t=2.23, p=0.03, n=114) (Fig. 3.3; Appendix 1.2A). Food consumption was greatest in Dolly (88.86 ± 4.83 kg/week) and decreased (in order) in Thandi (63.28 ± 12.55 kg/week), Simo (58.93 ± 4.75 kg/week), Dimple (51.78 ± 3.59 kg/week) and Domino (38.13 ± 5.68 kg/week). As described above, food intake was positively correlated with weight for the majority of animals (Table 3.2). Thandi's overall food consumption was significantly reduced when water temperatures, during the preceding month, were warmer. However, this contrasted with positive relationships between food consumption and water temperature for Domino (Table 3.3).

Table 3.3: Correlation coefficients (r), levels of significance (*: p<0.05, **: p<0.01, ***: p<0.001) and sample sizes (n) for significant relationships between dietary parameters and water temperature for the five captive dolphins (Data are presented as r*(n)) (NS: non-significant (p>0.05)).

Parameter		Dimple	Dolly	Thandi	Simo	Domino
D1	T1	NS	NS	NS	NS	0.54**(22)
	T2	NS	NS	NS	NS	0.53**(22)
	T3	NS	NS	-0.42**(34)	NS	0.61**(22)
E1	T1	NS	NS	NS	0.36*(35)	NS
	T2	NS	NS	NS	0.36*(34)	NS
	T3	NS	NS	NS	0.46**(35)	0.49*(22)
E2	T1	0.39*(25)	NS	NS	0.38*(35)	NS
	T2	NS	NS	NS	0.37*(34)	NS
	T3	0.40*(25)	NS	NS	0.35*(35)	0.44*(22)
E3	T3	NS	NS	NS	NS	0.46*(21)

D1: Total food consumption of week prior to blood sample, E1: Dietary energy intake of day prior to the blood sample; E2: Mean daily energy intake of week in which blood was sampled, E3: Mean daily energy intake of week prior to the blood sample, T1: Mean water temperature of week prior to the blood sample, T2: Mean water temperature of month in which blood was sampled, T3: Mean water temperature of month prior to the blood sample.

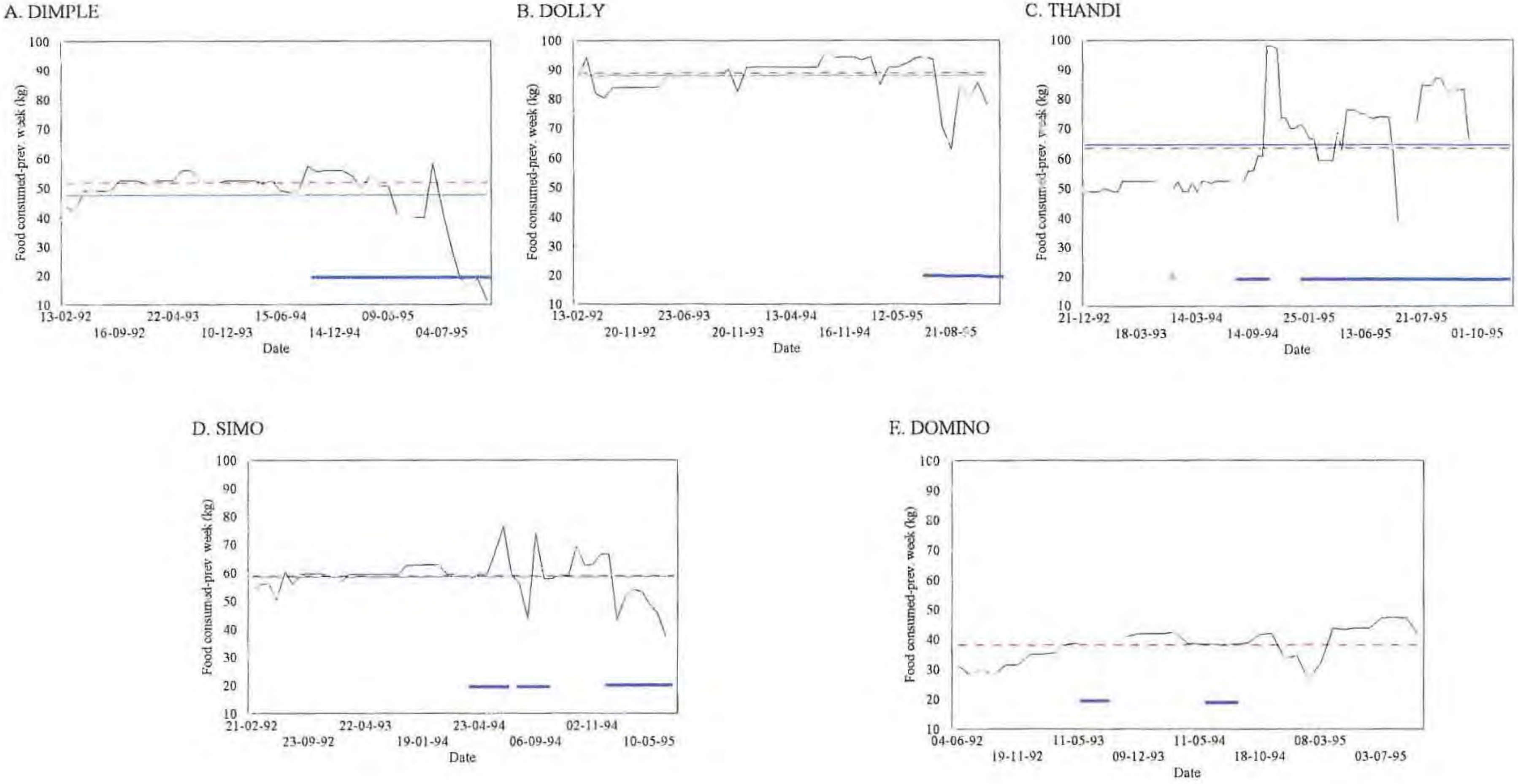


Figure 3.3: Total food consumed (kg) (—) in the week prior to blood sampling for all five captive dolphins (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the overall mean total weight of food consumed (—)and the "normal" (see text) mean weight of food consumed (- - -) in the week prior to blood sampling for the duration of the study (*: parturition; — : extended periods of illness and/or treatment or period of aberrant feeding and/or behaviour).

Changes in dietary energy intake paralleled those of total food intake (Fig. 3.4-3.7). Fluctuations in energy consumption were greatest for Thandi (Fig. 3.4-3.7C; Appendix 1.2). Increased variability in energy intake could, in most cases, be associated with periods of illness and/or treatment. Outlying low levels of energy intake were recorded for most animals, but most frequently for Dimple and Simo (Appendix 1.2).

Mean levels of energy intake for the different time periods were significantly different between the five animals (ANOVA, Dietary energy intake of day prior to blood sample: $F=198.97$, $p<0.001$, $n=234$; Mean dietary energy intake of week in which blood was sampled: $F=235.58$, $p<0.001$, $n=225$; Mean dietary energy intake of week: $F=242.62$, $p<0.001$, $n=233$ and month: $F=356.72$, $p<0.001$, $n=247$ prior to blood sample). Only Dimple and Thandi had similar mean dietary energy intakes (Two sample t-tests, $p>0.005$). For all but one dietary energy category, Dolly's mean energy intake was significantly higher than all animals, followed by Simo, Dimple and Thandi. Domino consumed significantly less dietary energy than all four other animals (Two sample t-test; $p\leq0.001$) (Fig. 3.4-3.7; Appendix 1.2). In contrast, Simo's mean level of energy intake in the long-term (i. e. the month prior to the blood sample) (40 490.00 ± 2 108.00 kJ/day) was significantly higher than most other animals, except Dolly (61 240.00 ± 5162 kJ/day). Energy intake was positively related to water temperature in Dimple, Simo and Domino, although relationships were not consistent between animals. Neither Dolly and/or Thandi showed any significant correlations between dietary energy intake and water temperature (Table 3.3).

PHYSIOLOGICAL PARAMETERS

There were no significant differences, for all five animals, between total recorded data (inclusive of outliers) and "normal" data from which outliers had been removed (Mann Whitney U-test; $p<0.05$). Therefore, although the figures in the following section represent all data points (including outliers), only "normal" data, were used in analyses. Mean values from both data sets are included for comparative purposes. In addition, although not used in the analyses, data recorded prior to the study was included for further comparison.

The number and nature of significant relationships between morphological parameters (weight; blubber thickness); physiological parameters (leukocytic and haematological parameters) and age, temperature and diet were varied and not consistent between animals. In the majority of cases, relationships were based on data from one or two animals. Only "normal" data were used in the fitting of best-fit curves. Outliers were plotted on graphs as two separate categories. The first of these included results outlying with regards to the dependent variable, i.e. the blood parameter. The second category included results that were not significantly different in terms of the blood parameter itself, but were associated with outlying values of the independent variable, i.e. temperature, diet, weight, etc.

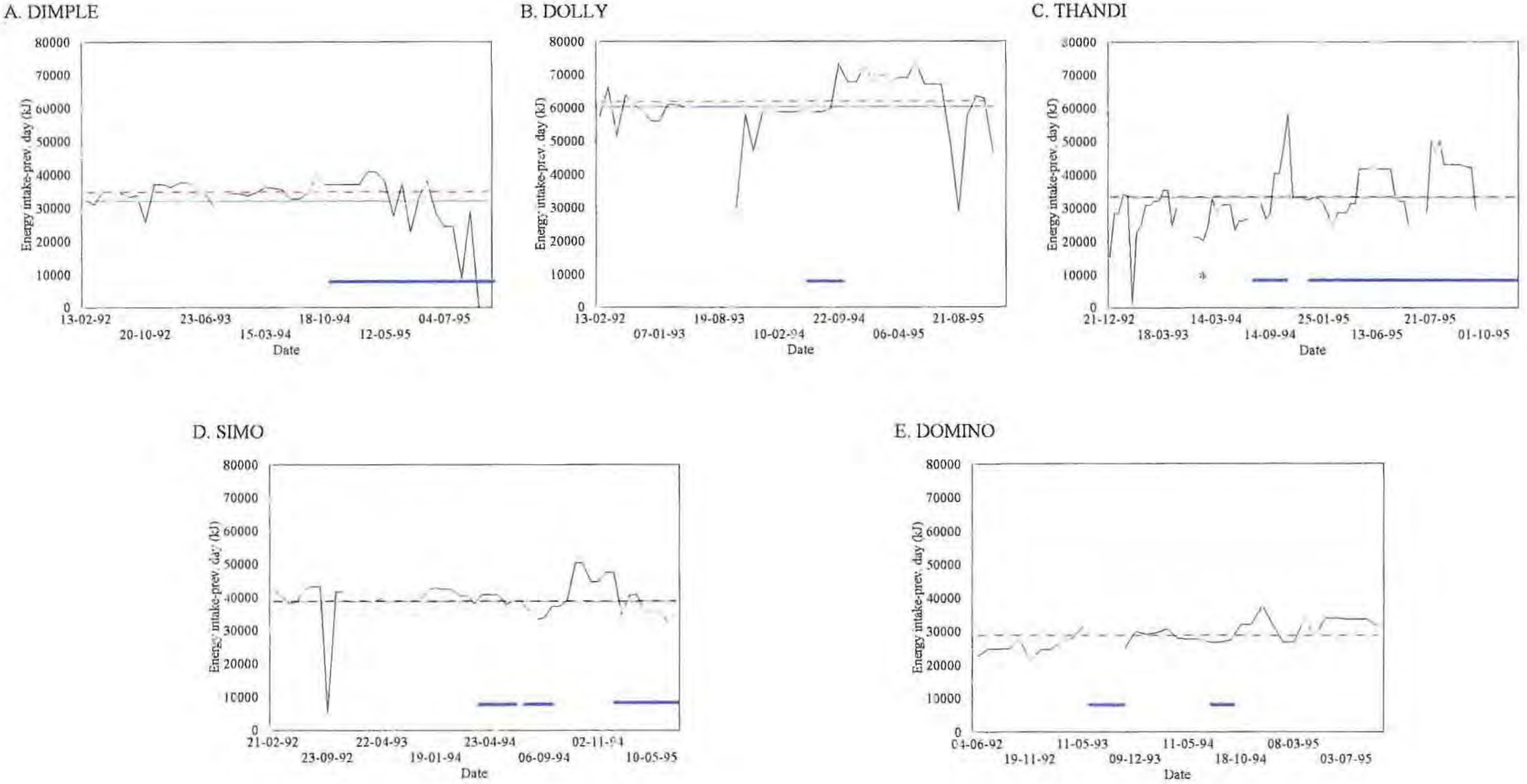


Figure 3.4: Dietary energy intake (kJ) of the day prior to blood sampling (—) for all five captive animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the overall mean energy intake of the previous day (—) and the "normal" (see text) mean energy intake (- -) of the day prior to blood sampling between 1992 and 1995 (*: parturition; —: extended periods of illness and/or treatment or periods of aberrant feeding and/or behaviour).

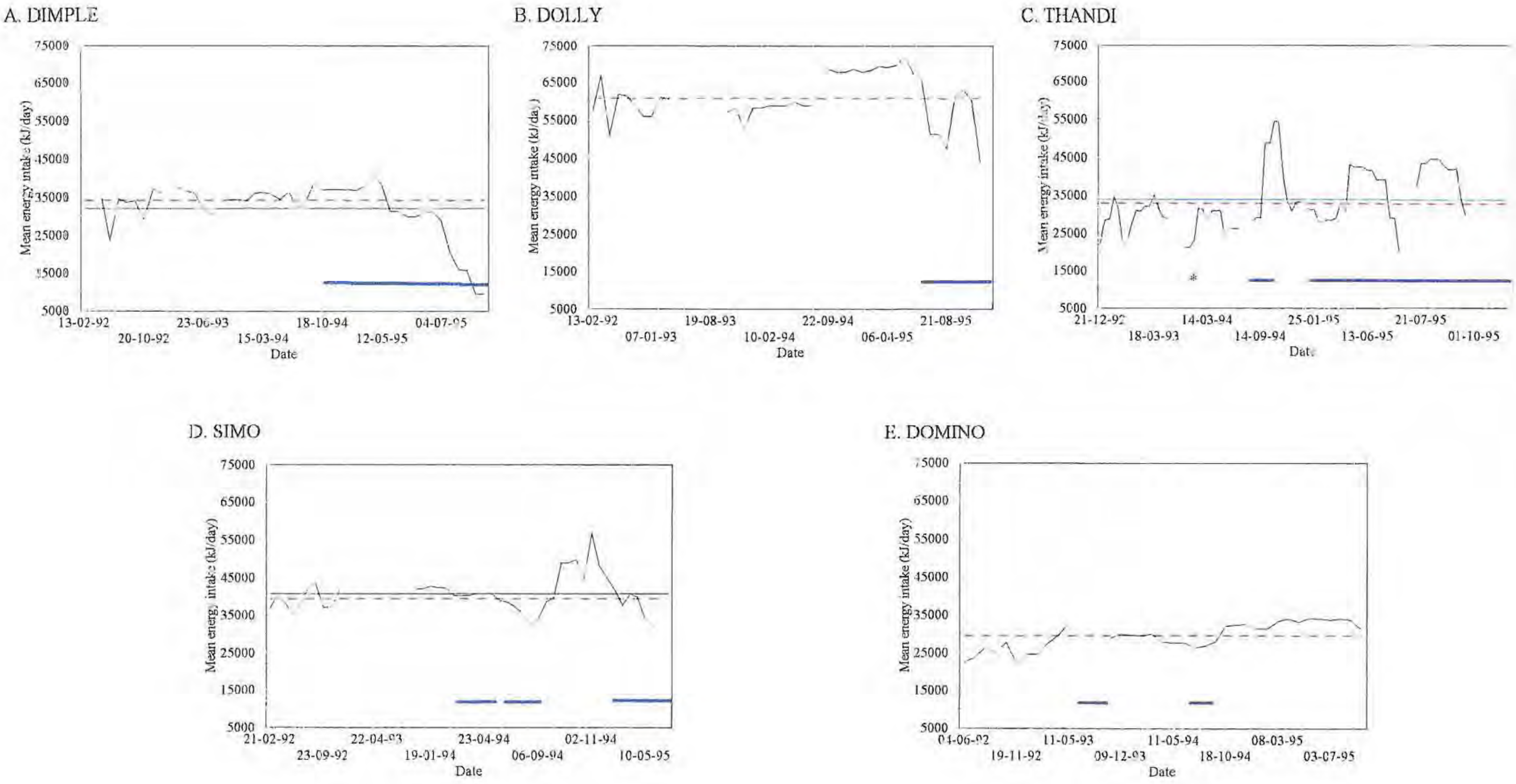
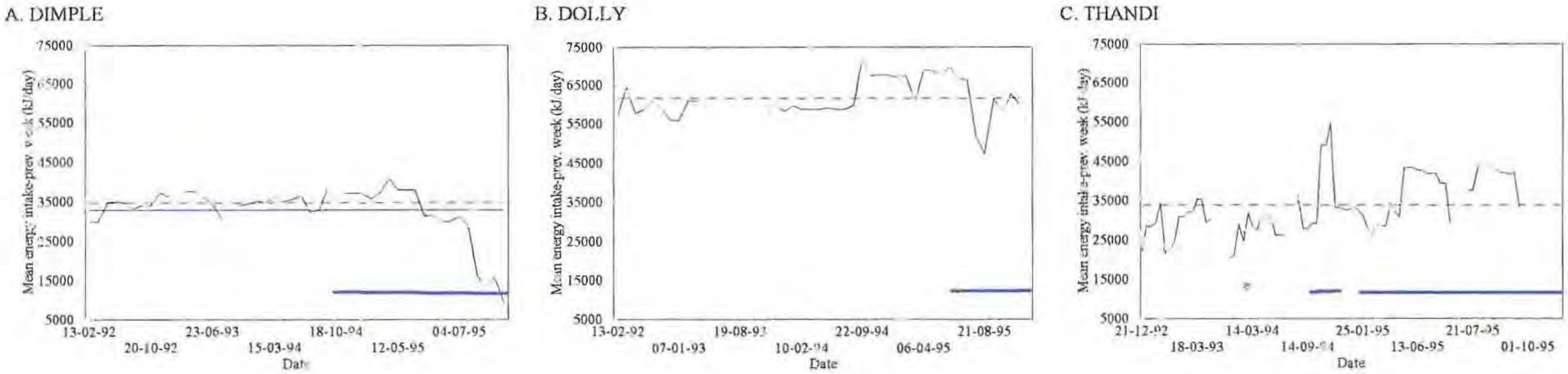


Figure 3.5: Mean daily dietary energy intake (kJ/day) for the week in which blood was sampled (—) for all five captive animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the overall mean daily energy intake (—) and the "normal" (see text) mean daily energy intake (- - -) for the week in which blood was sampled between 1992 and 1995 (*: parturition; ▬ : extended period of illness and/or treatment or period of aberrant feeding and/or behaviour).



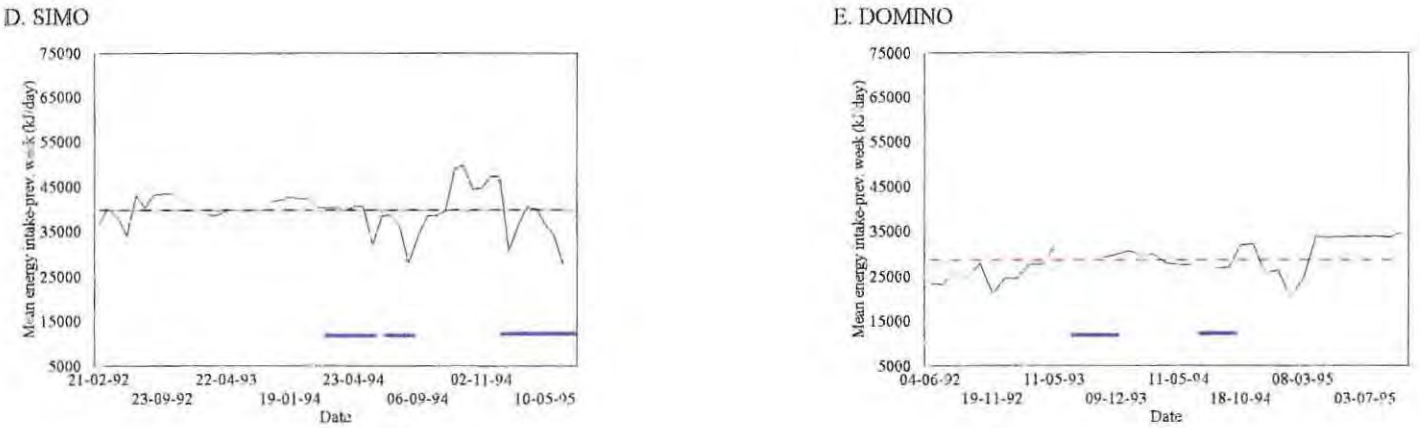


Figure 3.6: Mean daily dietary energy intake (kJ/day) of the week prior to blood sampling (—) for all five captive dolphins (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the overall mean daily energy intake (—) and the "normal" (see text) mean daily energy intake (- - -) of the week prior to blood sampling between 1992 and 1995 (*: parturition; ▬: extended periods of illness and/or treatment or periods of aberrant behaviour and/or feeding).

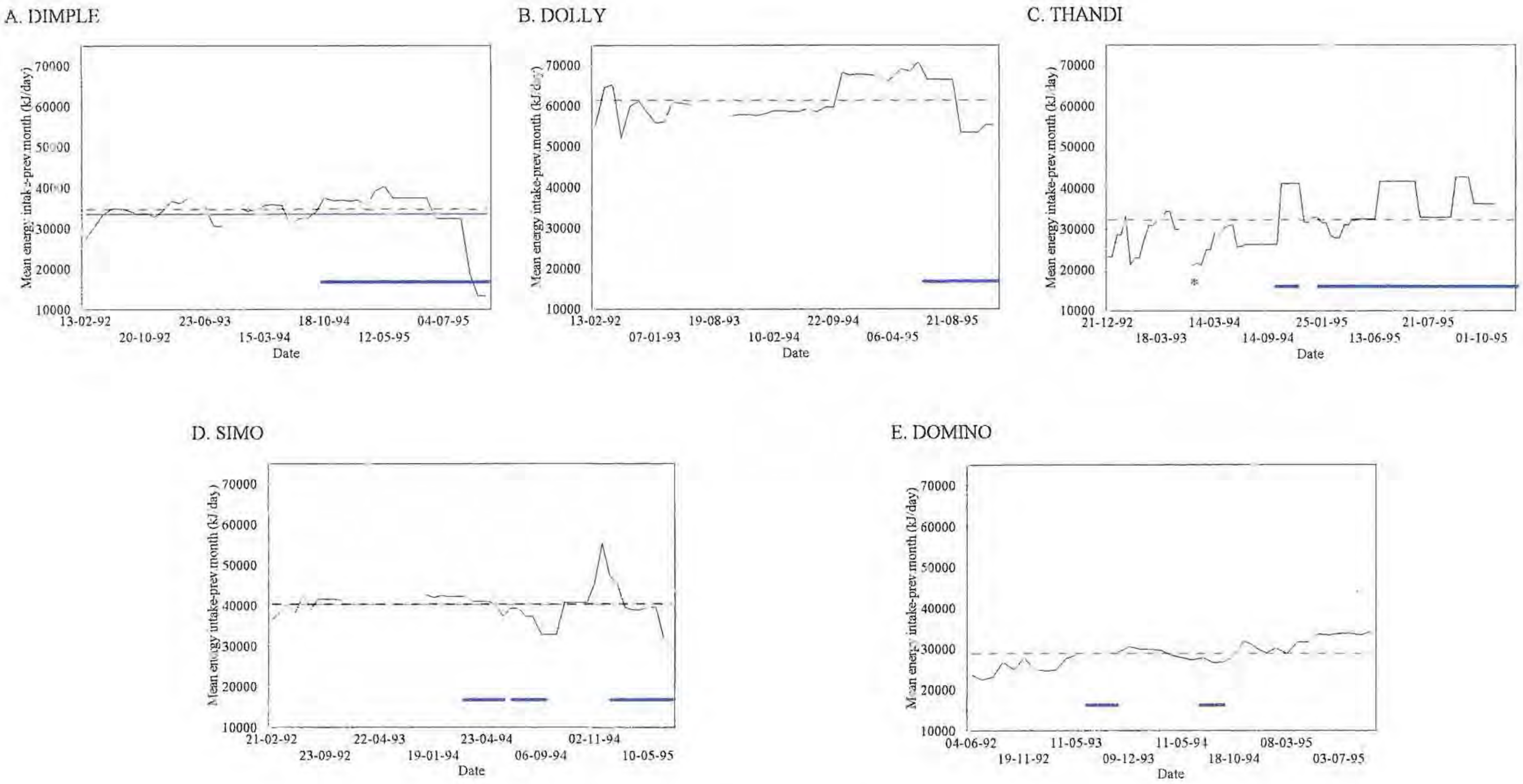


Figure 3.7: Mean daily dietary energy intake (kJ/day) for the month prior to blood sampling (—) for all five captive dolphins (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the overall mean daily energy intake (—) and the "normal" (see text) mean daily energy intake (- - -) for the month prior to blood sampling between 1992 and 1995 (*: parturition; ▬: extended periods of illness and/or treatment or period of aberrant feeding and/or behaviour).

Despite the exclusion of obvious outliers using statistical methods, results indicated that data were highly variable and that some data points included in the analyses may be associated with "unrecorded" abnormal or ill health episodes. However, subjective records of behaviour were not sufficient to remove these points and, for this reason, these data were included. As a result, the best-fit curves plotted to these data may reflect more complex relationships than actually exist. The equations for the curves of best fit are listed in Appendix 2.1. The type of curve and associated coefficients for the respective equation are listed for each animal in Appendix 2.2.

Leukocytic parameters

a. White blood cell (WBC) count

There were no significant differences in the mean WBC counts of each of the five animals before and after outliers were removed from the data (Table 3.4; Fig. 3.8). In addition, four animals showed no marked differences between the means obtained during the study and those recorded prior to the study. Thandi, however, had an overall mean WBC count of 14.53 ± 8.36 x10^9 cells/l during the study, which was approximately 50 % higher than the mean recorded previously (7.23 ± 4.07 x10^9 cells/l) (Fig. 3.8C). The range of data for four of the animals was fairly restricted (~5-10 x10^9 cells/l) but Thandi's data were highly variable (Appendix 1.3A). As a consequence of this high variability, no outliers were identified from Thandi's data (Appendix 1.3A). Outlying high WBC counts were identified from Dimple, Dolly and Simo (Appendix 1.3A), and were associated with periods of illness in all three animals, particularly Thandi (Fig. 3.8C). An increased WBC count was associated with pregnancy in both Dolly (Fig. 3.8B) and Thandi (Fig. 3.8C).

Age did not appear to have a significant effect on WBC count. Thandi was the only animal with a "normal" mean WBC count (13.53 ± 7.08 x10^9 cells/l) significantly higher than all four other animals (Two sample t-test; $p<0.001$). Dimple (8.64 ± 1.60 x10^9/l), Dolly (8.04 ± 1.78 x10^9/l) and Simo (7.86 ± 2.06 x10^9/l) had similar WBC counts (Two sample t-test, $p>0.005$). Dimple and Domino (7.59 ± 1.03 x10^9/l) had significantly different mean WBC counts ($t=3.51$, $p<0.001$, $df=82$) (Table 3.4). Overall, WBC counts were similar for four of the five animals, increased variability in Thandi's data resulting in a higher mean WBC count (Fig. 3.9). Specifically, WBC counts were significantly correlated with age during the study in two animals, Thandi and Domino (Table 3.5). Thandi's WBC count was relatively stable (~5-10 x10^9 cells/l) between the ages of 22 and 24 years, after which numbers of WBC's and data variability increased (Fig. 3.10A). Although WBC counts for Domino were highly variable, levels were generally higher (6.50-9.50 x10^9 cells/l) prior to age three years (Fig. 3.10B).

Table 3.4a: Mean leukocytic and haematological values and associated data for the captive bottlenose dolphin 'Dimple'.
Table A: Mean values calculated using all results obtained between 1992 and 1995
Table B: "Normal" mean values calculated from data exclusive of statistical outliers (box-whisker plot).

		Table A					**Table B**				
PARAMETER	**UNITS**	**MEAN**	**STD**	**RANGE**	**90% RANGE**	**N**	**MEAN**	**STD**	**RANGE**	**90% RANGE**	**N**
White blood cells (WBC)	x10^9/l	9.32	3.26	6.40-25.30	8.53-10.11	48	8.64	1.60	6.40-12.70	8.24-9.04	45
Neutrophils	%	65.85	6.60	54.00-85.00	64.26-67.45	48	64.78	5.22	54.00-75.00	63.47-66.09	45
Monocytes	%	5.36	3.45	1.00-16.00	4.52-6.21	47	5.13	3.10	1.00-12.00	4.36-5.90	46
Lymphocytes	%	23.63	5.61	6.00-36.00	22.27-24.98	48	23.71	3.43	17.00-31.00	22.82-24.61	42
Eosinophils	%	4.89	2.57	0.00-12.00	4.26-5.53	46	4.73	2.36	0.00-9.00	4.14-5.32	45
Red blood cells (RBC)	x10^12/l	4.04	0.23	3.57-4.64	3.98-4.09	48	4.01	0.20	3.57-4.47	3.96-4.06	46
Haemoglobin (HB)	g/dl	17.91	0.99	15.70-20.50	17.67-18.16	48	17.85	0.80	16.40-19.70	17.65-18.05	45
Packed cell volume (PCV)	l/l	0.50	0.03	0.46-0.58	0.50-0.51	48	0.50	0.02	0.46-0.55	0.49-0.50	43
Mean cell volume (MCV)	fl	124.90	2.54	122.00-133.00	124.30-125.50	48	124.40	1.69	122.00-129.00	124.00-124.80	45
Mean cell haemoglobin (MCH)	pg	44.36	0.60	42.70-46.00	44.22-44.51	48	44.40	0.45	43.30-45.20	44.28-44.51	45
Mean cell haemoglobin conc. (MCHC)	g/dl	35.50	0.87	32.90-37.30	35.29-35.71	48	35.65	0.66	34.30-37.30	35.49-35.82	45
Platelets	x10^9/l	92.14	22.46	47.00-129.00	83.90-100.40	22	92.14	22.46	47.00-129.00	83.90-100.4	22
Reticulocyte count	%	3.81	2.26	0.60-12.20	3.26-4.36	47	3.02	0.98	3.57-4.47	3.96-4.06	46
Corrected index	%	2.12	1.18	0.40-6.50	1.83-2.40	47	1.79	0.65	0.40-3.50	1.62-1.96	42

Table 3.4b: Mean leukocytic and haematological values and associated data for the captive bottlenose dolphin 'Dolly'.
Table A: Mean values calculated using all results obtained between 1992 and 1995
Table B: "Normal" mean values calculated from data exclusive of statistical outliers (box-whisker plot).

		Table A					**Table B**				
PARAMETER	**UNITS**	**MEAN**	**STD**	**RANGE**	**90% RANGE**	**N**	**MEAN**	**STD**	**RANGE**	**90% RANGE**	**N**
White blood cells (WBC)	x10^9/l	9.48	4.90	4.60-31.00	8.28-10.68	47	8.04	1.78	4.60-12.40	7.57-8.50	42
Neutrophils	%	71.67	8.60	48.00-86.00	156.90-158.60	46	71.67	8.60	48.00-86.00	69.55-73.80	46
Monocytes	%	3.63	2.33	1.00-12.00	3.05-4.21	46	3.30	1.73	1.00-8.00	2.86-3.73	44
Lymphocytes	%	21.87	7.06	7.00-39.00	20.12-23.62	46	21.87	7.06	7.00-39.00	20.12-23.62	46
Eosinophils	%	2.73	1.76	0.00-8.00	2.24-3.22	37	2.58	1.54	0.00-6.00	2.15-3.02	36
Red blood cells (RBC)	x10^12/l	4.60	1.41	3.83-5.13	4.54-4.67	47	4.62	0.23	4.06-5.13	4.56-4.68	46
Haemoglobin (HB)	g/dl	18.09	1.27	14.30-20.20	17.78-18.40	47	18.32	0.95	15.90-20.20	18.08-18.56	44
Packed cell volume (PCV)	l/l	0.52	0.03	0.42-0.58	0.51-0.53	47	0.52	0.02	0.47-0.58	0.52-0.53	42
Mean cell volume (MCV)	fl	112.80	3.69	105.00-120.00	111.90-113.70	47	112.80	3.69	105.00-120.00	111.90-113.70	47
Mean cell haemoglobin (MCH)	pg	39.36	1.55	36.30-41.60	34.73-35.13	47	39.36	1.55	36.30-41.60	38.98-39.73	47
Mean cell haemoglobin conc. (MCH	g/dl	34.93	0.83	32.60-36.70	34.73-35.13	47	34.98	0.67	33.30-36.40	34.81-35.15	44
Platelets	x10^9/l	132.90	40.64	42.00-212.00	119.80-146.00	28	136.30	37.22	74.00-212.00	124.10-148.50	27
Reticulocyte count	%	3.17	1.41	1.10-8.80	2.81-3.53	43	2.80	0.84	1.10-5.60	2.57-3.02	38
Corrected index	%	1.80	0.22	1.20-1.90	1.40-1.65	42	1.67	0.50	0.70-2.90	1.53-1.80	40

Table 3.4c: Mean leukocytic and haematological values and associated data for the captive bottlenose dolphin 'Thandi'.
Table A: Mean values calculated using all results obtained between 1992 and 1995
Table B: "Normal" mean values calculated from data exclusive of statistical outliers (box-whisker plot).

		Table A					**Table B**				
PARAMETER	**UNITS**	**MEAN**	**STD**	**RANGE**	**90% RANGE**	**N**	**MEAN**	**STD**	**RANGE**	**90% RANGE**	**N**
White blood cells (WBC)	x10^9/l	14.53	8.36	4.80-39.40	13.07-15.99	91	13.53	7.08	4.80-27.80	12.27-14.80	87
Neutrophils	%	70.98	10.12	48.00-92.00	69.18-72.77	88	70.98	10.12	48.00-92.00	69.18-72.77	88
Monocytes	%	4.06	2.37	1.00-11.00	3.63-4.49	85	3.83	2.07	1.00-9.00	3.45-4.21	82
Lymphocytes	%	19.00	9.54	2.00-41.00	17.31-20.69	88	19.00	9.54	2.00-41.00	17.31-20.69	88
Eosinophils	%	4.45	2.93	0.00-13.00	3.88-5.01	74	4.45	2.93	0.00-13.00	3.88-5.01	74
Red blood cells (RBC)	x10^12/l	4.04	0.38	3.08-4.79	3.98-4.11	91	4.07	0.35	3.14-4.79	4.00-4.13	89
Haemoglobin (HB)	g/dl	16.52	1.60	12.40-19.50	16.24-16.80	91	16.90	1.17	14.20-19.50	16.69-17.12	82
Packed cell volume (PCV)	l/l	0.47	0.05	0.35-0.56	0.46-0.48	91	0.47	0.04	0.36-0.56	0.47-0.48	89
Mean cell volume (MCV)	fl	116.30	3.77	110.00-132.00	115.70-117.00	90	116.10	3.40	110.00-124.00	115.50-116.70	89
Mean cell haemoglobin (MCH)	pg	40.86	1.21	38.80-43.50	40.65-41.08	91	40.86	1.21	38.80-43.50	40.65-41.08	91
Mean cell haemoglobin conc. (MCI	g/dl	35.10	0.91	30.20-36.60	34.94-35.26	91	35.18	0.74	31.50-36.60	35.05-35.31	89
Platelets	%	109.00	51.33	1.00-260.00	97.52-120.50	56	111.60	28.59	52.00-181.00	104.70-118.50	48
Reticulocyte count	%	5.03	3.08	0.40-14.20	4.48-5.58	86	4.43	2.22	0.40-10.20	4.02-4.85	80
Corrected index	%	2.53	1.37	0.20-6.40	2.29-2.78	85	2.38	1.20	0.20-6.40	2.16-2.60	81

Table 3.4d: Mean leukocytic and haematological values and associated data for the captive bottlenose dolphin 'Simo'.
Table A: Mean values calculated using all results obtained between 1992 and 1995
Table B: "Normal" mean values calculated from data exclusive of statistical outliers (box-whisker plot).

		Table A					**Table B**				
PARAMETER	**UNITS**	**MEAN**	**STD**	**RANGE**	**90% RANGE**	**N**	**MEAN**	**STD**	**RANGE**	**90% RANGE**	**N**
White blood cells (WBC)	x10^9/l	8.61	4.12	4.90-31.90	7.66-9.56	53	7.86	2.06	4.90-13.00	7.37-8.35	50
Neutrophils	%	63.71	11.77	41.00-85.00	60.98-66.45	52	63.71	11.77	41.00-85.00	60.98-66.45	52
Monocytes	%	5.02	3.28	2.00-22.00	4.24-5.81	49	4.53	1.98	2.00-9.00	4.05-5.02	47
Lymphocytes	%	21.69	7.53	7.00-37.00	19.92-23.45	51	21.69	7.53	7.00-37.00	19.92-23.45	51
Eosinophils	%	9.78	6.26	0.00-24.00	8.30-11.27	50	8.63	5.07	0.00-18.00	7.38-9.89	46
Red blood cells (RBC)	x10^12/l	4.57	0.43	3.50-5.35	4.47-4.67	53	4.58	0.33	3.76-5.04	4.50-4.66	47
Haemoglobin (HB)	g/dl	17.98	1.81	13.60-21.00	17.56-18.39	53	18.60	1.10	16.30-21.00	18.33-18.88	45
Packed cell volume (PCV)	l/l	0.51	0.05	0.39-0.62	0.5-0.53	53	0.53	0.03	0.45-0.62	0.52-0.54	43
Mean cell volume (MCV)	fl	112.70	2.95	107.00-121.00	112.00-113.40	53	112.10	2.27	107.00-117.00	111.60-112.70	49
Mean cell haemoglobin (MCH)	pg	39.40	1.18	36.70-41.70	39.13-39.67	53	39.45	1.12	37.60-41.70	39.19-39.71	52
Mean cell haemoglobin conc (MCH	g/dl	34.95	0.88	31.40-37.00	34.75-35.16	53	34.98	0.68	33.50-36.30	34.82-35.14	51
Platelets	x10^9/l	162.40	44.29	32.00-249.00	150.80-174.10	41	165.70	39.56	100.00-249.00	155.20-176.20	40
Reticulocyte count	%	4.78	2.33	1.60-9.40	4.24-5.32	52	4.78	2.33	1.60-9.40	4.24-5.32	52
Corrected index	%	2.64	1.12	1.00-5.20	2.37-2.90	51	2.64	1.12	1.00-5.20	2.37-2.90	51

Table 3.4e: Mean leukocytic and haematological values and associated data for the captive bottlenose dolphin 'Domino'.
Table A: Mean values calculated using all results obtained between 1992 and 1995
Table B: "Normal" mean values calculated data exclusive of statistical outliers (box-whisker plot).

		Table A					**Table B**				
PARAMETER	**UNITS**	**MEAN**	**STD**	**RANGE**	**90% RANGE**	**N**	**MEAN**	**STD**	**RANGE**	**90% RANGE**	**N**
White blood cells (WBC)	x10^9/l	7.59	1.03	5.50-9.60	7.31-7.87	39	7.59	1.03	5.50-9.60	7.31-7.87	39
Neutrophils	%	55.38	11.12	31.00-75.00	52.38-58.39	39	55.38	11.12	31.00-75.00	52.38-58.39	39
Monocytes	%	3.43	1.79	1.00-7.00	2.94-3.93	37	3.43	1.79	1.00-7.00	2.94-3.93	37
Lymphocytes	%	31.51	7.90	21.00-48.00	29.38-33.65	39	31.51	7.90	21.00-48.00	29.38-33.65	39
Eosinophils	%	10.03	5.18	1.00-11.00	8.59-11.46	37	10.03	5.18	1.00-20.00	8.59-11.46	37
Red blood cells (RBC)	x10^12/l	4.33	0.34	3.49-5.14	4.24-4.42	39	4.33	0.28	3.80-4.91	4.25-4.41	37
Haemoglobin (HB)	g/dl	17.32	1.41	13.90-21.20	16.94-17.70	39	17.31	1.16	15.10-19.40	16.99-17.63	37
Packed cell volume (PCV)	l/l	0.49	0.04	0.40-0.58	0.48-0.50	39	0.49	0.03	0.42-0.53	0.48-0.50	35
Mean cell volume (MCV)	fl	113.80	1.77	111.00-119.00	113.30-114.20	39	113.60	1.57	111.00-118.00	113.20-114.10	38
Mean cell haemoglobin (MCH)	pg	40.00	0.85	37.20-41.50	39.77-40.23	39	40.08	0.72	38.50-41.50	39.88-40.27	38
Mean cell haemoglobin conc. (MCH	g/dl	35.17	0.80	33.00-36.50	34.95-35.39	39	35.23	0.73	33.40-36.50	35.03-35.43	38
Platelets	x10^9/L	180.50	41.96	98.00-245.00	167.00-194.00	28	198.80	23.92	166.00-245.00	190.00-207.60	22
Reticulocyte count	%	5.21	2.32	1.50-12.20	4.58-5.83	39	5.02	2.04	1.50-9.80	4.46-5.58	38
Corrected index	%	2.79	1.15	0.70-6.00	2.47-3.10	39	2.70	1.04	0.70-5.00	2.42-2.98	38

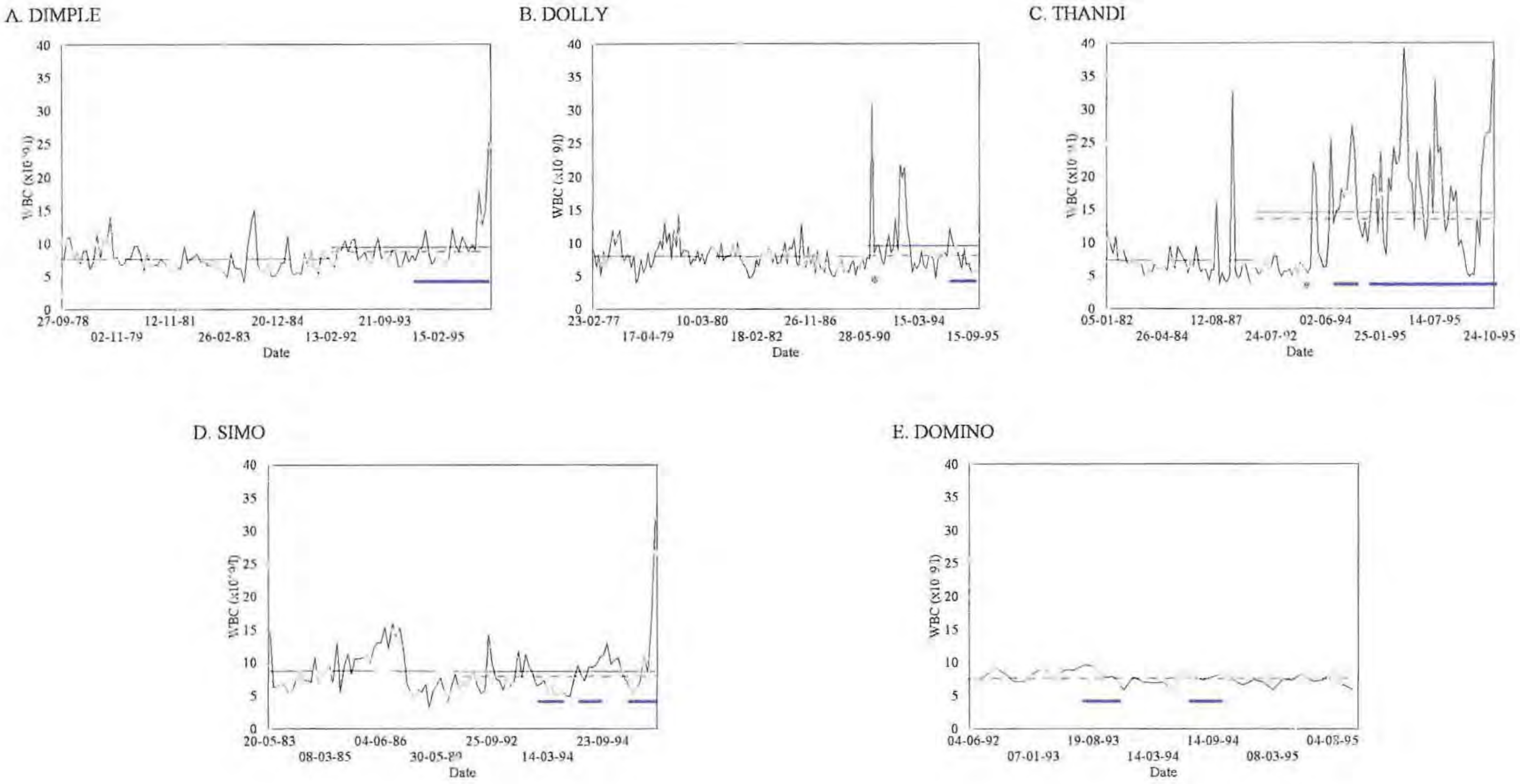


Figure 3.8: White blood cell (WBC) count (x10 9/L) (—) for all five captive animals (A. Dimple; B. Dolly; C. Thandi, D. Simo; E. Domino). Also shown, for each animal, is the overall mean WBC count for all records prior to the study (- -), the overall mean WBC count (—) and the "normal" (see text) mean WBC count (- - -) for the study between 1992 and 1995 (*: parturition; —: extended periods of illness and/or treatment or period of aberrant feeding and/or behaviour).

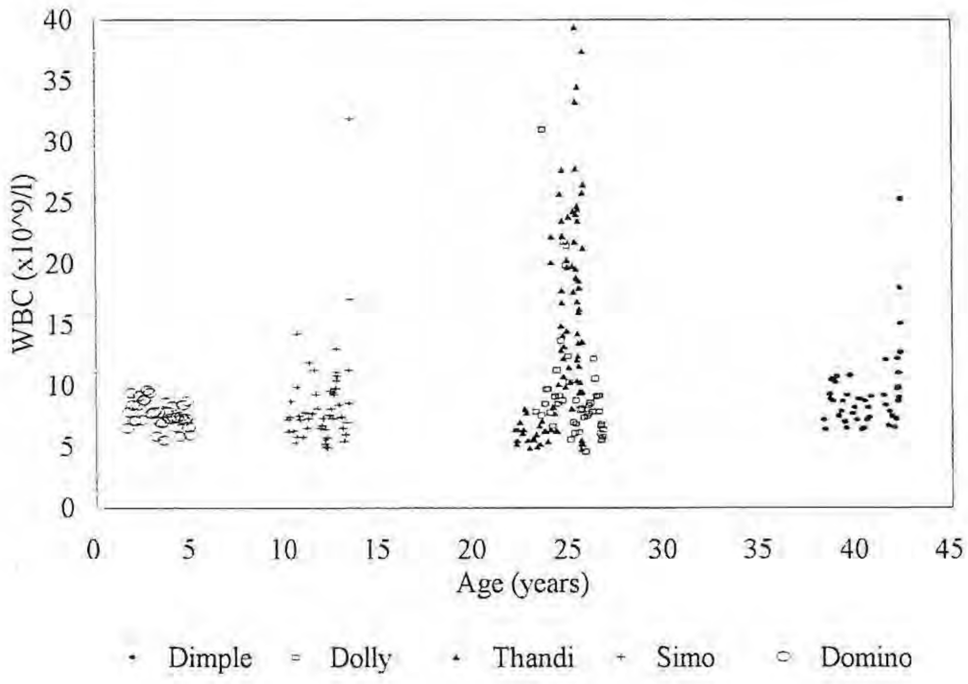
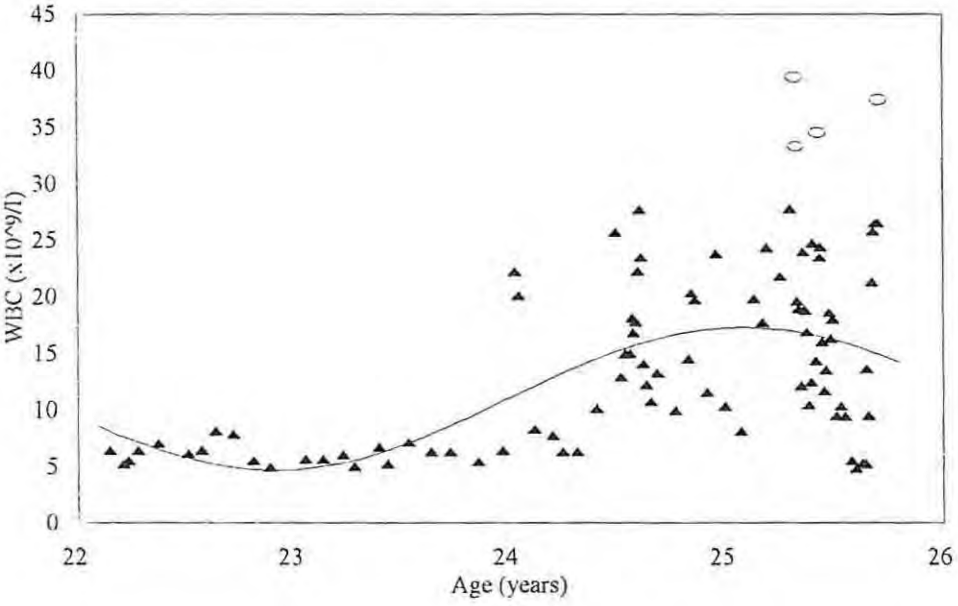


Figure 3.9: Relationship between age and white blood cell (WBC) counts, recorded between 1992 and 1995, for the five captive dolphins.

A. THANDI



B. DOMINO

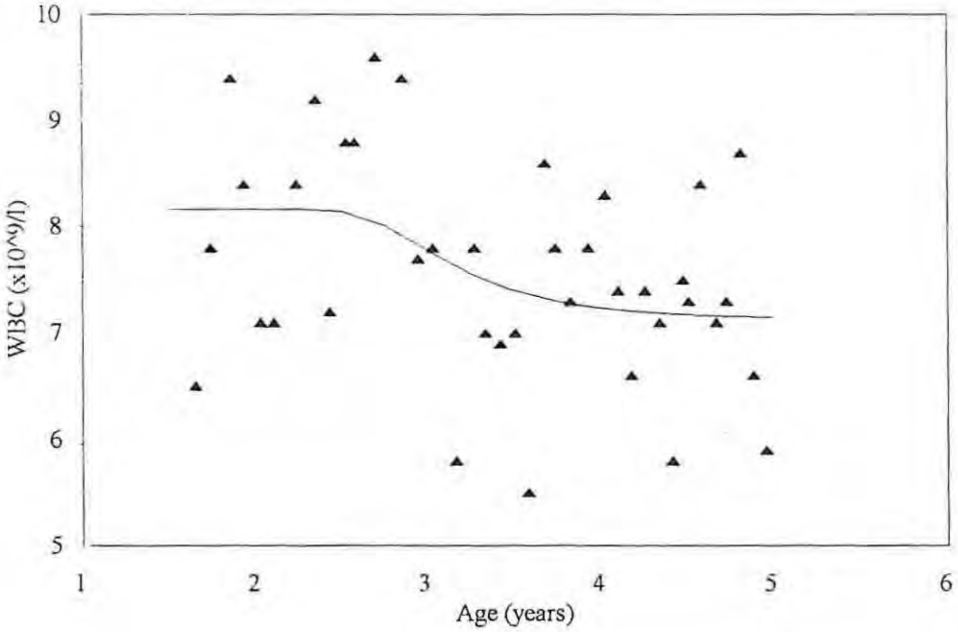


Figure 3.10: Curves of best fit ( ) for animals (A. Thandi; B. Domino) with significant correlations ($p<0.05$) between white blood cells (WBC) and age. Included in the graph are all "normal" (see text) data ( ) and statistically-determined outliers on the y-axis ( ).

Table 3.5: Correlation coefficients (r), levels of significance (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$) and sample sizes (n) for significant relationships between white blood cell (WBC) counts and independent variables for the five captive dolphins (Data are presented as r*(n)) (NS: non-significant ($p>0.05$)).

Variable	Dimple	Dolly	Thandi	Simo	Domino
Age	NS	NS	0.55***(85)	NS	-0.34*(39)
Weight	NS	NS	-0.28*(57)	NS	NS
D1	0.49**(40)	NS	0.26*(69)	NS	NS
E1	-0.39**(41)	NS	NS	NS	NS
E2	-0.43**(40)	NS	NS	-0.43**(38)	NS
Month	0.33*(45)	NS	NS	0.50***(50)	NS
T1	-0.55***(45)	NS	NS	-0.48***(50)	NS
T2	-0.56***(45)	NS	NS	-0.52***(49)	NS
T3	-0.62***(45)	NS	NS	-0.60***(50)	NS

D1: Total food consumption of week prior to blood sample, E1: Dietary energy intake of day prior to the blood sample; E2: Mean daily energy intake of week in which blood was sampled, T1: Mean water temperature of week prior to blood sample, T2: Mean water temperature of month in which blood was sampled, T3: Mean water temperature of month prior to blood sample (T3).

White blood cells were not significantly affected by changes in body weight for any of the five captive dolphins. Although WBC data from Thandi was negatively correlated with total body weight (Table 3.5), data were highly variable, showing no distinct trend (Fig. 3.11). WBC counts, associated with significantly low total body weights (< 155 kg), were within the "normal" WBC range for this animal. None of the animals showed any relationship between WBC and blubber thickness.

The WBC counts of Dimple and Thandi were positively correlated with total food consumption of the week prior to blood sampling (Table 3.5). However, data were variable and no clear trend could be distinguished. In addition, outlying food consumption levels (< 40 kg/week; > 90kg/week) did not seem to affect WBC count, with results remaining within the "normal" range. WBC's were only correlated with the dietary energy intake of the day preceding the blood sample for Dimple (Table 3.5), with WBC's apparently decreasing as energy intake increased (Fig. 3.12). Outlying increased WBC counts were all associated with low levels of energy intake. Both Dimple and Simo showed significant negative correlations between WBC count and the mean energy intake of the week in which blood was sampled (Table 3.5) (Fig. 3.13). Dimple's WBC count decreased (~ 11.00-6.50 x10^9 cells/l) energy intake increased between 27 000 kJ/day and 42 000 kJ/day, with all outlying WBC counts (>15.00 x10^9 cells/l) associated with significantly low levels of energy intake (<25 000 kJ/day) (Fig. 3.13A). The relationship between these two parameters was more complex for Simo (Fig. 3.13B). WBC counts were generally high (8.50-11.50 x10^9 cells/l) at energy intake levels of between 30 000 and 35 000 kJ/day. As energy intake increased above 35 000 kJ/day WBC

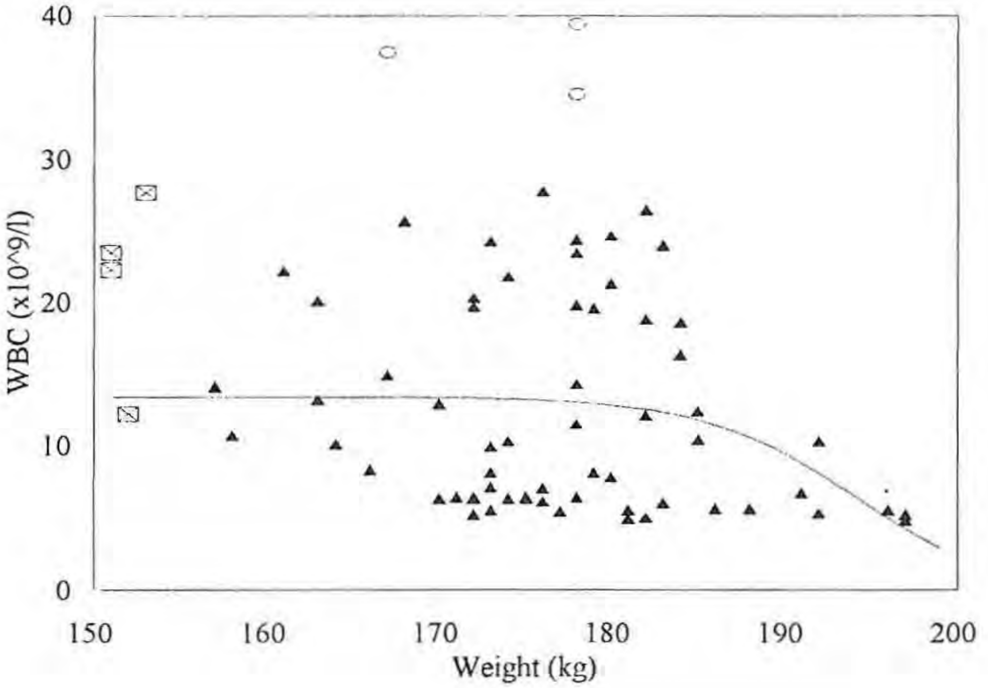
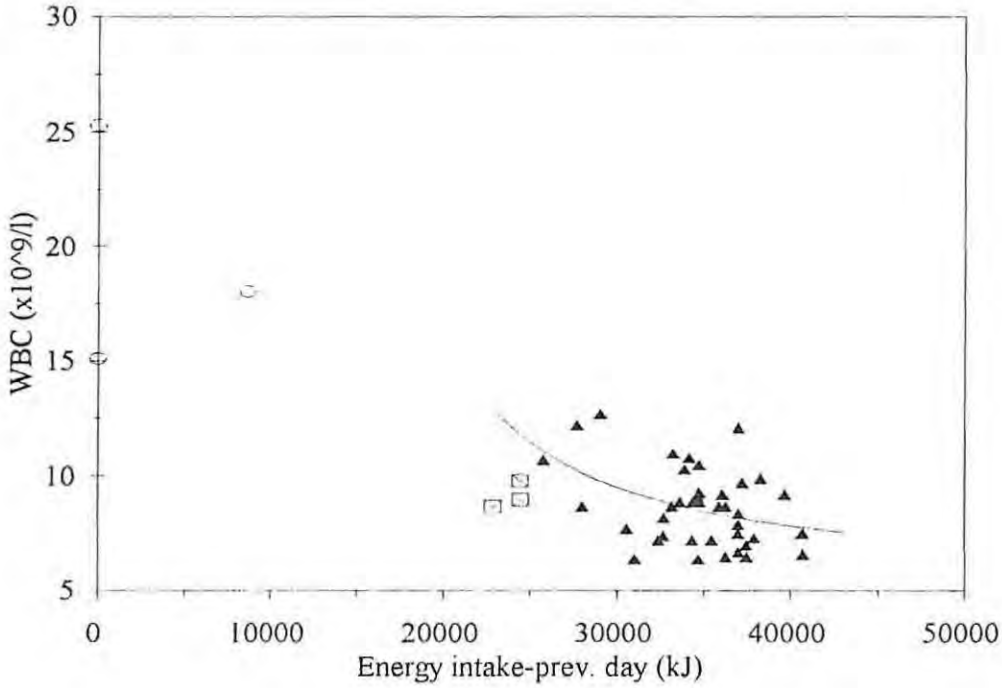


Figure 3.11: Curve of best fit (—) for Thandi with a significant correlation ($p<0.05$) between white blood cells (WBC) and weight. Included in the graph are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis ○).

A.



B.

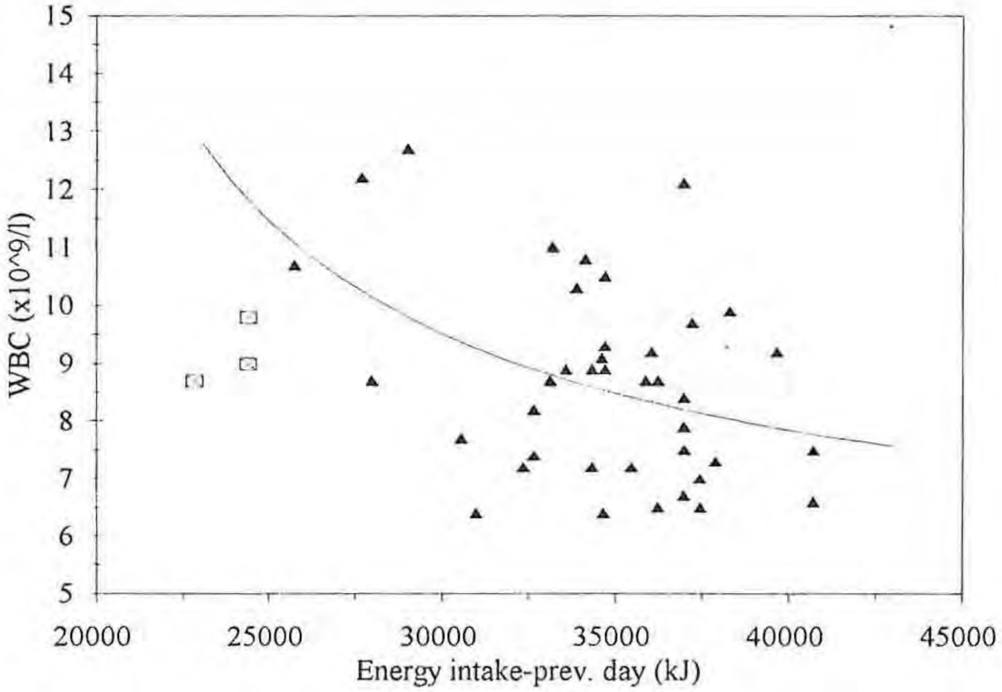
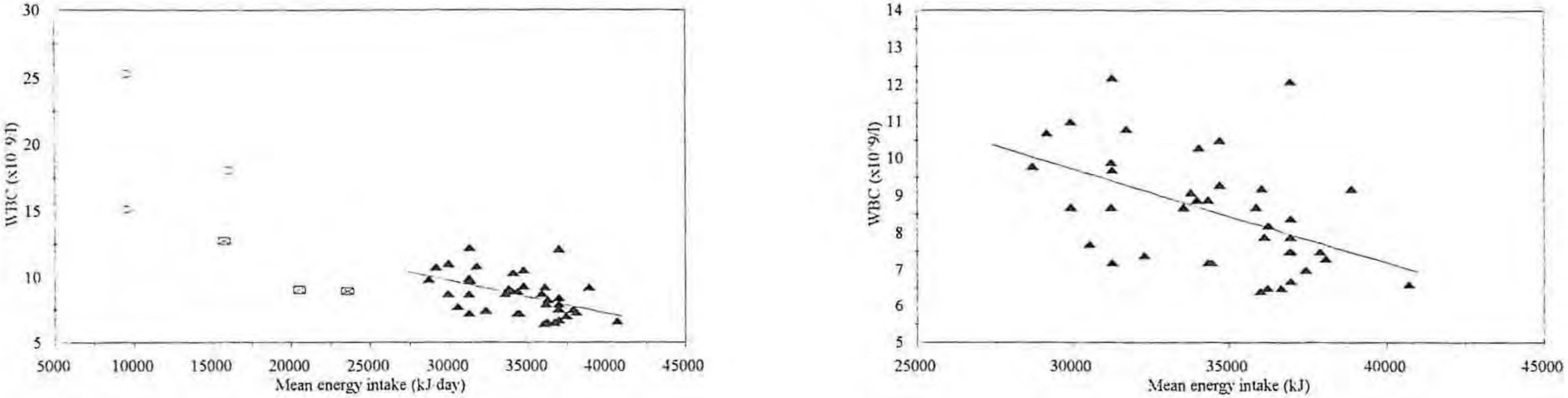


Figure 3.12: Curves of best fit (—) for Dimple (A & B) with a significant correlation ($p<0.05$) between white blood cells (WBC) and the dietary energy intake of the day prior to the blood sample. Included in the graph are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ⊠ ; y-axis: ○).

A. DIMPLE (a)

(b)



B. SIMO (a)

(b)

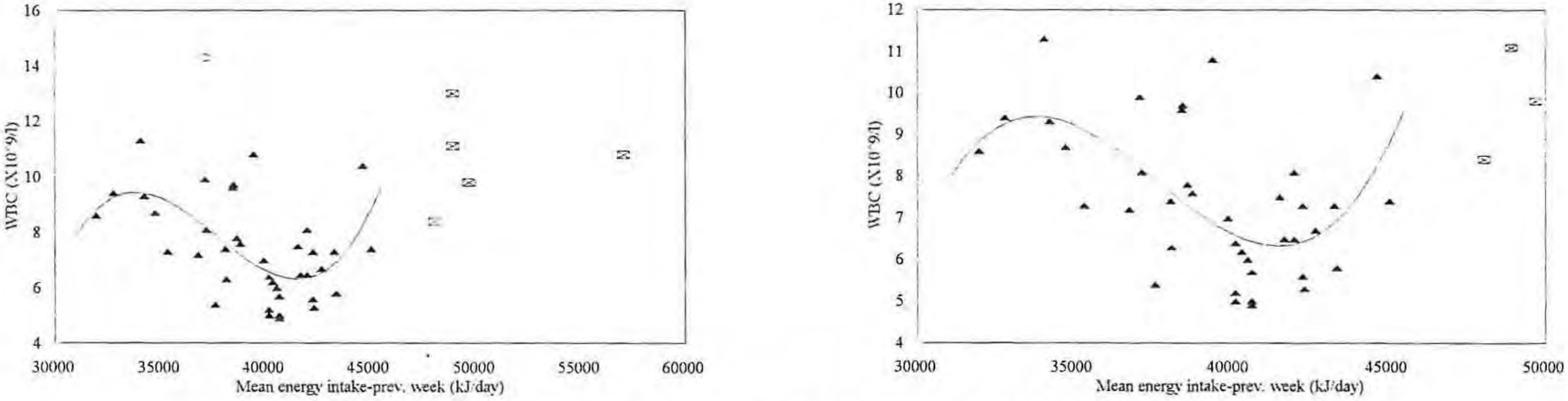


Figure 3.13: Curves of best fit (—) for animals (A. Dimple (a & b); B. Simo (a & b)) with significant correlations ($p<0.05$) between white blood cells (WBC) and the mean daily dietary energy intake of the week in which blood was sampled. Included in the graph are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

counts decreased (~5.00 to 7.00 x10^9 cells/l). Above energy intake levels of 45 000 kJ/day, WBC counts again appeared to increase, although limited data makes this trend difficult to define. WBC counts corresponding to energy intake levels above the norm (>47 500 kJ/day) were high (>8.00 x10^9 cells/l) but remained within the normal WBC range (3.13B.b). WBC counts were not significantly related to dietary energy intake in the longer-term.

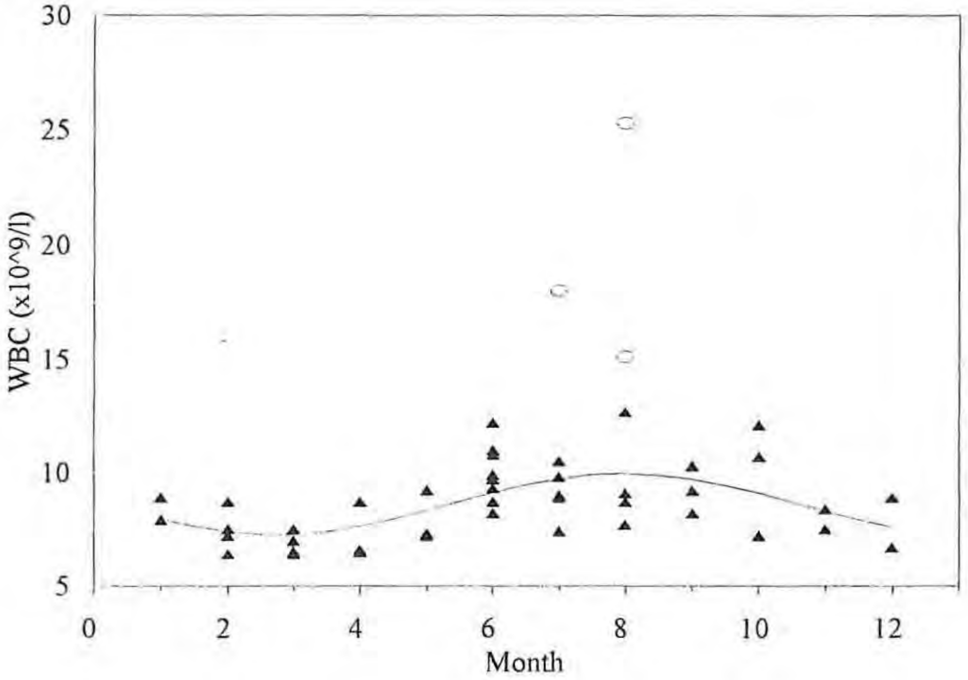
White blood cell counts were positively correlated with the month of the year for Dimple and Simo (Table 3.5) (Fig. 3.14). WBC's were relatively stable at low levels (~6-9 x10^9 cells/l) between January and April (summer-autumn), increasing slightly (from 7.00 x10^9 cells/l to 12.00 x10^9 cells/l) between June and October. In the final two months of the year, WBC levels began to decrease again to their original levels. High outliers, from both animals, were only identified in the winter months (Fig. 3.14). In addition, Dimple's and Simo's WBC counts were significantly correlated with all three categories of water temperature (Table 3.5) (Fig. 3.15). Although the relationships were not identical, all showed a similar trend. WBC counts were generally higher and more variable at water temperatures below 21°C. Outliers, for both Dimple and Simo, were identified only at water temperatures below 20°C (Fig. 3.15).

b. Neutrophils

Neutrophil levels were generally highly variable (Appendix 1.3B; Fig. 3.16). Dimple was the only animal for which neutrophil levels remained relatively stable (Fig. 3.16A). As a result of this high variability, very few outlying neutrophil values were identified (Appendix 1.3B). Values above 80 %, in the case of Dimple only, were classified as significantly high (Box-whisker plot). These increased neutrophil values were associated with a period of illness at the end of the study (Fig. 3.16A). No similar associations could be identified for other animals (Fig. 3.16). Mean neutrophil levels were between 60 % and 70 % for all animals, except Domino (55.38 ± 11.12 %) (Table 3.4). Domino's mean neutrophil count was significantly lower than all other animals (Two sample t-test; $p<0.0001$) (Table 3.4e). Dolly (71.67 ± 8.60 %) and Thandi (70.98 ± 10.12 %) ($t=0.40$; $p=0.69$; $df=132$) had significantly higher mean neutrophil levels. Dimple (64.78 ± 5.22 %) and Simo (63.71 ± 11.77 %) had similar mean neutrophils counts ($t=0.56$; $p=0.58$; $df=95$), although these were significantly lower than Dolly and Thandi (Table 3.4).

Overall, neutrophil values appeared to increase with age, being lowest in Domino and highest in Thandi and Dolly (Fig. 3.17). However, Dimple's neutrophil data illustrated that neutrophils may be reduced in elderly animals. More specifically, neutrophils were positively correlated with age during the study in Thandi and Simo (Table 3.6). The age-related increase in neutrophils, evident for Thandi, was due primarily to an increase in variability in the latter half of the study (Fig.

A. DIMPLE



B. SIMO

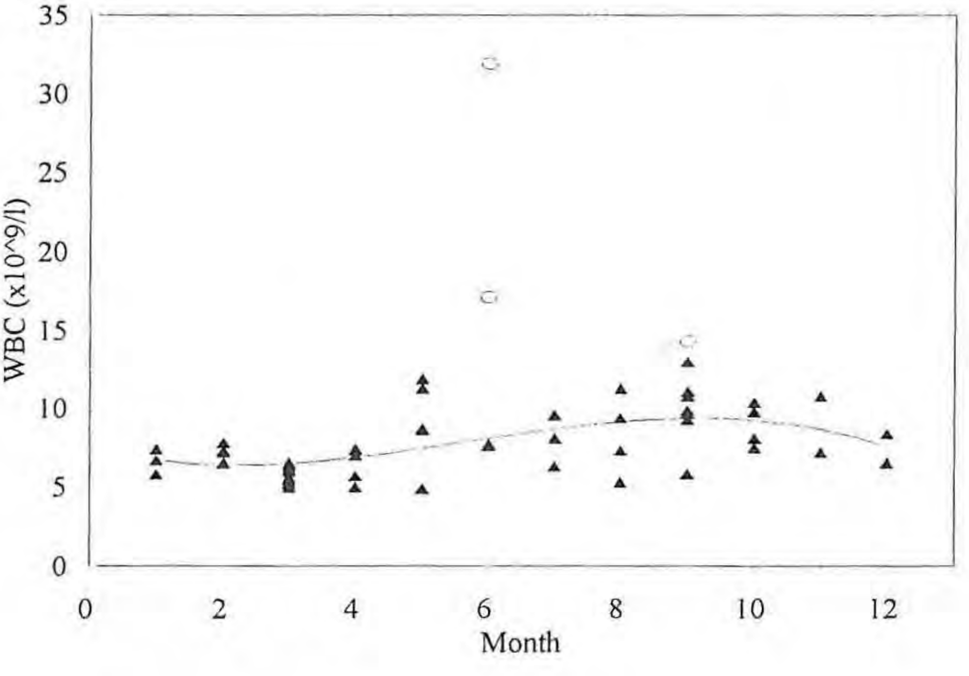


Figure 3.14: Curves of best fit (—) for animals (A. Dimple; B. Simo) with significant correlations ($p<0.05$) between white blood cells (WBC) and the month of the year. Included in the graph are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

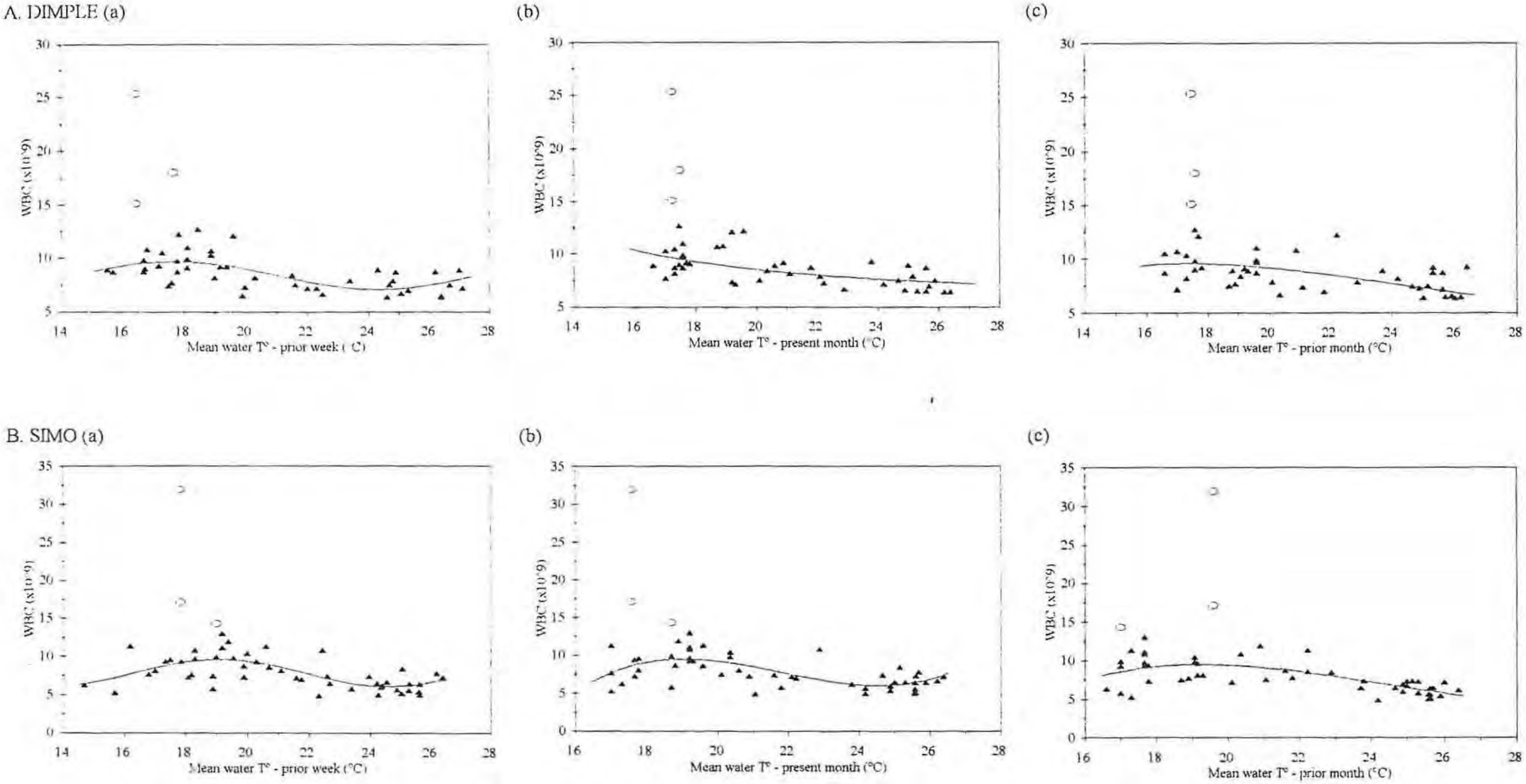


Figure 3.15: Curves of best fit (—) for animals (A. Dimple; B.Simo) with significant correlations ($p<0.05$) between white blood cells (WBC) and the mean water temperature of the week prior to the blood sample (a), the mean water temperature of the month in which blood was sampled (b) and the mean water temperature of the month prior to the blood sample (c). Included in the graph are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

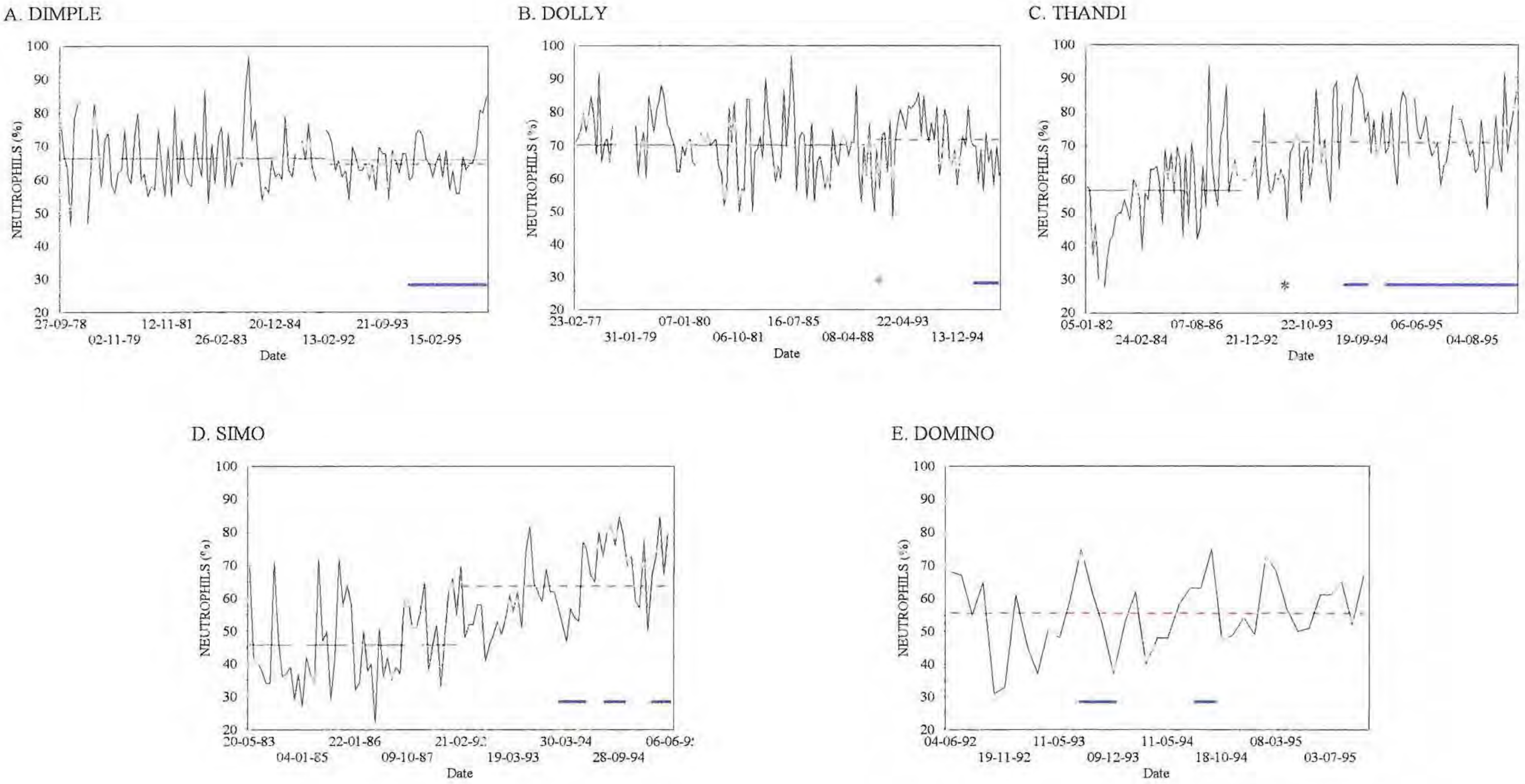


Figure 3.16: Neutrophil levels (%) (——) for all five captive dolphins (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino) Also shown, for each animal, is the mean neutrophil level for all records prior to the study (– –); the overall mean neutrophil level (——) and the "normal" (see text) mean neutrophil level (- - -) for the study between 1992 and 1995 (*: parturition; ▬: extended periods of illness and/or treatment or period of aberrant feeding and/or behaviour).

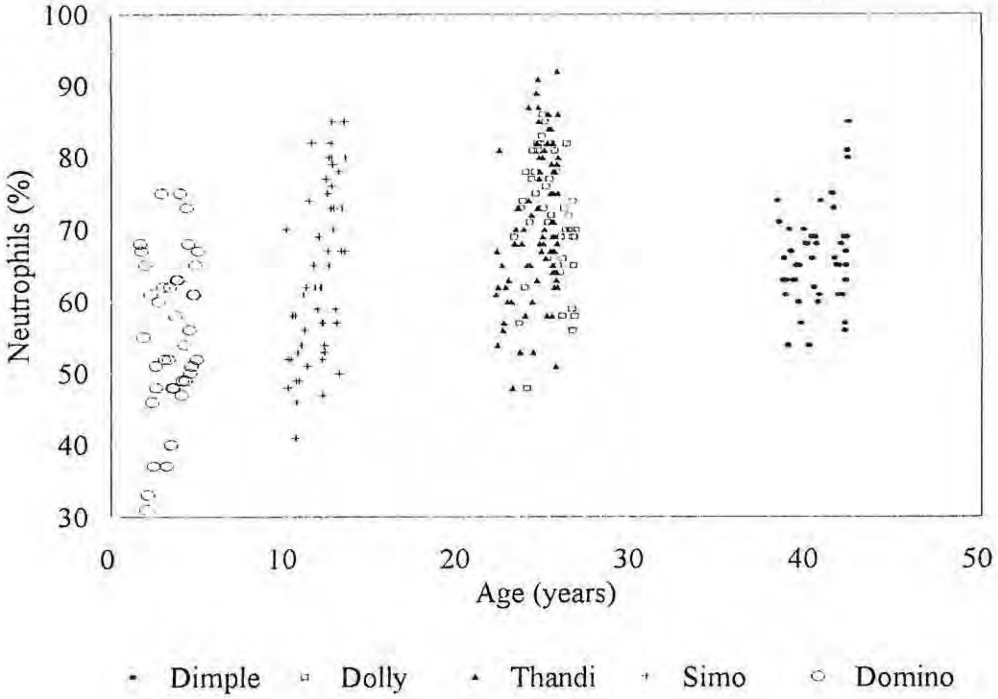


Figure 3.17: Relationship between neutrophil percentage and age of the five captive dolphins for records obtained between 1992 and 1995.

3.18A). In contrast, to this, Simo's neutrophil levels increased steadily from approximately 50 % to 80 % between the ages of 10 and 14 years (Fig. 3.18B). Neutrophils were negatively correlated with weight for Dolly and Thandi (Table 3.6) (Fig. 3.19). Data were variable, but high neutrophil values were recorded predominantly at lower weights, for both animals. Outlying low total body weights, recorded for Thandi (< 155 kg), were associated with high, but "normal" neutrophil levels (> 79 %) (Fig. 3.19B).

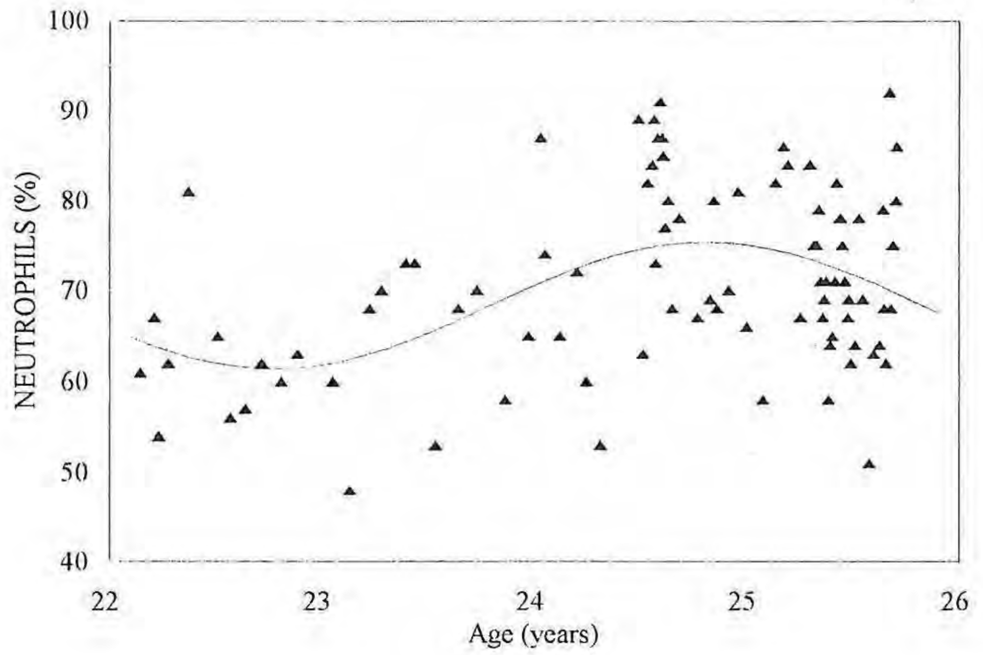
Table 3.6: Correlation coefficients (r), levels of significance (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$) and sample sizes (n) for significant relationships between neutrophils and independent variables for the five captive dolphins (Data are presented as r*(n)) (NS: non-significant ($p>0.05$)).

Variable	Dimple	Dolly	Thandi	Simo	Domino
Age	NS	NS	0.35**(86)	0.58***(52)	NS
Weight	NS	-0.29*(43)	-0.31*(57)	NS	NS
E1	NS	NS	NS	-0.46**(44)	NS
E3	NS	NS	NS	-0.37*(42)	NS
E4	-0.32*(43)	NS	NS	NS	NS
T1	NS	NS	NS	-0.37**(52)	-0.35*(39)
T2	NS	NS	NS	-0.28*(51)	-0.40**(39)
T3	NS	NS	NS	-0.31*(52)	-0.32*(39)

E1: Dietary energy intake of day prior to blood sample; E3: Mean daily energy intake of week and month (E4) prior to blood sample, T1: Mean water temperature of week prior to blood sample, T2: Mean water temperature of month in which blood was sampled, T3: Mean water temperature of month prior to blood sample.

None of the five animals exhibited significant relationships between neutrophil levels, basic levels of food consumption and/or recent dietary energy intake. However, Simo's neutrophil data were negatively correlated with the mean daily energy intake of the week prior to the blood sample (Table 3.6). Although the trend was not distinct, neutrophils appeared to decrease with increased energy intake (Fig. 3.20A). Data variability was highest (45-85 %) at mean energy intake levels around 40 000 kJ/day. Neutrophil levels measured during periods of aberrant dietary energy intake were high, but "normal". Similarly, Dimple's neutrophil data were negatively correlated with the mean dietary energy intake of the month prior to the blood sample (Table 3.6). Although data were variable (~53-75 %), neutrophils were generally increased (60-75 %) at energy intake levels below 33 000 kJ/day (Fig. 3.20C). Outlying high neutrophil levels (>80 %) were related to outlying low (<20 000 kJ/day) energy intake levels (Fig. 3.20B).

A. THANDI



B. SIMO

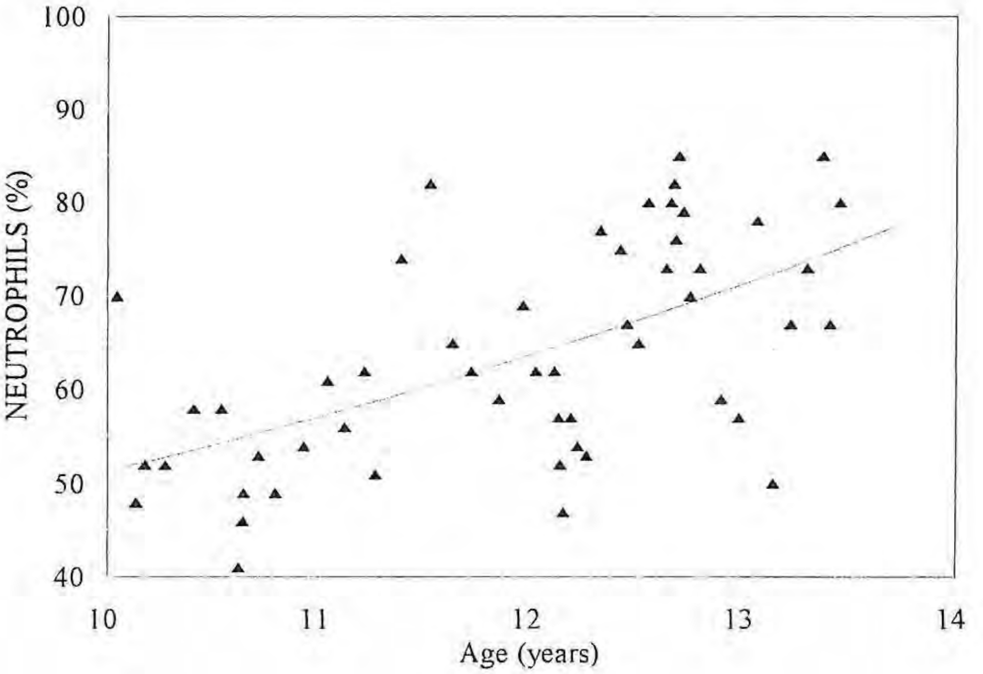


Figure 3.18: Curves of best fit (—) for animals (A. Thandi; B. Simo) with significant correlations ($p<0.05$) between neutrophils and age during the study.

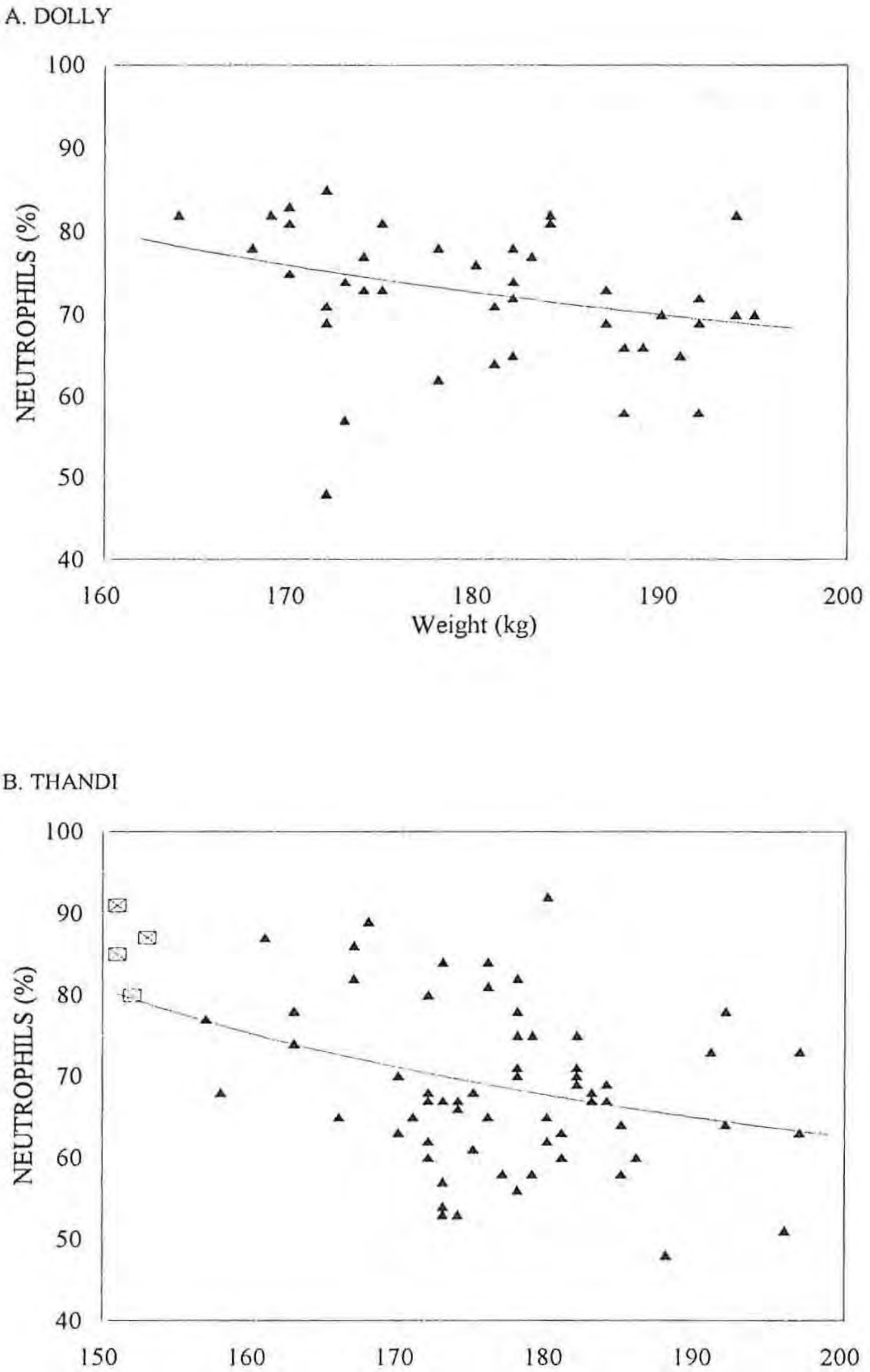
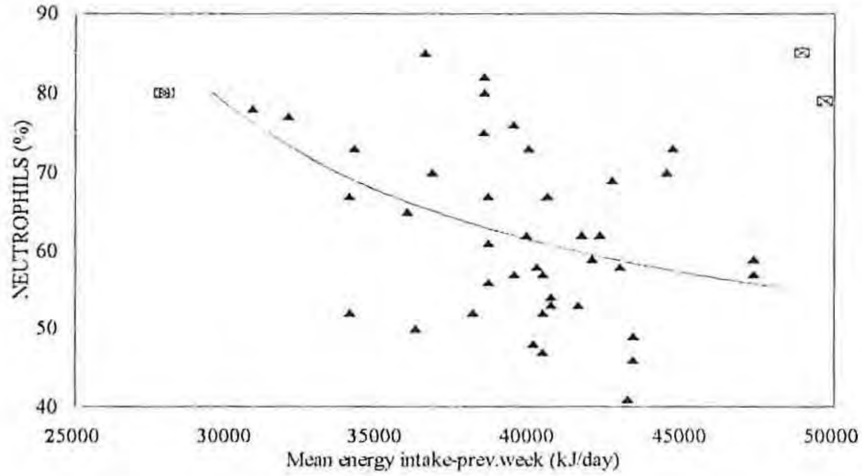
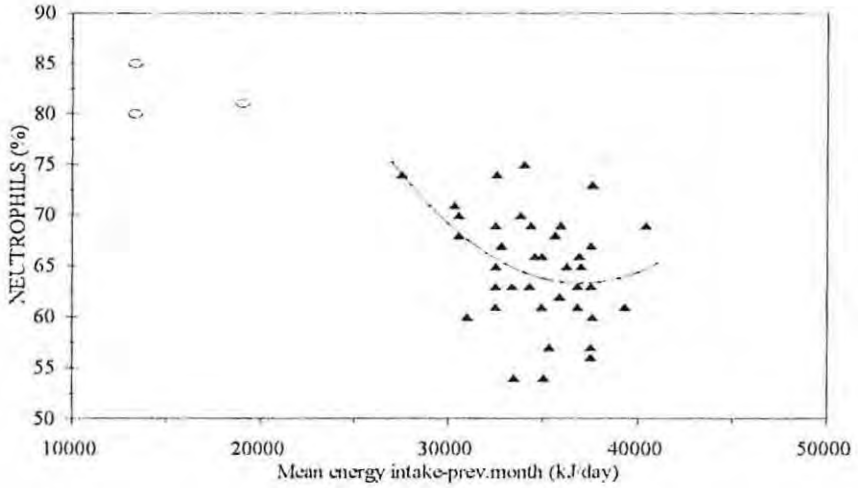


Figure 3.19: Curves of best fit (—) for animals (A. Dolly; B. Thandi) with significant correlations ($p<0.05$) between neutrophils and weight. Included in the graph are all "normal" (see text) data (▲) and statistically determined outliers on the x-axis (☒).

A. SIMO



B. DIMPLE



C. DIMPLE

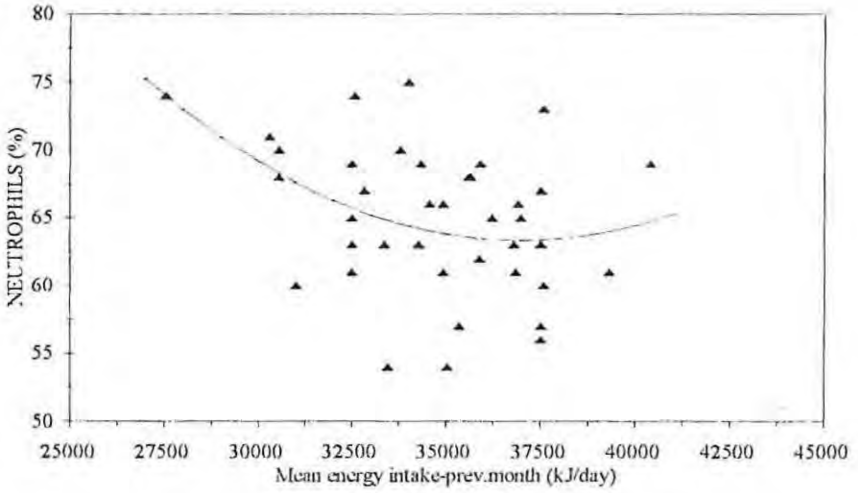


Figure 3.20: Curves of best fit for animals (A. Simo; B & C. Dimple) with significant correlations ($p<0.05$) between neutrophils and the mean daily dietary energy intake of the week (A) and month (B & C) prior to the blood sample. Included in the graph are all "normal" (see text) data (—) and statistically determined outliers (x-axis: ⊠ ; y-axis: ○).

Neutrophils were negatively correlated with water temperature for the two male dolphins, Simo and Domino (Table 3.6). High data variability complicated these relationships for both animals. However, in the short-term (mean water temperature of the week prior to the blood sample; and month in which the blood sample was taken), it appeared that neutrophils were reduced at water temperatures between 22°C and 24°C; and were highest below 19°C and above 25°C (Fig. 3.21).

c. Eosinophils

The proportion of eosinophils in the leukocyte differential varied substantially in all animals (Fig. 3.22). Eosinophil fluctuations during the study were most marked for Simo (Appendix 1.3E; Fig. 3.22D). The restricted ranges of eosinophil data from both Dimple and Dolly resulted in the removal of outliers greater than 9 % and 6 %, respectively (Appendix 1.3E). There did not appear to be any relationship between eosinophil fluctuations and periods of illness and/or treatment (Fig. 3.22). Mean eosinophil levels recorded for Thandi and Simo, during the study, were markedly lower than those recorded previously (Fig. 3.22). Domino's mean eosinophil level (10.03 ± 5.18 %) was significantly lower than all animals, except Simo (8.63 ± 5.07 %) ($t=1.24$; $p=0.22$; $df=81$). Dimple (4.73 ± 2.36 %) and Thandi (4.45 ± 2.93 %) had similar mean eosinophil levels ($t=0.56$; $p=0.58$; $df=117$). These were significantly higher than that of Dolly (2.58 ± 1.54 %) (Two sample t-test; $p<0.0001$) (Table 3.4).

Overall, age-related changes in eosinophil levels were difficult to characterise (Fig. 3.23). The two younger, male dolphins had significantly higher, and more variable, eosinophil levels than the three older female animals. However, within the three female animals, eosinophils did not appear to change with age. Simo was the only animal for which the proportion of eosinophils was significantly correlated with age during the study (Table 3.7). Eosinophils appeared to decrease steadily with the increase in age over the study period (Fig. 3.24). This cell type was not significantly correlated with weight and/or blubber thickness ($p>0.05$).

Dimple was the only animal for which eosinophils were positively correlated with the total food consumption of the week prior to blood sampling (Table 3.7). Although the trend was indistinct, eosinophils tended to be reduced (2-6 %) at food consumption levels of less than 51 kg/week (Fig. 3.25B). However, outlying low levels of food intake (<42 kg/week) did not appear to have a marked effect on eosinophil proportions, as all eosinophil values measured during periods of reduced food intake were classified as "normal" (Fig. 3.25A). Both Dolly and Simo's eosinophil data were positively correlated with the energy intake of the day prior to the blood sample and the mean daily energy intake of the week in which blood was sampled (Table 3.7). For the former correlation, data were highly variable and specific trends were difficult to identify. Significantly reduced energy intake on the day prior to blood sampling (by Dolly) did not have a marked effect on eosinophil levels, although a significantly high eosinophil value was associated with greater

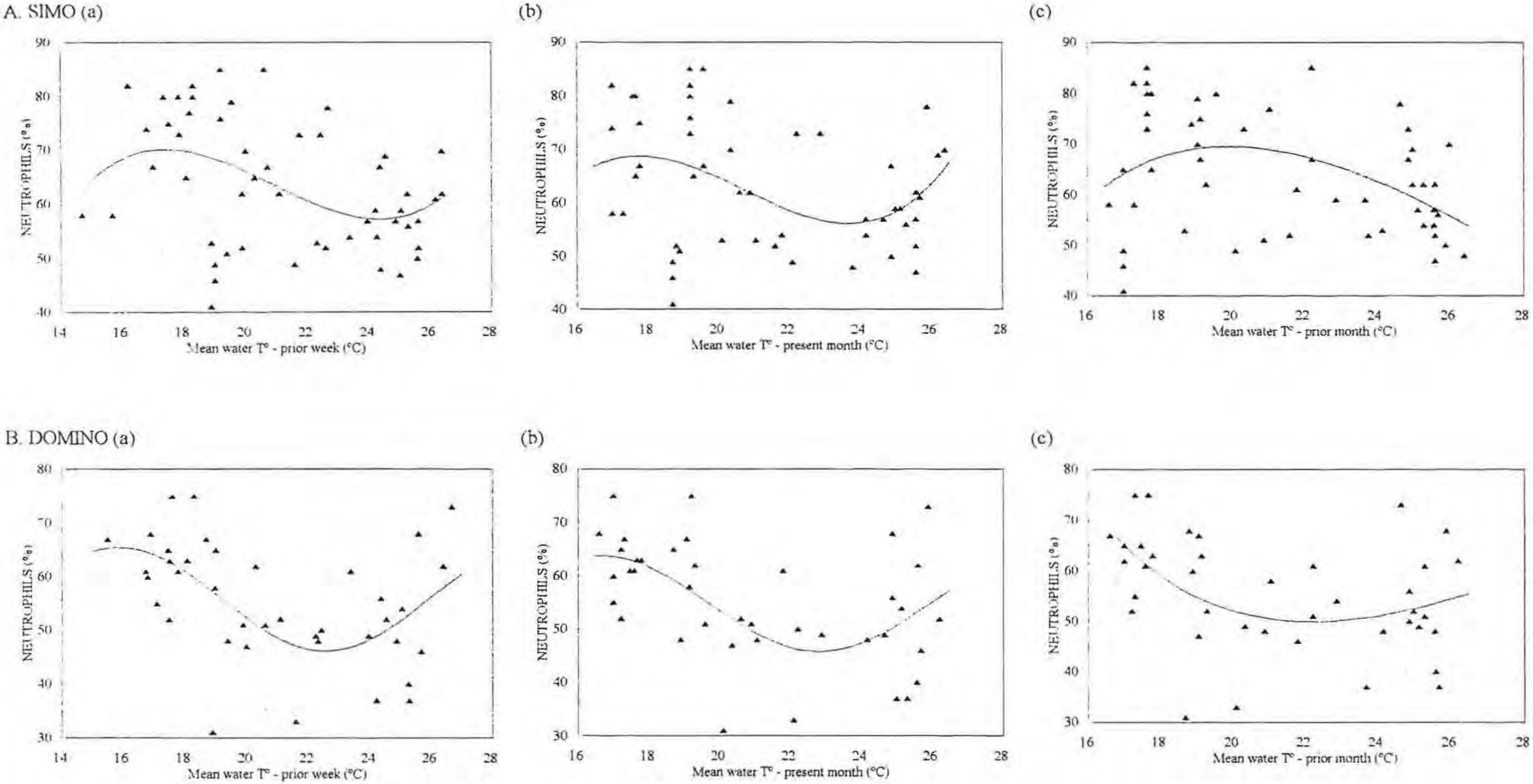


Figure 3.21: Curves of best fit (—) for animals (A. Simo; B. Domino) with significant correlations ($p<0.05$) between neutrophils and the mean water temperature of the week prior to the blood sample (a), the mean water temperature of the month in which blood was sampled (b) and the mean water temperature of the month prior to the blood sample (c). Included in the graphs are all "normal" (see text) data (▲).

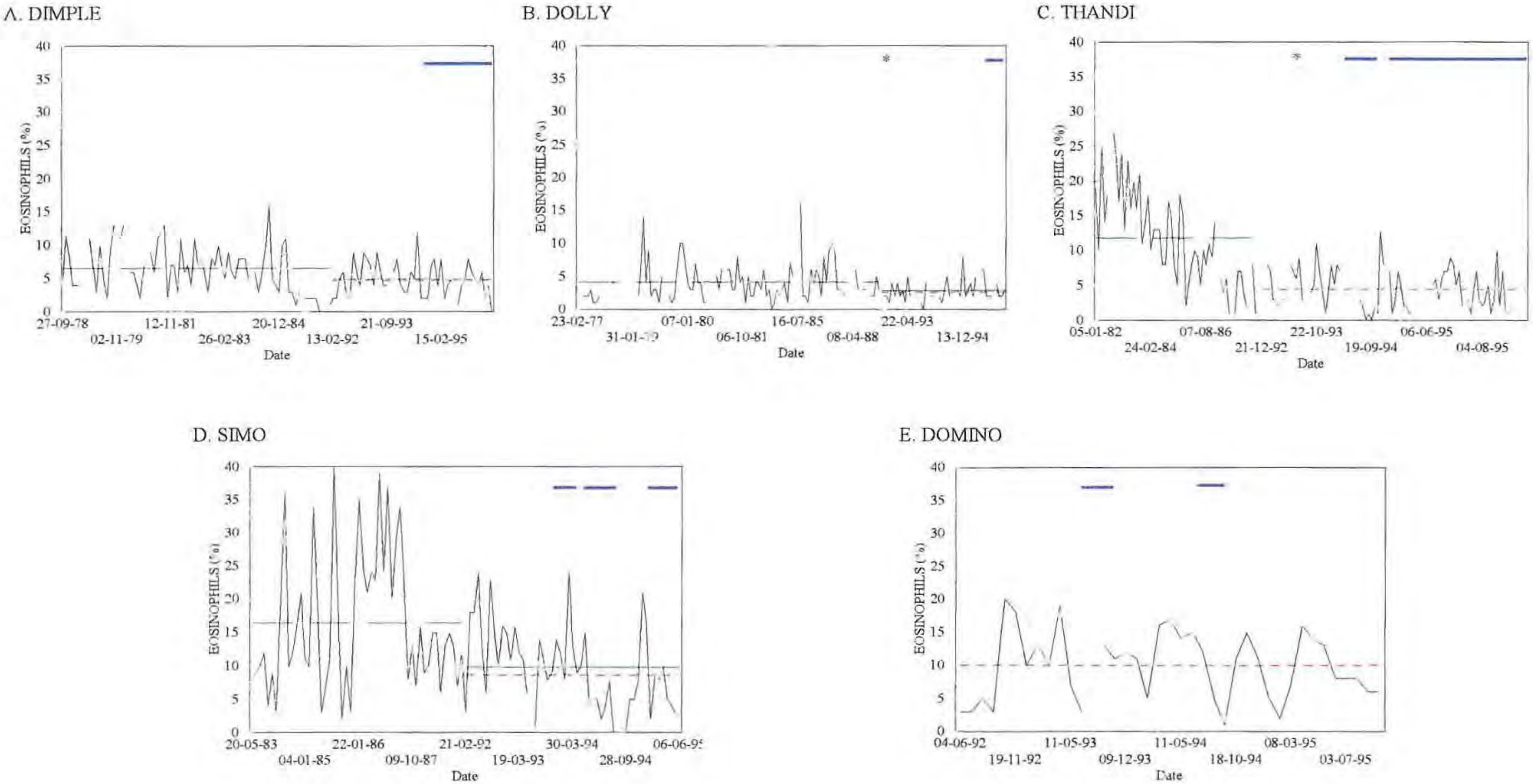


Figure 3.22: Levels of eosinophils (%) (—) for all five captive dolphins (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the mean eosinophil level for all records prior to the study (- -), the overall mean eosinophil level (—) and the "normal" (see text) mean eosinophil level (- - -) for the study between 1992 and 1995 (*: parturition; —: extended periods of illness and/or treatment or period of aberrant feeding and/or behaviour).

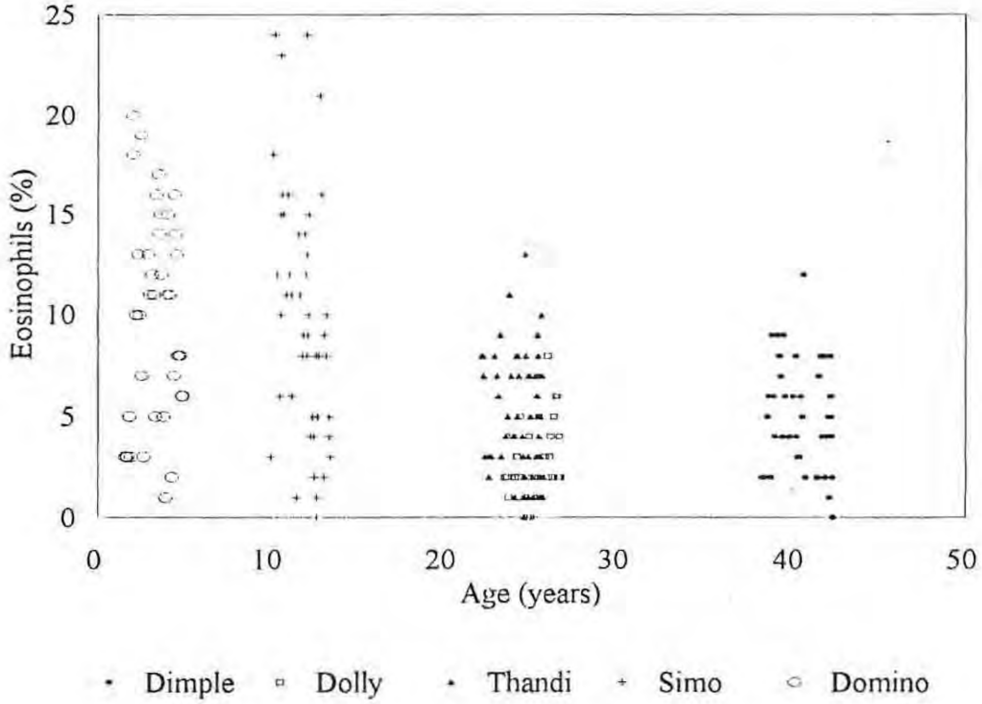


Figure 3.23: Relationship between eosinophil percentage and age for the five captive dolphins. from records obtained between 1992 and 1995.

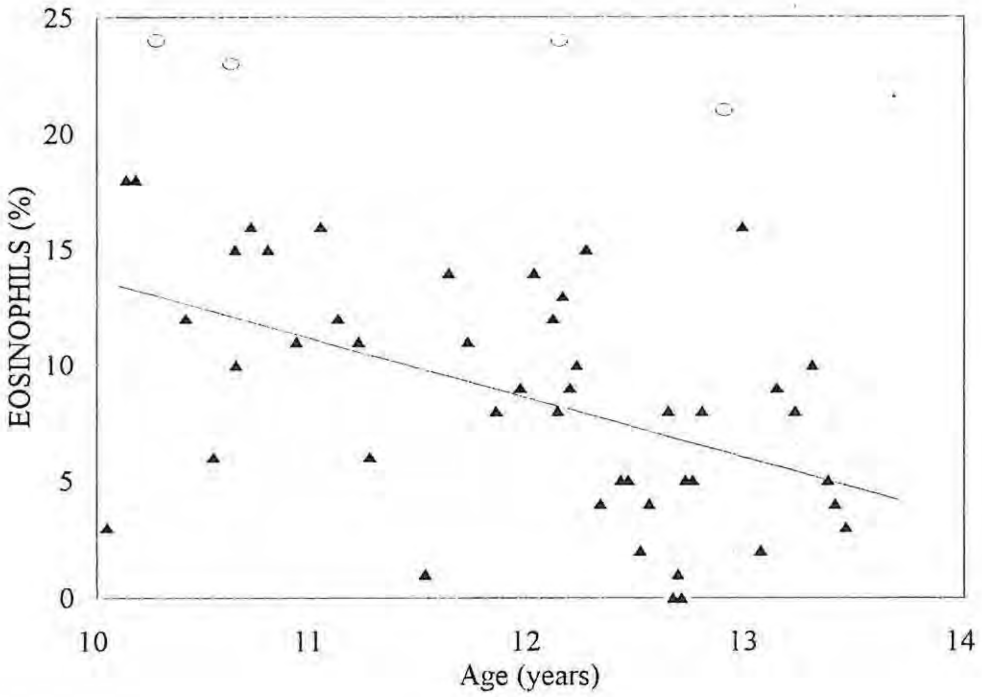
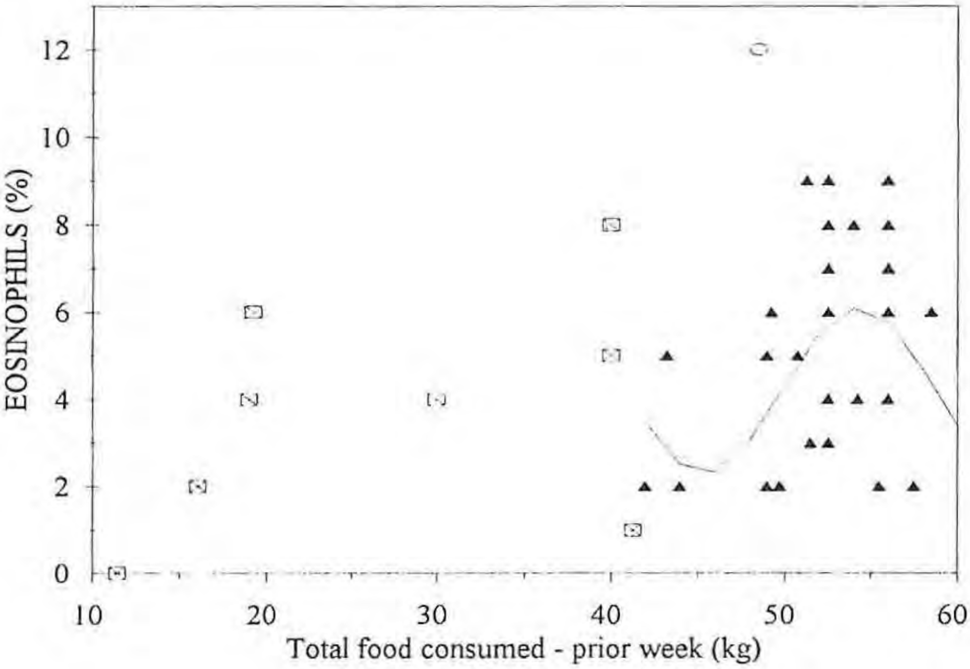


Figure 3.24: Curve of best fit (—) for Simo with a significant correlation ($p<0.05$) between age and eosinophils. Included in the graph are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

A.



B.

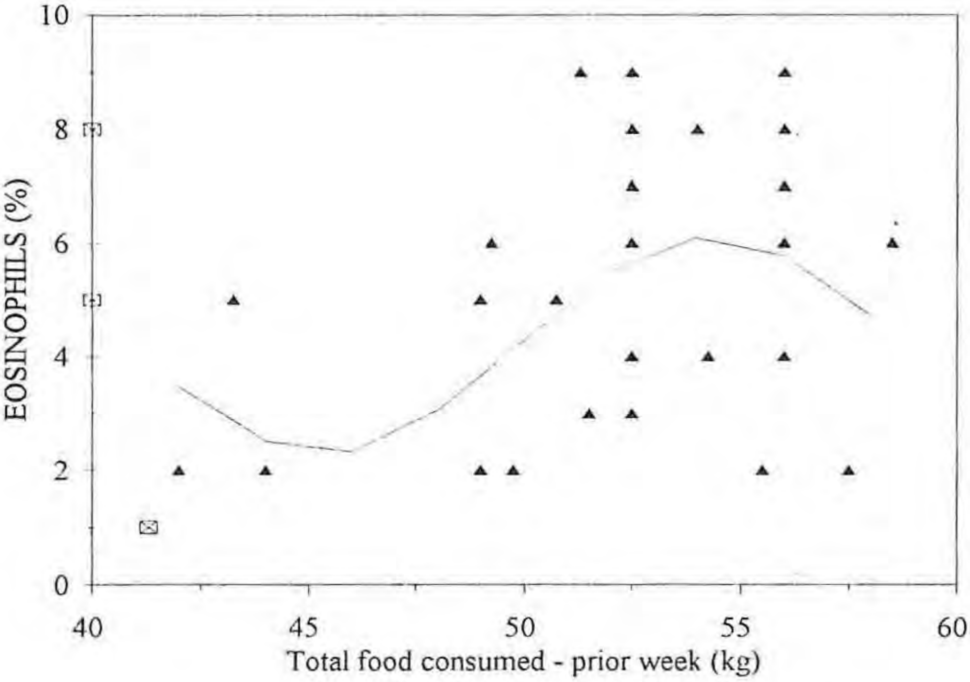


Figure 3.25: Curves of best fit (—) for Dimple with significant correlations ($p<0.05$) between eosinophils and total food consumed in the week prior to blood sampling. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

intake of dietary energy (Fig. 3.26). For the latter correlation, eosinophil levels of both animals were generally lower at reduced mean levels of energy intake (Fig. 3.27).

Table 3.7: Correlation coefficients (r), levels of significance (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$) and sample sizes (n) for significant relationships between eosinophils and independent variables for the five captive dolphins (Data are presented as r*(n)) (NS: non-significant ($p>0.05$)).

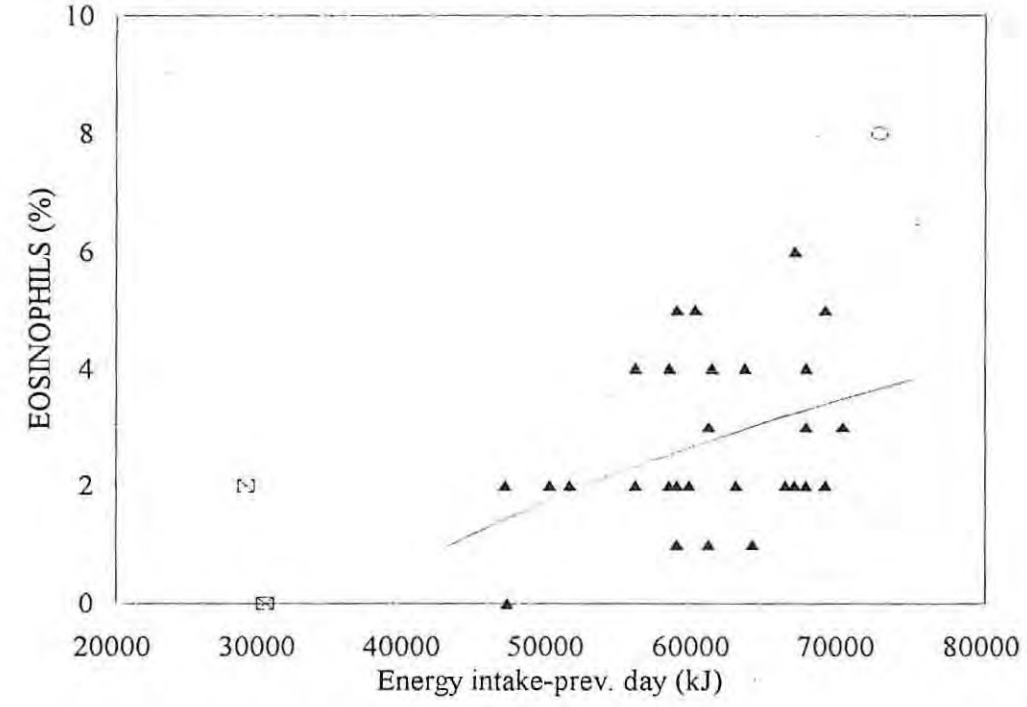
Variable	Dimple	Dolly	Thandi	Simo	Domino
Age	NS	NS	NS	-0.50***(46)	NS
D1	0.36*(35)	NS	NS	NS	NS
E1	NS	0.36*(32)	NS	0.38*(39)	NS
E2	NS	0.40*(33)	NS	0.36*(35)	NS
E3	NS	NS	NS	0.49**(34)	NS
E4	NS	0.37*(36)	NS	NS	NS
T1	NS	NS	NS	0.43**(45)	0.33*(37)
T2	NS	NS	NS	0.37**(45)	0.37*(37)
T3	NS	NS	NS	0.35*(46)	0.38*(37)

D1: Total food consumption of week prior to blood sample, E1: Dietary energy intake of day prior to blood sample; E2: Mean daily energy intake of week in which blood was sampled, E3: Mean daily energy intake of week and month (E4) prior to blood sample, T1: Mean water temperature of week prior to blood sample, T2: Mean water temperature of month in which blood was sampled, T3: Mean water temperature of month prior to blood sample (T3).

Eosinophils were also related to mean daily energy intake in the longer-term (i. e. the week and month prior to the blood sample) (Table 3.7). Simo's eosinophil levels increased significantly (~ 1 % to 15 %) with increased mean energy intake (30 000-47500 kJ/day) (Fig. 3.28A). Levels of eosinophils measured when energy intake was reduced below this range were low (< 5%) but "normal". Dolly's data showed that increased eosinophil levels were associated with greater mean daily energy intake during the month prior to the blood sample (Table 3.7; Fig. 3.28B).

Eosinophil data from both Simo and Domino were positively correlated with water temperature (Table 3.7) (Fig. 3.29). This relationship was clearest for Domino, particularly pertaining to short-term changes in water temperature. Eosinophils appeared to increase in number (~0-15 %) as water temperature increased between 14°C and 22°C. However, there appeared to be a decrease in eosinophil levels once water temperatures increased above 24°C (Fig. 3.29B).

A.



B.

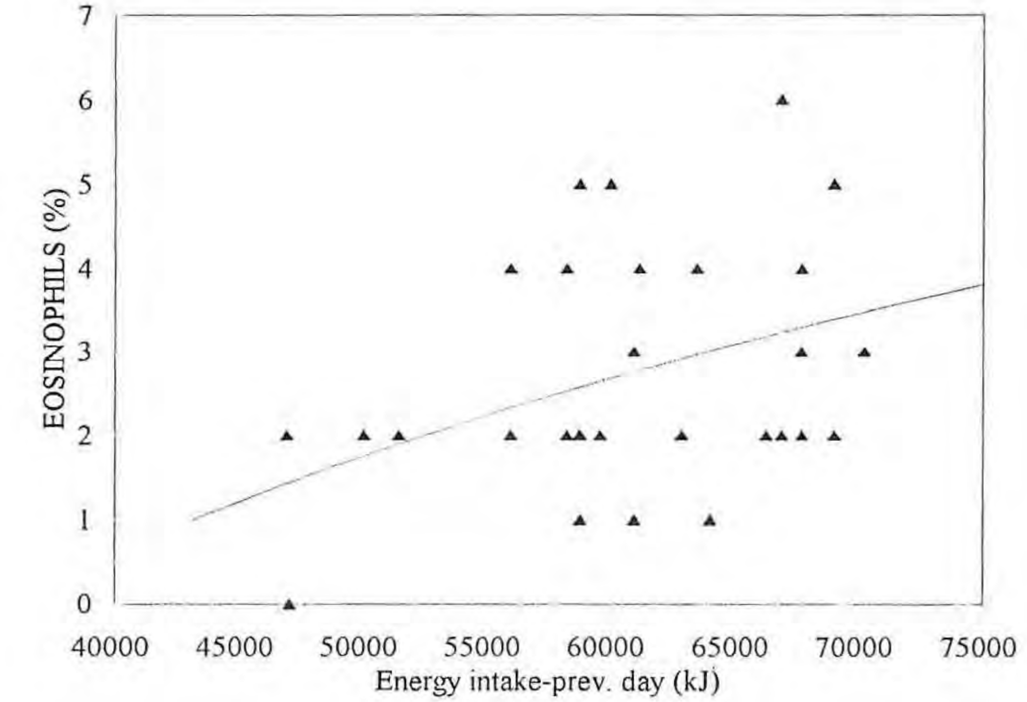
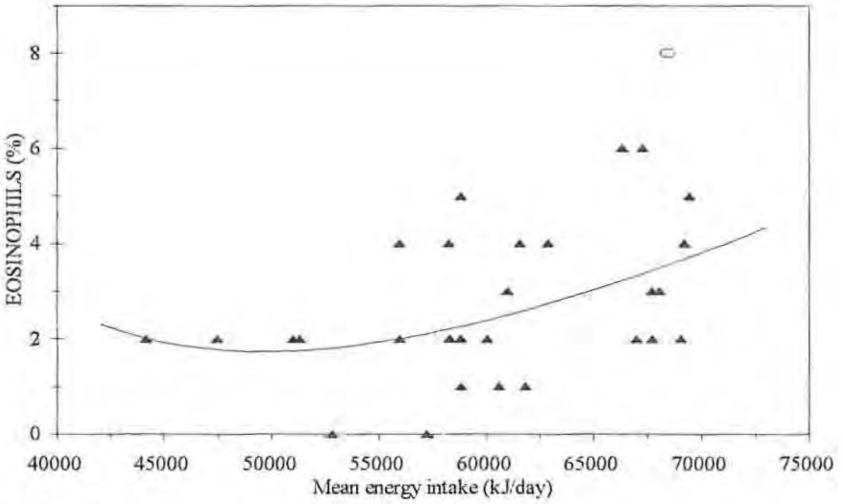
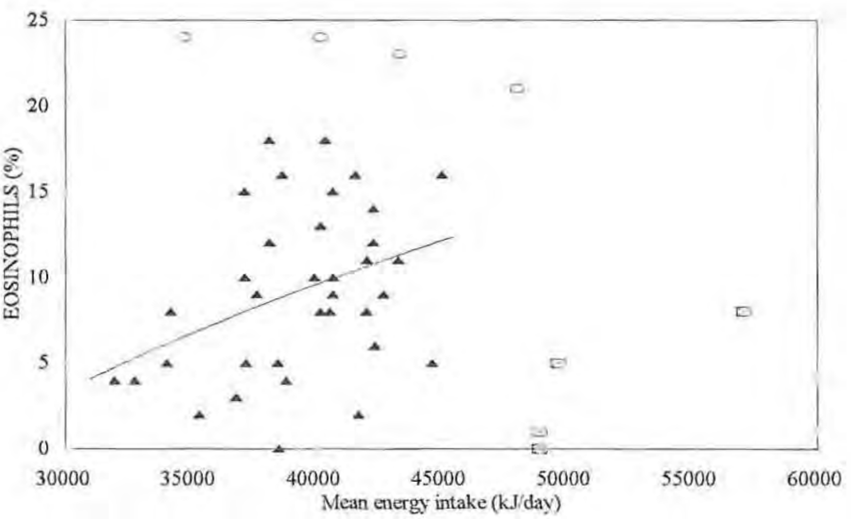


Figure 3.26: Curves of best fit (—) for Dolly (A & B) with significant correlations ($p<0.05$) between eosinophils and the dietary energy intake of the day prior to the blood sample. Included in the graph are all "normal" (see text) data (▲) and statistically determined outliers (x-axis ☒ ; y-axis: ○).

A. DOLLY



B. SIMO (a)



(b)

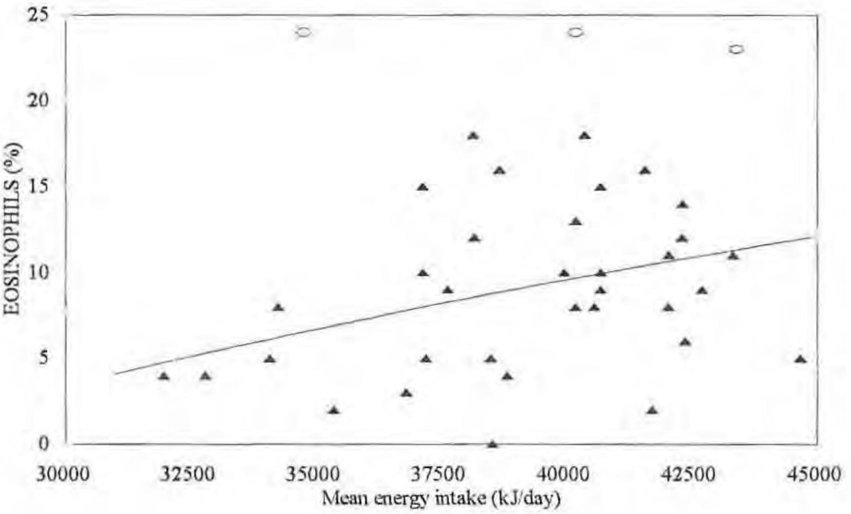
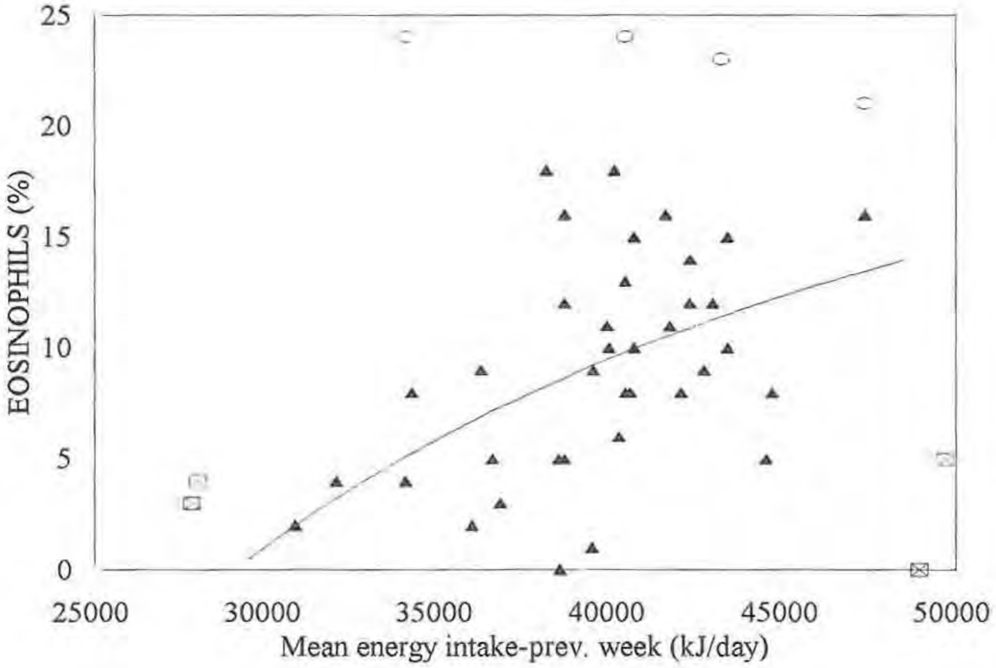


Figure 3.27: Curves of best fit (—) for animals (A. Dolly; B. Simo (a & b)) with significant correlations ($p<0.05$) between eosinophils and the mean daily dietary energy intake of the week in which blood was sampled. Included in the graph are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

A. SIMO



B. DOLLY

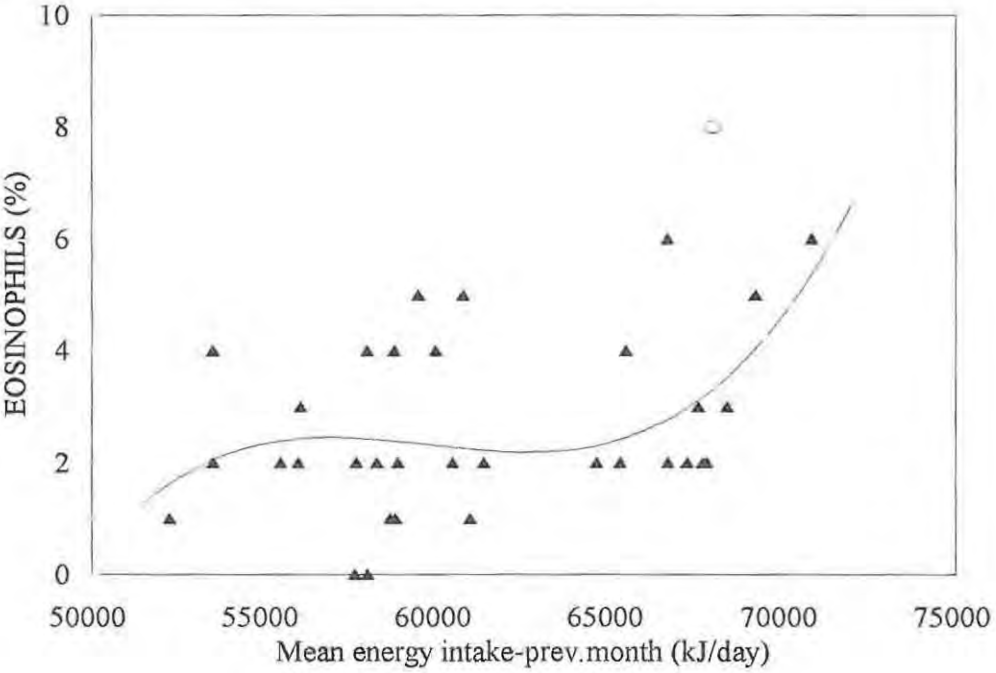


Figure 3.28: Curves of best fit for animals (A. Simo; B. Dolly) with significant correlations ($p<0.05$) between eosinophils and the mean daily energy intake of the week (A) and month (B) prior to the blood sample. Included in the graph are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

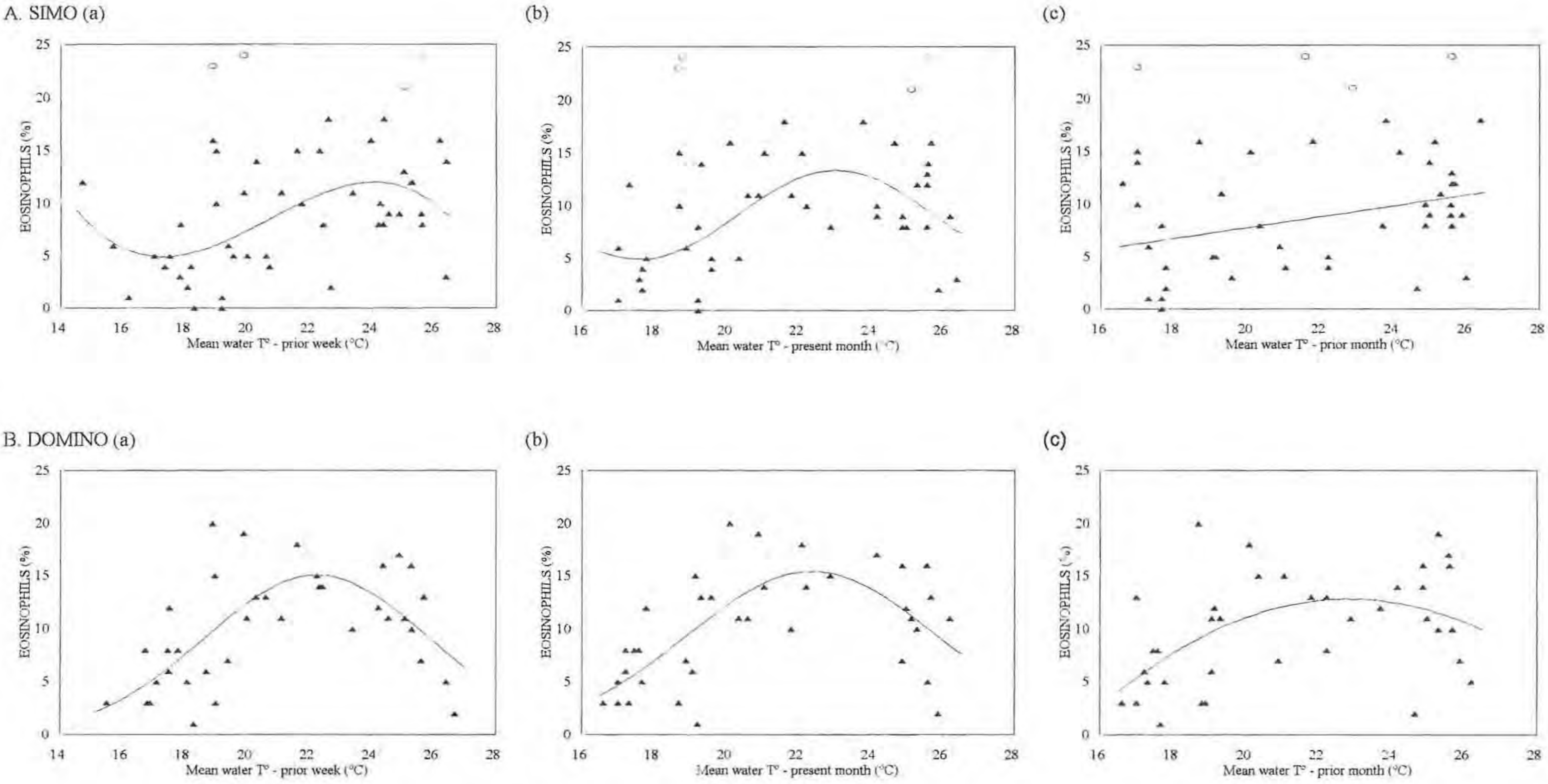


Figure 3.29: Curves of best fit (—) for animals (A. Simo; B. Domino) with significant correlations ($p<0.05$) between eosinophils and the mean water temperature of the prior week (a), the month in which blood was sampled (b) and the prior month (c). Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

Haematological parameters

a. Red blood cell (RBC) count

Mean RBC counts for all animals during the study ranged between 4.00 x10^{12} cells/l and 5.00 x10^{12} cells/l (Fig. 3.30; Table 3.4), similar to RBC counts recorded prior to the study. Outlying low and/or high RBC counts were measured in data from Dimple, Dolly and Simo (Appendix 1.4A). Marked reductions in RBC count were associated with illness in both Thandi and Simo (Fig. 3.30C & D). RBC counts from Dimple became more variable during the period of illness at the end of the study (Fig. 3.30A). Dolly (4.62 ± 0.23 x10^{12} cells/l) and Simo (4.58 ± 0.33 x10^{12} cells/l) had significantly higher mean RBC counts than other animals (t=0.71; p=0.48; df=91). Domino's mean RBC level (4.33 ± 0.28 x10^{12} cells/l) was also significantly higher than Dimple (4.01 ± 0.20 x10^{12} cells/l) and Thandi (4.07 ± 0.35 x10^{12} cells/l) (Two sample t-test; $p<0.001$) (Table 3.4).

Overall, there did not appear to be a marked change in RBC counts with age (Fig. 3.31). However, as an elderly animal, Dimple's RBC counts were slightly below those of the other four animals. Although RBC counts from Dimple, Dolly and Thandi were significantly correlated with age during the study (Table 3.8), none of these trends could be ascribed to specific age-associated biological trends. RBC's were significantly correlated with total body weight for Dolly and Thandi (Table 3.8). Dolly's RBC data were variable, but counts were slightly higher at increased body weight (Fig. 3.32A). In contrast, this relationship for Thandi was more complex, with RBC counts apparently reduced (~3.30-4.00 x10^{12} cells/l) at weights approximating 180 kg (Fig. 3.32B).

Table 3.8: Correlation coefficients (r), levels of significance (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$) and sample sizes (n) for significant relationships between red blood cell (RBC) counts and independent variables for the five captive dolphins (Data are presented as r*(n)) (NS: non-significant ($p>0.05$)).

Variable	Dimple	Dolly	Thandi	Simo	Domino
Age	0.35*(46)	0.30*(46)	-0.29*(87)	NS	NS
Weight	NS	0.47**(42)	-0.40**(57)	NS	NS
Blubber thickness	NS	0.63*(10)	NS	NS	0.66*(12)
D1	-0.42**(41)	0.37*(38)	-0.59***(69)	NS	NS
E1	NS	NS	-0.40***(72)	NS	NS
E2	NS	NS	-0.47***(69)	NS	NS
E3	NS	NS	-0.44***(73)	NS	NS
E4	NS	0.37*(43)	-0.29**(84)	NS	NS
Month	NS	-0.29*(46)	NS	NS	NS
T1	NS	NS	0.40***(87)	NS	NS
T2	NS	NS	0.46***(86)	NS	NS
T3	NS	NS	0.37***(87)	NS	NS

D1: Total food consumption of week prior to blood sample, E1: Dietary energy intake of day prior to blood sample; E2: Mean daily energy intake of week in which blood was sampled, E3: Mean daily energy intake of

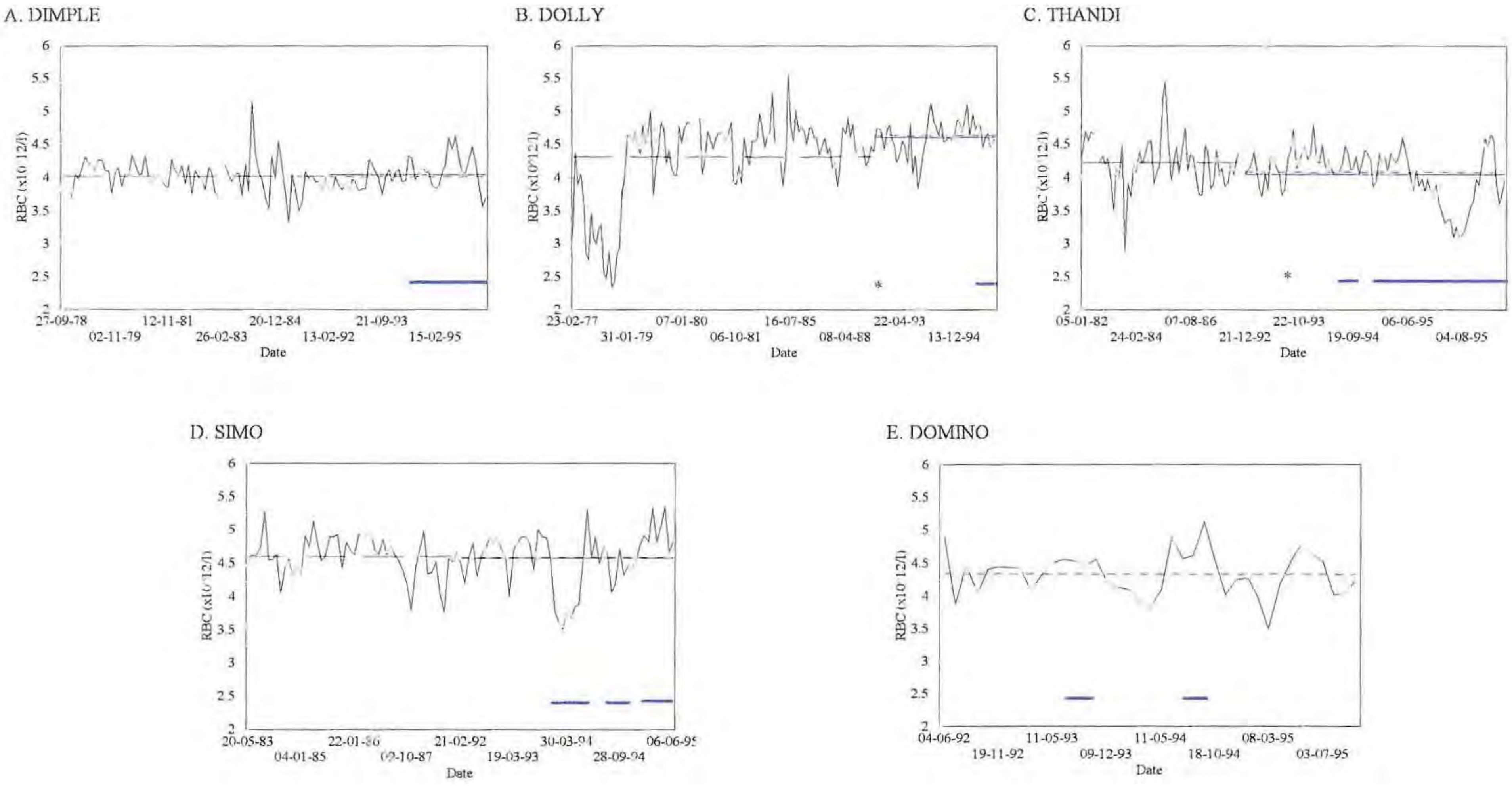


Figure 3.30: Red blood cell (RBC) counts (x10^12/L) (—) for all five captive animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino) Also shown, for each animal, is the mean RBC count for all records prior to the study (···), the overall mean RBC count (—) and the "normal" (see text) mean RBC count (- - -) for the study between 1992 and 1995 (*: parturition; —: extended periods of illness and/or treatment or period of aberrant feeding and/or behaviour).

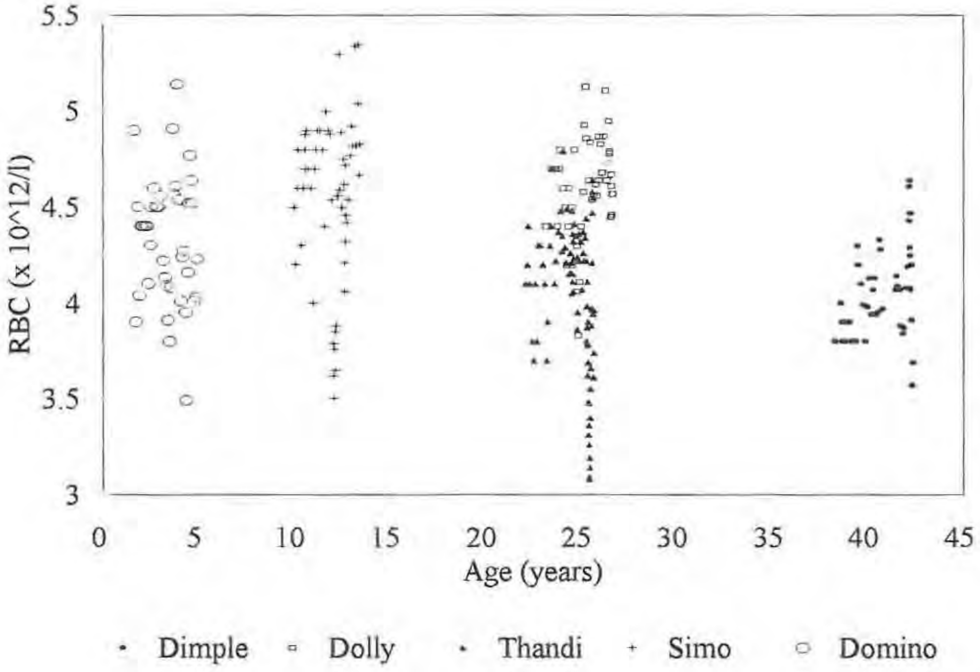
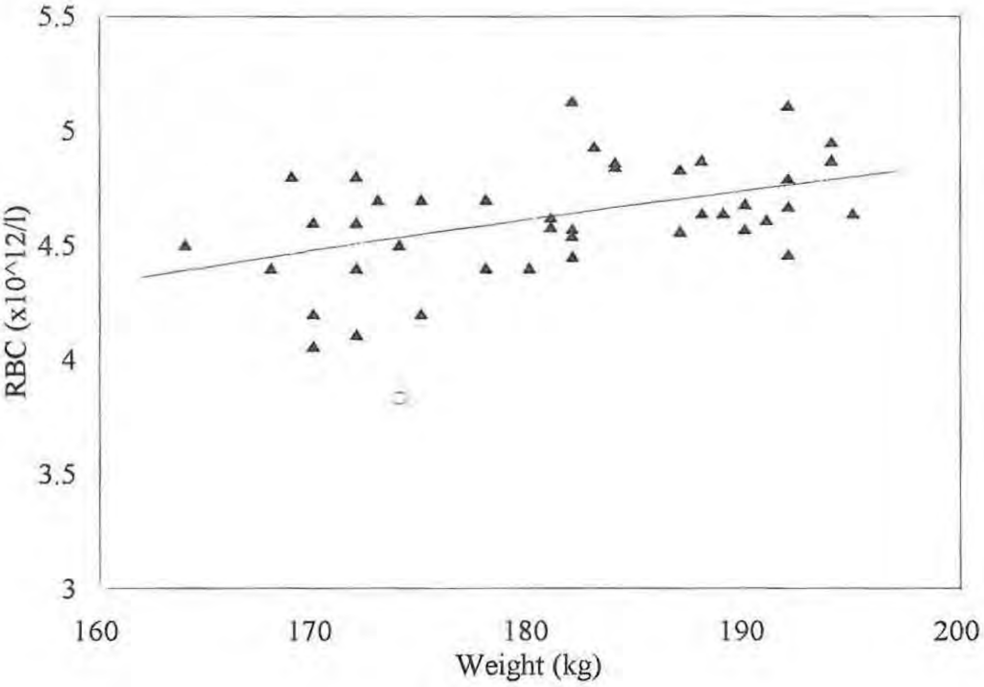


Figure 3.31: Relationship between red blood cell (RBC) count and age, for the five captive dolphins, from records obtained between 1992 and 1995.

A. DOLLY



B. THANDI

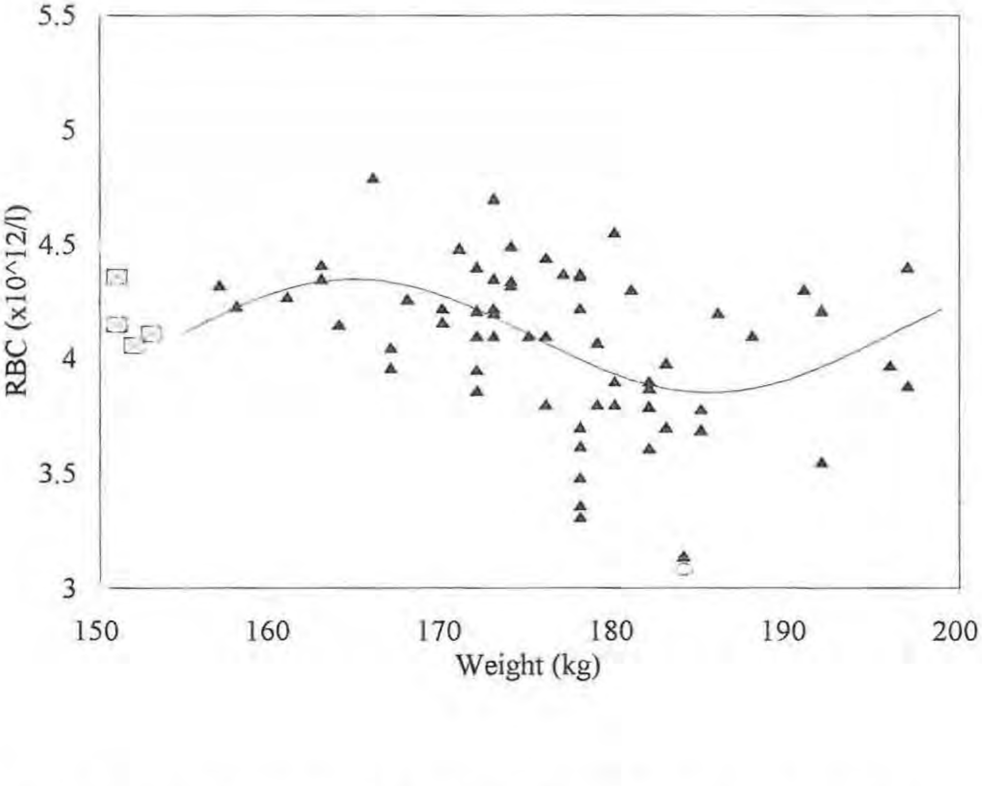


Figure 3.32: Curves of best fit (—) for animals (A. Dolly; B. Thandi) with significant correlations ($p<0.05$) between red blood cells (RBC) and total body weight. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers. (x-axis: ☒ ; y-axis: ○).

week and month (E4) prior to blood sample, T1: Mean water temperature of week prior to blood sample, T2: Mean water temperature of month in which blood was sampled, T3: Mean water temperature of month prior to blood sample.

Red blood cell counts from both Dolly and Domino were positively related to blubber thickness (Table 3.8), with limited measurements illustrating that RBC's were increased when blubber was thicker. This relationship for Domino was not as distinct as that of Dolly (Fig. 3.33). A significantly high blubber thickness (24.50 mm) was associated with a low, but "normal", RBC count for Dolly (Fig. 3.33).

Red blood cell counts were significantly correlated with a number of dietary parameters (Table 3.8). RBC's were significantly correlated with the total amount of food consumed in the week prior to blood sampling for Dimple, Dolly and Thandi, but relationships were inconsistent (Table 3.8). The relationships described for Dolly and Thandi were directly opposed to each other (Fig. 3.34). Dolly's RBC count was variable but appeared to be increased at greater levels of food consumption (Fig. 3.34A). RBC counts recorded after periods of reduced food consumption were, however, within the "normal" range for this animal. Thandi's RBC counts, in contrast, were relatively stable (3.75-4.50 $\times 10^{12}$ cells/l) when food consumption was between 50kg/week and 70 kg/week (Fig. 3.34B). Beyond this, RBC counts began to decline with increased food consumption, although RBC counts corresponding to significantly high food intake (>90 kg/week) were "normal".

In a similar context, Thandi was the only animal for which RBC counts were negatively correlated with the dietary energy intake of the day and week prior to the blood sample; as well as the mean energy intake of the week in which blood was sampled (Table 3.8). These relationships were similar (Fig. 3.35), although trends were complicated by data variability. RBC counts were generally variable at mean daily energy intake levels of between 15 000 kJ/day and 35 000 kJ/day, however, at energy intakes of between 35 000 kJ/day and 45 000 kJ/day, RBC counts appeared to be slightly reduced. The trend for the mean energy intake of the week prior to the blood sample was complicated by three increased RBC counts at levels of energy intake greater than 45 000 kJ. These energy intake levels were classified as outlying for particular dietary parameters (Fig. 3.35B) but not for others (Fig. 3.35A & C).

Red blood cell data from both Dolly and Thandi were significantly correlated with long-term dietary intake, i. e. the mean daily energy intake of the month prior to the blood sample (Table 3.8). Overall, RBC's from Dolly were predominantly more numerous (> 4.60 $\times 10^{12}$ cells/l) following long-term energy intake levels of greater than 65 000 kJ/day (Fig. 3.36A). The same data for Thandi were extremely variable and the decrease in RBC counts apparent at mean daily energy intake levels (of the previous month) greater than 40 000 kJ/day could not be verified (Fig. 3.36B).

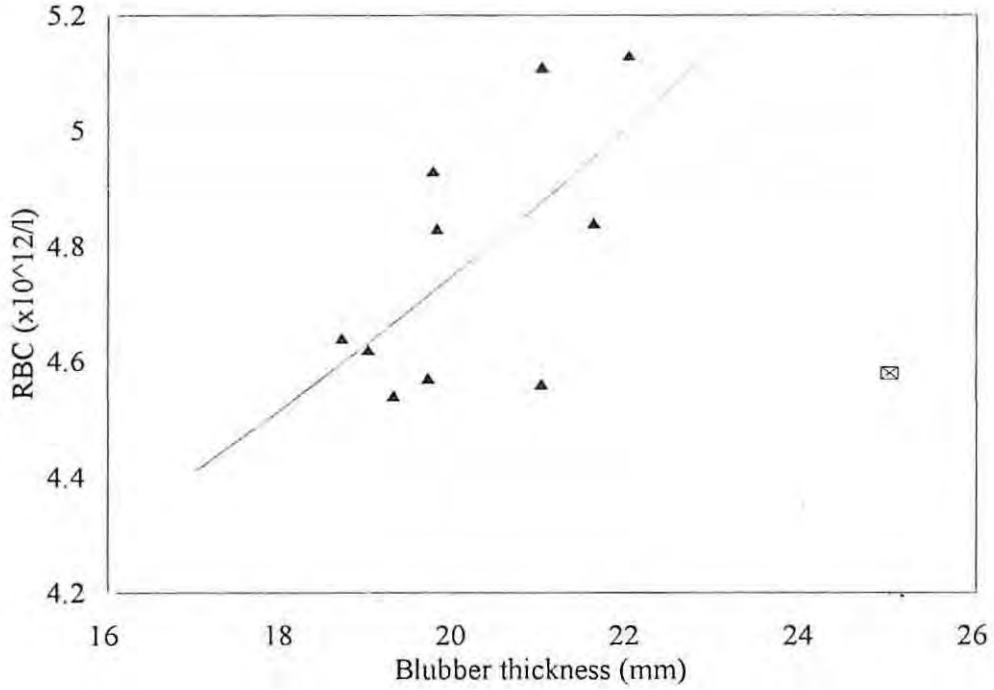
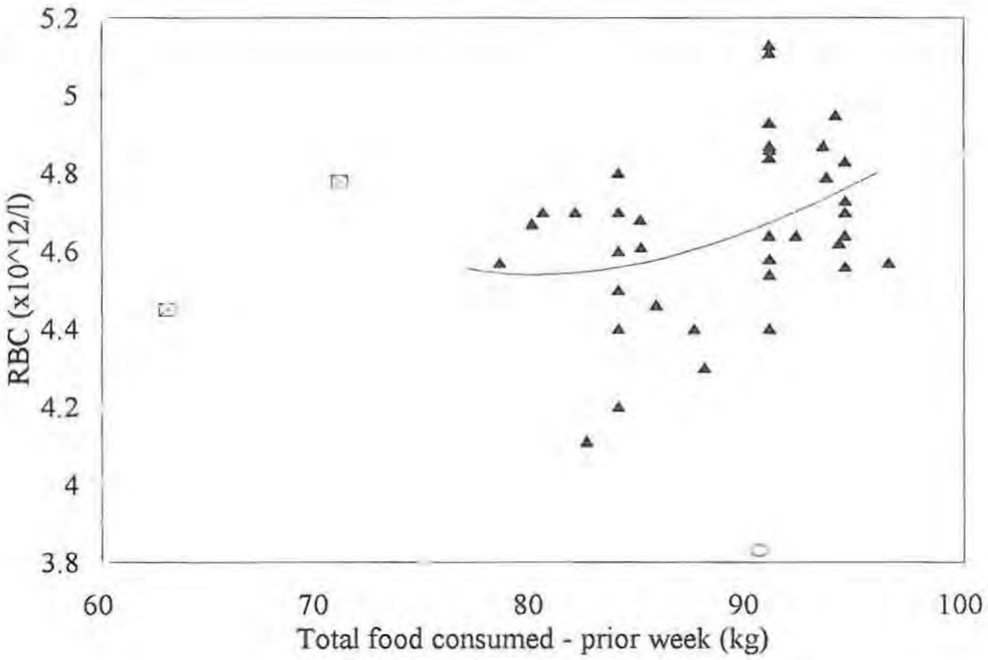


Figure 3.33: Curve of best fit (—) for Dolly with a significant correlation ($p<0.05$) between re blood cell (RBC) count and blubber thickness. Included in the graph are all "normal" (see text) data and statistically determined outliers on the x-axis (☒).

A. DOLLY



B. THANDI

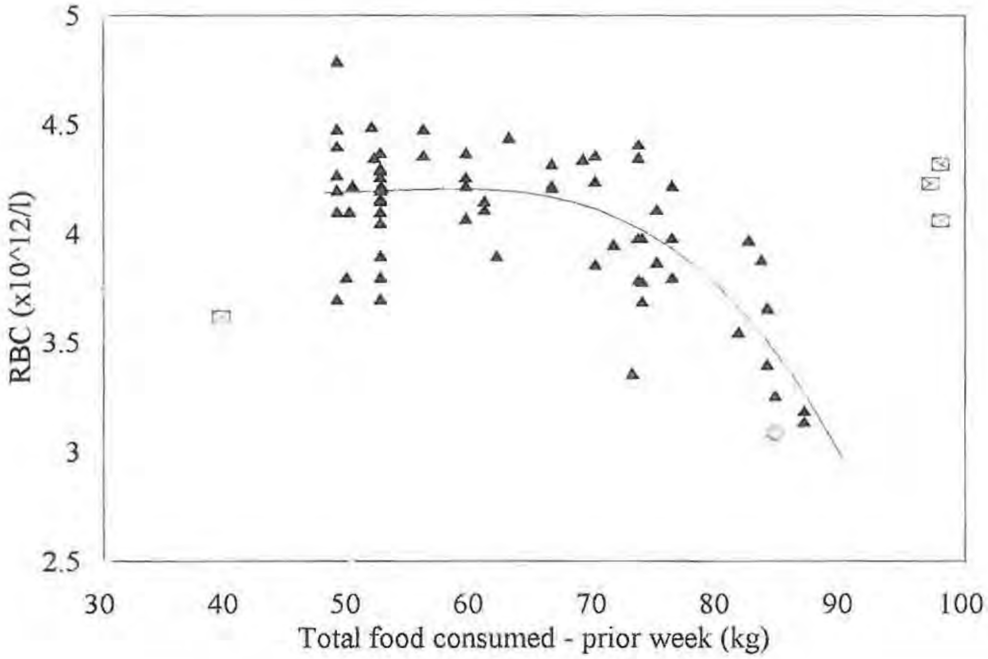


Figure 3.34: Curves of best fit (—) for animals (A. Dolly; B. Thandi) with significant correlations ($p<0.05$) between red blood cells (RBC) and total food consumed in the week prior to blood sampling. Included in the graph are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ⊠ ; y-axis: ○).

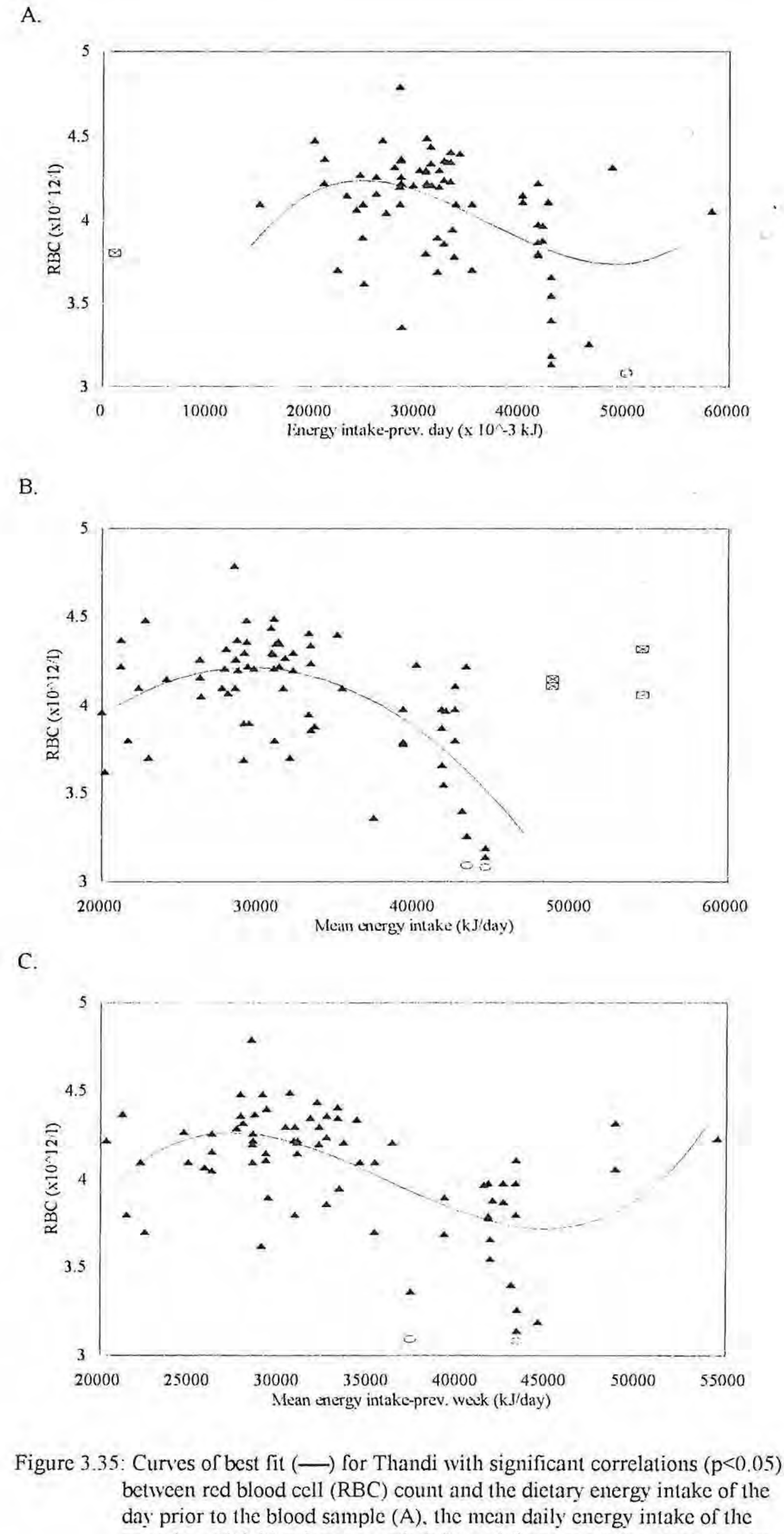
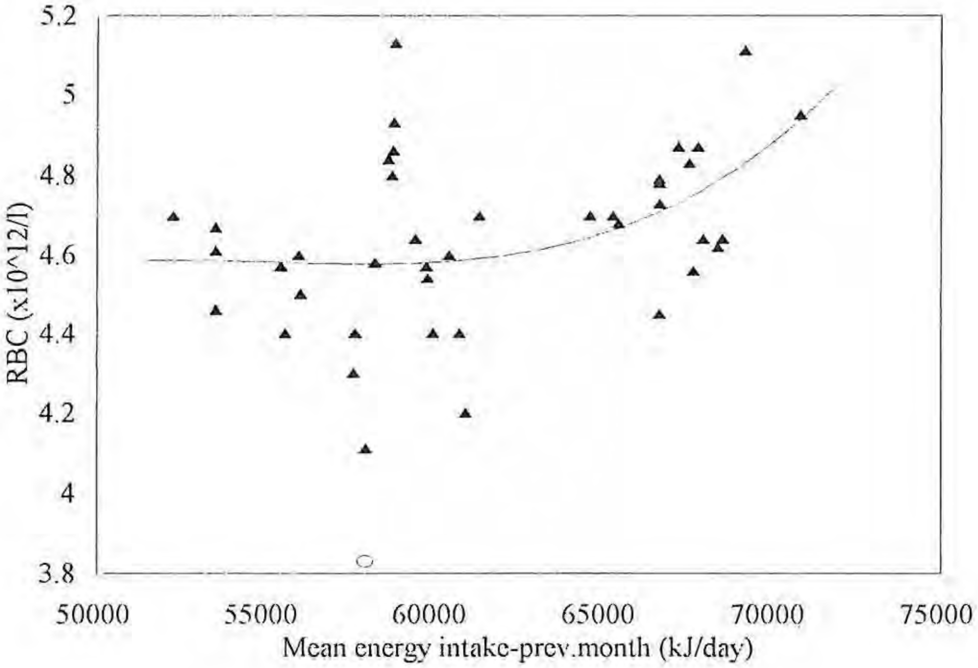


Figure 3.35: Curves of best fit (—) for Thandi with significant correlations ($p<0.05$) between red blood cell (RBC) count and the dietary energy intake of the day prior to the blood sample (A), the mean daily energy intake of the week in which blood was sampled (B) and the mean daily energy intake of the week prior to the blood sample (C). Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒; y-axis: ○).

A. DOLLY



B. THANDI

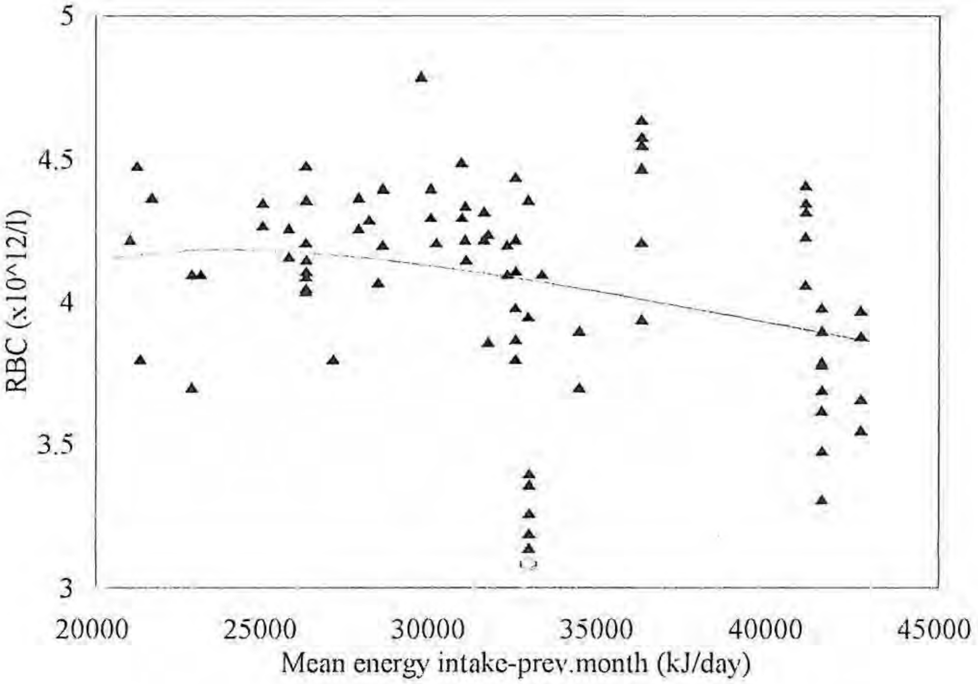


Figure 3.36: Curves of best fit (—) for animals (A. Dolly B. Thandi) with significant correlations ($p<0.05$) between red blood cell (RBC) count and the mean daily energy intake of the month prior to the blood sample. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

Seasonal changes in RBC count were evident only for Dolly, for which RBC's were negatively correlated with the month in which the blood was sampled (Table 3.8). Although data were variable over all months of the year, low RBC counts were recorded more frequently between August and November. A single outlying low RBC count was measured during this period. Thandi was the only animal for which RBC counts were positively correlated with water temperature (Table 3.8). RBC data for this animal were highly variable, particularly at water temperatures below 21°C (Fig. 3.37). Outlying low RBC counts ($<3.50 \times 10^{12}$ cells/l) were measured only at temperatures below 19°C, with RBC's remaining relatively stable ($\sim 3.80\text{-}4.50 \times 10^{12}$ cells/l) at water temperatures between 21°C and 26°C (Fig. 3.37).

b. Haemoglobin (HB)

Changes in HB concentration generally paralleled changes in RBC counts. All animals showed several fluctuations in HB levels during the study (Fig. 3.38), with variability being highest in data from Thandi and Simo (Fig. 3.38 C&D). Outlying values were identified in all animals (Appendix 1.4B). Dolly, Thandi and Simo all displayed a tendency for reduced HB levels during illness, but HB increased in Dimple and Domino during illness (Fig. 3.38). Thandi's mean HB concentration (16.90 ± 1.17 g/dl) was significantly lower than all other animals (Two sample t-test, $p>0.005$), with the exception of Domino (17.31 ± 1.16 g/dl) ($t=1.88$; $p=0.08$; $df=117$) (Two sample t-test; $p<0.01$) (Table 3.4). Dolly's mean HB level (18.32 ± 0.95 g/dl) was similar to Dimple's (17.85 ± 0.80 g/dl) and Simo's (18.60 ± 1.10 g/dl) but significantly higher than that of Thandi and Domino (Two sample t-test, $p<0.001$). Domino's mean HB level was similar to that of Dimple ($t=2.48$; $p=0.015$; $df=80$) and Thandi ($t=1.77$; $p=0.08$; $df=117$). (Table 3.4).

Overall, there was no marked change in HB concentration with age (Fig. 3.39). During the study, HB levels from three animals (Dimple, Dolly and Thandi) were significantly correlated with age (Table 3.9), but none of these were directly associated with biological changes in age and/or maturity status. Levels of HB were significantly correlated with weight for Dolly, Thandi and Simo (Table 3.9). However, data for all three animals were highly variable, particularly for Thandi, and HB levels were relatively stable over the majority of the weight range (Fig. 3.40). HB concentrations in both Dolly and Simo were slightly increased at weights greater than 185 kg (Fig. 3.40 A&C). In contrast, reduced HB levels were recorded at weights of approximately 185 kg in Thandi (Fig. 3.40B). Significantly reduced weight was not associated with outlying HB measures. Domino's HB levels were positively correlated with blubber thickness (Table 3.9). An increase of HB from approximately 16 g/dl to 19 g/dl was affiliated with an increase in blubber thickness from 17 mm to 24 mm (Fig. 3.41).

Thandi was the only animal for which HB was negatively correlated with total weekly food consumption (Table 3.9; Fig. 3.42). Outlying low HB concentrations (<14.00 g/dl) were measured

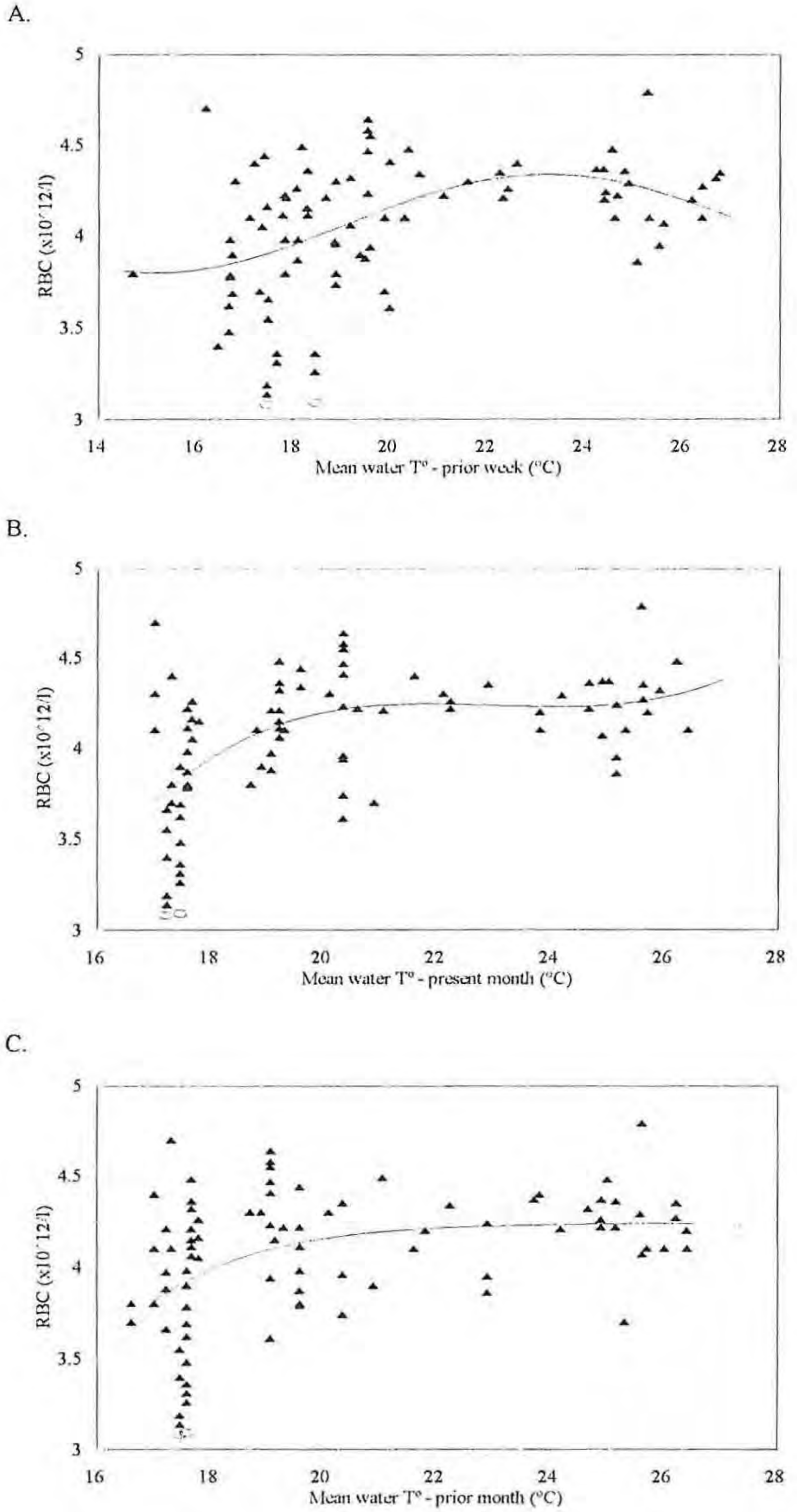


Figure 3.37: Curves of best fit (—) for Thandi with significant correlations ($p<0.05$) between red blood cells (RBC) and the mean water temperature of the prior week (A); the month in which blood was sampled (B) and the prior month (C). Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

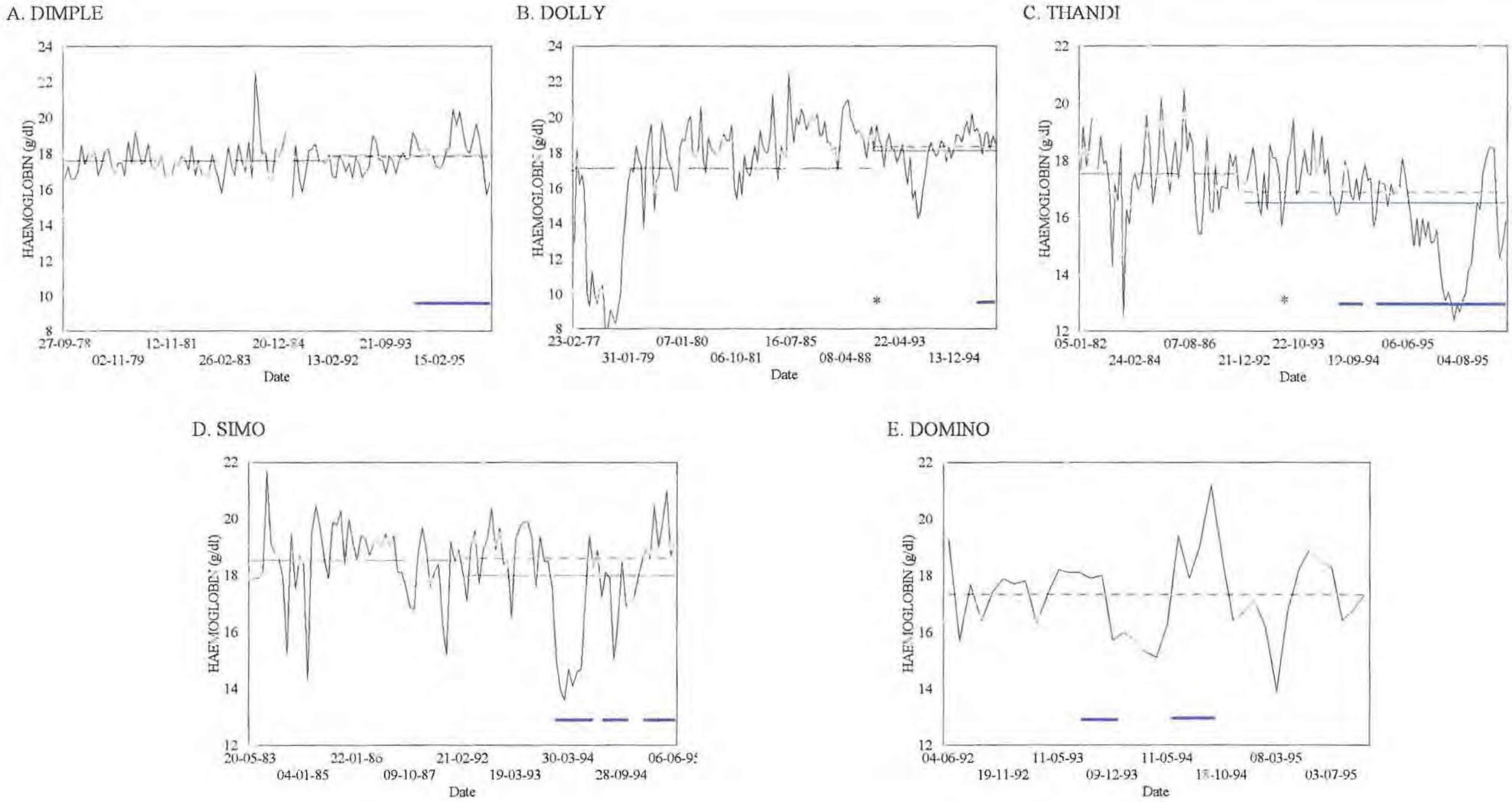


Figure 3.38: Haemoglobin (HB) levels (g/dl) (—) for all five captive animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the mean HB level for all records prior to the study (- -); the overall mean HB level (—) and the "normal" (see text) mean HB level (- - -) for the study between 1992 and 1995 (*: parturition; —: extended periods of illness and/or treatment or periods of aberrant feeding and/or behaviour).

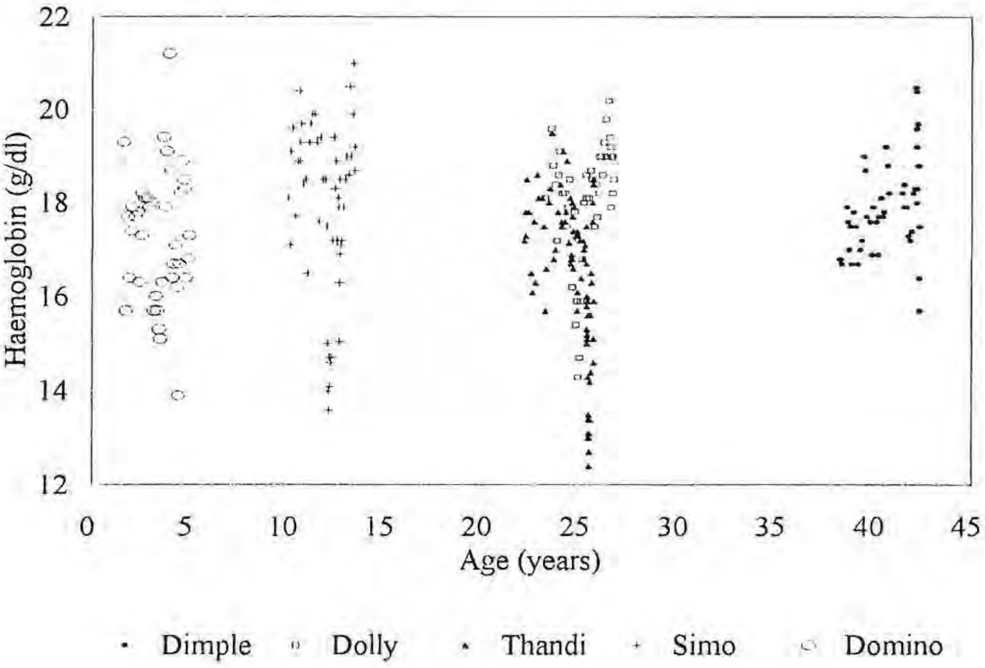
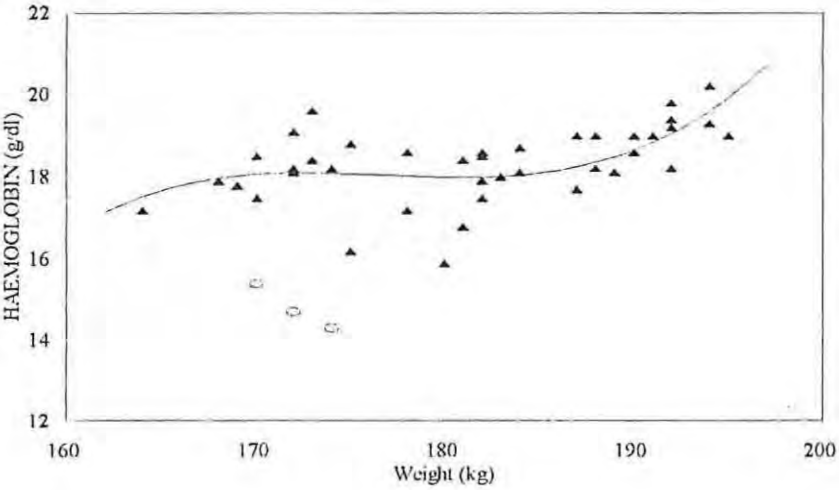
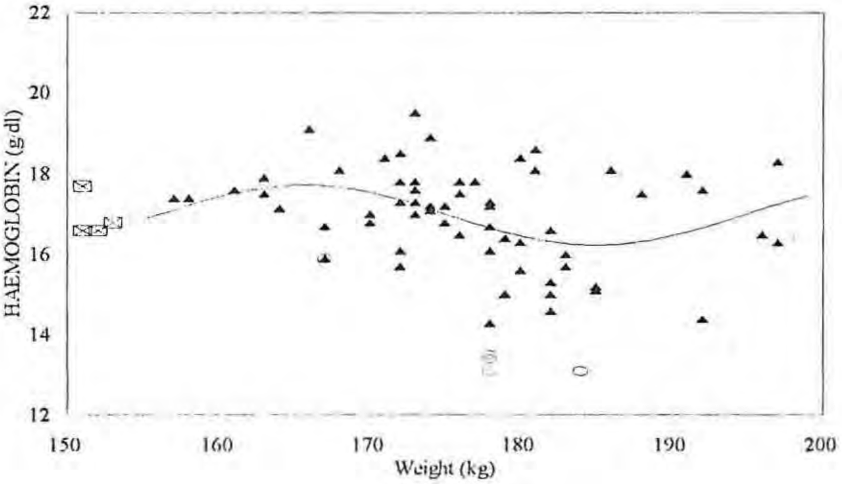


Figure 3.39: Relationship between haemoglobin (HB) concentration and age for the five captive dolphins, from records obtained between 1992 and 1995.

A. DOLLY



B. THANDI



C. SIMO

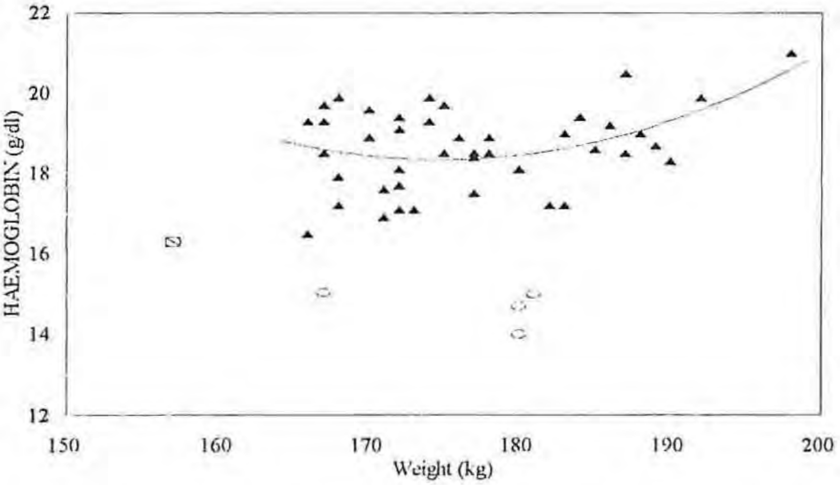


Figure 3.40: Curves of best fit (—) for animals (A. Dolly; B. Thandi; C. Simo) with significant correlations ($p<0.05$) between haemoglobin (HB) and weight. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

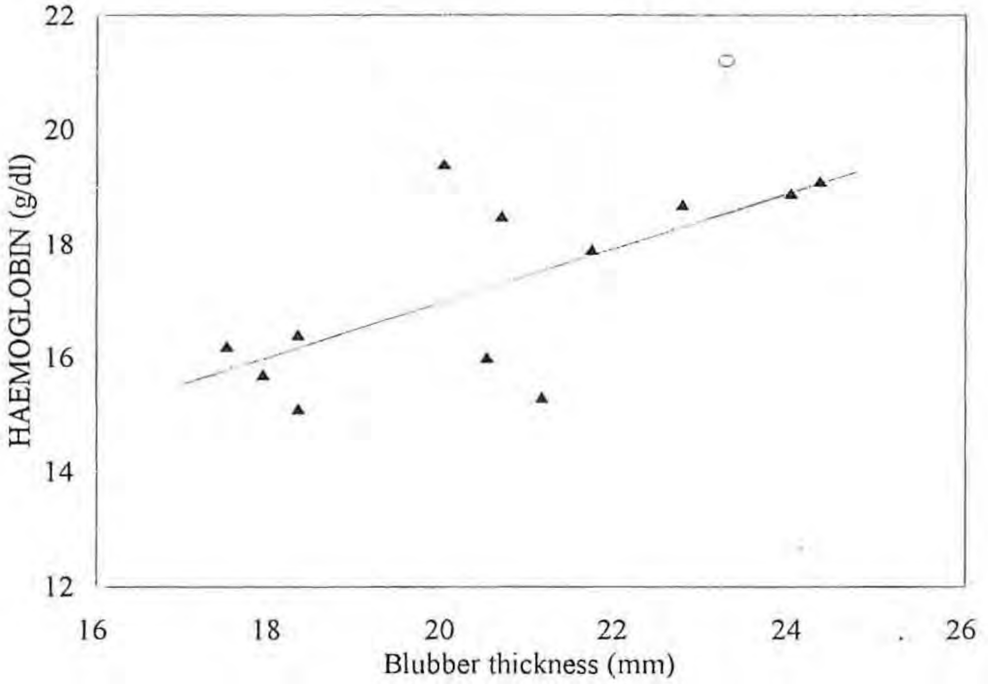


Figure 3.41: Curve of best fit (—) for Domino with a significant correlation ($p<0.05$) between haemoglobin (HB) concentration and blubber thickness. Included in the graph are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

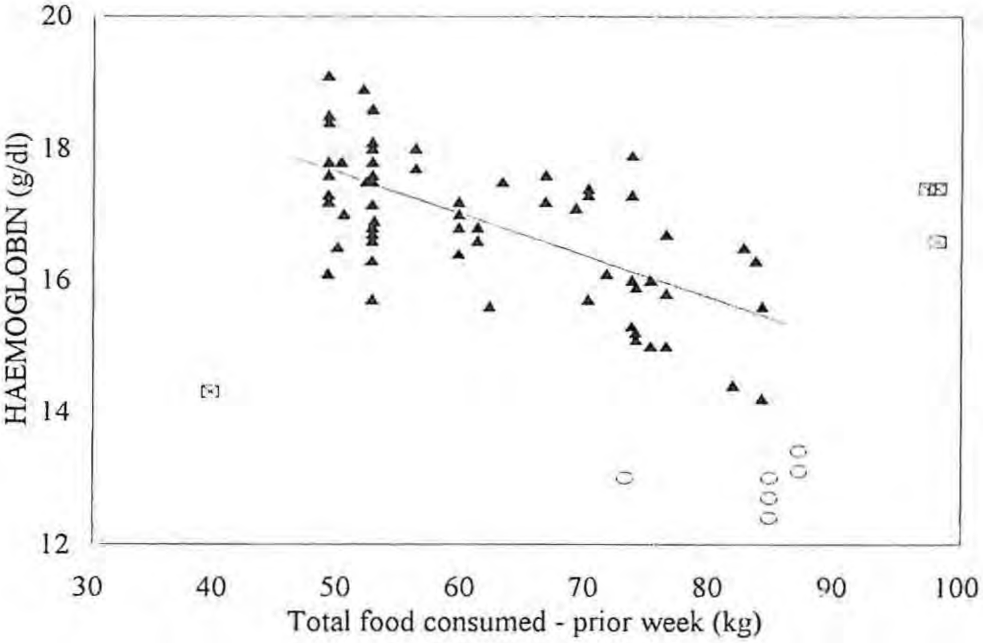


Figure 3.42: Curve of best fit (—) for Thandi with a significant correlation ($p<0.05$) between haemoglobin (HB) concentration and total food consumed in the week prior to blood sampling. Included in the graph are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ▨ ; y-axis: ○).

predominantly at increased levels of food consumption, although "normal" HB values were also measured when food intake was above 90 kg/week and below 40 kg/week. Similarly, Thandi's HB levels were negatively correlated with all categories of dietary energy intake (Table 3.9). In contrast, Dolly's HB levels were positively correlated with the mean daily energy intake of the month prior to the blood sample (Table 3.9). Although data were variable, relationships between Thandi's HB and energy intake in the short-term were similar, with reduced HB associated predominantly with greater short-term energy intake (Fig. 3.43). Relationships between HB and long-term energy intake for Dolly and Thandi were similar to those described for RBC counts.

Table 3.9: Correlation coefficients (r), levels of significance (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$) and sample sizes (n) for significant relationships between haemoglobin (HB) concentration and independent variables for the five captive dolphins (Data are presented as r*(n)) (NS: non-significant ($p>0.05$)).

Variable	Dimple	Dolly	Thandi	Simo	Domino
Age	0.46***(45)	0.35*(44)	-0.43***(80)	NS	NS
Weight	NS	0.46**(40)	-0.30*(57)	0.30*(42)	NS
Blubber thickness	NS	NS	NS	0.69**(12)	NS
D1	NS	NS	-0.46***(65)	NS	NS
E1	NS	NS	-0.41***(68)	NS	NS
E2	NS	NS	-0.41***(65)	NS	NS
E3	NS	NS	-0.45***(69)	NS	NS
E4	NS	0.37**(43)	-0.36**(77)	NS	NS
T1	-0.41**(45)	NS	0.34**(80)	NS	NS
T2	-0.40**(45)	NS	0.36**(79)	NS	NS
T3	NS	NS	0.26*(80)	NS	NS

D1: Total food consumption of week prior to blood sample, E1: Dietary energy intake of day prior to blood sample; E2: Mean daily energy intake of week in which blood was sampled, E3: Mean daily energy intake of week and month (E4) prior to blood sample, T1: Mean water temperature of week prior to blood sample, T2: Mean water temperature of month in which blood was sampled, T3: Mean water temperature of month prior to blood sample.

Haemoglobin was negatively correlated with water temperature for Dimple, but positively correlated for Thandi (Table 3.9). Data from Dimple showed that although HB values remained relatively stable (~16.5-20.0 g/dl) over a wide range of water temperatures, lower HB concentrations were more frequent at higher temperatures (Fig. 3.44A). In contrast, Thandi's HB tended to increase slightly with increasing water temperature (Fig. 3.44B). Outlying HB levels, in both animals, were associated with colder water temperatures.

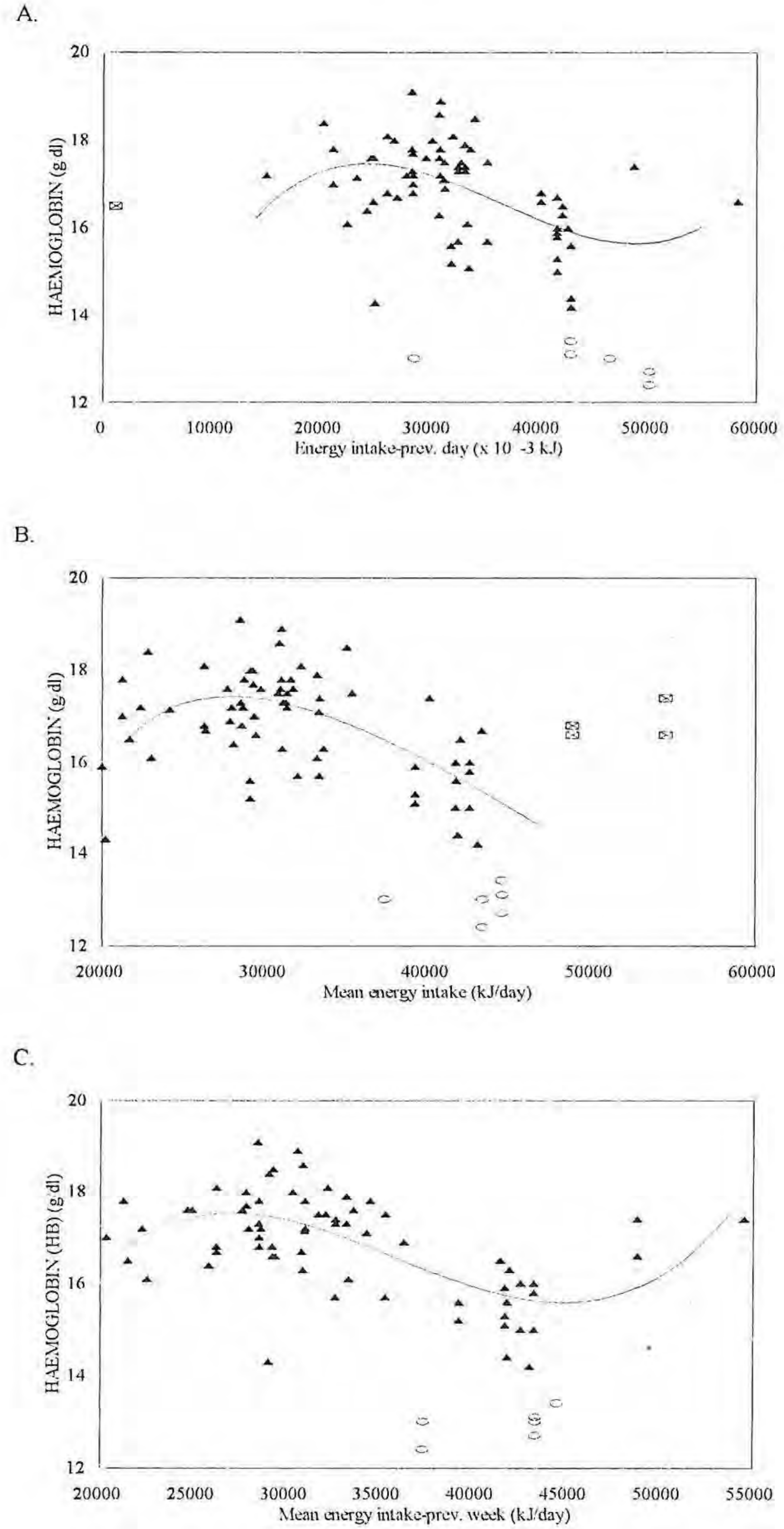


Figure 3.43: Curves of best fit (—) for Thandi with significant correlations ($p<0.05$) between haemoglobin (HB) concentration and the dietary energy intake of the day prior to the blood sample (A), the mean daily energy intake of the week in which blood was sampled (B) and the mean daily energy intake of the week prior to the blood sample (C). Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

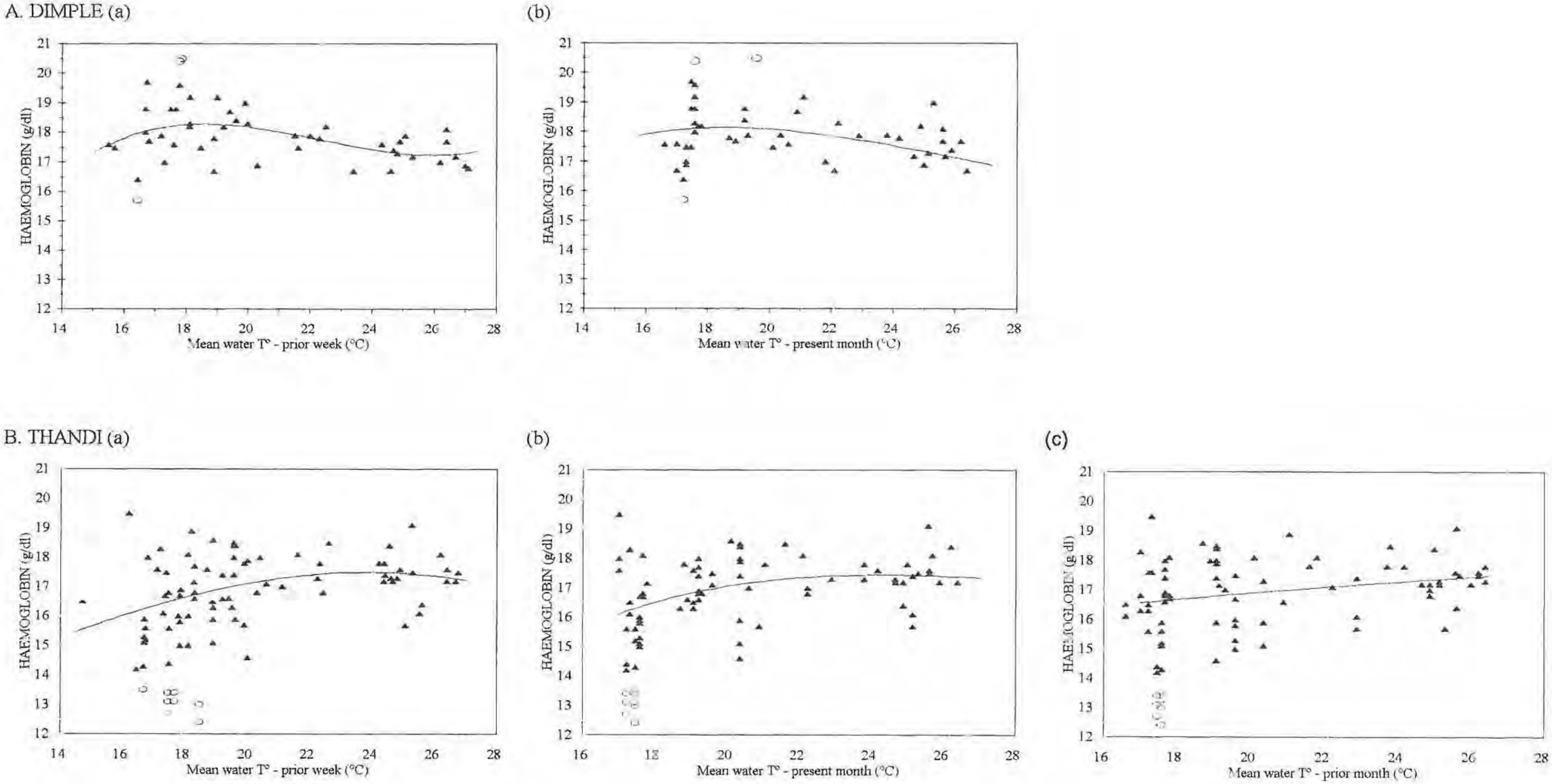


Figure 3.44: Curves of best fit (—) for animals (A. Dimple; B. Thandi) with significant correlations ($p<0.05$) between haemoglobin (HB) and the mean water temperature of the prior week (a); the month in which blood was sampled (b) and the prior month (c). Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

c. Packed cell volume (PCV)

Changes in PCV during the study mirrored the patterns described for RBC and HB (Fig. 3.45). Mean PCV values for all animals were between 0.45 l/l and 0.55 l/l (Table 3.4) and were generally similar to data recorded prior to 1992, although the mean PCV for Thandi declined slightly during the study. This was due primarily to several outlying low PCV values measured in the final stages of the study (Fig. 3.45C). Associations between PCV and illness, for other animals, were difficult to assess due to high variability. However, low PCV was classified as outlying more frequently than increased PCV (Appendix 1.4C). Dimple was the only animal for which elevated PCV's (> 0.56 l/l) were classified as outlying. Mean PCV values were significantly different between animals (F=27.50; p<0.001; n=251) (Table 3.4). Dolly (0.52 ± 0.02 l/l) and Simo (0.53 ± 0.03 l/l) had significantly higher mean PCV's, although those of Dimple (0.50 ± 0.02 l/l) and Domino (0.49 ± 0.03 l/l) were only slightly (but significantly) lower (Two sample t-test; p<0.001). Thandi had the lowest mean PCV (0.47 ± 0.04 l/l), which was significantly different from all animals except Domino (t=1.84; p=0.07; df=122) (Table 3.4).

Overall, PCV did not appear to change markedly with the age of captive dolphins (Fig. 3.46). During the study specifically, no meaningful age-related changes occurred in PCV, although data from both Dimple and Thandi were coincidentally correlated with age (Table 3.10). PCV was significantly related to weight in three animals: Dolly, Thandi and Simo (Table 3.10). However, although PCV values from both Dolly and Simo were slightly reduced at low weights, the data did not reflect any significant trends. In addition, PCV's associated with significantly reduced weights were within the "normal" range for each animal. Domino's PCV values were positively related to blubber thickness (Table 3.10). This relationship was similar to those described for Domino's RBC and HB data, with thicker blubber affiliated with increased PCV.

Table 3.10: Correlation coefficients (r), levels of significance (*: p<0.05, **: p<0.01, ***: p<0.001) and sample sizes (n) for significant relationships between packed cell volume (PCV) and independent variables for the five captive dolphins (Data are presented as r*(n)) (NS: non-significant (p>0.05)).

Variable	Dimple	Dolly	Thandi	Simo	Domino
Age	0.36*(43)	NS	-0.44***(87)	NS	NS
Weight	NS	0.32*(38)	-0.33**(57)	0.35*(40)	NS
Blubber thickness	NS	NS	NS	NS	0.78**(11)
D1	NS	NS	-0.67***(69)	NS	NS
E1	NS	NS	-0.46***(72)	NS	NS
E2	NS	NS	-0.51***(69)	NS	NS
E3	NS	NS	-0.48***(73)	NS	NS
E4	0.36*(38)	0.38**(43)	-0.34***(84)	NS	NS
T1	NS	NS	0.42***(87)	NS	-0.37*(35)
T2	NS	NS	0.46***(86)	NS	-0.43**(35)
T3	NS	NS	0.39***(87)	NS	NS

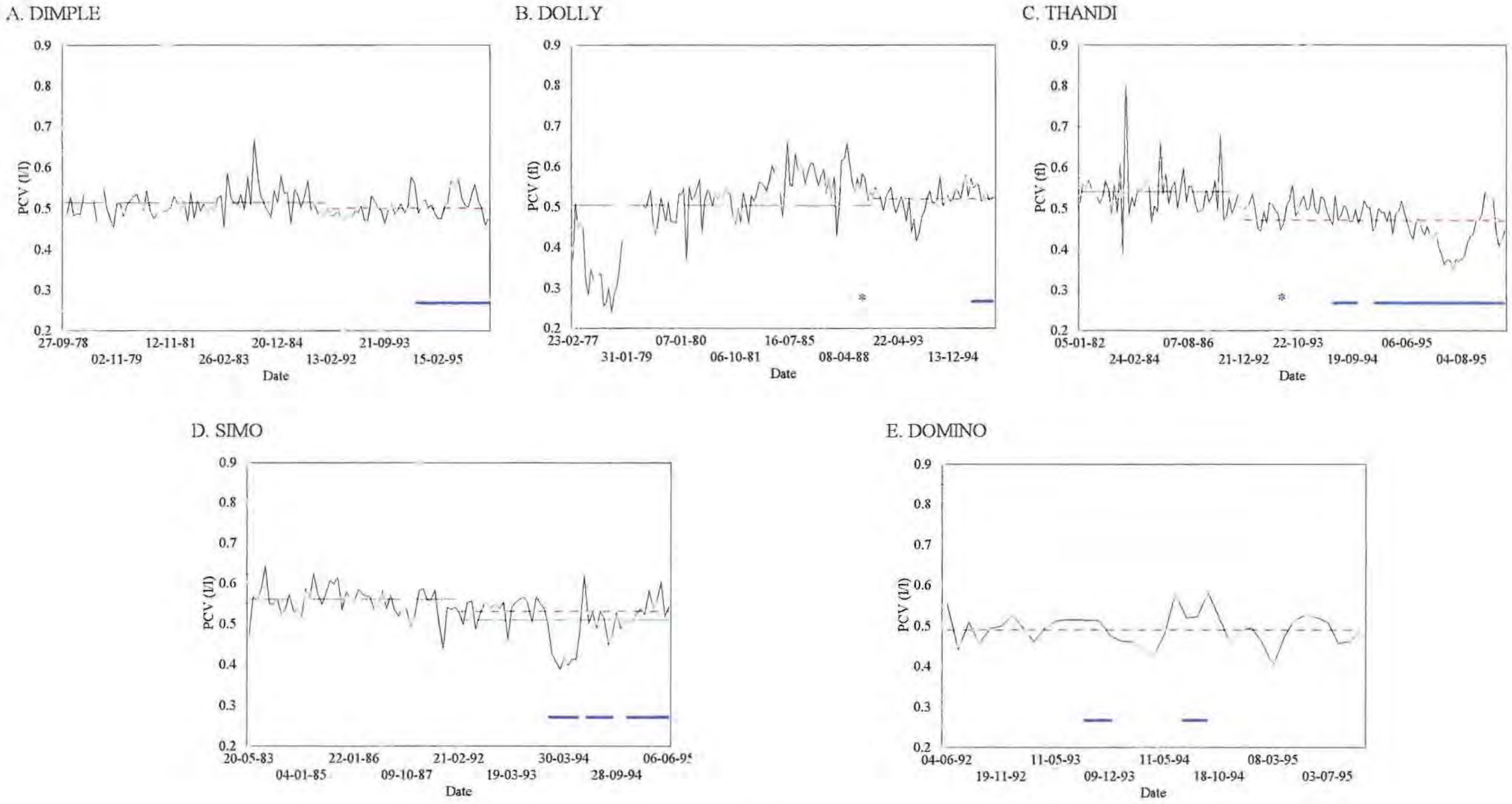


Figure 3.45: Packed cell volume (PCV) values (l/l) (——) for all five captive animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the mean PCV for all records prior to the study (——), the overall mean PCV (——) and the "normal" (see text) mean PCV (- - -) for the study between 1992 and 1995. (*: parturition; ▬: extended periods of illness and/or treatment or periods of aberrant behaviour and/or feeding).

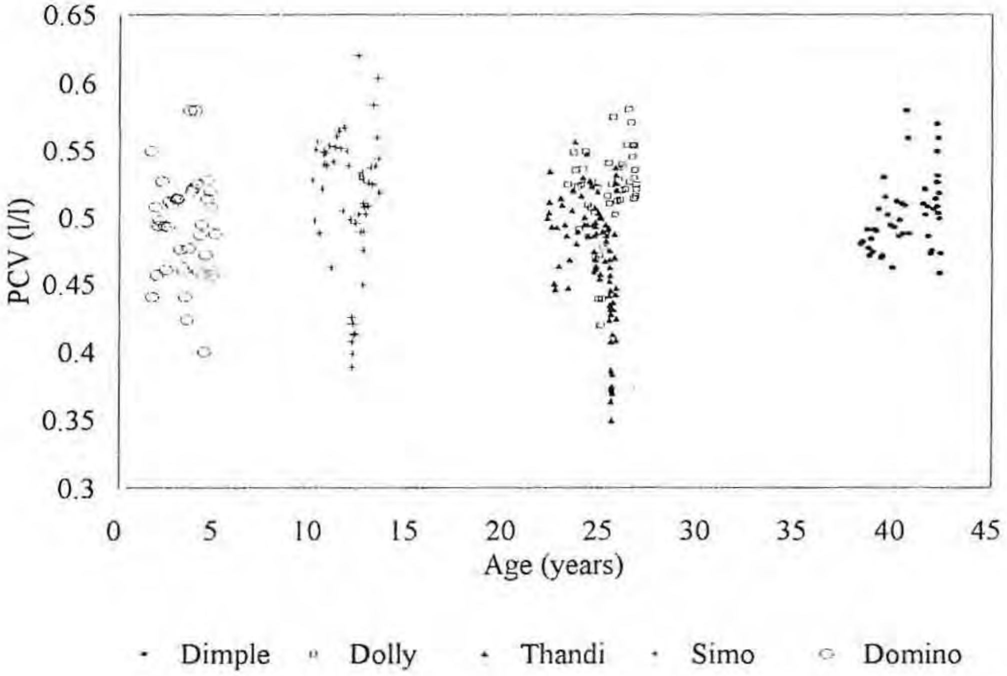


Figure 3.46: Relationship between packed cell volume (PCV) and age, for the five captive dolphins, from records obtained between 1992 and 1995.

D1: Total food consumption of week prior to blood sample, E1: Dietary energy intake of day prior to blood sample; E2: Mean daily energy intake of week in which blood was sampled, E3: Mean daily energy intake of week and month (E4) prior to blood sample, T1: Mean water temperature of week prior to blood sample, T2: Mean water temperature of month in which blood was sampled, T3: Mean water temperature of month prior to blood sample.

Relationships between PCV and dietary data were, in most cases, evident for Thandi only and were similar to those described for other haematological parameters (Table 3.10). PCV values were generally variable over a wide range of food and/or energy intake levels. However, in all cases, PCV was reduced at food and energy intake levels greater than 75 kg/week and 40 000 kJ/day, respectively (Fig. 3.47 & 3.48). Trends were complicated by extraordinary data in some cases.

Packed cell volume data from the three female dolphins were significantly correlated with the mean daily energy intake of the month preceding the blood sample (Table 3.10). Data from both Dimple and Dolly were positively correlated with this dietary parameter (Fig. 3.49 A&B), but Thandi's PCV data were negatively correlated with the same dietary parameter. Although Thandi's PCV appeared to decrease slightly at energy intake levels greater than 37 500 kJ/day, values were particularly variable at this point (Fig. 3.49C).

Data from Thandi and Domino showed PCV to be significantly correlated with water temperature, although Domino's data were not related to the mean water temperature of the month preceding the blood sample (Table 3.10). Relationships for Thandi were similar to those described for HB, with PCV's generally lower and more variable at temperatures below 20°C (Fig. 3.50A). PCV data for Domino varied (0.44-0.53 l/l) over the entire temperature range (15-27°C), but volumes decreased slightly at higher temperatures (Fig. 3.50B).

d. Mean cell volume (MCV)

Mean cell volumes for all five animals were less variable than other haematological parameters (Fig. 3.51). In addition, no specific fluctuations in MCV could be associated with aberrant periods of feeding and/or illness (Fig. 3.51). A declining trend in Dolly's MCV was observed following the birth of Domino in 1991 (Fig. 3.51B). Ranges of MCV data recorded during the study were restricted, but several outlying high MCV values were recorded (Appendix 1.4D). Mean MCV values, during the study, were generally lower than means calculated from previous records. Dolly (112.80 ± 3.69 fl) had a similar mean MCV to both Simo (112.10 ± 2.27 fl) (t=1.03; p=0.31; df=94) and Domino (113.60 ± 1.57 fl). Both Dimple (124.40 ± 1.69 fl) and Thandi (116.10 ± 3.40 fl) had significantly higher mean MCV's (Two sample t-test; p<0.001) (Table 3.4).

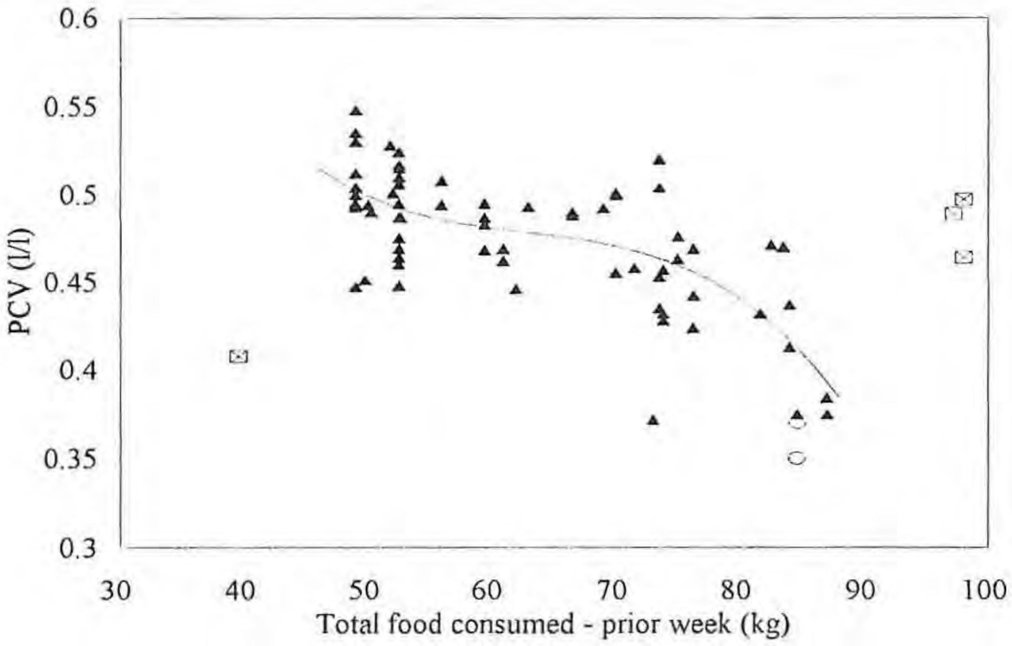


Figure 3.47: Curve of best fit (—) for Thandi with a significant correlation ($p<0.05$) between packed cell volume (PCV) and the total food consumed during the week prior to the blood sample. Included in the graph are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

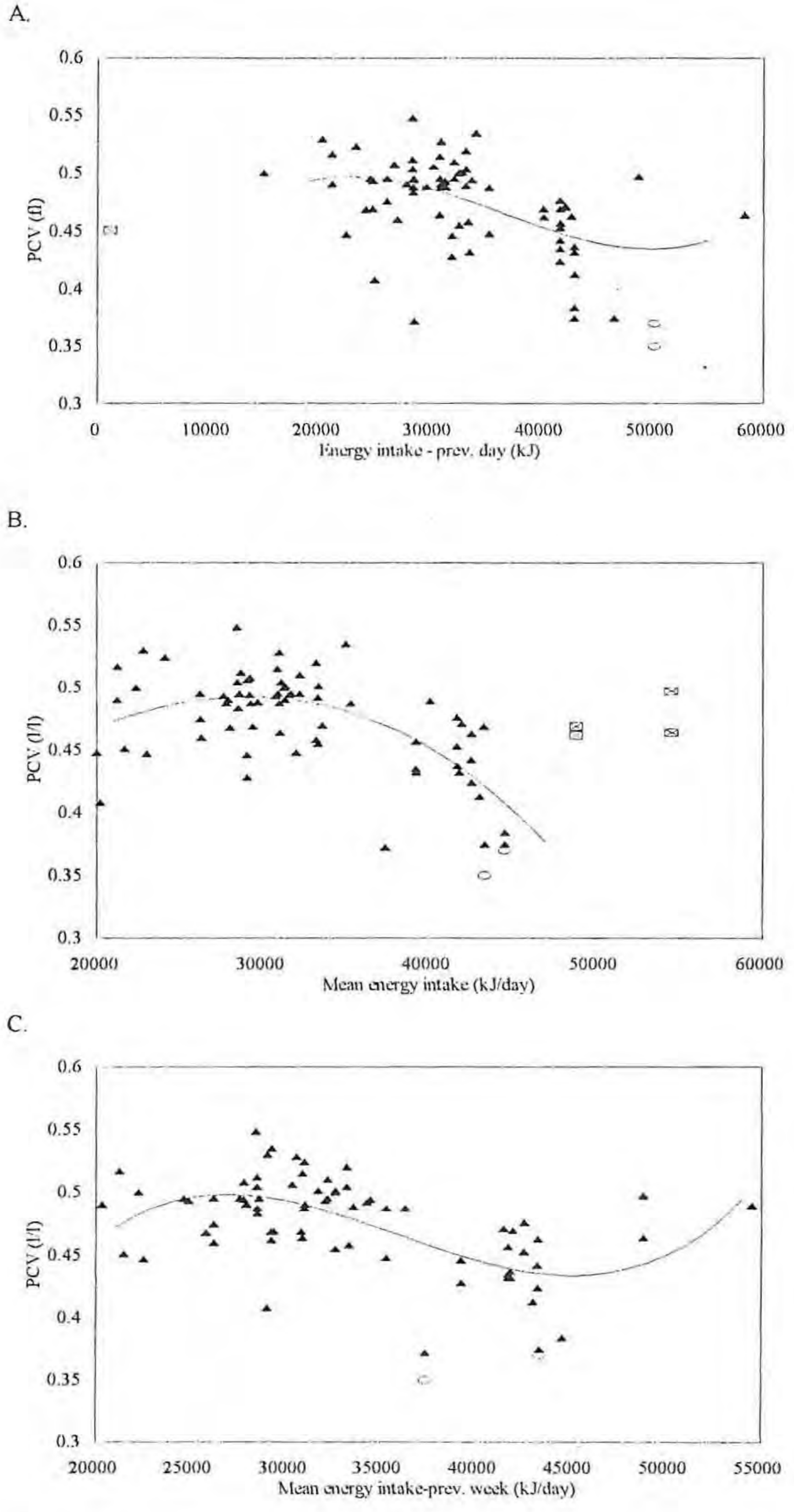


Figure 3.48: Curves of best fit (—) for Thandi with significant correlations ($p<0.$ between packed cell volume (PCV) and the dietary energy intake of th day prior to the blood sample (A), the mean daily energy intake of the week in which blood was sampled (B) and the mean daily energy intak of the week prior to the blood sample (C). Included in the graphs are a "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

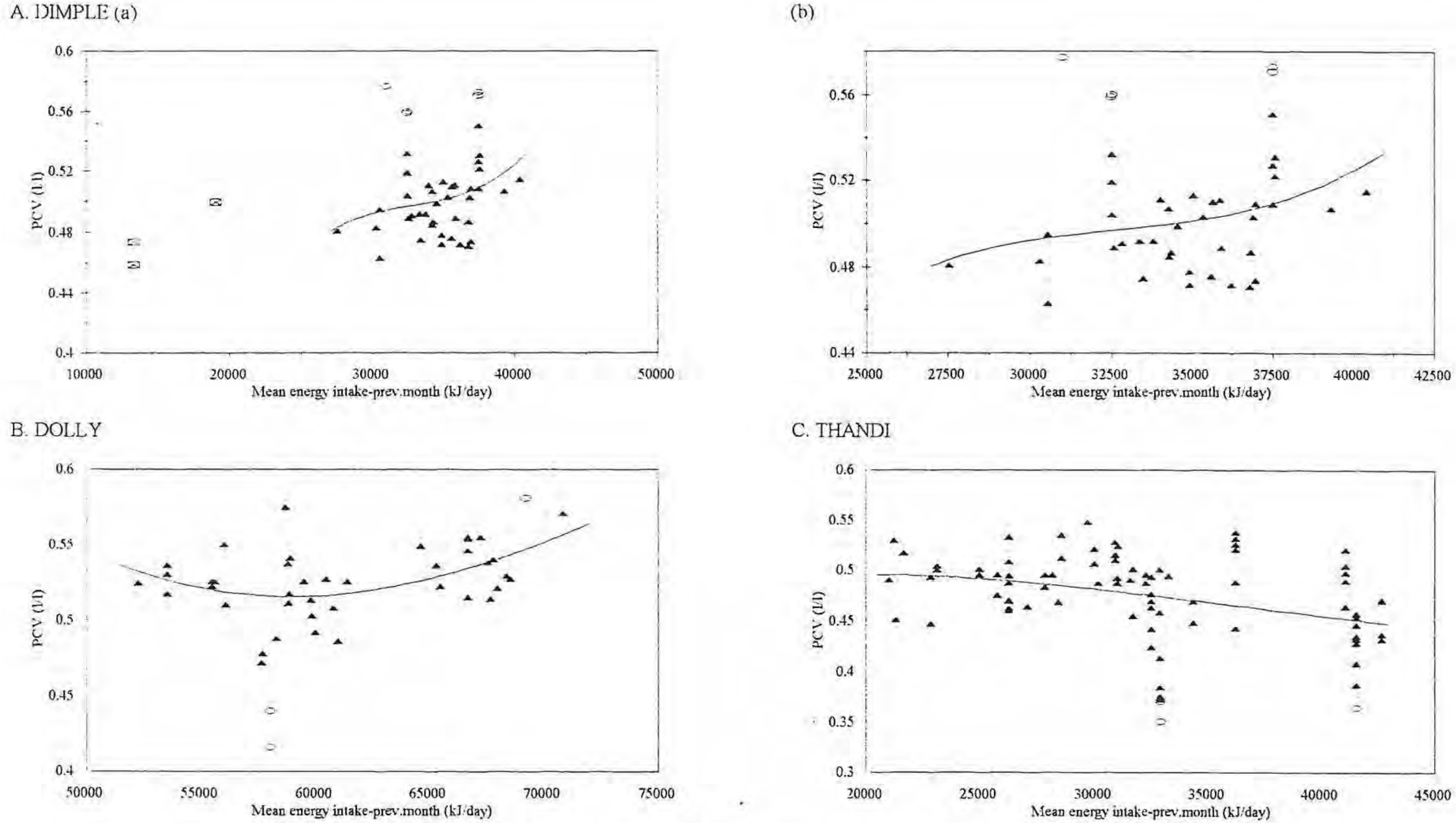


Figure 3.49: Curves of best fit (—) for animals (A. Dimple (a & b); B. Dolly; C. Thandi) with significant correlations ($p<0.05$) between packed cell volume (PCV) and the mean daily energy intake of the month prior to the blood sample. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

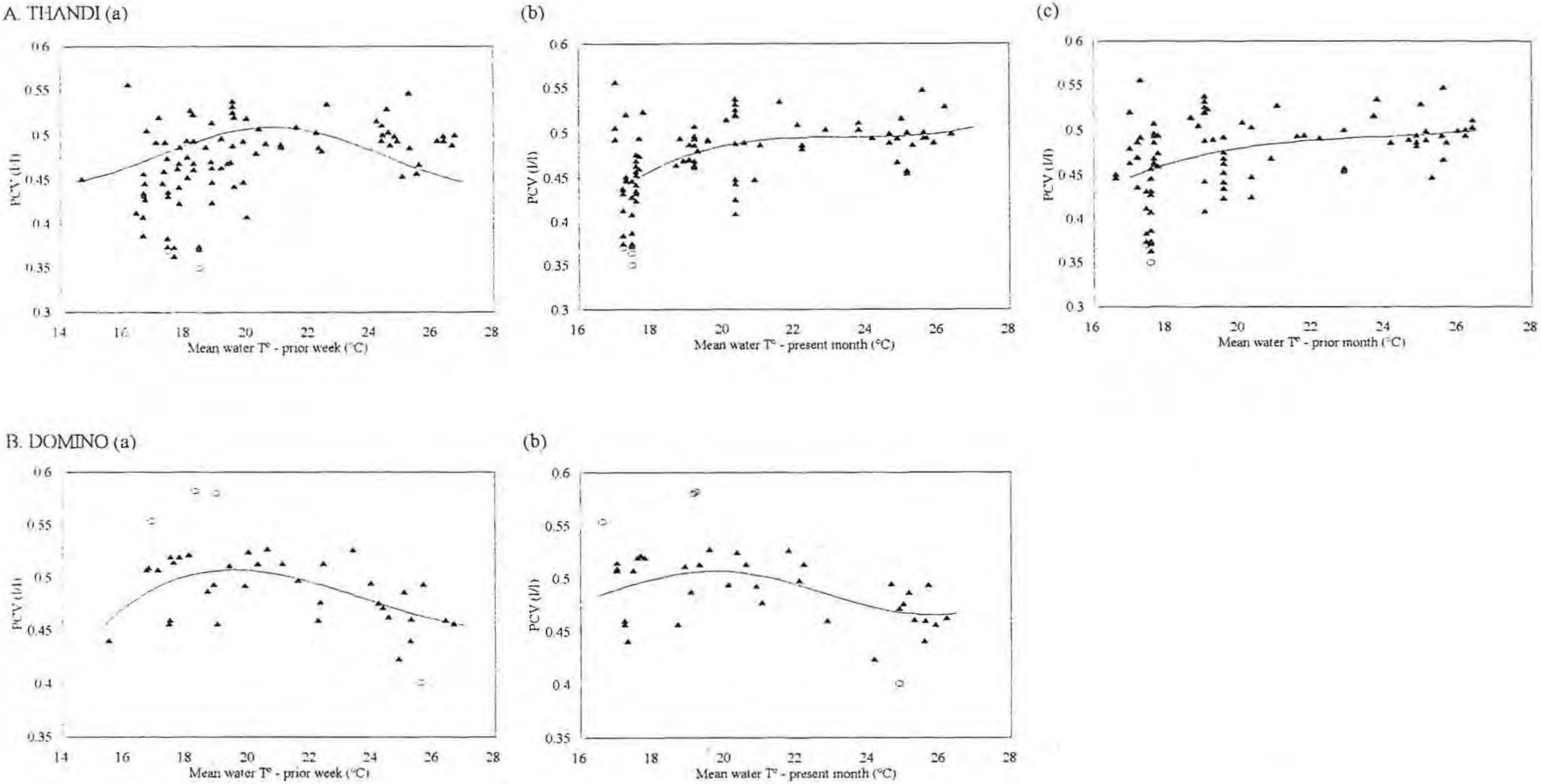


Figure 3.50: Curves of best fit (—) for animals (A. Thandi; B. Domino) with significant correlations ($p<0.05$) between packed cell volume (PCV) and the mean water temperature of the prior week (a); the month in which blood was sampled (b) and the prior month (c). Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

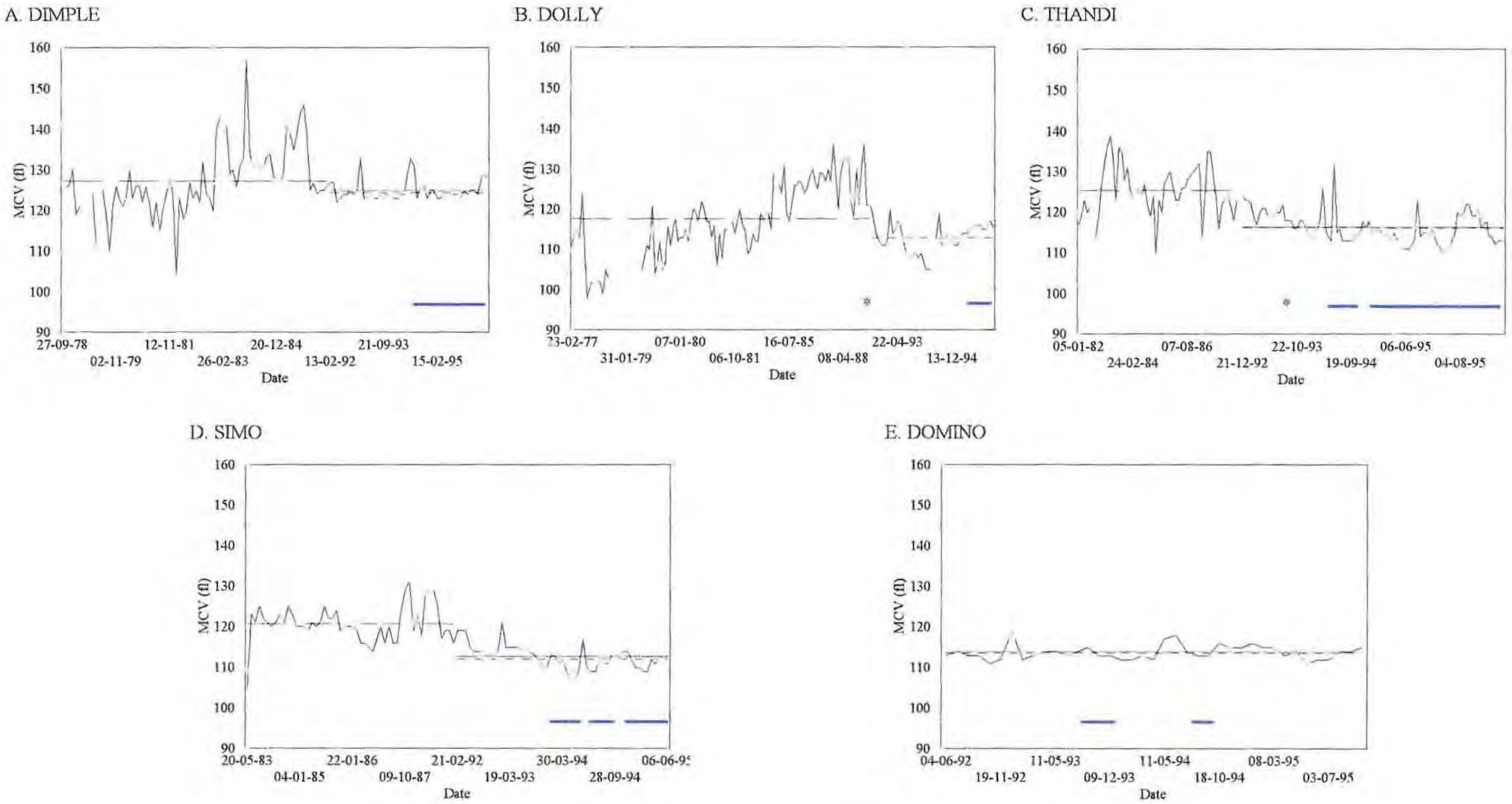


Figure 3.51: Mean cell volume (MCV) values (fl) (—) for all five captive animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the mean MCV for all records prior to the study (- · -); the overall mean MCV (—) and the "normal" (see text) mean MCV (- - -) for the study between 1992 and 1995 (*: parturition; —: extended periods of illness and/or treatment or periods of aberrant behaviour and/or feeding).

Overall, MCV appeared to change markedly only in elderly animals. Dimple, the oldest of the five animals by approximately 12 years, had significantly increased MCV values (Fig. 3.52). There were no biologically significant age-associated changes in MCV during the study. MCV data from the two male dolphins, Simo and Domino were significantly correlated with total body weight (Table 3.11). For Simo, MCV's were highest (~115 fl) at the lower weights (165-170 kg) and appeared to decrease as weight increased (Fig. 3.53A). The trend was complicated by extraordinary MCV values corresponding to weights greater than 190 kg. Domino's MCV was relatively stable (111-115 fl) over the weight range, although there appeared to be a slight increase as weight increased (Fig. 3.53B).

Mean cell volume was negatively correlated with blubber thickness, but only for Dimple (Table 3.11). Although samples were limited, MCV was reduced as blubber thickness increased from 19.4 mm to 24.0 mm. Outlying high MCV values were measured at blubber thicknesses of 21.0 and 21.3 mm (Fig. 3.54).

Table 3.11: Correlation coefficients (r), levels of significance (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$) and sample sizes (n) for significant relationships between mean cell volume (MCV) and independent variables for the five captive dolphins (Data are presented as r*(n)) (NS: non-significant ($p>0.05$)).

Variable	Dimple	Dolly	Thandi	Simo	Domino
Weight	NS	NS	NS	-0.42**(42)	0.42**(36)
Blubber thickness	-0.72*(10)	NS	NS	NS	NS
Month	NS	NS	NS	0.28*(49)	NS
T1	NS	-0.33*(47)	NS	-0.29*(49)	NS
T2	NS	-0.35*(47)	NS	-0.32*(48)	NS
T3	NS	NS	NS	-0.36*(49)	NS

T1: Mean water temperature of week prior to blood sample, T2: Mean water temperature of month in which blood was sampled, T3: Mean water temperature of month prior to blood sample.

Mean cell volume was not significantly related to any of the dietary parameters. Significant monthly changes in MCV were identified only for Simo (Table 3.11). Data were generally highly variable, but this variability appeared to be greatest during the first five months of the year. Outliers were also only measured during this period. In conjunction, MCV was negatively correlated with water temperature for both Dolly and Simo (Table 3.11). Low MCV values were measured more frequently at water temperatures above 23°C (Fig. 3.55). This applied to both Dolly and Simo, with the rate of decrease being similar for both animals.

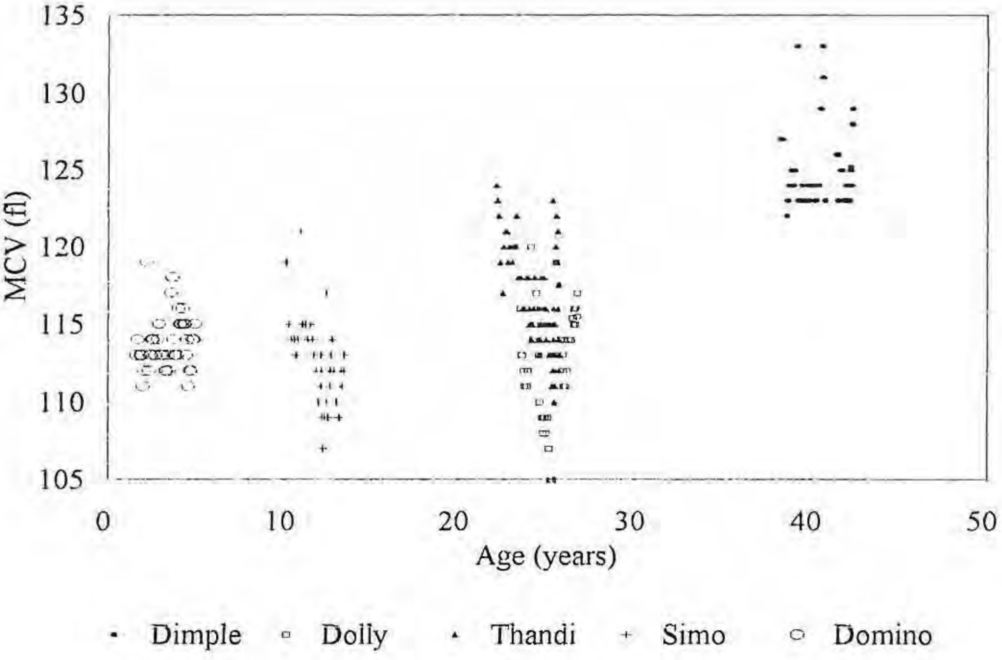
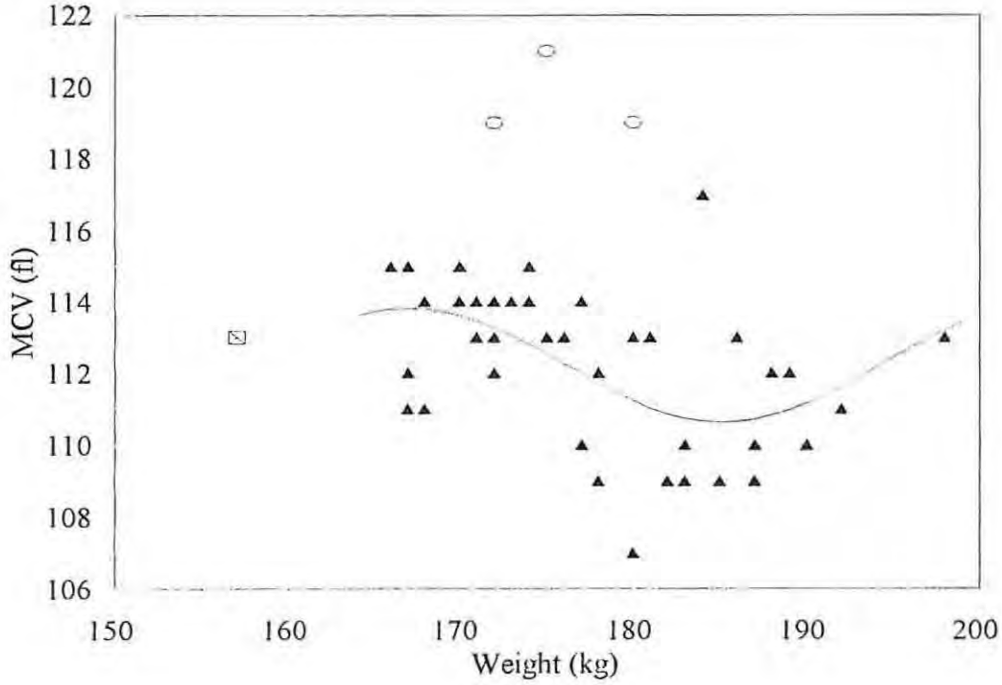


Figure 3.52: Relationship between mean cell volume (MCV) and age, for the five captive dolphins, from records obtained between 1992 and 1995.

A. SIMO



B. DOMINO

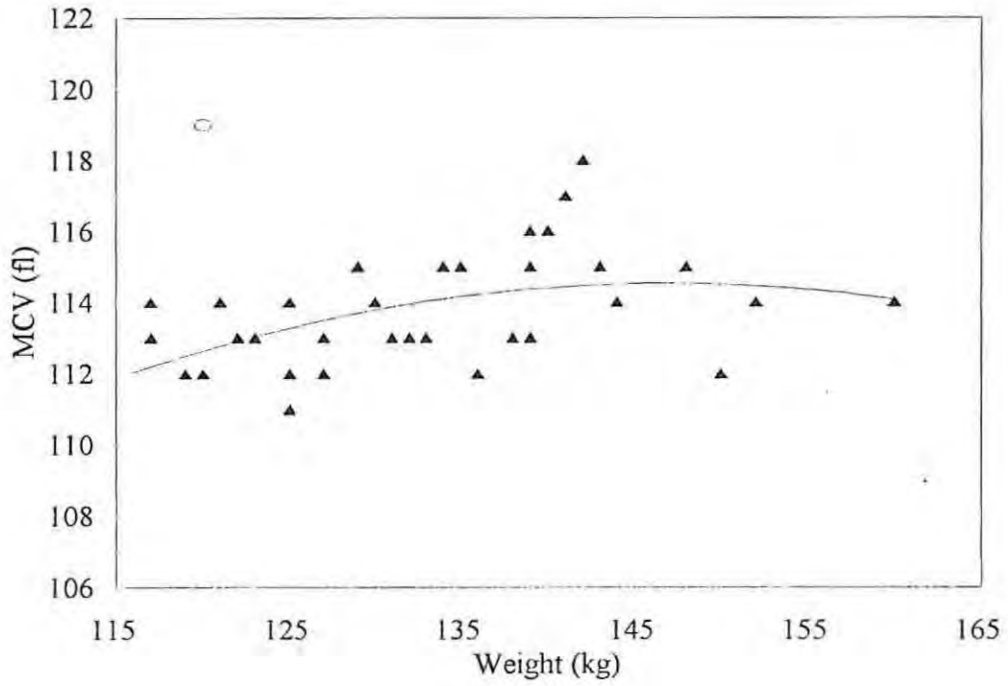


Figure 3.53: Curves of best fit (—) for animals (A. Simo; B. Domino) with significant correlations ($p<0.05$) between mean cell volume (MCV)and weight. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ⊠ ; y-axis: ○).

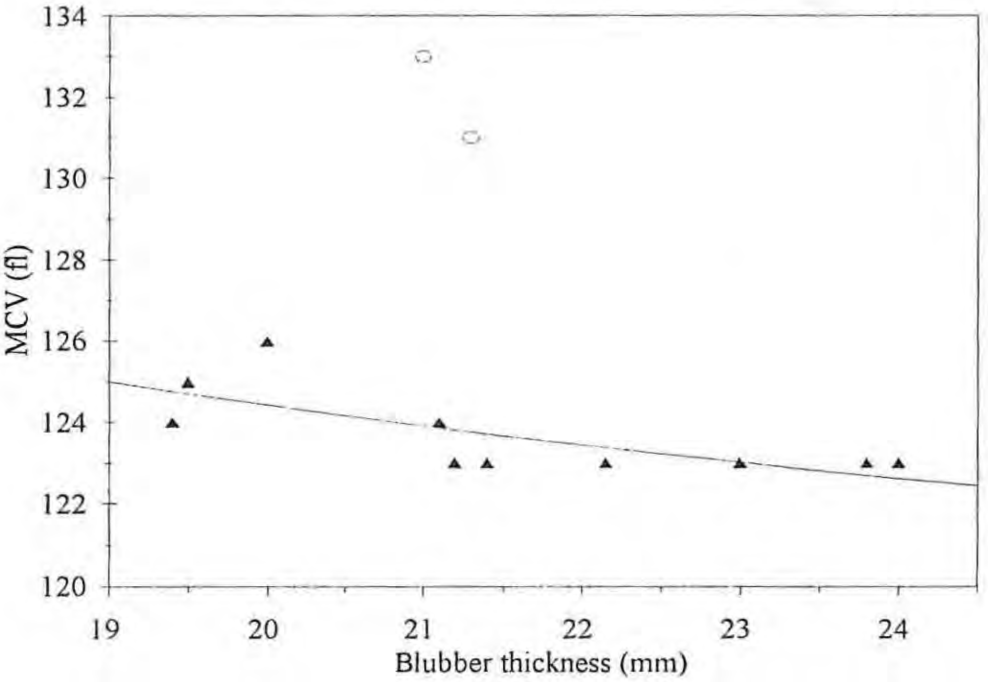


Figure 3.54: Curve of best fit (—) for Dimple with a significant correlation ($p<0.05$) between mean cell volume (MCV) and blubber thickness. Included in the graph are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

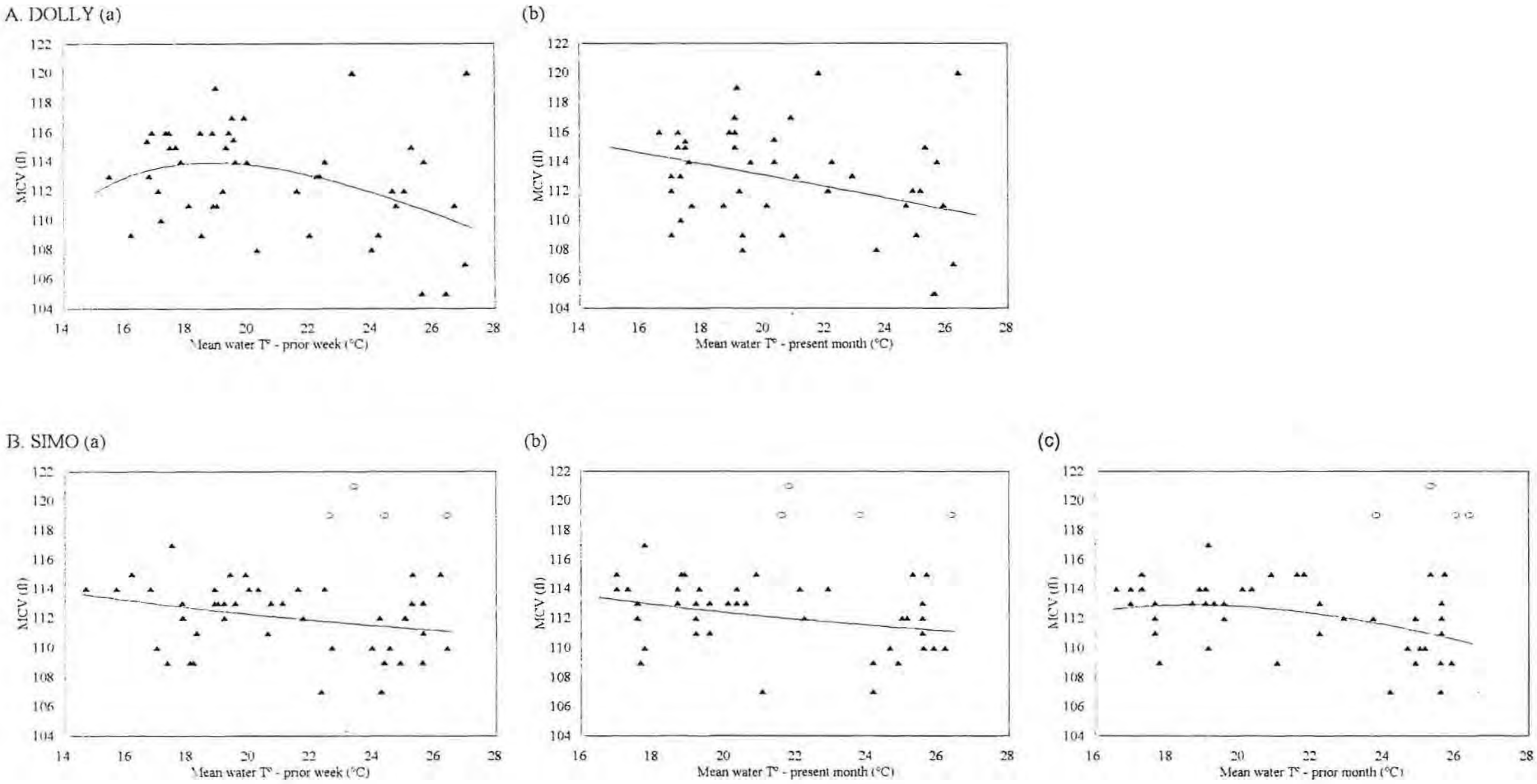


Figure 3.55: Curves of best fit (—) for animals (A. Dolly; B. Simo) with significant correlations ($p<0.05$) between mean cell volume (MCV) and the mean water temperature of the week prior to the blood sample (a); the month in which blood was sampled (b) and the month prior to the blood sample (c). Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

e. Platelet count

Platelet counts varied markedly for all five animals and irregular analyses prevented the description of any particular pattern (Fig. 3.56). Relationships between outlying platelet counts (Appendix 1.5C) and periods of illness were impossible to examine in light of irregular analyses (Fig. 3.56). Based on the data obtained during the study, all five animals had significantly different platelet levels (F=46.71; p<0.001; n=158; Two sample t-test; p<0.01) (Table 3.4). The significance of the difference between Dimple's and Thandi's mean platelet counts (t=-2.82; p=0.006; df=68) was reduced when adjusted (Bonferroni correction, p=0.005). Domino had the highest mean platelet count (198.80 ± 23.92 x10^9/l) with mean platelet levels being reduced (in order) in Simo, Dolly, Thandi and Dimple (Table 3.4).

Overall, platelet counts appeared to decrease in older animals (Fig. 3.57A). This trend was also evident for specific animals during the study. Both Dimple and Domino had platelet counts that were negatively correlated with the change in age during the study (Table 3.12). Limited data made this relationship for Dimple complex, but lower platelet levels (~40-70 x10^9 cells/l) were measured predominantly at the end of the study (estimated age 42 years) (Fig. 3.57B). Domino's platelet levels showed a similar, but less complex, decrease (~220-170 x10^9 cells/l) as age increased between 1.5 and 5 years (Fig. 3.57C).

Platelet counts from both Simo and Domino decreased significantly with increasing weight (Table 3.12) (Fig. 3.58). Domino's platelet level decreased from approximately 225 x10^9 cells/l to 170 x10^9 cells/l as weight increased from 115 kg to 142 kg (Fig. 3.58B). Platelet levels for Simo were more variable, but decreased by approximately 100 x10^9 cells/l as weight increased from 165 kg to 198 kg (Fig. 3.58A). There were no clear relationships between outlying values for these parameters.

Table 3.12: Correlation coefficients (r), levels of significance (*: p<0.05, **: p<0.01, ***: p<0.001) and sample sizes (n) for significant relationships between platelet count and independent variables for the five captive dolphins (Data are presented as r*(n)) (NS: non-significant (p>0.05)).

Variable	Dimple	Dolly	Thandi	Simo	Domino
Age	-0.46*(22)	NS	NS	NS	-0.62**(22)
Weight	NS	NS	NS	-0.51**(35)	-0.59**(21)
E1	NS	-0.62**(22)	0.36*(36)	NS	NS
E2	NS	-0.53**(33)	NS	NS	NS
E3	-0.46*(19)	-0.52**(22)	0.36*(37)	NS	NS
E4	NS	NS	0.30*(43)	NS	-0.57**(19)
Month	NS	NS	0.32*(48)	NS	NS

E1: Dietary energy intake of day prior to blood sample; E2: Mean daily energy intake of week in which blood was sampled, E3: Mean daily energy intake of week and month (E4) prior to blood sample.

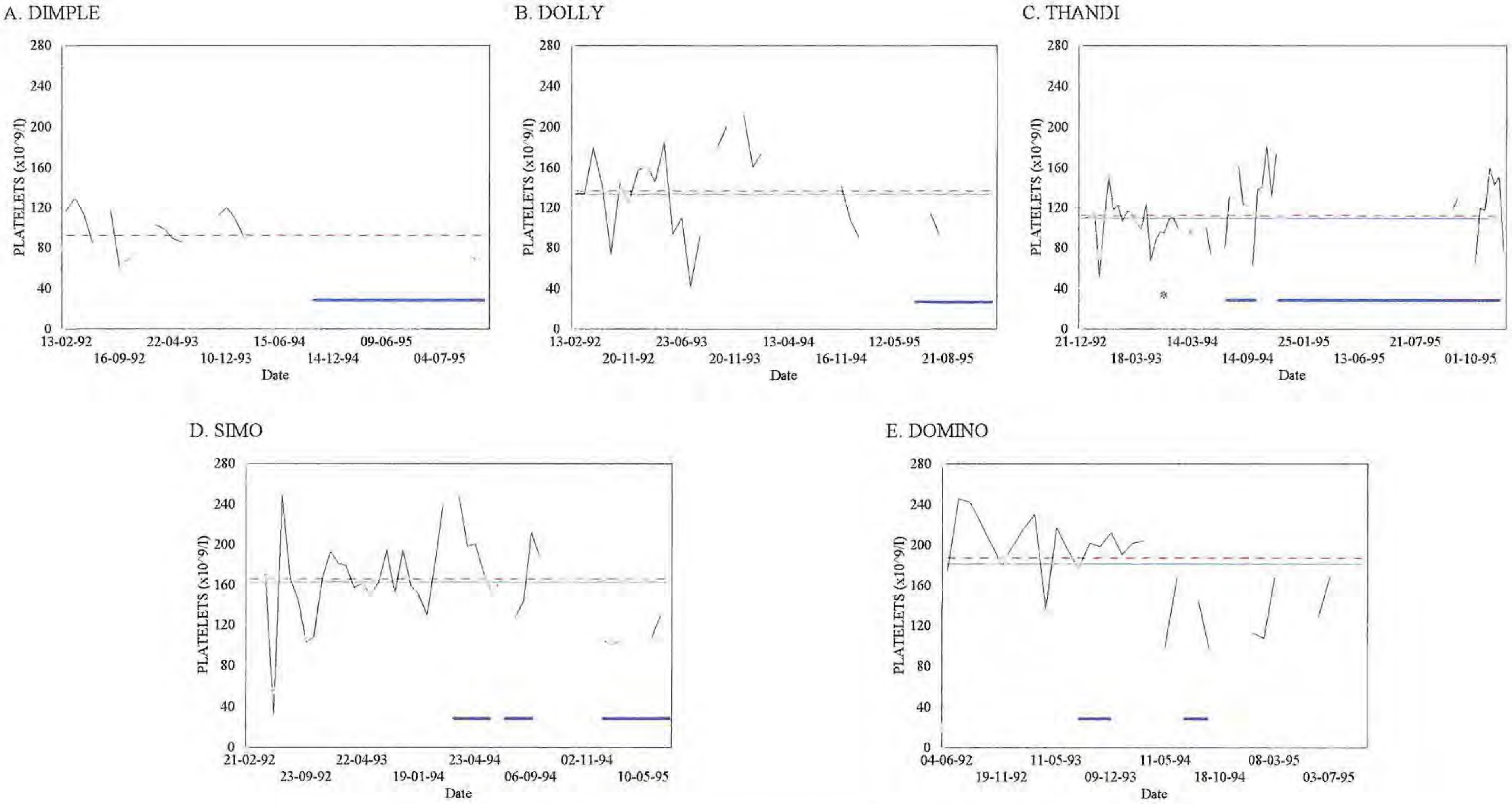
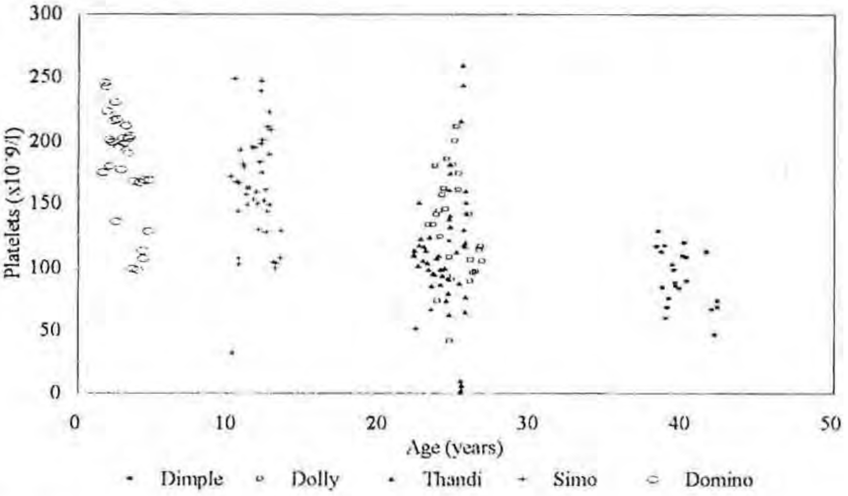
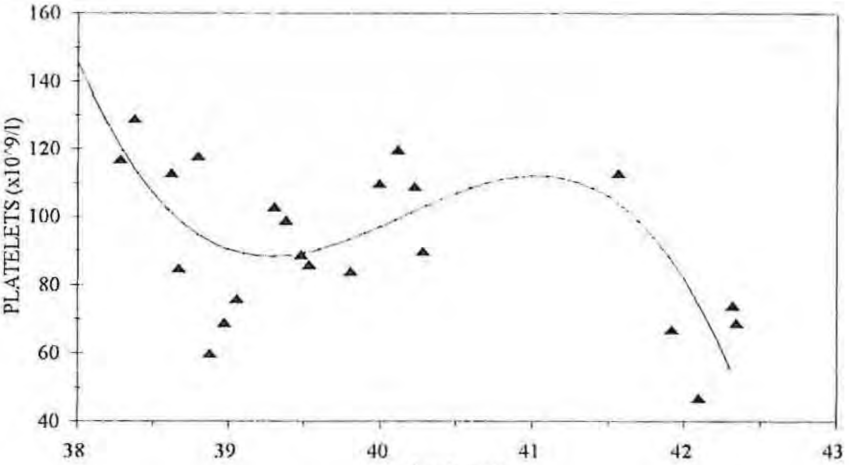


Figure 3.56: Platelet counts (x10^9/L) (—) for all five captive animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the overall mean platelet level (—) and the "normal" (see text) mean platelet count (- - -) for the study between 1992 and 1995 (*: parturition; ▬: extended periods of illness and/or treatment of periods of aberrant feeding and/or behaviour).

A.



B. DIMPLE



C. DOMINO

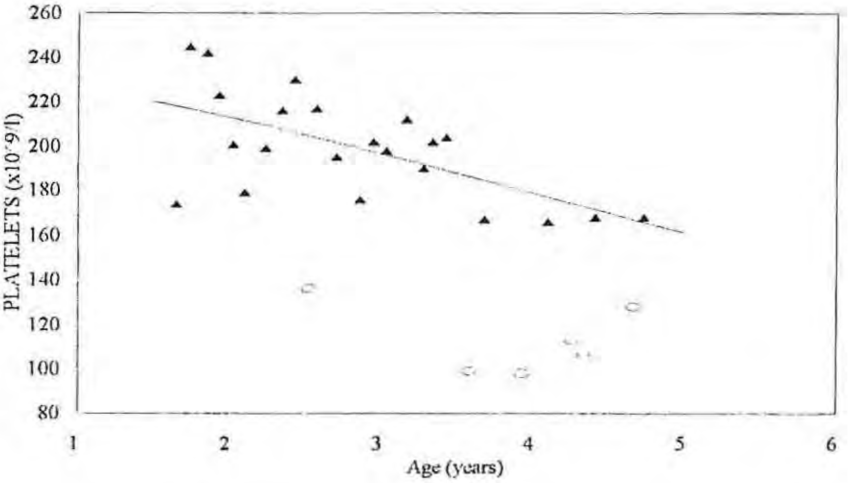
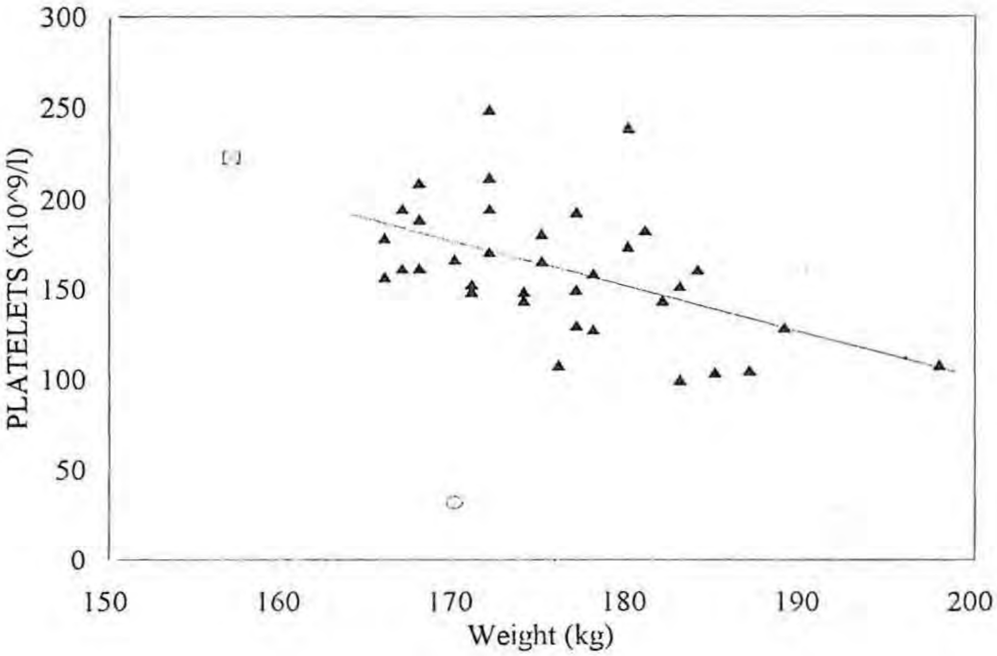


Figure 3.57: Overall relationship between age and platelet count for the five capti dolphins (A) and curves of best fit (—) for animals (B. Dimple; C. Domino) with significant correlations ($p<0.05$) between age and platelet count during the study. Included in the graphs (B & C) are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

A. SIMO



B. DOMINO

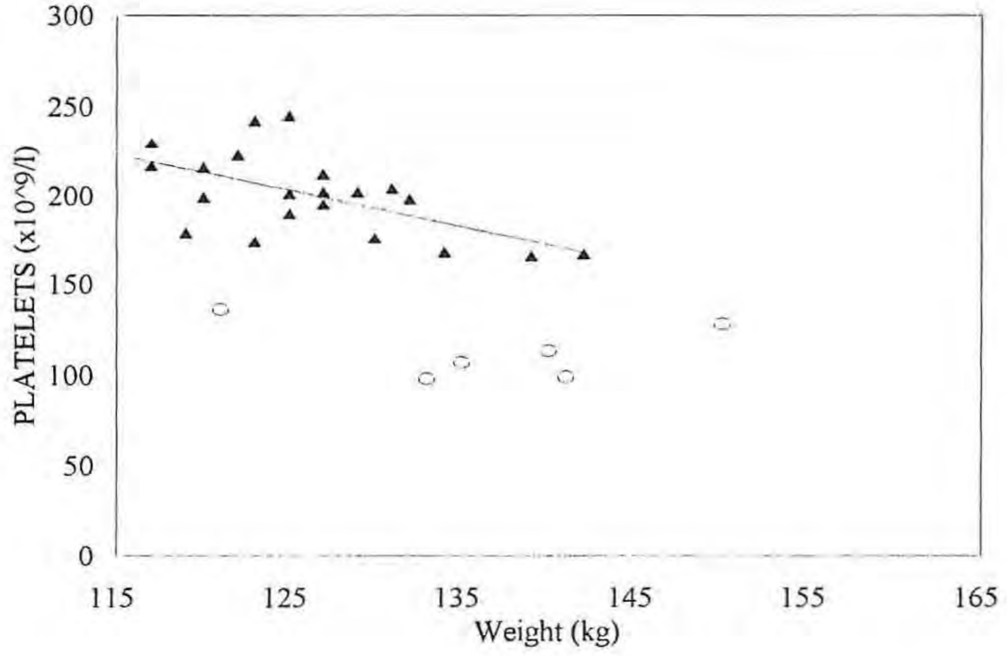


Figure 3.58: Curves of best fit (—) for animals (A. Simo; B. Domino) with significant correlations ($p<0.05$) between platelet count and weight. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

Although a number of animals had platelet counts that were significantly correlated with the various dietary parameters (Table 3.12), data were generally highly variable and the majority of these trends were difficult to clarify. Dolly's platelet count decreased steadily as energy intake increased (Fig. 3.59A). Platelet counts for Thandi, whose energy intake was slightly lower, were relatively stable, if variable (~75-130 x10^9 cells/l), between energy intake levels of 20 000 and 35 000 kJ (Fig. 3.59B). In addition, platelet counts from Domino and Thandi were significantly correlated with the mean daily energy intake of the month prior to the blood sample (Table 3.12) (Fig. 3.60). This trend, for Domino, was almost identical to those described for Dolly (Fig. 3.60A). Thandi's platelet counts appeared relatively stable over a wide range of energy intake levels (Fig. 3.60B). There were no clear relationships between outlying energy intake levels and platelet counts.

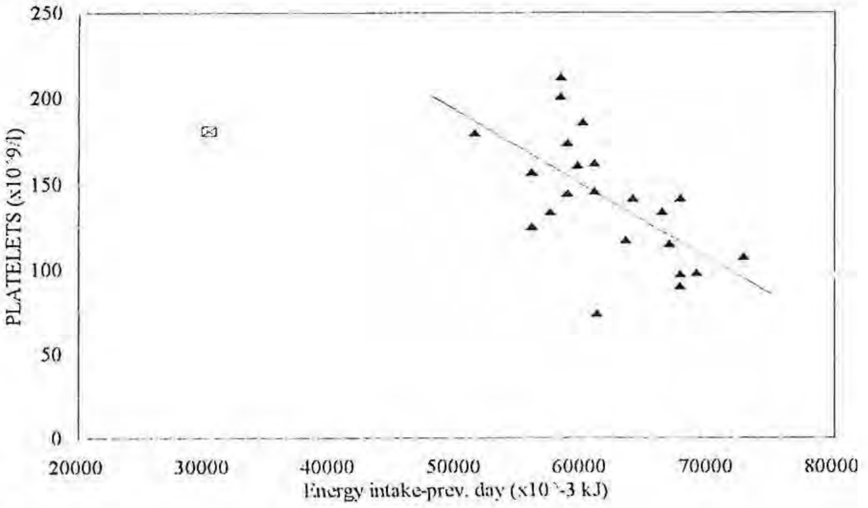
Although platelet levels were not significantly related to water temperature, data from Thandi showed that platelet numbers underwent significant seasonal changes (Table 3.12). Although data were relatively stable throughout the year, platelet counts appeared to decline in the winter. Outliers were recorded only during the winter months (Fig. 3.61).

f. Reticulocyte count / Corrected index

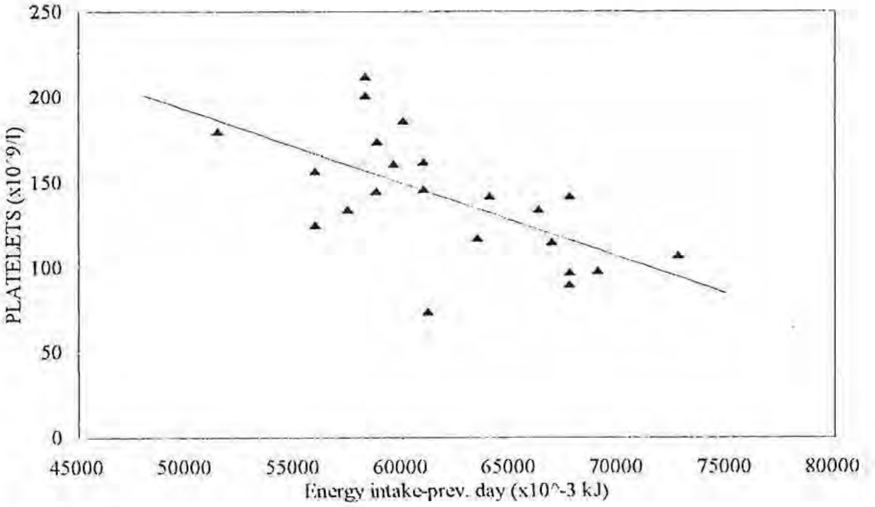
The trends of reticulocyte counts and associated corrected index were very similar (Fig. 3.62 & 3.63), as corrected index is calculated from reticulocyte count (See Materials and Methods). Few data existed for these parameters prior to 1992. Data from Thandi, Simo and Domino were particularly variable, although Simo was the only animal for which low outlying data were identified (Appendix 1.5D & E). Outlying high reticulocyte counts and corrected indices could be associated most clearly with periods of illness for Thandi and Simo (Fig. 3.62 & 3.63). Simo's reticulocyte count and corrected index remained markedly reduced during the final period of the study, which comprised a period of illness, eventually resulting in the animal's death. The relationships between the five animals were identical for these two parameters. Dimple (ret. count: 3.02 ± 0.98 % / cor. index: 1.79 ± 0.65 %) and Dolly (2.80 ± 0.84 % / 1.67 ± 0.50 %) had similar mean reticulocyte counts and corrected indices (Table 3.4). However, these were significantly lower than Thandi (4.43 ± 2.22 % / 2.38 ± 1.20 %), Simo (4.78 ± 2.33 % / 2.64 ± 1.12 %) and Domino (5.02 ± 2.04 % / 2.70 ± 1.04 %) (Two sample t-test; $p<0.001$) (Table 3.4).

Although mean reticulocyte counts and mean corrected index values increased with the age of the animals, overall, these parameters did not differ markedly between animals of different ages (Fig. 3.64). Variation in values was similar for all animals. During the study, reticulocyte counts and their associated corrected indices were negatively correlated with age in Dolly and Domino (Table 3.13). Reticulocyte counts for Dolly were relatively stable and any definite decline was difficult to

A. DOLLY (a)



(b)



B. THANDI

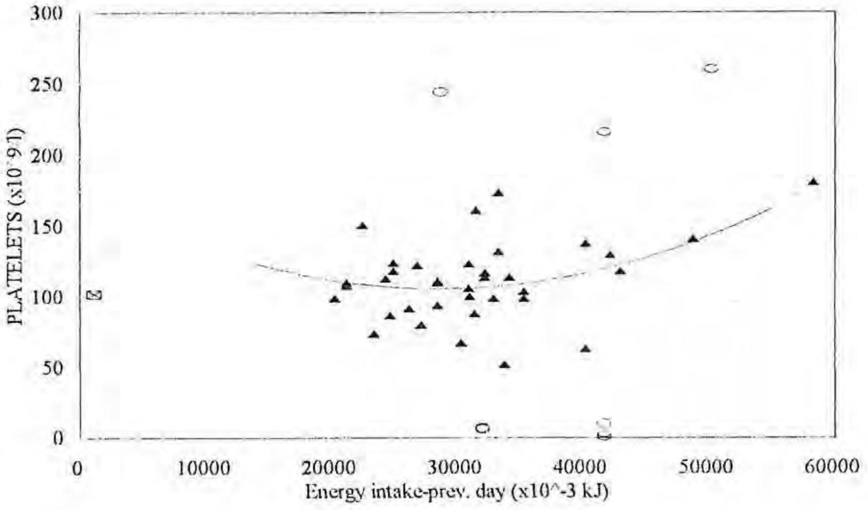
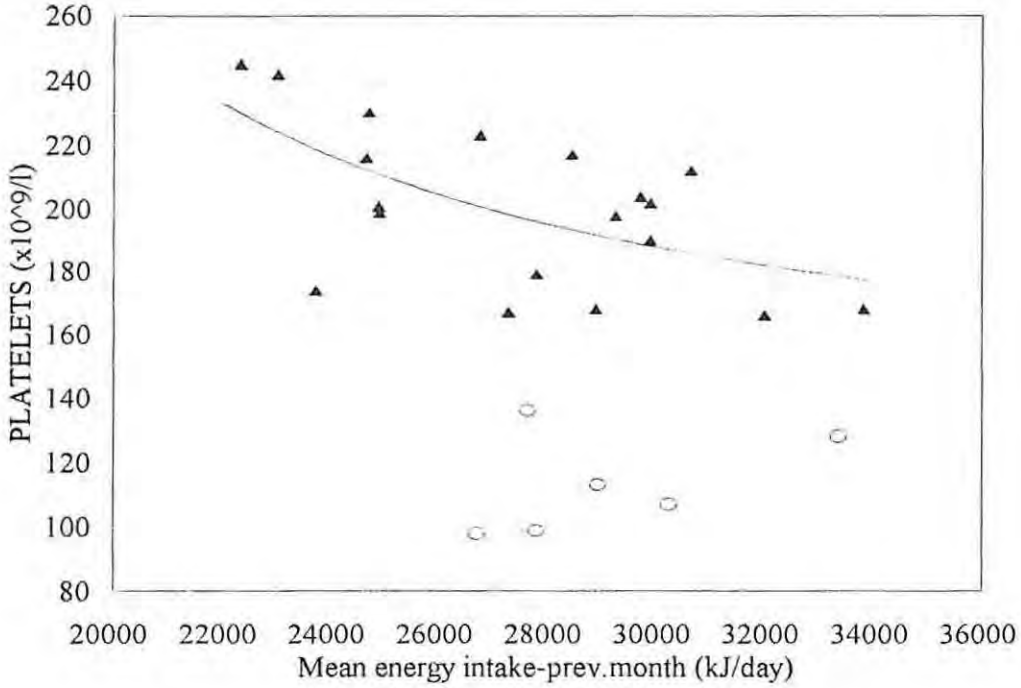


Figure 3.59: Curves of best fit (—) for animals (A. Dolly (a & b); B. Thandi) with significant correlations ($p<0.05$) between platelets and the dietary energy intake of the day prior to the blood sample. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

A. DOMINO



B. THANDI

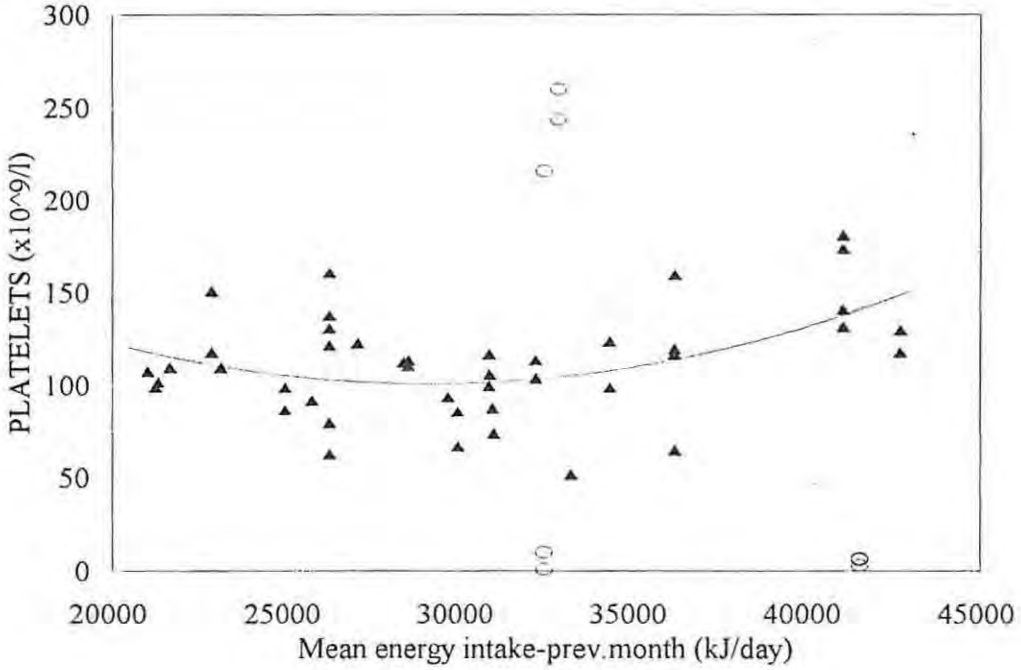


Figure 3.60: Curves of best fit (—) for animals (A. Domino; B. Thandi) with significant correlations ($p<0.05$) between platelet count and the mean daily energy intake of the month prior to the blood sample. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

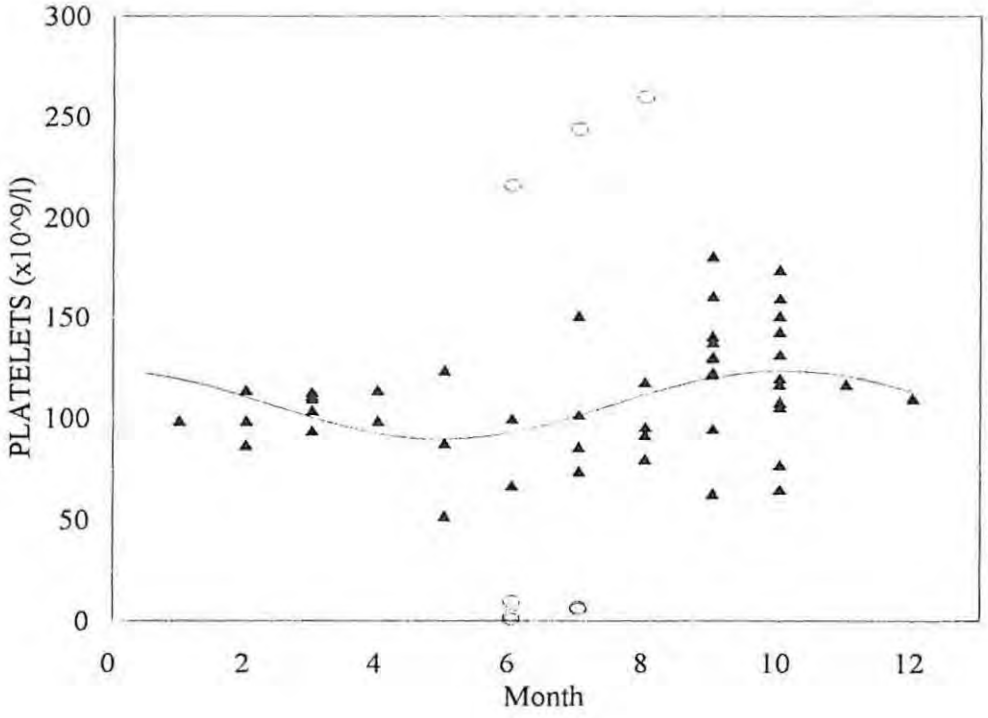


Figure 3.61: Curve of best fit (—) for Thandi with a significant correlation ($p<0.05$) between platelet count and the month of the year. Included in the graph are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

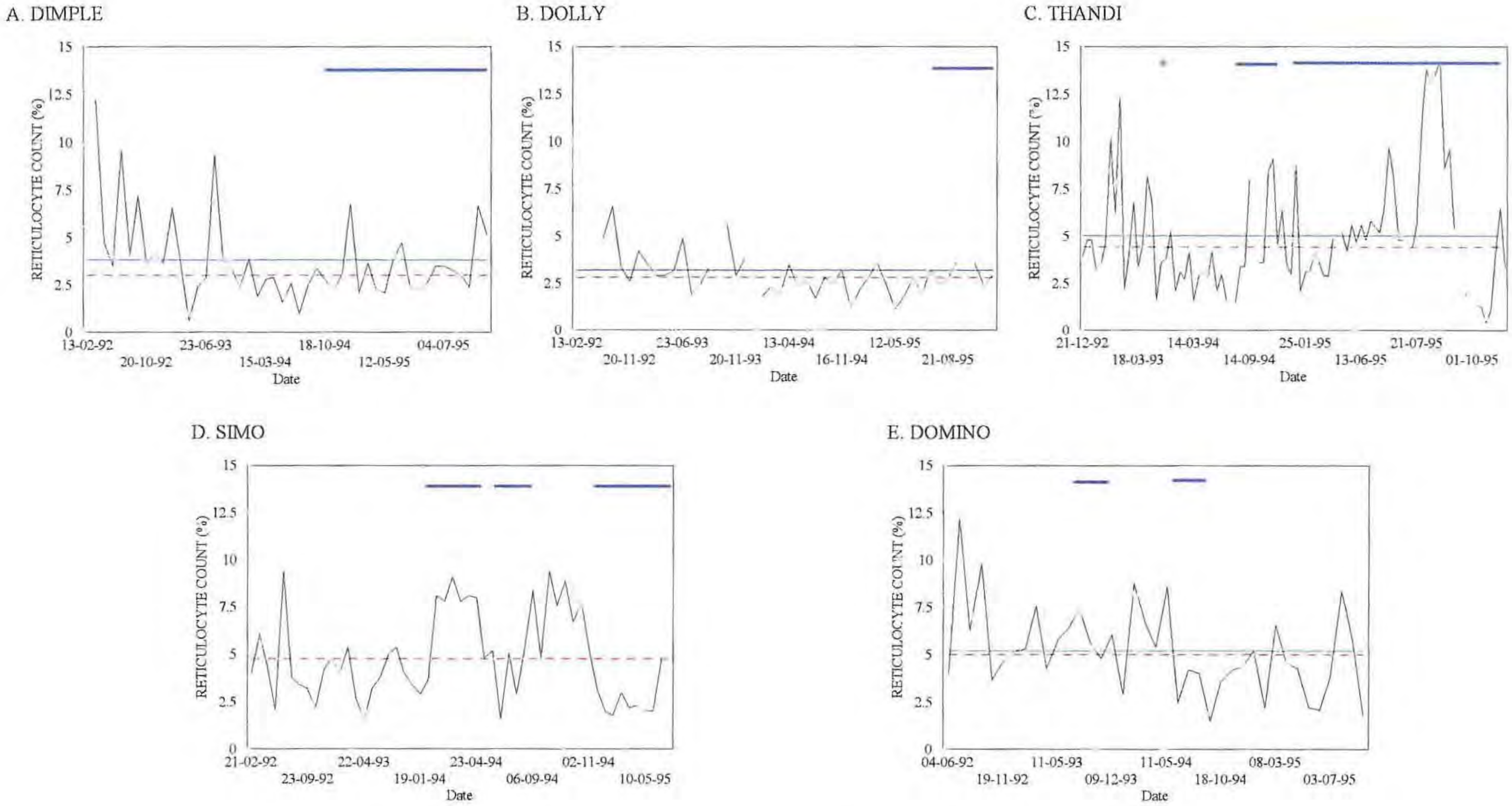


Figure 3.62: Reticulocyte counts (%) (—) for all five captive animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the overall mean reticulocyte count (—) and the "normal" (see text) mean reticulocyte count (- - -) for the study between 1992 and 1995 (*: parturition; ▬ : extended periods of illness and/or treatment or periods of aberrant feeding and/or behaviour).

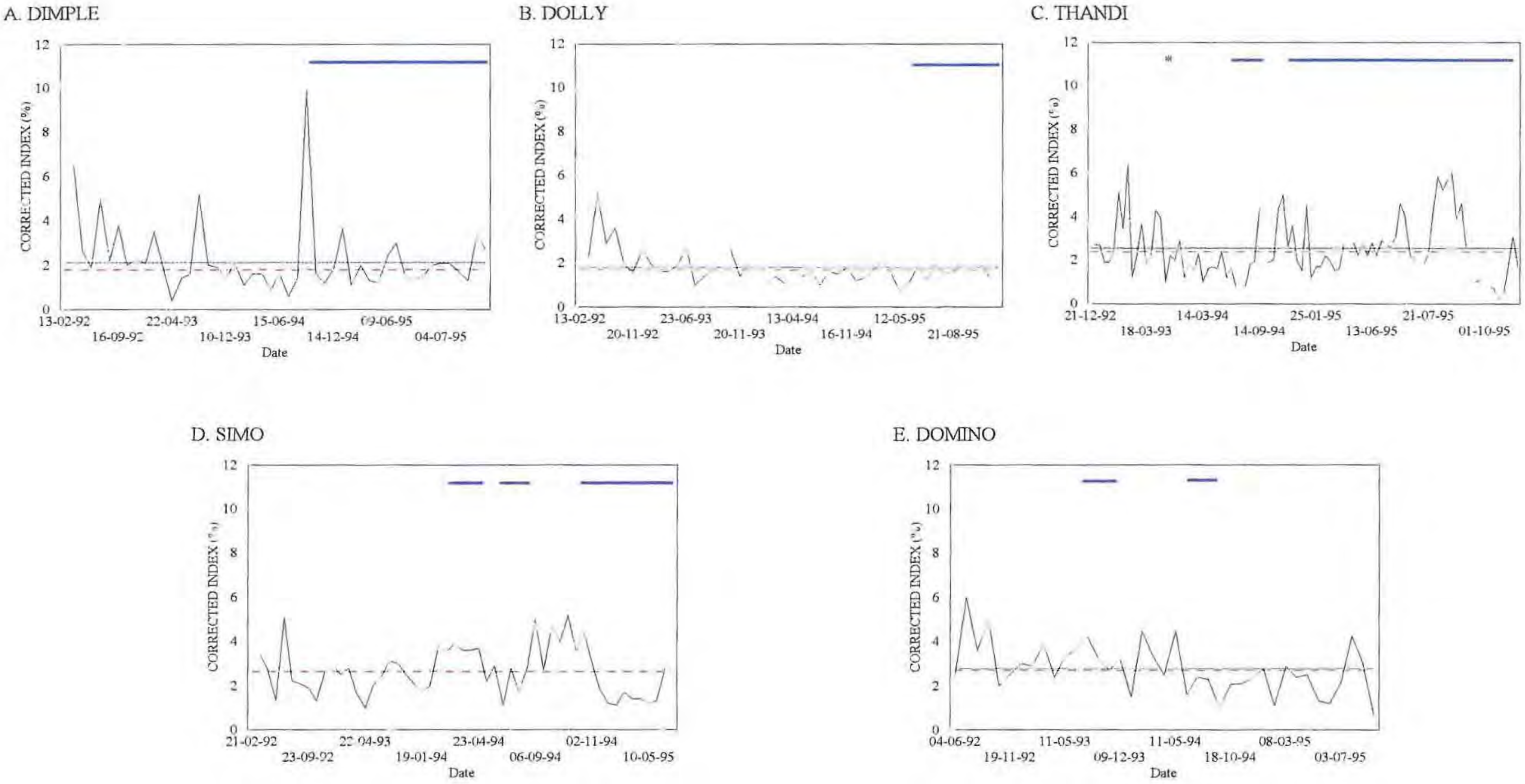
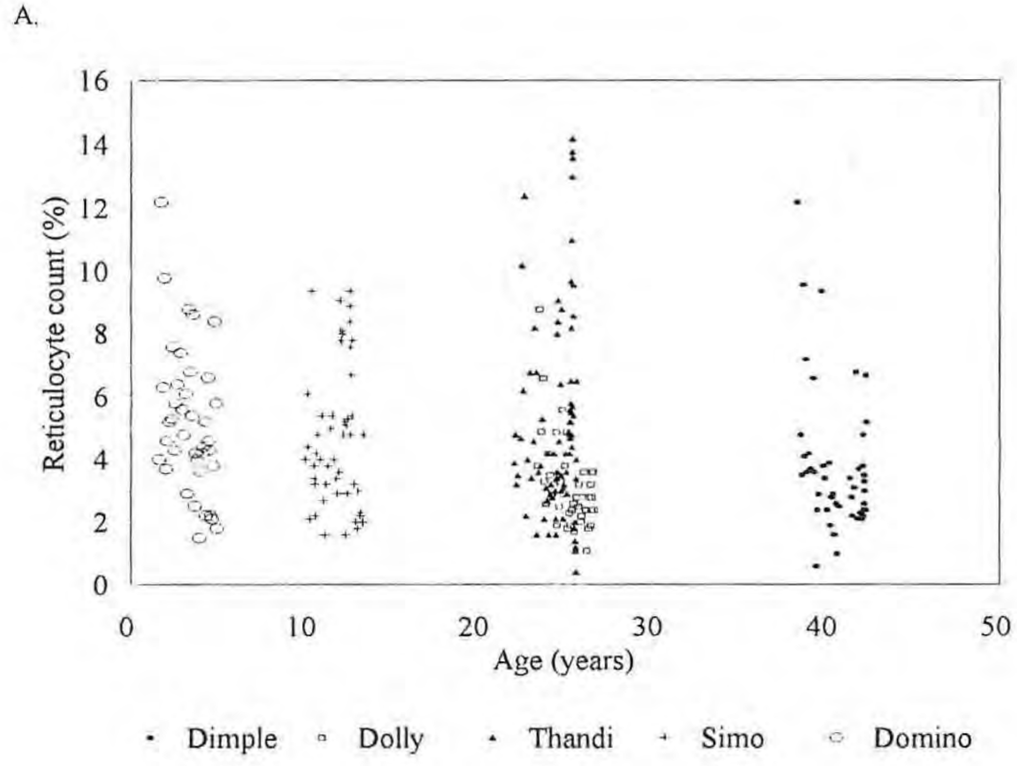


Figure 3.63: Corrected indices (%) (—) for all five captive animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the overall mean corrected index (—) and the "normal" (see text) mean corrected index (- - -) for the study between 1992 and 1995 (*: parturition; —: extended periods of illness and/or treatment or periods of aberrant feeding and/or behaviour).



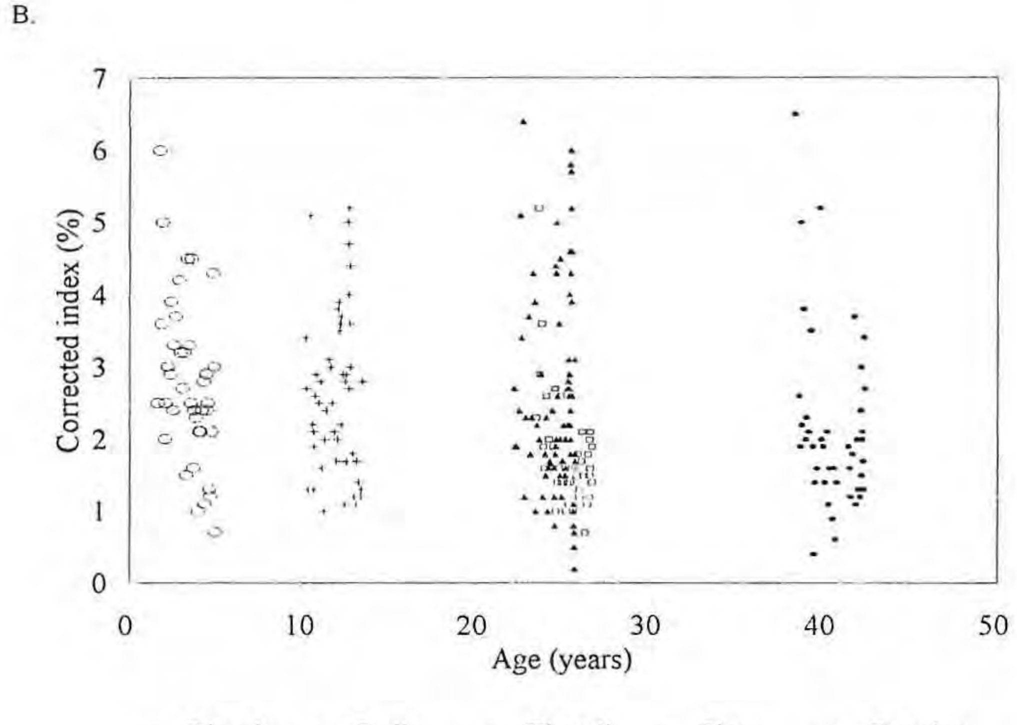


Figure 3.64: Relationships between reticulocyte count (A) and the corrected index (B) and age for the five captive dolphin, from records obtained between 1992 and 1995.

define (Fig. 3.65A). Domino's data for these two parameters were variable, but there appeared to be a decline in both reticulocyte counts (~6-4 %) and corrected indices (~3.5-2.5 %) as age increased from 1.5 to 5 years (Fig. 3.65B).

Table 3.13: Correlation coefficients (r), levels of significance (*: p<0.05, **: p<0.01, ***: p<0.001) and sample sizes (n) for significant relationships between reticulocyte count (a) and corrected index (b) and independent variables for the five captive dolphins (Data are presented as r*(n)) (NS: non-significant (p>0.05)).

Variable		Dimple	Dolly	Thandi	Simo	Domino
Age	a	NS	-0.32*(38)	NS	NS	-0.34*(38)
	b	NS	NS	NS	NS	NS
Weight		NS	-0.33*(36)	NS	-0.37**(45)	NS
		NS	-0.36*(38)	NS	-0.41**(44)	NS
D1		NS	NS	0.29*(62)	NS	NS
		NS	-0.33*(33)	NS	NS	NS
E2		NS	NS	0.26*(63)	NS	NS
		NS	NS	NS	NS	NS
E3		NS	NS	0.38**(66)	NS	NS
		NS	NS	0.29*(67)	NS	NS
E4		NS	NS	0.27*(75)	NS	NS
		NS	NS	NS	NS	NS
T1		NS	NS	-0.37*(79)	NS	NS
		NS	NS	NS	NS	NS
T2		NS	NS	-0.29**(78)	NS	NS
		NS	NS	-0.24*(79)	NS	NS
T3		-0.33*(40)	NS	-0.27*(79)	NS	NS
		-0.32*(42)	NS	-0.25*(80)	NS	NS

D1: Total food consumption of week prior to blood sample, E2: Mean daily energy intake of week in which blood was sampled, E3: Mean daily energy intake of week and month (E4) prior to blood sample, T1: Mean water temperature of week prior to blood sample, T2: Mean water temperature of month in which blood was sampled, T3: Mean water temperature of month prior to blood sample.

Both reticulocyte counts and corrected indices from Dolly and Simo were negatively correlated with total body weight (Table 3.13). Relatively stable data in the case of Dolly contrasted with highly variable data from Simo, making these relationships difficult to define (Fig. 3.66). Increased reticulocyte counts and corrected indices were more frequently measured, for both animals, at lower total body weights.

Counts of reticulocytes for Thandi were positively correlated with the total food consumption of the week prior to blood sampling (Table 3.13). However, this relationship appeared to be coincidental, as data were variable, with no apparent relationship between reduced food intake and

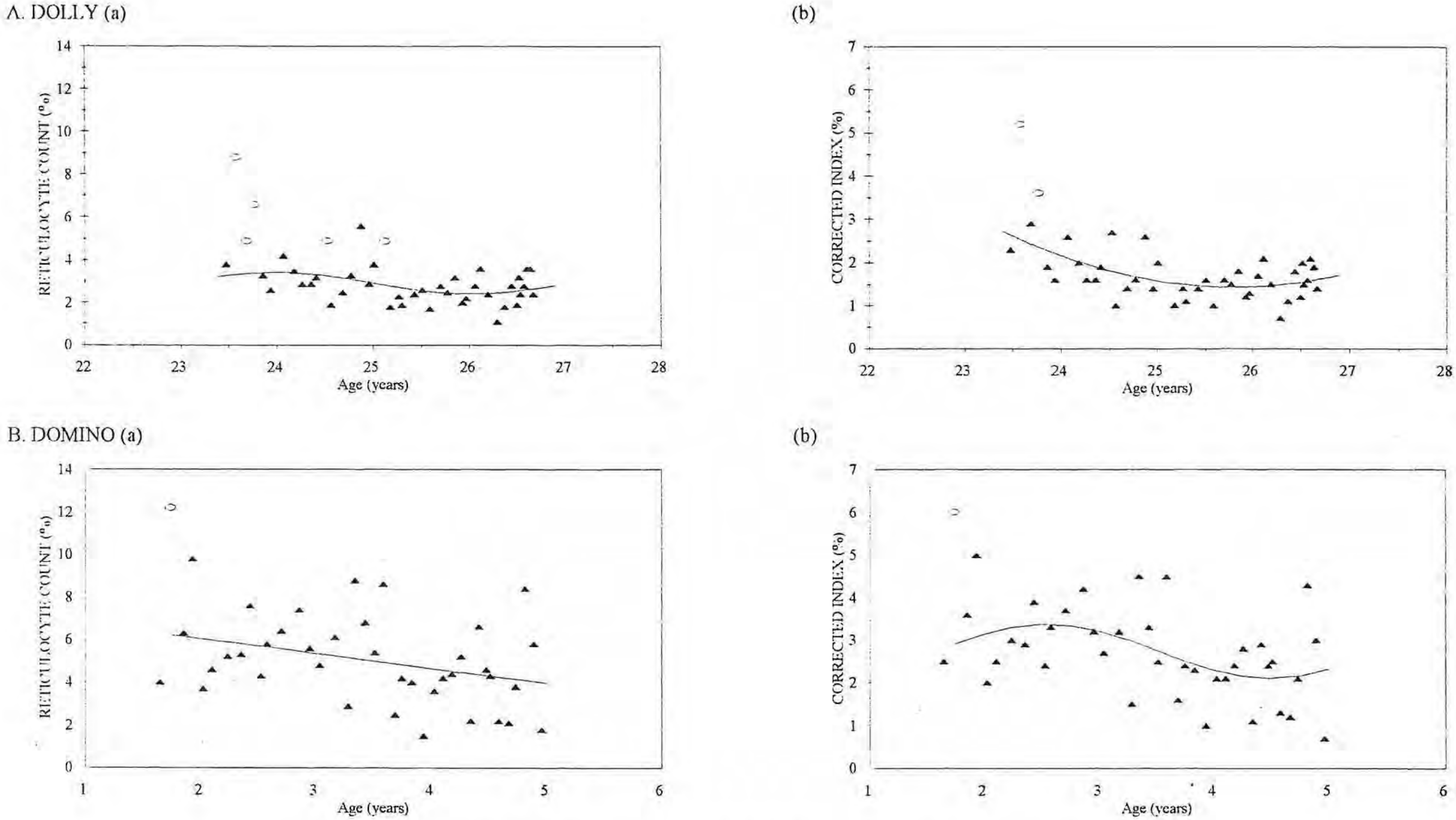


Figure 3.65: Curves of best fit (—) for animals (A. Dolly; B. Domino) with significant correlations ($p<0.05$) between reticulocyte counts (a), the corrected index (b) and age during the study. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

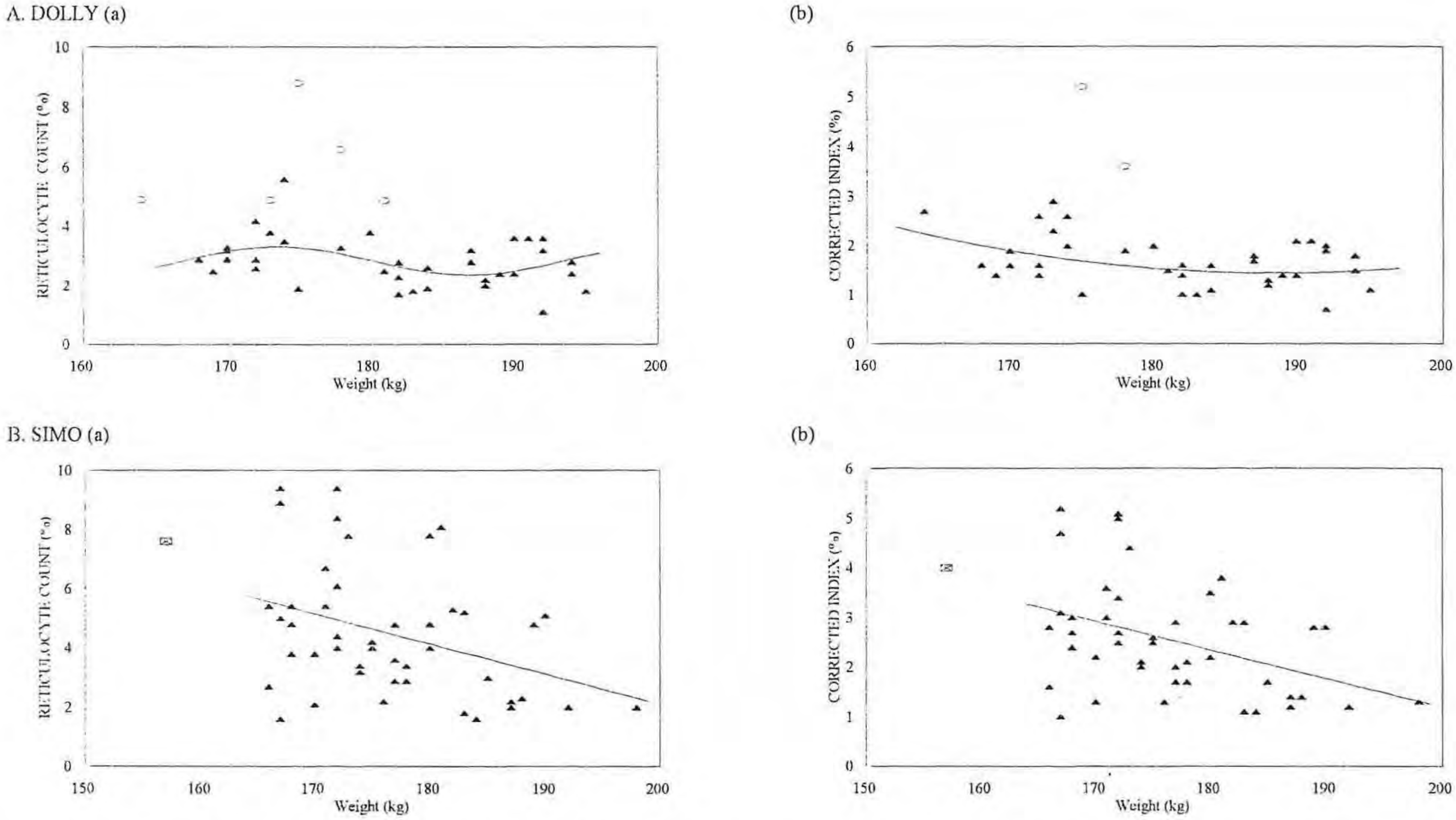


Figure 3.66: Curves of best fit (—) for animals (A. Dolly; B. Simo) with significant correlations ($p<0.05$) between reticulocyte counts (a), the corrected index (b) and total body weight. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ⊠ ; y-axis: ○).

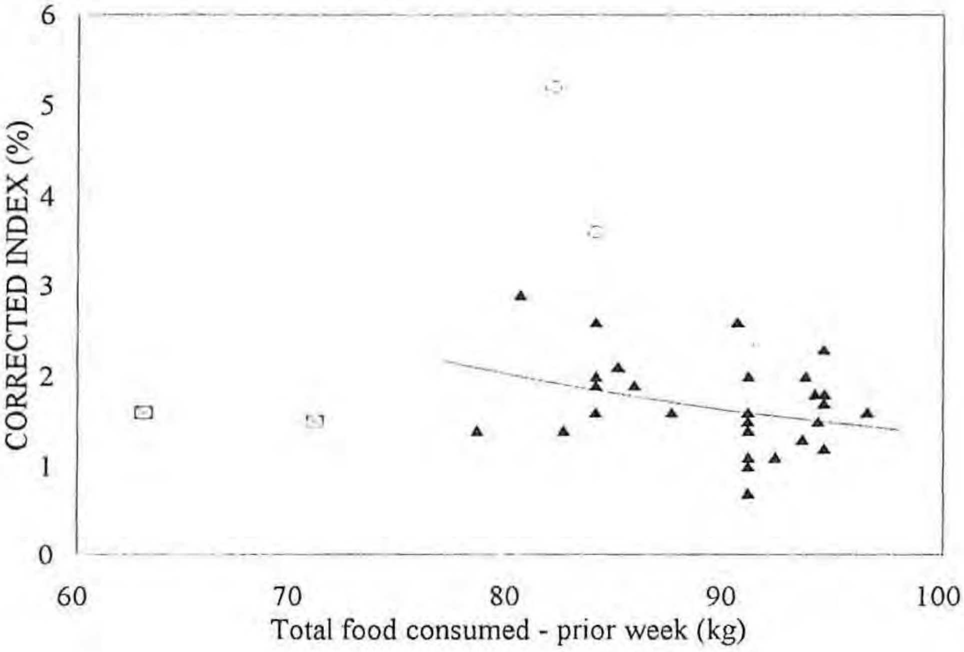
outlying reticulocyte counts. Outlying reticulocyte counts (> 11 %) were recorded mainly at high levels of food consumption (± 85kg/week). The corrected index was negatively related to this diet category for Dolly only (Table 3.13). Again, data were highly variable and a specific trend was difficult to define. However, values for this index did appear to be slightly increased when food consumption was lower. Outlying high corrected index values were associated with low levels of food consumption, although significantly reduced food intake was also associated with "normal" corrected index values (Fig. 3.67). Reticulocytes were positively related with the mean daily energy intake of the week in which blood was sampled, as well as the week and month prior to blood sampling (Table 3.13). However, these relationships were significant only for Thandi and data were too variable to elucidate further on these relationships.

Thandi was the only animal for which reticulocyte counts were negatively correlated with all three categories of water temperature. Data from Dimple was similarly related to the mean water temperature of the prior month (Table 3.13). Data from both animals were relatively stable and did not show any marked changes over the entire temperature range (Fig. 3.68). However, counts made at water temperatures lower than 20°C were more variable and slightly increased. Outlying high reticulocyte counts in Thandi's data were all measured at water temperatures below 20°C. Corrected indices from these two animals were identically related to water temperature.

DISCUSSION

The use of "curves of best fit" in the present study constituted a base-line analysis of how physiological parameters related to morphological, dietary and environmental variables in captive dolphins. It was hoped that certain of these trends could be used in a predicative capacity, such that aspects of physiology could be used to estimate morphological and nutritional condition and vice versa, in "condition models". The value of this being that oceanarium staff and marine mammal researchers could use a selection of accessible, easily available parameters to estimate those parameters which are less easily obtained. However, the results emphasised the large-scale variation typical of other haematological studies, both within and between individual animals. Inconsistencies made it impossible, within the realms of this study, to build models, which could be used for predictive purposes. Certain relationships indicated that the formation of these types of models is feasible and with larger numbers of animals and further analyses, these types of models could prove effective in predicting and monitoring condition in both captive and free-ranging marine mammals.

(a)



(b)

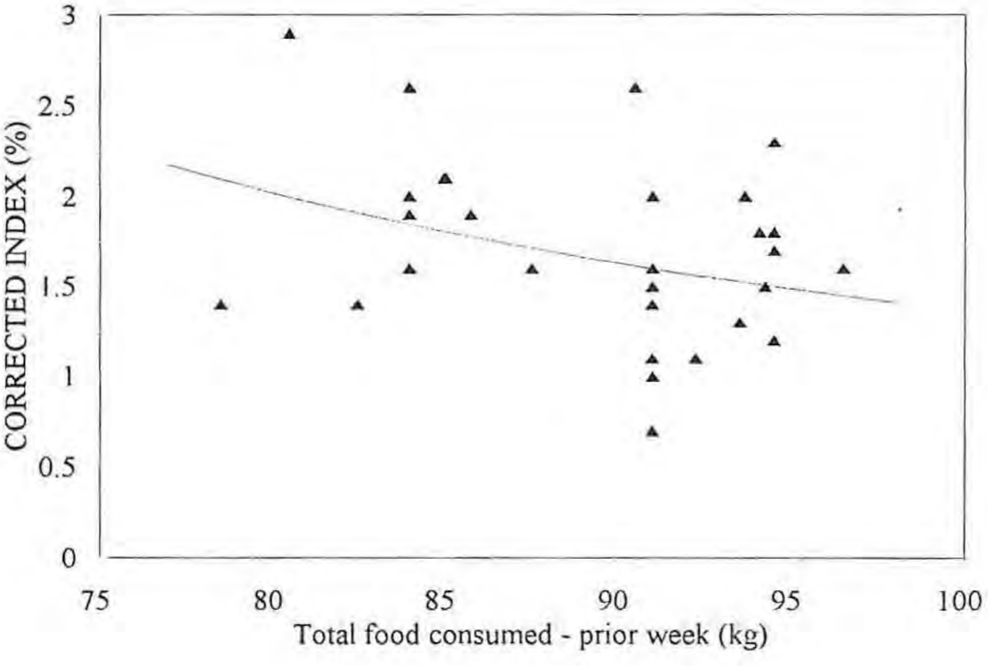


Figure 3.67: Curves of best fit (—) for Dolly (a & b) with a significant correlation ($p<0.05$) between the corrected index and total food consumed in the week prior to blood sampling. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

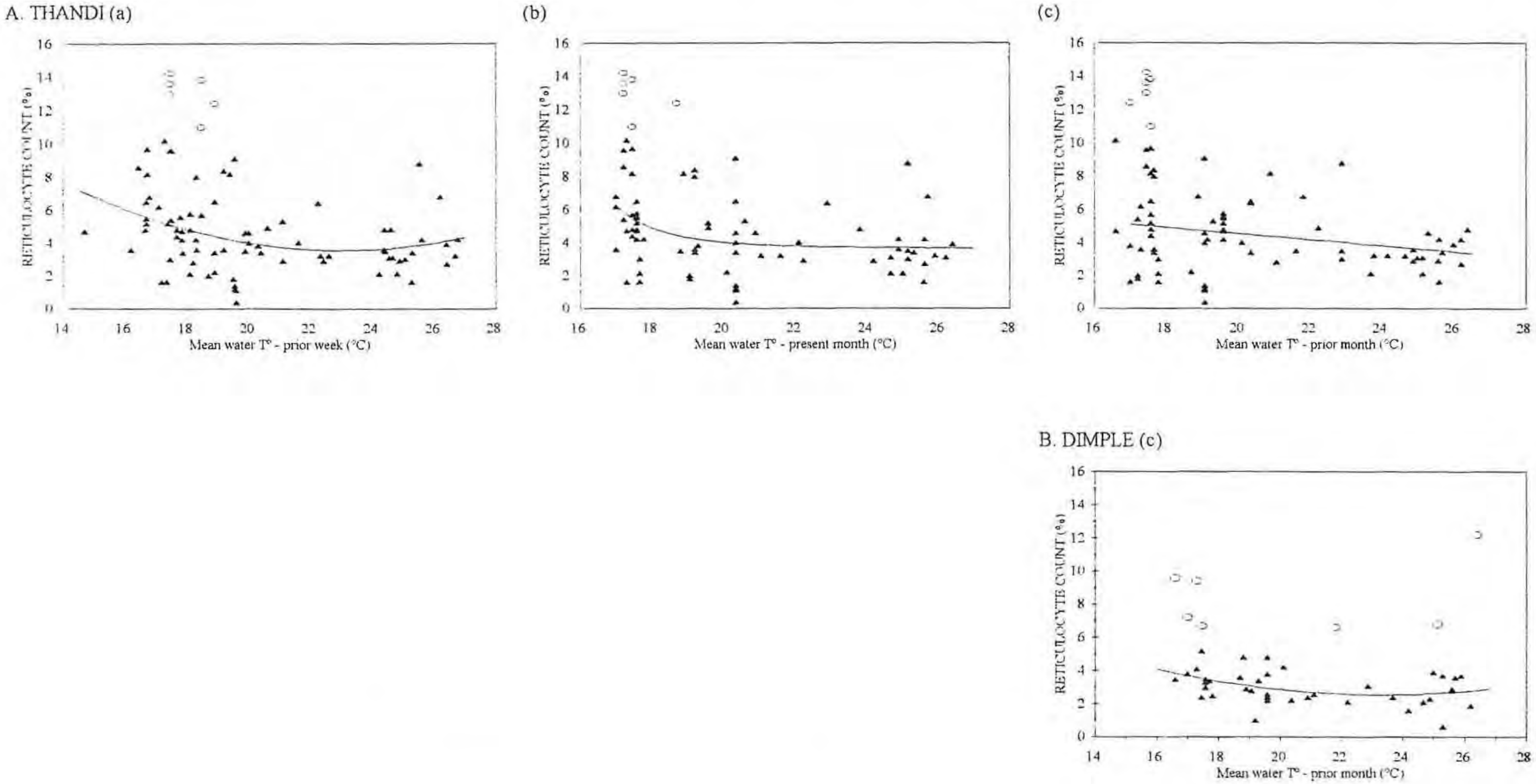


Figure 3.68: Curves of best fit (—) for animals (A. Thandi; B. Dimple) with significant correlations ($p<0.05$) between reticulocyte count and the mean water temperature of the prior week (a); the month in which blood was sampled (b) and the month prior to the blood sample (c). Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

The causes of the data variation were difficult to verify with the limited number of captive dolphins used in the current study. It may be a result of age and/or sex or it may reflect the different ways in which individuals respond physiologically to morphological, social and environmental changes. Several of the relationships between haematological parameters and age, weight, diet and water temperature were based on data from only one or two animals. However, they highlighted potential trends, which, with further investigation and a larger sample size, will serve as valuable indicators of the interactions between physiological and morphological condition, with respect to diet and environmental variables.

Only Thandi was pregnant during the study and, as discussed previously, several data from this animal were biased as a result of extended illness and treatment. Unfortunately, her pregnancy corresponded with that time at which her condition was optimised and was followed by the illness and treatment, which eventually led to her death. In this respect, it was impossible to properly investigate physiological and morphological changes that occurred with pregnancy in captive dolphins.

"Normal" mean WBC counts from the bottlenose dolphins used in this study were all, with the exception of Thandi, within the range of WBC levels reported in the literature for this and other cetaceans (Appendix 4). Thandi's frequent illness and treatment during the study resulted in an artificially elevated "normal" mean WBC level. WBC's are generally used as an indication of an animal's immunological integrity with leukocytosis (an increase in total WBC) being either physiological (fear, excitement, stress, etc.) or pathological, reflecting a disease condition or a combination of both (Ridgway *et al.* 1970, Medway and Geraci 1978, Cornell 1983, Clarke 1990, Bossart and Dierauf 1990). WBC counts from the animals used in this study were highly variable in all five animals except the youngest (Domino) and although the majority of outlying WBC counts could be associated with a known period of illness or medication, this was not always the case. Highly variable WBC counts, with fluctuations not always correlated with the obvious physical state of the animal, have proved problematic with other species of marine mammal (Ronald and Kay 1982, Cornell 1983).

The high variability and increased WBC count recorded for Thandi can also be attributed to the long-term, and sometimes severe, stress that this animal was exposed to during illness and treatment. Elevated WBC counts have been associated with different types of artificial stress in terrestrial (Rietkerk *et al.* 1994) and marine mammals (Lee *et al.* 1977, Medway and Geraci 1978, Medway and Cornell 1989, Bossart and Dierauf 1990, Thurman and van der Elst 1995). The increase in WBC during different types of stress has been attributed to tissue damage resulting from increased and/or excessive muscle damage.

Overall, WBC counts did not differ markedly with age. Although age-related changes in WBC have been recorded previously (Engelhardt 1979, Cornell 1983, Asper *et al.* 1990, Rietkerk *et al.* 1994, Nielsen 1995, Thurman and van der Elst 1995), the trends described by different authors are not consistent. There may be a change in WBC counts with changes in maturity status, as shown by Domino during this study (and Simo in years prior to the study), but further in-depth investigations are needed before these relationships can be clarified. This was emphasised by the fact that the increase in WBC for Simo contrasted with the slight decrease in WBC evident for Domino during a similar age period. The reduction in WBC at age three years may be an indication of a reduction in the suckling rate and the initiation of the animal's own immunological status. Although Domino was seen to suckle from Dolly throughout the study, the frequency of suckling was reduced during the latter half of the study (pers. comm. Oceanarium staff). Dierauf *et al.* (1981 in Bossart and Dierauf 1990) reported that harbour seal (*Phoca vitulina*) neonates are born with low WBC counts, but levels increase to within normal range (> 7.00 x10^9/l) within the first 10 days of life.

There was no defined relationship between weight, blubber thickness and WBC count during this study. In this respect, it appears that changes in morphological condition do not necessarily affect immunological condition and vice versa. However, it must be noted that blubber thickness measurements were limited and additional data may show that severe infections and consequent high WBC counts potentially relate to low blubber thickness measurements, at a stage when infection or disease has led to a deterioration of morphological condition. Although a direct relationship between high WBC counts and deteriorating morphological condition has not been documented, illness and infection is generally associated with a reduction in weight (Ridgway 1965, Ridgway and Fenner 1982).

The relationship between WBC and diet during this study was complex. Thandi was the only animal to show a significant correlation between the number of WBC and the level of food consumption during the week prior to the blood sample. The highly variable data prevented any definite conclusions concerning this relationship, although increased WBC counts were generally associated with elevated feeding levels (corresponding to "normal" mean levels of food intake for this animal). This trend was again biased by the frequent periods of illness and treatment, which this animal experienced. Food intake was generally increased and/or highly erratic during these periods and, for this reason, the relationships between diet and haematological parameters for this particular animal appeared to have been artificially reversed. Poor appetite was related to fluctuating WBC counts in a Baikal seal (*Pusa sibrica*) (Ronald and Kay 1982). However, during this study, excessively high or low feeding levels do not seem to have a direct affect on WBC counts, as records of WBC at these extremes remained within the normal range. The fact that outlying high WBC counts were associated with severely reduced food intake was directly related to the fact that ill animals generally "voluntarily" reduce their food intake when feeling "unwell".

However, this reduced food intake may also increase their susceptibility to further infection and disease processes. Many disorders can be directly attributed to malnutrition, which has been known to impair the immune system (Geraci 1981, Cates and Schroeder 1986).

The effect of dietary energy intake on WBC counts was difficult to assess due to highly variable data in cases. WBC counts did, however, appear to be related to diet in the short-term, on a daily and weekly basis. Generally, an increase in energy intake was followed by a decrease in WBC's, implying an increase in immunological integrity with improved diet status. This relationship may also reflect the complexity of interactions between diet, total body weight and immunological activity. Changes in WBC counts have been recorded as common during malnutrition in terrestrial mammals (Chandra *et al.* 1982 in Lochmiller *et al.* 1988), although WBC counts were not affected by diet in white-tailed deer fawns (Seal *et al.* 1978b). However, WBC progressively declined during fasting in gray wolves (*Canis lupus*) (DelGiudice *et al.* 1987b). This decline was attributed to the lack of adrenocorticoid elevations, normally associated with feeding and carbohydrate and protein metabolism, rather than nutritional deprivation. WBC's were affected by diet in harbour seals (Kuiken 1985), although it is unknown if the energy content of the diet played a role in these differences. The incidence of parasites or other immunologically challenging aspects resulting from the diet may be a contributing factor in the elevation of WBC count. Pollutants, for example, have been shown to suppress the immune system (de Swart *et al.* 1995) and this may be a factor that needs to be taken into account when examining the leukocytic parameters of wild animals, or captive animals fed on prey species caught in heavily polluted seas. The pollutant level of prey species fed to the animals used in this study was not monitored although all fish were caught commercially along the south-east coast of South Africa, where pollutant loads in marine species are relatively low (Cockcroft *et al.* 1991).

The apparent increase of WBC in winter months, evident for Dimple, reflects an increase in immunological activity during winter. An elderly animal may be more susceptible to infections and other pathogens during the winter months when exposed to colder water temperatures. The feasibility of this increased when WBC counts were considered in terms of water temperature. Both Dimple and Simo showed an increase in WBC counts at lower water temperatures, further reflecting that immunological efficiency may be reduced at colder water temperatures. Temperature-related changes in WBC counts have not been documented previously. However, bottlenose dolphins are reportedly thermoneutral at water temperatures around 22°C (Ross and Cockcroft 1990; Cockcroft 1991) and in this respect, it is feasible that these animals are immunologically more susceptible to infections at water temperatures below 22°C.

WBC differential counts are informative in that proportional changes in the different WBC types give an insight into the nature and type of the different physiological and/or pathological processes that may be involved in causing the total WBC count to rise or decline. Neutrophils increase with inflammation, stress and during exercise or excitement (Penington *et al.* 1978, Bossart and Dierauf 1990), trauma and metabolic imbalances (Penington *et al.* 1978) and infection-associated stress (Thurman and van der Elst 1995). Animals used in this study showed highly variable neutrophil levels but mean levels were within the range of neutrophil values recorded in the literature (Appendix 4). However, all animals, except Domino, had mean neutrophil levels that were higher than the means reported for bottlenose dolphins. This would seem to reflect that the captive dolphins used in this study were more stressed, excited and/or exposed to a higher level of infection-associated influences than those animals used in previous studies. However, in light of the possible age-associated increase in neutrophils documented in this study and others (Lochmiller *et al.* 1985c, Asper *et al.* 1990, Rietkerk *et al.* 1994, Nielsen 1995), it is more feasible that the elevated neutrophil levels indicated that the animals used in this study were predominantly older than those in the literature, for which the majority of ages are unknown. De Monte and Pilleri (1972) pointed out that little is known of the variability of the individual classes of WBC cell types at different ages in cetaceans.

Although the significant increase in neutrophils observed in Thandi during the study may be a continuation of a long term age-related trend, as described above, it is more likely to be an indication of the higher incidence of illness, erratic behaviour and stress during the latter half of the study (Medway and Geraci 1964, 1978, Bossart and Dierauf 1990, Thurman and Van der Elst 1995). Simo, on the other hand, showed a steady increase in the neutrophil proportion between the ages of 10 and 14 years, similar to the increase in Thandi's neutrophil levels between the ages of 12 and 15 years. As the incidence of illness for Simo was minimal during the study, it is possible that this trend was related to the long-term increase in neutrophils. The mean neutrophil level for Domino was still below that of Simo, although this may change as Domino grows from adolescence to maturity. The decreased number of neutrophils at higher weights in Dolly and Thandi reflected a reduced susceptibility to infection at higher weights. Greater total body weight for any particular animal is generally associated with an increased (and improved) condition (Ridgway 1965, Ridgway and Fenner 1982) and an implied reduced susceptibility to infections.

Although ill-defined, the relationship between neutrophils and dietary energy intake indicated that neutrophils decreased with increased energy intake. This was similar to the relationship described for total WBC count and dietary energy intake. High levels of neutrophils are a sign of infection (Thurman and van der Elst 1995), thus this trend could be interpreted in terms of increased energy intake improving overall condition and consequently reducing the chance of infection. Although few data exist on the relationship between diet and the WBC differential in marine mammals, feed restriction of black-tailed jackrabbits (*Lepus californicus*) resulted in an increase in neutrophils

(Henke and Demarais 1990). Neutrophils were also significantly related to diet composition for collared peccaries (Lochmiller *et al.* 1988).

Data from the two male captive dolphins illustrated that lower levels of neutrophils could generally be attributed to water temperatures between 20°C and 24°C. This temperature range corresponds to the thermoneutral zone of 22°C for bottlenose dolphins (Ross and Cockcroft 1990, Cockcroft 1991). This implied that bottlenose dolphins may be physiologically more "comfortable" within this temperature range and as a result their immunological status is more stable. The animals are consequently less susceptible to disease and infection within this temperature range. This type of influence has not been documented previously in marine mammals.

Marine mammals typically have a high percentage of eosinophils. This has been attributed to the almost universal parasitism in marine mammals (Tomilin 1957, Ridgway 1965, 1972, Bossart and Dierauf 1990). However, literature also shows there to be a high level of circulating eosinophils in marine mammal blood, without clinical evidence of parasitism (Bogdanova and Lebedev 1973, Medway and Geraci 1978). Clinically, eosinophilia has been related to allergies and diseases leading to decomposition of body protein (Thurman and van der Elst 1995). Levels of eosinophils measured during this study were generally lower than those recorded for bottlenose dolphins in the literature (Appendix 4). Although the small sample size prevented any investigation of sexual differences, the three female dolphins had mean eosinophil levels that were significantly lower than those of the two male dolphins. Higher eosinophil levels in female dolphins have also been recorded in bottlenose dolphins, Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) (Ridgway *et al.* 1970), and dusky dolphins (*L. obscurus*) (Fothergill and Jogessar 1986) (Appendix 4). However, it is also possible that these differences were related to age, rather than sex, of the animals used in the current study. Eosinophils were lowest in the younger two animals. Kuiken (1985) found that eosinophils were higher in older harbour seals. Long-term trends show that eosinophils for Thandi and Simo apparently changed with age, paralleling those described for neutrophils. Taking all five animals into account, the results of this study show that eosinophil levels are higher in juvenile animals and decrease throughout adolescence until levels stabilise at maturity. There are limited data available pertaining to potential age and sex differences of WBC cell types, although an adolescent harbour porpoise (*Phocoena phocoena*) had a significantly greater percentage of eosinophils than its adult counter part (Kastelein *et al.* 1990) (Appendix 4).

Thandi's eosinophil levels were artificially increased by the periods of illness and treatment she was exposed to during the study, thus increasing her eosinophil level above that of Dolly (the older of the two). Drug administrations and acute infections reportedly increase eosinophils (Penington *et al.* 1978). Stress reportedly depresses the eosinophil proportion (Medway and Geraci 1964, 1978, Ridgway 1965, Medway and Moldovan 1966, Geraci and Medway 1973, Medway 1983). In this respect, it appeared that long term illness and medical treatment of Thandi caused eosinophil levels to rise.

The association between eosinophils and diet was not consistent between animals, although the trend was similar throughout. Although data were variable, all feeding and energy intake categories indicated that the percentage of eosinophils was higher at greater levels of energy intake. It must be noted, however, that additional measurements are needed at lower food intakes to further elucidate this trend. The increase in eosinophils corresponds to the proportional decrease in neutrophils described previously. Proportional changes in eosinophils and neutrophils during periods of reduced food intake have been associated with nutritional stress in feed-restricted black-tailed jackrabbits causing an increase in cortisol production and a subsequent depression of the immune system (Henke and Demarais 1990). Harbour seals fed plaice and flounder (Kuiken 1985) had higher percentages of eosinophils than those fed on mackerel. Although the energy contents of these species of fish are unknown, it is possible that energy intake by harbour seals had an effect on levels of eosinophils. The effects of pollutants, which may influence eosinophils, have been discussed previously for WBC.

The relationship between eosinophils and water temperature was similar in nature to that described for neutrophils. An increase in eosinophils coincides with water temperatures between 22°C and 24°C, the same temperature range at which neutrophil levels are the lowest. Seasonal changes in eosinophils in collared peccaries were interpreted as a response to variation in type and degree of parasitism (Lochmiller *et al.* 1985c). Proportional changes in neutrophils and eosinophils in mule deer (*Odocoileus hemionus*) have been related to stress experienced during cold temperatures (Anderson *et al.* 1970). The effects of stress on marine mammal eosinophils are well documented (Medway and Geraci 1964, 1978, Ridgway 1965, Medway and Moldovan 1966, Geraci and Medway 1973, Medway 1983) and reduced eosinophil proportions measured in water temperatures outside of the thermoneutral zone reflected increased stress in these environments.

The emotional and physical state of the animal at time of bleeding has an important effect on haematological factors, particularly HB (Lane *et al.* 1972, De Monte and Pilleri 1988, Thurman and van der Elst 1995, Castellini *et al.* 1996). This makes interpretation of haematological data difficult and fluctuations are not always explained by clinical data. Marine mammals have the lowest RBC numbers when compared to other mammals (Lenfant 1969, Bossart and Dierauf 1990). However, within the cetaceans or pinnipeds, RBC values are highly variable (Lenfant 1969, Medway and Geraci 1978). RBC, HB and PCV have been used predominantly as physiological indications of diving and swimming capabilities in marine mammals (Lenfant 1969, Ridgway *et al.* 1970, Medway and Geraci 1978, Bossart and Dierauf 1990, Horning and Trillmich 1997).

Red blood cell, HB and PCV values for the five animals used in this study were within the ranges recorded for these parameters in the literature, although mean HB and PCV values were higher than most of the means recorded for bottlenose dolphins (Appendix 4). RBC, HB and PCV data from this study appeared not to be age- or sex-related. Taking into account the behaviour and medical records for these animals, the pattern indicated that the animals with the least incidence of illness, treatment and/or erratic behaviour during the study had the highest mean RBC, HB and PCV levels. This suggestion concurs with Thurman and van der Elst (1995) who report that very fit animals have higher RBC counts.

In conjunction with the higher RBC, HB and PCV values described above, MCV values were generally lower than those in the literature (Appendix 4). Similarly, mean MCV values for particular animals in the study were inversely related to RBC, HB and PCV. MCV is an indication of the size and quality of the RBC (Bossart and Dierauf 1990) and is ordinarily larger in marine mammals than terrestrial mammals and is considered to be a respiratory adaptation. Compared to the other haematological parameters, which may be influenced by a number of factors, MCV is an absolute quantity remaining practically constant in the individual and varies only with the other biological values (De Monte and Pilleri 1988). This explains the inverse relationship between these parameters for the five captive animals. Fit animals, in good overall condition, are characterised by high RBC, HB and PCV and low MCV (Thurman and van der Elst 1995).

No specific long-term age related trends in RBC, HB and PCV could be identified for any of the five animals, although data from two of the older animals (Thandi and Dimple) were generally below those of the other three animals. These reductions were associated with a marked increase in MCV, for Dimple particularly. Although some of these changes are probably due to illness, in the case of Dimple, they may also be indicative of an age-associated change in diving physiology and oxygen transport in elderly animals. In most species of marine mammal, RBC counts have been shown to be higher in adults. This has been attributed to the gain in weight and learning to dive as animals grow (Geraci 1971, Lane *et al.* 1972, Bossart and Dierauf 1990, Horning and Trillmich 1997). In neonatal elephant seals (*Mirounga leonina*) and harbour seals, RBC parameters change in direct proportion to the age of the animal. Decreases in RBC counts with associated increases in MCV, beginning at five to eight weeks of age, parallel the animals exposure to water and diving (Bryden and Lim 1969, McConnell and Vaughan 1983, Roletto and Dougherty 1983 in Bossart and Dierauf 1990). A fall in PCV during the first weeks of life has been demonstrated in elephant seals (Bryden and Lim 1969). This was attributed to rapid body growth exceeding the rate of RBC production. A similar trend, which occurs later and lasts longer, has been described in harp seals (*Pagophilus groenlandicus*) (Geraci 1971). Lower HB and PCV values have been recorded in older southern elephant seals (Lane *et al.* 1972) and harbour seals (Kuiken 1985). Several other authors have also recorded haematological correlations with age (Engelhardt 1977, Geraci and Smith

1975). Age-related changes in haematology have also been described for terrestrial mammals such as collared peccaries (Lochmiller *et al.* 1985c) and mountain gazelle (*Gazella gazella*). For the latter species, RBC, HB and PCV increased with age to peak in juveniles and then decreased to adult levels (Rietkerk *et al.* 1994). MCV, however, was lowest in infants, increasing to maximum levels in adults.

Data from Dolly and Simo, the two fittest animals with the least incidence of illness during the study, indicate that increased RBC, HB and PCV values were associated with increased body weight. The slight increase in RBC, HB and PCV implied an improvement in haematological status when the animal was heavier and in greater overall morphological condition. This corresponded with a negative relationship between Simo's MCV and weight. Increased HB and PCV values in belugas (*Delphinapterus leucas*) are assumed to be an indication of better physical condition and a greater level of activity (Ridgway *et al.* 1984). The direct influence of weight on RBC, HB and PCV in marine mammals has not been investigated. Seasonal changes in weight have, however, been correlated with similar patterns in RBC, HB and PCV for black bears (*Ursus americanus*) (Hellgren *et al.* 1993). Cameron and Luick (1972 in Hellgren *et al.* 1993) found that increases in body condition (fat reserves and lean body mass) of reindeer (*Rangifer tarandus*) were accompanied by increases in RBC and PCV. Increased PCV was significantly correlated with condition in bighorn sheep (*Ovis canadensis*) (Franzmann 1972) and impala (*Aepyceros melampus*) (Gallivan *et al.* 1995). Wolf pups (*Canis lupus*) with higher HB levels were presumed to be in a better state of health (Seal *et al.* 1975). Mule deer similarly showed increased HB and PCV with improved physical condition or nutritive status (Anderson *et al.* 1970).

It seemed extraordinary that the female dolphins exhibited significant correlations between weight and RBC, HB and PCV; but only the male dolphins had significant correlations between weight and MCV. Although sex-based haematological differences are inconsistent in the literature (Appendix 4), it is possible, that there is a difference in haematological functioning between male and female dolphins. Results from net-caught bottlenose dolphins reflected a similar sex-related difference in haematology (see Chapter 6). Sex differences in haematology have also been described for Galápagos fur seals (*Arctocephalus galapagoensis*) (Horning and Trillmich 1997), mountain gazelle (Rietkerk *et al.* 1994), black bears (Hellgren *et al.* 1997) and foxes (*Vulpes velox*) (Mainka 1988).

Changes in haematology are also affected by plasma volume. For example, plasma volume increases in white tailed deer during summer, at least partially because of the role of plasma water in dissipating heat through increased vascularisation of the skin (Jacobsen 1978 in Hellgren *et al.* 1993). Changes in total body water and water compartmentalisation associated with alterations in stored body fat also contribute to changes in red cell volume. Changes in plasma volume must be taken into account when considering and interpreting changes in RBC, HB and PCV.

The present study showed convincingly that haematological status increased with increased blubber thickness. Increases in haematological parameters and blubber thickness have been related to improved physical condition and fitness (Ridgway *et al.* 1970, Ridgway and Fenner 1982, Ognetov 1990, Costa *et al.* 1993, Thurman and van der Elst 1995). In another context, harbour seals that had pupped the year prior to blood sampling, were found to have lower HB and PCV (Kuiken 1985). Although blubber thickness was not examined for these animals, it is possible that these reduced values were associated with reduced blubber thickness, as a result of the mobilisation of fat stores to produce milk.

The number and nature of significant relationships between diet and RBC, HB and PCV was not consistent. Thandi's data showed a decrease in RBC, HB and PCV with increased food and all aspects of energy intake. However, these trends were again probably due to the incidence of illness in this animal. Food intake of this animal was usually increased above the set quota by Oceanarium staff during treatment, and although these values are within the calculated "normal" range, they were associated with periods of illness and treatment. The reverse relationship between these parameters reflected a clinical problem, rather than a direct relationship with nutritional condition. In this context, when assessing condition, low haematological values corresponding to a high level of food consumption and energy intake should be used as an indicator of a potential clinical problem. Intestinal malabsorption and associated poor nutrition reportedly lead to reduced RBC and HB (Bossart and Dierauf 1990). HB levels and PCV typically increase during periods of low or no food intake, reflecting dehydration (Medway and Geraci 1978, Clarke 1990, Thurman and van der Elst 1995).

Data from Dolly and Dimple reflected increased RBC, HB and PCV values at higher levels of food and energy intake. Combining these trends with the relationships between weight and RBC, HB and PCV, it would seem reasonable to assume that an increase in haematological values would occur with increased food intake due to the improved state of hydration and overall physical condition (Ridgway *et al.* 1970, Ridgway and Fenner 1982, Ognetov 1990, Costa *et al.* 1993, Thurman and van der Elst 1995). Literature for terrestrial mammals shows that the relationship between diet and haematological parameters is complex. Dietary restriction resulting in decreased RBC, HB and PCV has been reported for numerous terrestrial mammals (Seal *et al.* 1978b, Lochmiller *et al.* 1985b, Henke and Demarais 1990, Wolkers *et al.* 1994a). Although apparently not affected by diet during the current study, MCV of male collared peccaries was significantly reduced during dietary restrictions (Lochmiller *et al.* 1985c). In contrast, white-tailed deer fed a "poor quality" diet showed an increase in HB and PCV, ascribed to haemoconcentration and weight loss (Warren *et al.* 1982). The haematology of white-tailed deer fawns was significantly affected by the energy and protein content of the diet (Seal *et al.* 1978b). HB levels and RBC counts were increased and reduced, respectively at low levels of protein intakes. MCV was increased, thus allowing the cells to contain more HB. A reduced energy intake also resulted in a low RBC count. Low RBC coupled with increased MCV was apparently caused by decreased food intake of bobcats (*Felis rufus*) (Knick *et al.* 1993). In the same study, high RBC, HB and PCV was associated with decreased water intake, also described for fasted wolves (DelGiudice *et al.* 1987b) and badgers (*Taxidea taxus*) (Harlow and Seal 1981 in Knick *et al.* 1993). Increased PCV in "feed-restricted" red deer (*Cervus elaphus*) was attributed to dehydration and decreased absolute plasma volume (Wolkers *et al.* 1994a). Similarly, reduced PCV in feed-restricted jackrabbits was possibly an effect of increased water consumption, to compensate for reduced food intake (Henke and Demarais 1990). The effect of diet on haematological parameters has also been reported for black bears for which RBC counts were higher and MCV's lower in "good years" (Hellgren *et al.* 1993). Reduced HB values were recorded from Chacma baboons (*Papio ursinus*) feeding on a diet low in fat and protein (Melton and Melton 1982).

The influence of diet on the haematology of marine mammals has not been investigated to any great extent. McConnell and Vaughn (1983) reported that haematological parameters may be reduced as a result of the fish fed to captive animals being frozen and thawed. This process is known to break down and destroy many vitamins. If the diet is not supplemented, vitamin deficiencies may result in haematological changes. However, the diets of the dolphins used in the present study were supplemented daily and no deficiencies were suspected. Another factor that must be taken into account when considering condition is parasitism, as this may have an effect on haematology, either directly or indirectly. Parasitism was found to have an effect on PCV in impala (Gallivan *et al.* 1995) and rainbow trout (*Onchorhynchus mykiss*) (Rand and Cone 1990). This was related to parasites causing anemia and weight loss. The authors of the former study pointed out that this effect might be exaggerated in animals on a low nutritional plane. Thus, PCV may be a useful indicator of loss of condition owing to factors other than diet deficiency.

Haematological changes in accordance with changes in water temperature were difficult to verify. The fact that many of these relationships were based on data from Thandi complicated the matter further, as these data were biased, as discussed previously. In other animals, which were less frequently exposed to illness and treatment during the study, data implied that haematological values were slightly increased when water temperatures were reduced. This relationship could mean either greater activity and haematological performance in the winter months, due primarily to increased thermoregulatory demands; or it may be related to slight changes in diet as a result of Oceanarium staff increasing the energy content of the diet during the winter months. Higher levels of RBC, HB and PCV and lower MCV in collared peccaries during winter was ascribed to increased erythropoetic activity (Lochmiller *et al.* 1985b), in turn, related to seasonal changes in plasma volume dynamics (haemoconcentration, expansion) or dietary changes. In terrestrial mammals, it is usually a combination of temperature and seasonal changes in diet and forage quality that influences RBC and HB, which generally peak in winter (Lochmiller *et al.* 1985b, c, Hellgren *et al.* 1993). In black bears these alterations mirror a change in weight. The haematological increases from summer to autumn have been related to increased food intake, improved nutritional condition and possible changes in erythropoiesis (in preparation for hibernation) (Hellgren *et al.* 1993). In addition, variation in food supply further affected the amplitude of these seasonal fluctuations. Endogenous rhythms have also been suggested to play a role in seasonal changes in haematology in terrestrial mammals (Franzmann and Schwartz 1988, Hellgren *et al.* 1989, 1993, Knick *et al.* 1993).

Mountain gazelle, however, show an increase in RBC and decrease in MCV during summer, and not winter as described above (Rietkerk *et al.* 1994). The authors attributed this to heat stress. Decreases in RBC, HB and PCV were observed prior to moulting in Baikal (Ronald and Kay 1982) and harp seals (Ronald *et al.* 1969 in Ronald and Kay 1982, Engelhardt 1977 in Engelhardt 1979). These decreases were ascribed to the high levels of physiological stress, which occur during moult. The complexities of RBC, HB, PCV and MCV dynamics have been discussed previously and it seems apparent that different species have developed differing haematological mechanisms to adapt to their environment. RBC, HB, PCV and MCV collaborate physiologically in order to optimise the oxygen carrying capacity of the blood with respect to fluctuating food quality and quantity, season and temperature and external and internal water sources.

Results from this study showed that platelet counts decreased with age. This overall decrease was also evident within the data of specific animals, implying that this decline may be ongoing throughout the animal's life. Alternatively, there may be several maturity-related changes, although without additional data these potential changes are difficult to define. The two male dolphins revealed a relationship between increasing weight and declining platelet numbers. As these animals generally increased in weight as the study progressed, this trend is certainly age-related. Bossart and Dierauf (1990) previously emphasised the need for baseline platelet values of marine mammals, as there is a severe lack of indications of platelet levels in bottlenose dolphins and/or other marine mammal species. This has prevented the comparison of data from this study. Platelets are important in blood clotting and increases can be associated with medication, certain vitamin deficiencies and cases of poisoning. Thurman and van der Elst (1995) report that increases are usually clinically insignificant.

Platelets were significantly related with all aspects of dietary energy intake, although this was not consistent for all five animals. Overall, the majority of relationships showed that platelets were reduced at higher levels of energy intake. However, this decrease was usually small, with most values appearing relatively stable over a wide range of energy intake levels. Thandi, was the exception to this, her platelet numbers appeared to increase slightly with increasing energy intake. Again, it is suspected that the reasons for this are related to increased energy intake during periods of illness and/or treatment.

For the present study, mean reticulocyte counts reflected an age-related pattern. Although values recorded from the five animals overlapped extensively, mean counts were lowest in the two oldest animals (Dimple and Dolly), reflecting a lower turnover of RBC's. The fact that Thandi, Simo and Domino had ever increasing numbers of reticulocytes reflected that this turnover, and the production of new RBC's, declines with age. This was reinforced by the significant negative correlations between age, reticulocyte counts and corrected indices for Dolly and Domino. Reticulocytes are immature, replacement RBC's, which are released by the bone marrow in response to a need for new RBC as a result of blood loss, haemolytic diseases or in remission of other types of anemia (Thurman and van der Elst 1995). Reticulocytes represent the new RBC population and are therefore an important indicator of marrow activity and the status of erythropoetic functioning. There is a severe lack of data on marine mammal reticulocytes in the literature. Although RBC counts were generally higher in older animals, it would appear that their turnover is reduced as the animal ages.

The relationship between weight and reticulocyte count implies that counts decrease with increasing weight. This may reflect a prolonging of the lives of circulating RBC's as a result of an improvement in physical condition (resulting from an increase in weight). Elevated numbers of reticulocytes have been associated with anemia in dolphins (Medway and Geraci 1978) and it seems likely that improved overall condition includes the 'health' of circulating RBC's. Consequently, the rate of RBC breakdown and production is optimised, reducing the need for reticulocytes. In the same context, relationships between the corrected index and reticulocyte counts and the mean energy intake of the prior month, respectively reflected that there was a reduced need for reticulocytes when nutritional condition is improved.

Reticulocyte counts and corrected indices were slightly increased and more variable at temperatures below 20°C. In addition, high outliers for both parameters were also only measured at temperatures below 20°C, suggesting that the turnover rate of RBC's was higher at lower temperatures. This higher turnover rate may be related to the increased level of activity, which occurs in winter, in order to compensate for colder water temperatures. The production of RBC's is stimulated by a greater oxygen demand stemming mainly from the muscles used for locomotion (De Monte and Pilleri 1988). Higher reticulocyte counts in free-living seals, compared to captive animals, has been related to a more active life in the wild (McConnell and Vaughan 1983).

Summary

Summarising the results of the present study, a haematological "index of condition" was established for captive bottlenose dolphins (Table 3.14). This index is provided as an indication of haematological condition and the nature of physiological changes, which occur with respect to fluctuations in the animal's condition.

Table 3.14: Haematological condition index for captive bottlenose (A: optimal condition, B: precautionary, C: less than ideal condition).

		'Condition factor'		
Parameter		**A**	**B**	**C**
WBC (x 10^9 cells/l)		6.4-9.9	10.0–20.1	> 20.5
Neutrophils (%)	Juvenile	48-63	< 40, > 70	
	Adolescent	53.5-73.5	< 52, > 75	
	Adult	64–78	< 60, >80	
Eosinophils	Juv./Adol.	5-14	>18	
	Mature	2-7	>8	
RBC (x10^{12} cells/l)		4.1–4.8	< 3.8	< 3.5
HB (g/dl)		16.3–19.3	14.0-16.0	< 13.5
PCV (l/l)		0.46-0.55	<0.42, >0.60	
MCV (fl)		111-119	120-127	> 128
	(Elderly)	123-125	126-128	> 128
Platelets (x 10^9 cells/l)*	Juv./Adol.	148-198	< 120, > 220	
	Mature	85-150	< 80, > 170	< 40, > 220
Reticulocyte count (%)*	Juv./Adol.	4.1-5.9	6.0-10.0	> 10.0
	Mature	2.8-4.5	5.0-7.0	>8.0
Corrected index (%)*	Juv./Adol.	2.3-3.2	3.5-5.0	> 5.5
	Mature	1.5-2.5	3.0-4.5	>5.0

In conclusion, haematological parameters of captive dolphins appeared to be better indicators of overall physical condition and nutritional status than leukocytic parameters. Although WBC, neutrophils and eosinophils exhibited some important trends, these were more clearly related to clinical changes in health, than to changes in condition due to dietary and environmental factors. Haematological parameters such as RBC, HB, PCV and MCV demonstrated strong and important relationships with all aspects of the animal's life and provided a valuable indication of condition with respect to the environment and responses to it. The relationships between diet and haematological parameters illustrated that RBC, HB and PCV were more useful as short-term indicators of nutritional condition. A change in erythropoetic function resulting from changes in nutritional plane has rapid effects on haematological values because the RBC population represents a continuum and a significant proportion of the population is renewed daily (Hellgren *et al.*, 1993). Weight, on the other hand, was a better long-term indicator of nutritional status. Significant correlations between total body weight and the mean daily energy intake of the week and month prior to the blood sample highlighted the potential of weight as a long-term indicator of condition and nutritional status. The importance of blubber thickness measurements as a morphological index of condition was confirmed by this study. The limited number of relationships between haematological condition and morphological condition was, to a certain extent, expected as they respectively reflect short- and long-term changes in condition. However, the interesting correlations between blubber thickness and RBC, HB, PCV and MCV are potentially valuable in combining morphological and physiological assessments of condition in a predictive capacity. The highly complex interactions between haematology, diet, temperature and weight demand that all possible influential factors be taken into account when considering haematological and overall physical condition. The lack of data on platelet and reticulocyte counts for marine mammals was frustrating but both parameters are functionally vital and show potential in the assessment of condition.

CHAPTER 4:

MORPHOLOGICAL AND PHYSIOLOGICAL INDICATORS OF CONDITION IN CAPTIVE BOTTLENOSE DOLPHINS (*Tursiops truncatus*):

II. BLOOD CHEMISTRY.

INTRODUCTION

Studies on the blood chemistry parameters of terrestrial mammals and the influence of environmental, social and seasonal factors have been highly successful, with many authors identifying particular parameters which are useful in assessing nutritional condition and general physiological, as well as physical, health. Blood chemistry components, found to be most useful in the monitoring of terrestrial mammal metabolism and nutritional condition, include blood urea nitrogen (BUN), the BUN:creatinine ratio, serum alkaline phosphotase (ALP) (Franzmann 1972, Seal *et al.* 1978, Lochmiller *et al.* 1986, Dinkines *et al.* 1991, Wolkers *et al.* 1994a) and the ratio of serum albumin to globulin (Lochmiller *et al.* 1985b). A number of these parameters have subsequently been related to the condition of the environment as a whole, and used as indicators of habitat quality (Seal and Hoskinson 1978, Seal *et al.* 1978a, Warren *et al.* 1981, 1982, Lochmiller *et al.* 1985b, 1985c, 1988, Dinkines *et al.* 1991, Sams *et al.* 1998).

Marine mammal physiological studies were initiated as early as 1902 (Joliet 1902 in De Monte and Pilleri 1988). All these early studies were based on opportunistic investigations of stranded and/or whaling specimens. However, the advent of oceanariums and dolphinariums and the keeping of marine mammals in captivity made marine mammal physiology easier to investigate. Analyses of blood chemistry components have played a vital role in these physiological studies.

The majority of early marine mammal physiological studies provided baseline values of blood chemistry components, in conjunction with results of haematological tests (Medway and Geraci 1965, Ridgway 1965, Andersen 1968, White *et al.* 1976, Medway and Geraci 1978, Engelhardt 1979, Irvine *et al.* 1980, Ronald and Kay 1982, Cornell, 1983, Kuiken 1985). More recently, the need for successful maintenance of animals in captivity has resulted in extensive use of blood chemistry parameters in the assessment of marine mammal health (Asper *et al.* 1990, Clarke 1990, Fothergill *et al.* 1991, Thurman and van der Elst 1995). The carefully controlled captive environment has allowed for investigations into age-, sex- and certain reproductive-related changes in blood chemistry (Asper *et al.* 1990). Consequently, captive animals have provided valuable blood chemistry data, which have proved useful in comparative field studies (Boice and Roberts 1966, Nelson 1970, De Monte and Pilleri 1972, 1977, Bogdanova and Lebedev 1973, Allen *et al.* 1976, Geraci *et al.* 1979, Irvine *et al.* 1980, Nielsen and Andersen 1982, McConnell and Vaughan 1983, Medway 1983, Medway and Cornell 1989, Kastelein *et al.* 1990, Montoya-Ospina 1994, Heidel *et al.* 1996).

The relationship between morphological parameters such as weight and blubber thickness, diet and temperature are relatively well known (for example, Costa *et al.* 1993) but little is known of the influences of these factors on blood chemistry parameters. Terrestrial studies have shown that body condition not only reflects the nutritional status of an animal but also the influence of animal-habitat interactions (Virgl and Messier 1993). Morphological condition is typically easier to assess than physiological condition, both in captive and free-ranging animals, and the establishment of interactions between physiology and morphology would allow for interpretation of physiological condition from a morphological perspective (Ridgway and Fenner 1982, Ridgway and Kohin 1995).

Although morphological parameters have long been used as marine mammal condition indices (Eberhardt and Sniff 1977, Lockyer *et al.* 1985, Lockyer 1986, 1993a), similar physiological studies of the condition of marine mammals have only recently been carried out. However, these studies emphasise the importance and value of condition indices in assessing food resource quality and overall environmental health (Croxall *et al.* 1988, Costa *et al.* 1989, Castellini *et al.* 1993, Gales *et al.* 1994, Worthy and Abend 1997). Increasing coastal development and escalating degradation of marine habitats has put pressure on marine food resources and, as a result, populations of many marine species are in decline. In this respect, techniques used to assess the physiological and morphological condition of apex marine predators need to be developed, in order to effectively monitor and manage the status of marine habitats and biodiversity.

Captive animals, because of their controlled environment, provide an ideal opportunity to examine and monitor changes in blood chemistry parameters according to morphological, dietary, social and environmental changes. From these investigations in a controlled environment, base-line values, trends and relationships can be established for comparison with animals in the wild.

The aims of this study were as follows:

- To produce base-line blood chemistry values for healthy captive bottlenose (*Tursiops truncatus*) dolphins,

- to identify and investigate the effect of age, weight, blubber thickness, water temperature and diet on the blood chemistry of captive bottlenose dolphins,
- to produce an index of physiological condition, based on blood chemistry parameters, for captive bottlenose dolphins.

MATERIALS AND METHODS

Blood chemistry analyses paralleled the leukocytic and haematological analyses described in Chapter 3. Materials and methods used to assess and monitor the animals environment, behaviour, diet and feeding as well as morphological parameters such as weight and blubber thickness were identical to those described in the Materials and Methods section of Chapter 3 and are therefore not repeated in this chapter.

PHYSIOLOGICAL - BLOOD SAMPLING AND ANALYSES

Blood samples used in the analysis of blood and serum chemistry were collected at the same time as samples for haematological analysis (Chapter 3). Silicone coated vac-u-test ® tubes were used to sample blood for chemistry tests. Separate samples were drawn into potassium oxalate/sodium fluoride tubes for plasma glucose analysis. Samples were sent directly to the chemistry laboratories at the Port Elizabeth branch of the South African Institute for Medical Research for analysis. All analyses were done within 4 hours of sampling.

Blood chemistry analyses were carried out using an automated Cobas Mira S (Roche), using the following tests (Anon. 1992):

Liver-associated enzymes and total bilirubin

Aspartate aminotransferase (AST)	Jendrassik-Graff method
Alanine aminotransferase (ALT)	(same as above)
Total bilirubin	(same as above)

Liver-, muscle- and kidney-associated parameters

Creatinine:	Jaffe alkaline picrate method
Urea; BUN:	Enzymatic ultraviolet test, urease method
Alkaline phosphotase (ALP):	IFCC method (Kinetic colorimetric test)
Creatine phosphokinase (CPK):	"Optimised standard method" conforming to the recommendations of the Deutsche Gesellschaft fur Klinische Chemie.

Lactate dehydrogenase (LDH):	(same as above)
Proteins and electrolytes	
Total protein:	Biuret method
Albumin:	Bromcresol green dye method
Globulin:	(calculated: total protein-albumin)
Sodium; potassium:	Ion selective electrode method
Calcium:	Cresalphthalein method
Lipids	
Cholesterol (and associated lipids, see below)	Enzymatic CHOD/PAP method
Triglycerides	
HDL-cholesterol	
LDL-cholesterol	
Cholesterol:HDL-cholesterol	(Calculated)
Glucose:	Glucose oxidase method

Additional parameters used during the study included calculated ratios between certain parameters (BUN, ALP, CPK, LDH, sodium) and creatinine, as well as the ratio between albumin and globulin.

DATA ANALYSES

Data analyses for blood and serum chemistry results were identical to those described for haematology data in Chapter 3. Data from 1992 to 1995 were used in the analyses. For each animal, overall mean values were obtained for each blood chemistry parameter, using all recorded data points. Minimum-maximum ranges and 90 % confidence limits were also calculated.

As described in Chapter 3, outlying data were distinguished from "normal" data using box-whisker plots and Wilk-Shapiro Rankit plots (Zar 1984). Outlying data points were removed from the data and mean values, minimum-maximum ranges and 90 % confidence limits were calculated for "normal" data. Differences between complete data sets (inclusive of outliers) and "normal" data (exclusive of outliers) were examined for statistical differences using the Mann-Whitney U-test (Zar 1984). Differences and similarities between mean values of blood chemistry parameters for the five animals were analysed as described in Chapter 3.

Relationships between "normal" seasonal, environmental, dietary, morphological and physiological parameters, for each animal, were examined using multiple correlation analysis. The number of significant relationships ($p \leq 0.05$) between chemistry parameters and independent variables such as diet, water temperature, etc. were counted and only those blood parameters having more than 15 significant relationships with independent variables were used in the attempt to form a condition index.

Similar to statistical analyses for haematological data and independent variables (Chapter 3), multivariate statistics (cluster analyses, stepwise linear regression) were used in an attempt to better interpret interactions between blood chemistry parameters and independent variables. However, the complexity and inconsistency of results, due predominantly to the age and sex composition of the study group of captive dolphins, precluded the conclusive use of these types of statistics in the current study. Physiological data from a more comprehensive group of captive dolphins is needed to validate the use of multivariate statistics in interpreting interactions between blood chemistry and influential factors, such as age, diet and water temperature.

Significant relationships between "normal" blood chemistry, morphological (weight; blubber thickness) and age, temperature and diet data were assessed, for each animal, using curves of best fit (Curve Expert 1.1). These analyses were identical to those described in Chapter 3.

RESULTS

The results presented in this chapter correspond with samples used for haematological analyses (Chapter 3). Blood chemistry parameters (with less than 15 significant correlations with independent variables), and thus excluded from the current study, were potassium, calcium, total protein, glucose and serum lipids.

There were no significant differences, for all five animals, between total recorded blood chemistry data (inclusive of outliers) and "normal" data from which outliers had been removed (Mann Whitney U-test; $p<0.05$). The morphological parameters of weight and blubber thickness, associated dietary parameters and water temperature data have been described in Chapter 3. As blood chemistry analyses coincided with haematological analyses, the weight, blubber thickness, diet and environmental data from Chapter 3 are used in further comparisons with blood chemistry parameters.

Equations and coefficients for the curves of best fit are listed, for each animal in Appendix 2.1 and Appendix 2.2, respectively. As discussed in Chapter 3, results show the data to be highly variable (despite the exclusion of obvious outliers) For this reason, some of the curves may reflect more complex relationships than actually exist.

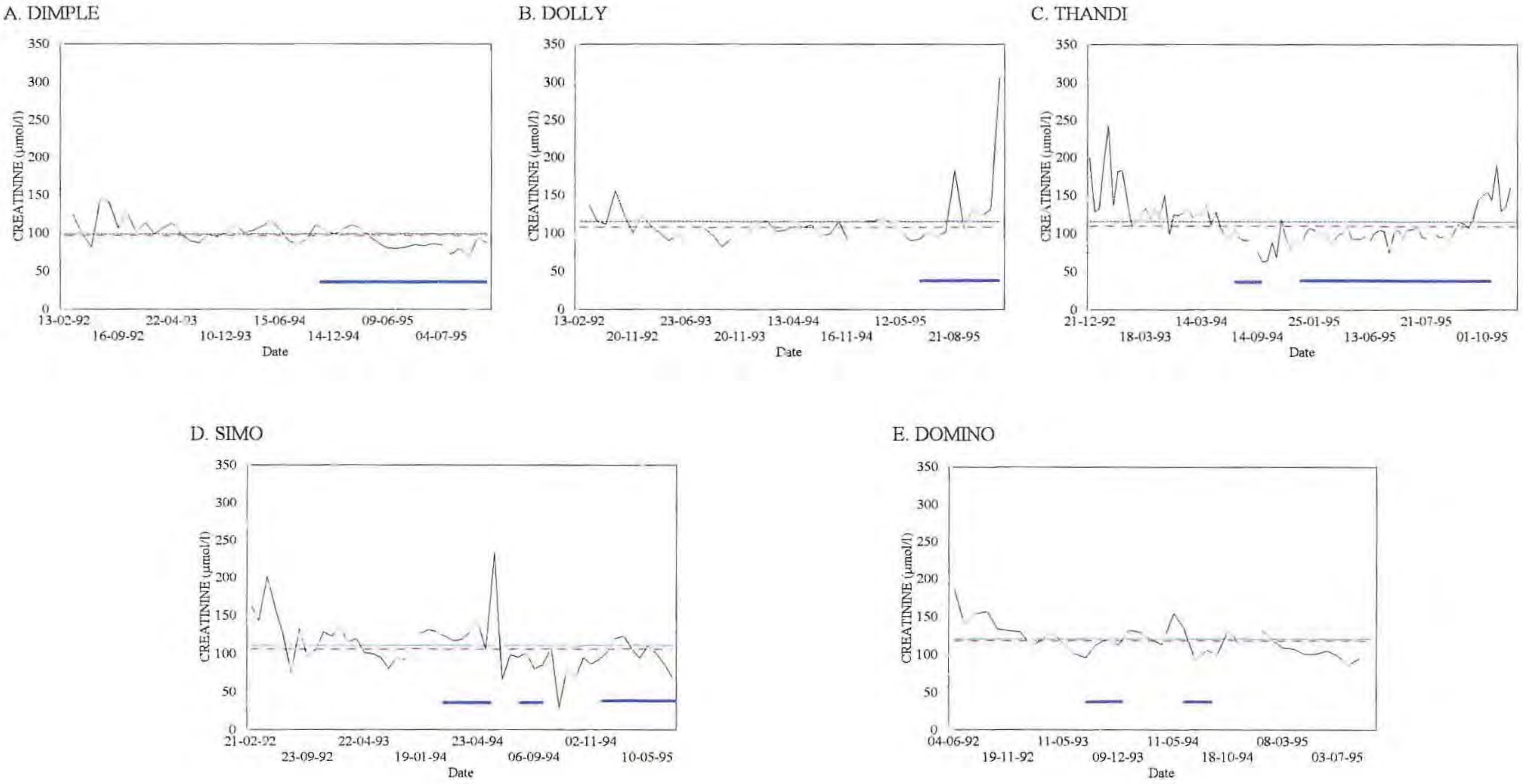
LIVER-, MUSCLE- AND KIDNEY-ASSOCIATED PARAMETERS

a. Creatinine

Creatinine fluctuations could generally be associated with periods of illness and/or treatment (Fig. 4.1). Few data existed for periods prior to the study, preventing any comparison with previous records. The range of creatinine values measured from all five animals was similar, and relatively restricted. Outlying high creatinine levels were identified in data from all five animals. However, only Simo's data contained outlying low creatinine levels (Appendix 3.1A). Dimple's "normal" mean creatinine (97.09 ± 13.37 μmol/l) was significantly lower than that of all other animals (Two sample t-test; $p \leq 0.001$), except Simo (106.10 ± 20.22 μmol/l) ($t=-2.50$; $p=0.01$; $df=90$). Dolly (107.60 ± 12.94 μmol/l), Thandi (109.00 ± 21.32 μmol/l), Simo and Domino (115.20 ± 14.73 μmol/l) had similar mean creatinine levels (Two sample t-test, Bonferroni corrected $p>0.005$) (Table 4.1).

"Normal" mean levels of creatinine were highest in the juvenile animal, relatively stable in the adolescent and adult animals and significantly reduced in the elderly animal. However, there was a large amount of overlap between animals (Fig. 4.2A). During the study, creatinine was negatively correlated with age in four animals: Dimple, Thandi, Simo and Domino (Table 4.2). However, only in Simo and Domino was this related directly to biological changes in age (Fig. 4.2). Creatinine, from both animals, showed a gradual and slight decrease in concentration as the study progressed. A significant relationship between total body weight and creatinine was evident only for Domino (Table 4.2). This trend was similar to the relationship between age and creatinine, but additional measurements at heavier weights were needed to validate this relationship.

The relationships between creatinine and food consumption were complicated by variable data, and the nature of the relationships varied between animals. Creatinine levels for Dolly and Thandi were negatively correlated with total food intake (Table 4.2), with creatinine concentrations being slightly increased during periods of lower food intake. In addition, reduced food intake was associated with outlying high creatinine levels in both animals (Fig. 4.3). Domino's creatinine levels were also negatively correlated with total food consumption and all categories of dietary energy intake. These relationships were almost identical to the trend between weight and creatinine for this animal, illustrating that creatinine concentrations were relatively stable over a wide range of food and energy intake levels, declining above 42 kg/week and 32 500 kJ/day, respectively (Fig. 4.3C & 4.4B). Additional data were needed to verify the decline in creatinine levels at increased food consumption and/or energy intake.



A. DIMPLE

B. DOLLY

C. THANDI

D. SIMO

E. DOMINO

Figure 4.1: Levels of creatinine (µmol/l) (——) for all five captive animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the overall mean creatinine level (——) and the "normal" (see text) mean creatinine level (- - -) for the study between 1992 and 1995 (——: extended period of illness and/or treatment or period of aberrant feeding and/or behaviour).

Table 4.1a: Mean blood chemistry values and associated data for the captive bottlenose dolphin 'Dimple'.
Table A: Mean values calculated using all results obtained between 1992 and 1995
Table B: "Normal" mean values calculated from data exclusive of statistical outliers (box-whisker plot).

		Table A					**Table B**				
PARAMETER	**UNITS**	**MEAN**	**STD**	**RANGE**	**90% RANGE**	**N**	**MEAN**	**STD**	**RANGE**	**90% RANGE**	**N**
Aspartate aminotransferase (AST)	U/l	645.20	289.70	81.00-1151.00	573.50-717.00	46	657.80	280.10	334.00-1151.00	587.60-727.90	45
Alanine aminotransferase (ALT)	U/l	201.60	110.10	54.00-477.00	174.60-228.60	47	195.60	103.30	54.00-399.00	170.00-221.20	46
Total bilirubin	umol/l	9.18	12.64	1.00-47.00	6.08-12.28	47	5.83	5.06	1.00-18.00	4.53-7.12	43
Creatinine	umol/l	99.09	16.22	68.00-148.00	95.11-103.10	47	97.09	13.37	68.00-128.00	93.74-100.40	45
Blood urea nitrogen (BUN)	mmol/l	7.29	0.95	4.40-9.00	7.05-7.52	47	7.41	0.75	5.60-9.00	7.23-7.60	45
BUN:creatinine		0.07	0.01	0.05-0.13	0.07-0.08	47	0.07	0.01	0.05-0.09	0.07-0.08	46
Alkaline phosphotase (ALP)	U/l	343.00	122.90	141.00-623.00	312.90-373.10	47	343.00	122.90	141.00-623.00	312.90-373.10	47
ALP:creatinine		3.63	1.68	1.36-8.65	3.22-4.04	47	3.54	1.40	1.44-6.41	3.18-3.90	43
Lactate dehydrogenase (LDH)	U/l	1406.00	482.90	669.00-2748.00	1288.00-1524.00	47	1406.00	482.90	669.00-2748.00	1288.00-1524.00	47
LDH:creatinine		15.07	7.40	5.92-40.41	13.26-16.88	47	14.52	6.43	5.92-30.14	12.93-16.11	46
Creatine phosphokinase (CPK)	U/l	165.80	88.90	81.00-442.00	143.80-187.80	46	163.70	67.06	101.00-343.00	145.50-182.10	38
CPK:creatinine		1.82	1.27	0.65-6.50	1.50-2.13	46	1.57	0.84	0.65-3.61	1.36-1.79	43
Total protein	g/l	73.20	4.18	65.00-83.00	71.30-75.10	15	73.08	2.60	69.00-77.00	71.79-74.36	13
Albumin	g/l	35.87	1.89	33.00-41.00	35.01-36.72	15	35.50	1.29	33.00-37.00	34.89-36.11	14
Globulin	g/l	37.33	3.11	29.00-42.00	35.92-38.75	15	37.93	2.17	34.00-42.00	36.90-38.95	14
Albumin:globulin		0.97	0.09	0.85-1.24	0.93-1.01	15	0.95	0.05	0.85-1.03	0.92-0.97	14
Glucose	mmol/l	5.55	0.80	3.90-7.00	5.21-5.89	17	5.55	0.80	3.90-7.00	5.21-5.89	17
Sodium	mmol/l	156.00	2.93	149.00-163.00	155.30-156.70	47	156.00	2.93	149.00-163.00	155.30-156.70	47
Sodium:creatinine		1.62	0.26	1.05-2.40	1.55-1.68	47	1.60	0.24	1.05-2.17	1.54-1.66	46
Potassium	mmol/l	3.50	0.40	2.10-4.10	3.41-3.60	47	3.56	0.30	3.00-4.10	3.48-3.63	45
Calcium	mmol/l	2.25	0.09	2.04-2.39	2.20-2.29	12	2.26	0.07	2.15-2.39	2.23-2.30	11
Cholesterol	mmol/l	6.48	1.90	4.00-10.80	5.54-7.42	13	6.12	1.45	4.00-8.20	5.37-6.87	12
Triglycerides	mmol/l	3.78	1.76	0.41-6.54	2.91-4.65	13	4.36	1.16	2.94-6.54	3.72-5.00	11
HDL-Cholesterol	mmol/l	3.41	0.52	2.73-4.13	3.11-3.71	10	3.41	0.52	2.73-4.13	3.11-3.71	10
LDL-Cholesterol	mmol/l	1.76	1.26	0.09-3.67	0.98-2.55	9	1.76	1.26	0.09-3.67	0.98-2.55	9
Cholesterol:HDL-Cholesterol		1.98	0.38	1.60-2.60	1.74-2.21	9	1.98	0.38	1.60-2.60	1.74-2.21	9

Table 4.1b: Mean blood chemistry values and associated data for the captive bottlenose dolphin 'Dolly'.
Table A: Mean values calculated using all results obtained between 1992 and 1995
Table B: "Normal" mean values calculated from data exclusive of statistical outliers (box-whisker plot).

		Table A					**Table B**				
PARAMETER	**UNITS**	**MEAN**	**STD**	**RANGE**	**90% RANGE**	**N**	**MEAN**	**STD**	**RANGE**	**90% RANGE**	**N**
Aspartate aminotransferase (AST)	U/l	188.10	27.12	152.00-277.00	181.30-195.00	44	183.80	19.17	154.00-227.00	178.7-188.90	40
Alanine aminotransferase (ALT)	U/l	35.89	7.46	15.00-55.00	34.00-37.78	44	36.37	6.81	21.00-55.00	34.63-38.12	43
Total bilirubin	umol/l	2.40	1.34	0.00-5.00	2.04-2.76	40	2.40	1.34	0.00-5.00	2.04-2.76	40
Creatinine	umol/l	115.00	34.81	83.00-306.00	106.10-123.80	44	107.60	12.94	83.00-138.00	104.20-111.00	41
Blood urea nitrogen (BUN	mmol/l	9.37	1.39	7.30-16.40	9.03-9.72	45	9.21	0.90	7.30-10.90	8.99-9.44	44
BUN:creatinine		0.09	0.02	0.03-0.20	0.08-0.09	44	0.09	0.01	0.05-0.12	0.08-0.09	41
Alkaline phosphotase (ALP)	U/l	433.00	224.20	94.00-822.00	376.20-489.8	44	433.00	224.20	94.00-822.00	376.20-489.80	44
ALP:creatinine		3.84	2.05	1.08-7.80	3.32-4.36	44	3.84	2.05	1.08-7.80	3.32-4.36	44
Lactate dehydrogenase (LDH)	U/l	1011.00	161.50	741.00-1531.00	970.20-1052.00	44	999.00	141.90	741.00-1374.00	962.60-1035.00	43
LDH:creatinine		9.17	1.88	3.11-12.46	8.69-9.65	44	9.31	1.65	6.33-12.46	8.89-9.73	43
Creatine phosphokinase (CPK)	U/l	149.20	22.88	108.00-212.00	143.40-155.00	44	146.30	18.81	108.00-189.00	141.40-151.20	42
CPK:creatinine		1.36	0.30	0.45-2.06	1.28-1.44	44	1.36	0.25	0.85-1.97	1.30-1.43	42
Total protein	g/l	72.08	3.23	67.00-77.00	70.41-73.76	12	72.08	3.23	67.00-77.00	70.41-73.76	12
Albumin	g/l	45.92	2.97	40.00-51.00	44.38-47.46	12	46.45	2.42	43.00-51.00	45.13-47.78	11
Globulin	g/l	26.17	3.54	23.00-35.00	24.33-28.00	12	25.36	2.29	23.00-29.00	24.11-26.62	11
Albumin:globulin		1.79	0.27	1.14-2.13	1.64-1.93	12	1.85	0.19	1.57-2.13	1.74-1.95	11
Glucose	mmol/l	4.64	1.24	2.50-6.60	4.05-5.22	14	4.64	1.24	2.50-6.60	4.05-5.22	14
Sodium	mmol/l	157.80	3.50	151.00-166.00	156.90-158.60	45	157.80	3.50	151.00-166.00	156.90-158.60	45
Sodium:creatinine		1.44	0.25	0.53-1.92	1.38-1.50	44	1.48	0.19	1.03-1.92	1.43-1.53	42
Potassium	mmol/l	4.14	0.40	3.40-5.00	4.04-4.24	45	4.14	0.40	3.40-5.00	4.04-4.24	45
Calcium	mmol/l	2.37	0.12	2.22-2.58	2.31-2.43	12	2.37	0.12	2.22-2.58	2.31-2.43	12
Cholesterol	mmol/l	3.18	0.32	2.70-3.80	3.02-3.34	13	3.18	0.32	2.70-3.80	3.02-3.34	13
Triglycerides	mmol/l	0.48	0.15	0.26-0.68	0.41-0.56	13	0.48	0.15	0.26-0.68	0.41-0.56	13
HDL-cholesterol	mmol/l	2.11	0.44	1.49-2.79	1.86-2.36	10	2.11	0.44	1.49-2.79	1.86-2.36	10
LDL-cholesterol	mmol/l	0.79	0.26	0.30-1.15	0.64-0.94	10	0.84	0.20	0.50-1.15	0.72-0.97	9
Cholesterol:HDL-cholesterol		1.52	0.22	1.20-1.90	1.40-1.65	10	1.52	0.22	1.20-1.90	1.40-1.65	10

Table 4.1c: Mean blood chemistry values and associated data for the captive bottlenose dolphin 'Thandi'.
Table A: Mean values calculated using all results obtained between 1992 and 1995
Table B: "Normal" mean values calculated from data exclusive of statistical outliers (box-whisker plot).

		Table A					**Table B**				
PARAMETER	**UNITS**	**MEAN**	**STD**	**RANGE**	**90% RANGE**	**N**	**MEAN**	**STD**	**RANGE**	**90% RANGE**	**N**
Aspartate aminotransferase (AST)	U/l	205.20	107.90	107.00-678.00	186.20-224.20	89	176.80	54.74	107.00-344.00	166.70-186.90	81
Alanine aminotransferase (ALT)	U/l	37.18	25.08	13.00-155.00	32.76-41.60	89	33.04	15.24	13.00-76.00	30.29-35.79	85
Total bilirubin	µmol/l	3.43	3.08	0.00-24.00	2.86-3.99	82	2.87	1.37	0.00-5.00	2.61-3.13	78
Creatinine	µmol/l	115.20	31.30	63.00-243.00	109.60-120.70	87	109.00	21.32	63.00-161.00	105.00-112.90	81
Blood urea nitrogen (BUN)	mmol/l	11.13	1.67	7.20-17.10	10.83-11.43	88	11.06	1.55	7.20-14.50	10.79-11.34	87
BUN:creatinine		0.11	0.08	0.04-0.77	0.10-0.13	86	0.10	0.03	0.04-0.16	0.10-0.11	83
Alkaline phosphotase (ALP)	U/l	383.90	239.30	116.00-1049.00	341.50-426.40	88	347.30	191.90	116.00-873.00	312.30-382.30	83
ALP:creatinine		3.37	1.89	0.77-9.80	3.04-3.71	86	3.24	1.67	0.77-8.05	2.93-3.54	84
Lactate dehydrogenase (LDH)	U/l	1089.00	442.90	483.00-3696.00	1010.00-1167.00	88	1034.00	305.80	483.00-1895.00	978.40-1089.00	85
LDH:creatinine		10.26	5.59	3.45-34.31	9.26-11.26	86	9.08	3.38	3.45-15.36	8.45-9.71	80
Creatine phosphokinase (CPK)	U/l	225.80	603.00	61.00-5769.00	119.00-332.70	88	148.90	58.23	61.00-300.00	138.20-159.60	82
CPK:creatinine		1.89	3.24	0.43-30.20	1.31-2.47	86	1.41	0.74	0.43-3.38	1.27-1.54	81
Total protein	g/l	81.23	7.61	59.00-92.00	78.87-83.59	30	82.96	3.72	77.00-89.00	81.66-84.26	24
Albumin	g/l	40.97	4.01	34.00-49.00	39.70-42.23	29	40.97	4.01	34.00-49.00	39.70-42.23	29
Globulin	g/l	40.14	6.26	25.00-50.00	38.16-42.12	29	40.68	5.64	31.00-50.00	38.86-42.50	28
Albumin:globulin		1.05	0.19	0.78-1.38	0.99-1.10	29	1.04	0.17	0.78-1.36	0.99-1.10	27
Glucose	mmol/l	6.72	1.51	3.10-9.60	6.13-7.30	20	6.91	1.28	4.70-9.60	6.40-7.41	19
Sodium	mmol/l	156.10	3.28	149.00-168.00	155.50-156.60	88	155.90	3.03	149.00-164.00	155.40-156.50	87
Sodium:creatinine		1.45	0.34	0.64-2.49	1.38-1.51	85	1.41	0.29	0.64-2.13	1.36-1.47	82
Potassium	mmol/l	3.85	0.43	2.00-5.20	3.77-3.93	88	3.84	0.33	3.10-4.60	3.78-3.90	85
Calcium	mmol/l	2.83	0.13	1.99-2.59	2.34-2.42	29	2.41	0.10	2.19-2.59	2.38-2.44	27
Cholesterol	mmol/l	4.05	1.00	2.60-7.00	3.73-4.37	29	4	0.81	2.80-5.60	3.73-4.26	27
Triglycerides (TGL)	mmol/l	0.85	0.34	0.29-1.60	0.74-0.96	28	0.78	0.22	0.54-1.26	0.71-0.86	24
HDL-cholesterol	mmol/l	2.33	0.79	0.71-3.37	2.06-2.60	25	2.53	0.59	1.55-3.73	2.31-2.75	22
LDL-cholesterol	mmol/l	1.45	0.65	0.36-2.94	1.22-1.67	25	1.32	0.51	0.36-2.33	1.14-1.50	23
Cholesterol:HDL-cholesterol		1.82	0.59	1.08-3.90	1.62-2.03	25	1.70	0.24	1.30-2.20	1.61-1.79	22

Table 4.1d: Mean blood chemistry values and associated data for the captive bottlenose dolphin 'Simo'.
Table A: Mean values calculated using all results obtained between 1992 and 1995
Table B: "Normal" mean values calculated from data exclusive of statistical outliers (box-whisker plot).

				Table A					**Table B**		
PARAMETER	**UNITS**	**MEAN**	**STD**	**RANGE**	**90% RANGE**	**N**	**MEAN**	**STD**	**RANGE**	**90% RANGE**	**N**
Aspartate aminotransferase (AST)	U/l	417.30	853.40	13.00-5360.00	221.00-613.60	53	240.80	73.38	129.00-420.00	223.10-258.60	48
Alanine aminotransferase (ALT)	U/l	115.30	355.40	19.00-2350.00	33.54-197.10	53	41.71	19.43	19.00-113.00	37.00-46.41	48
Total bilirubin	µmol/l	3.87	6.24	1.00-40.00	2.24-5.34	46	2.40	1.35	1.00-7.00	2.05-2.74	43
Creatinine	µmol/l	111.10	32.99	29.00-234.00	103.40-118.70	52	106.10	20.22	67.00-144.00	101.10-111.00	47
Blood urea nitrogen (BUN)	mmol/l	10.48	1.43	6.40-15.70	10.15-10.80	53	10.45	1.12	7.80-12.60	10.19-10.72	51
BUN:creatinine		0.11	0.05	0.04-0.37	0.09-0.12	52	0.10	0.03	0.04-0.18	0.09-0.11	51
Alkaline phosphotase (ALP)	U/l	390.40	247.30	105.00-1266.00	333.50-447.30	53	304.40	133.30	105.00-561.00	271.00-337.80	45
ALP:creatinine		3.54	4.09	0.96-14.72	2.99-4.09	52	3.15	1.30	0.96-6.34	2.84-3.46	50
Lactate dehydrogenase (LDH)	U/l	1265.00	1364.00	571.00-9230.00	951.00-1579.00	53	936.80	229.70	571.00-1583.00	881.20-992.50	48
LDH:creatinine		13.33	15.75	3.58-96.15	9.67-16.99	52	8.40	3.04	3.59-18.22	7.64-9.16	45
Creatine phosphokinase (CPK)	U/l	178.10	71.10	100.00-535.00	161.70-194.40	53	171.20	51.07	100.00-296.00	159.30-183.10	52
CPK:creatinine		1.88	1.46	0.63-8.62	1.54-2.22	52	1.63	0.74	0.63-3.25	1.45-1.80	50
Total protein	g/l	61.17	8.13	45.00-68.00	54.48-67.86	6	63.50	0.58	63.00-64.00	62.86-64.18	4
Albumin	g/l	40.80	4.38	34.00-46.00	36.62-44.98	5	41.33	1.16	40.00-42.00	39.39-43.28	3
Globulin	g/l	19.80	5.89	11.00-26.00	14.18-25.42	5	19.80	5.89	11.00-26.00	14.18-25.42	5
Albumin:globulin		2.12	0.65	1.62-3.09	1.59-2.83	5	2.21	0.65	1.62-3.09	1.59-2.83	5
Glucose	mmol/l	4.69	1.44	3.10-7.40	4.04-5.35	15	3.93	0.54	3.10-4.70	3.63-4.22	11
Sodium	mmol/l	157.90	2.93	148.00-165.00	157.20-158.50	53	157.80	2.24	153.00-163.00	157.20-158.30	50
Sodium:creatinine		1.57	0.66	0.69-5.45	1.42-1.72	52	1.49	0.37	0.69-2.31	1.41-1.58	51
Potassium	mmol/l	3.84	0.47	3.20-5.80	3.73-3.95	53	3.76	0.32	3.20-4.40	3.69-3.84	50
Calcium	mmol/l	2.30	0.05	2.24-2.36	2.24-2.35	4	2.30	0.05	2.24-2.36	2.24-2.36	4
Cholesterol	mmol/l	2.87	0.47	2.50-3.40	2.07-3.66	3	2.87	0.47	2.50-3.40	2.07-3.67	3
Triglycerides	mmol/l	0.49	0.10	0.37-0.55	0.31-0.67	3	0.49	0.10	0.37-0.55	0.31-0.67	3
HDL-Cholesterol	mmol/l	1.95	0.35	1.66-2.34	1.36-2.54	3	1.95	0.35	1.66-2.34	1.36-2.54	3
LDL-Cholesterol	mmol/l	0.69	0.26	0.39-0.87	0.25-1.13	3	0.69	0.26	0.39-0.87	0.25-1.13	3
Cholesterol:HDL-Cholesterol		1.47	0.15	1.30-1.60	1.21-1.72	3	1.47	0.15	1.30-1.60	1.21-1.72	3

Table 4.1e: Mean blood chemistry values and associated data for the captive bottlenose dolphin 'Domino'.
Table A: Mean values calculated using all results obtained between 1992 and 1995
Table B: "Normal" mean values calculated data exclusive of statistical outliers (box-whisker plot).

		Table A					**Table B**				
PARAMETER	**UNITS**	**MEAN**	**STD**	**RANGE**	**90% RANGE**	**N**	**MEAN**	**STD**	**RANGE**	**90% RANGE**	**N**
Aspartate aminotransferase (AST)	U/l	184.40	31.78	143.00-269.00	175.50-193.20	37	170.30	14.94	143.00-198.00	165.60-175.00	29
Alanine aminotransferase (ALT)	U/l	33.22	9.25	20.00-56.00	30.65-35.78	37	31.38	7.06	20.00-43.00	29.33-33.43	34
Total bilirubin	µmol/l	2.65	1.91	1.00-11.00	2.09-3.20	34	2.39	1.22	1.00-5.00	2.03-2.76	33
Creatinine	µmol/l	120.40	21.05	86.00-187.00	114.50-126.20	37	115.20	14.73	86.00-140.00	110.80-119.50	33
Blood urea nitrogen (BUN	mmol/l	8.76	0.56	7.80-10.10	8.61-8.92	37	8.76	0.56	7.80-10.10	8.61-8.92	37
BUN:creatinine		0.08	0.01	0.05-0.11	0.07-0.08	37	0.07	0.01	0.05-0.09	0.07-0.08	35
Alkaline phosphotase (ALP)	U/l	982.10	311.60	510.00-1835.00	895.60-1069.00	37	958.40	280.20	510.00-1484.00	879.50-1037.00	36
ALP:creatinine		8.16	2.14	4.81-12.12	7.56-8.75	37	8.16	2.14	4.81-12.12	7.56-8.75	37
Lactate dehydrogenase (LDH)	U/l	973.00	140.00	778.00-1290.00	934.20-1012.00	37	973.00	140.00	778.00-1290.00	934.20-1012.00	37
LDH:creatinine		8.39	2.18	4.21-12.67	7.78-8.99	37	7.64	1.04	5.86-9.64	7.30-7.98	27
Creatine phosphokinase (CPK)	U/l	222.40	50.04	116.00-309.00	208.50-236.20	37	228.40	44.29	151.00-309.00	215.70-241.00	35
CPK:creatinine		1.90	0.55	0.96-2.95	1.72-2.05	37	1.90	0.55	0.96-2.95	1.75-2.05	37
Total protein	g/l	63.14	4.95	56.00-72.00	59.51-66.78	7	61.67	3.33	56.00-66.00	58.93-64.40	6
Albumin	g/l	43.57	2.30	40.00-60.00	41.88-45.26	7	43.57	2.30	40.00-46.00	41.88-45.26	7
Globulin	g/l	19.57	4.28	16.00-28.00	16.43-22.71	7	18.17	2.32	16.00-22.00	16.26-20.07	6
Albumin:globulin		2.30	0.43	1.57-2.71	2.00-2.60	7	2.54	0.15	2.30-2.71	2.39-2.68	5
Glucose	mmol/l	4.62	1.52	1.80-6.80	3.74-5.50	10	4.93	1.22	3.50-6.80	4.18-5.69	9
Sodium	mmol/l	156.10	3.18	151.00-164.00	155.20-157.00	37	156.10	3.18	151.00-164.00	155.20-157.00	37
Sodium:creatinine		1.33	0.22	0.84-1.85	1.27-1.39	37	1.33	0.19	1.00-1.68	1.28-1.39	35
Potassium	mmol/l	3.84	0.41	3.20-4.70	3.72-3.96	37	3.84	0.41	3.20-4.70	3.73-4.00	37
Calcium	mmol/l	2.43	0.05	2.38-2.48	2.35-2.52	3	2.44	0.05	2.38-2.48	2.35-2.52	3
Cholesterol	mmol/l	3.34	0.47	2.70-4.00	2.89-3.79	5	3.33	0.16	3.20-3.50	3.08-3.59	3
Triglycerides	mmol/l	0.39	0.21	0.12-0.70	0.19-0.59	5	0.31	0.14	0.12-0.44	0.15-0.48	4
HDL-cholesterol	mmol/l	2.29	0.46	1.70-2.96	1.85-2.73	5	2.12	0.31	1.70-2.43	1.75-2.49	4
LDL-cholesterol	mmol/l	0.84	0.12	0.72-0.99	0.72-0.95	5	0.84	0.12	0.72-0.99	0.72-0.95	5
Cholesterol:HDL-cholesterol		1.48	0.08	1.40-1.60	1.40-1.56	5	1.48	0.08	1.40-1.60	1.40-1.56	5

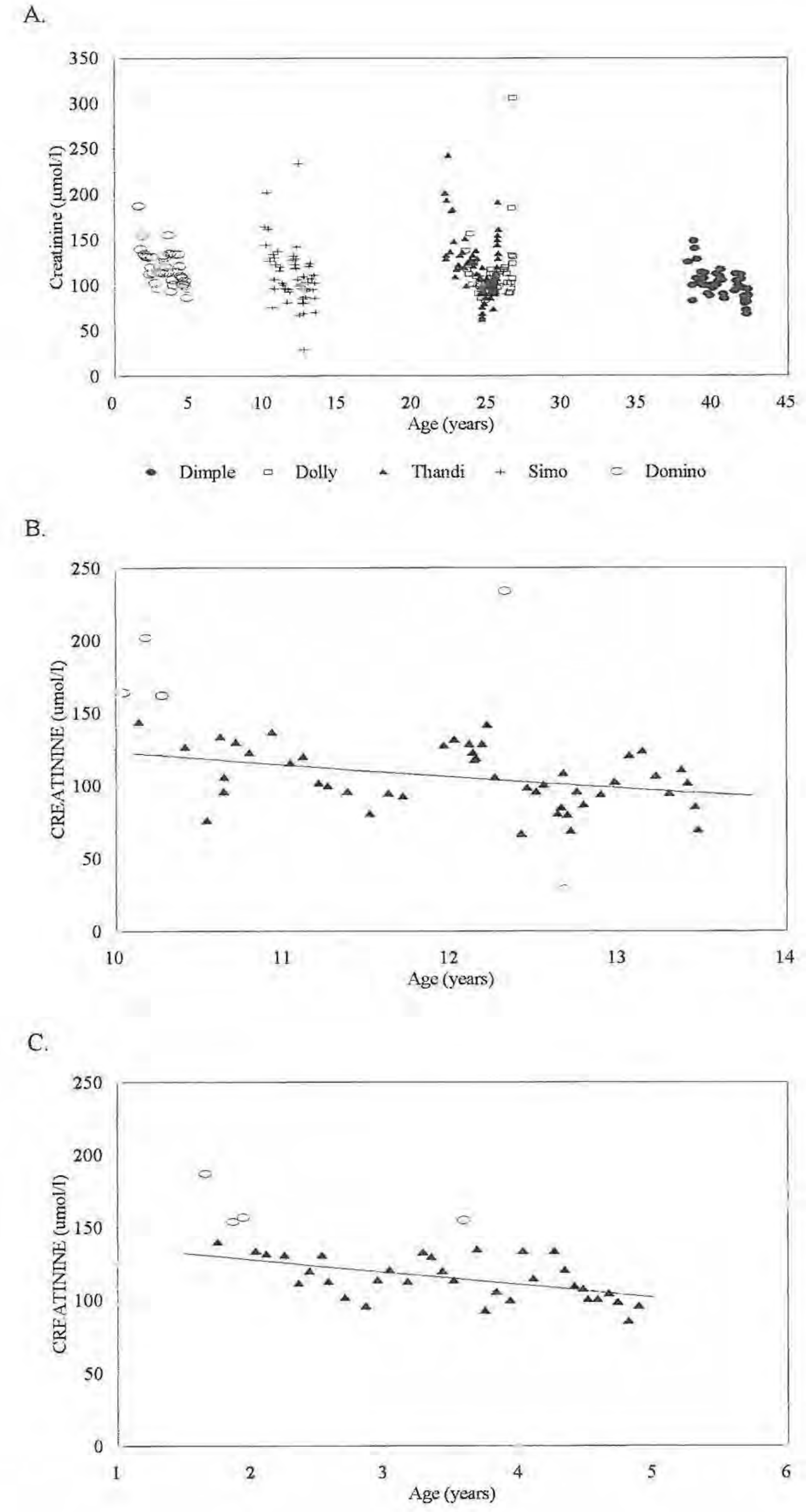


Figure 4.2: Relationship between levels of serum creatinine and age for the five dolphins (A) and curves of best fit ( ) for Simo (B) and Domino (C) with significant correlations ($p<0.05$) between creatinine and age for the study period 1992-1995. Included in the graphs are all "normal" (see text) data ( ) and statistically determined outliers on the y-axis ( ).

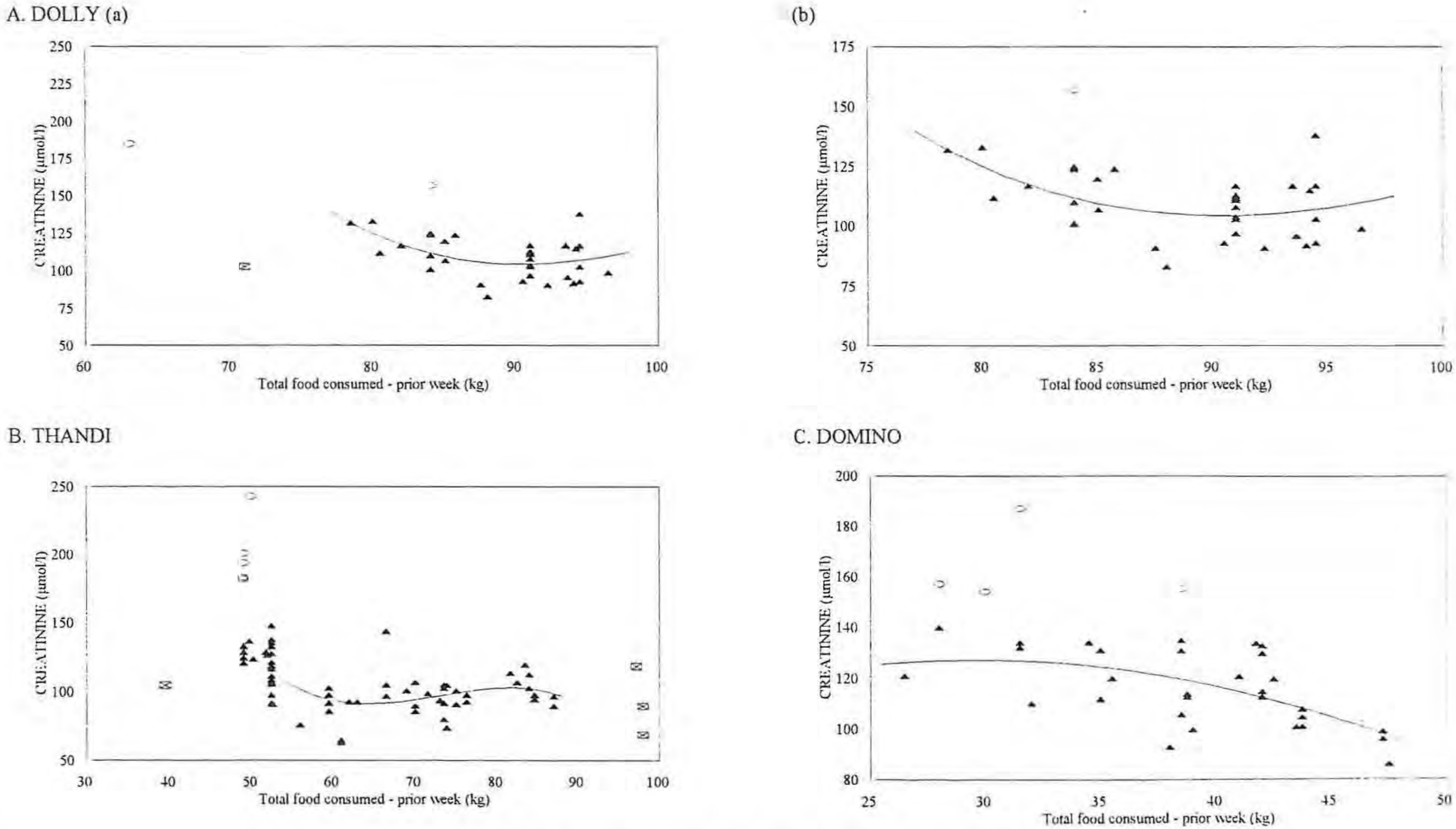
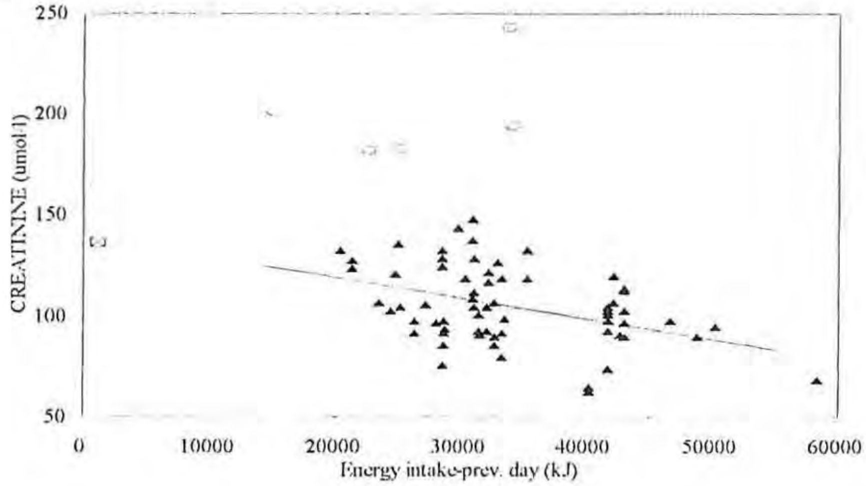
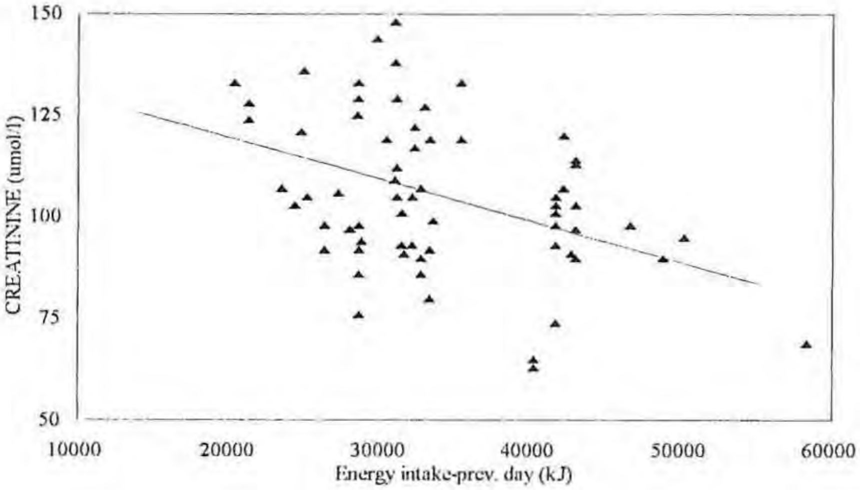


Figure 4.3 : Curves of best fit ( ) for animals (A. Dolly (a & b); B. Thandi; C. Domino) with significant correlations (p<0.05) between creatinine and total food consumed in the week prior to the blood sample. Included in the graphs are all "normal" (see text) data ( ) and statistically determined outliers (x-axis: ; y-axis:).

A. THANDI (a)



(b)



B. DOMINO

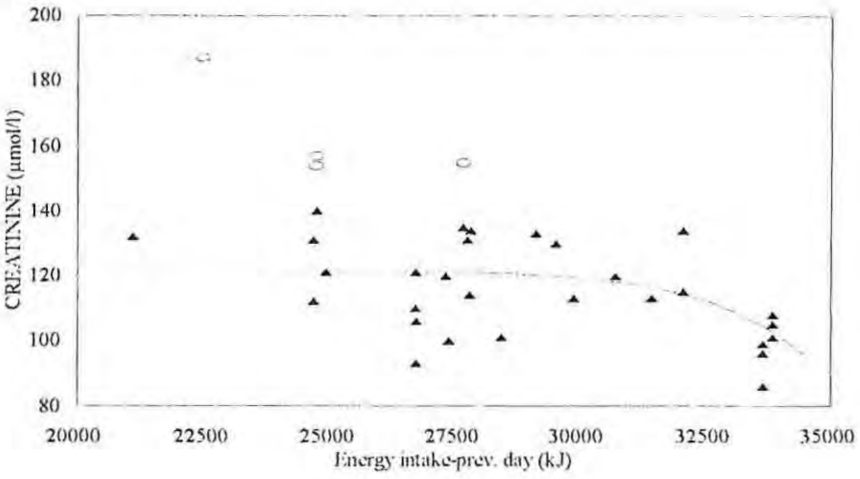


Figure 4.4: Curves of best fit ( ) for animals (A. Thandi (a & b); B. Domino) wit significant correlations ($p<0.05$) between creatinine and the dietary energy intake of the day prior to the blood sample. Included in the graphs are all "normal" (see text) data ( ) and statistically determine outliers (x-axis: y-axis:).

Table 4.2: Correlation coefficients (r), levels of significance (*:p<0.05, **: p<0.01, ***: p<0.001) and sample sizes (n) for significant relationships between serum creatinine and independent variables for the five captive dolphins (Data are presented as r*(n)) (NS: non-significant (p>0.05)).

Parameter	Dimple	Dolly	Thandi	Simo	Domino
Age	-0.55***(45)	NS	-0.3**(79)	-0.38**(47)	-0.54***(33)
Weight	NS	NS	NS	NS	-0.52**(31)
D1	NS	-0.38*(36)	-0.44***(63)	NS	-0.56***(30)
E1	NS	NS	-0.42***(66)	NS	-0.42*(30)
E2	0.46**(37)	NS	-0.32**(63)	NS	-0.47**(30)
E3	NS	NS	-0.28*(67)	NS	-0.54**(29)
E4	NS	NS	NS	NS	-0.57***(30)
Month	NS	NS	NS	-0.52***(47)	NS
T1	0.61***(45)	NS	NS	0.58***(47)	NS
T2	0.63***(45)	NS	NS	0.58***(47)	0.41*(33)
T3	0.61***(45)	NS	NS	0.57***(47)	NS

D1: Total food consumption of week prior to blood sample; E1: Dietary energy intake of day prior to blood sample; E2-E4: Mean daily energy intake of week in which blood was sampled, and week and month prior to blood sample, respectively, T1: Mean water temperature of week prior to blood sample, T2: Mean water temperature of month in which blood was sampled, T3: Mean water temperature of month prior to blood sample.

Thandi's creatinine levels were negatively correlated with the dietary energy intake of the day prior to the blood sample (Table 4.2). Although data were highly variable, creatinine levels measured at greater energy intake were predominantly lower than those measured at lower energy intakes (Fig. 4.4). Creatinine measurements from Dimple, Thandi and Domino were all significantly related to the mean daily dietary energy intake of the week in which blood was sampled (Table 4.2). The negative relationship between these two parameters for Thandi and Domino were almost identical to those described for total food consumption. In contrast, Dimple's creatinine levels appeared to increase (~80-110 μmol/l) as mean energy intake increased (~27 500-36 000 kJ/day) (Fig. 4.5). This trend was, however, complicated by reduced creatinine levels corresponding with energy intake levels above 37 500 kJ/day. Creatinine levels measured during periods of significantly reduced energy intake remained within the "normal" range for this animal (Fig. 4.5a).

Interpretation of significant relationships between serum creatinine and the mean daily energy intake of the week and/or month preceding the blood sample was precluded by variable data (Fig. 4.6A). Domino was the only animal for which the mean daily energy intake of the preceding month was negatively correlated with serum creatinine. Although creatinine concentrations were variable (~90-130 μmol/l) between energy intake levels of 26 000 kJ/day and 32 000 kJ/day, creatinine

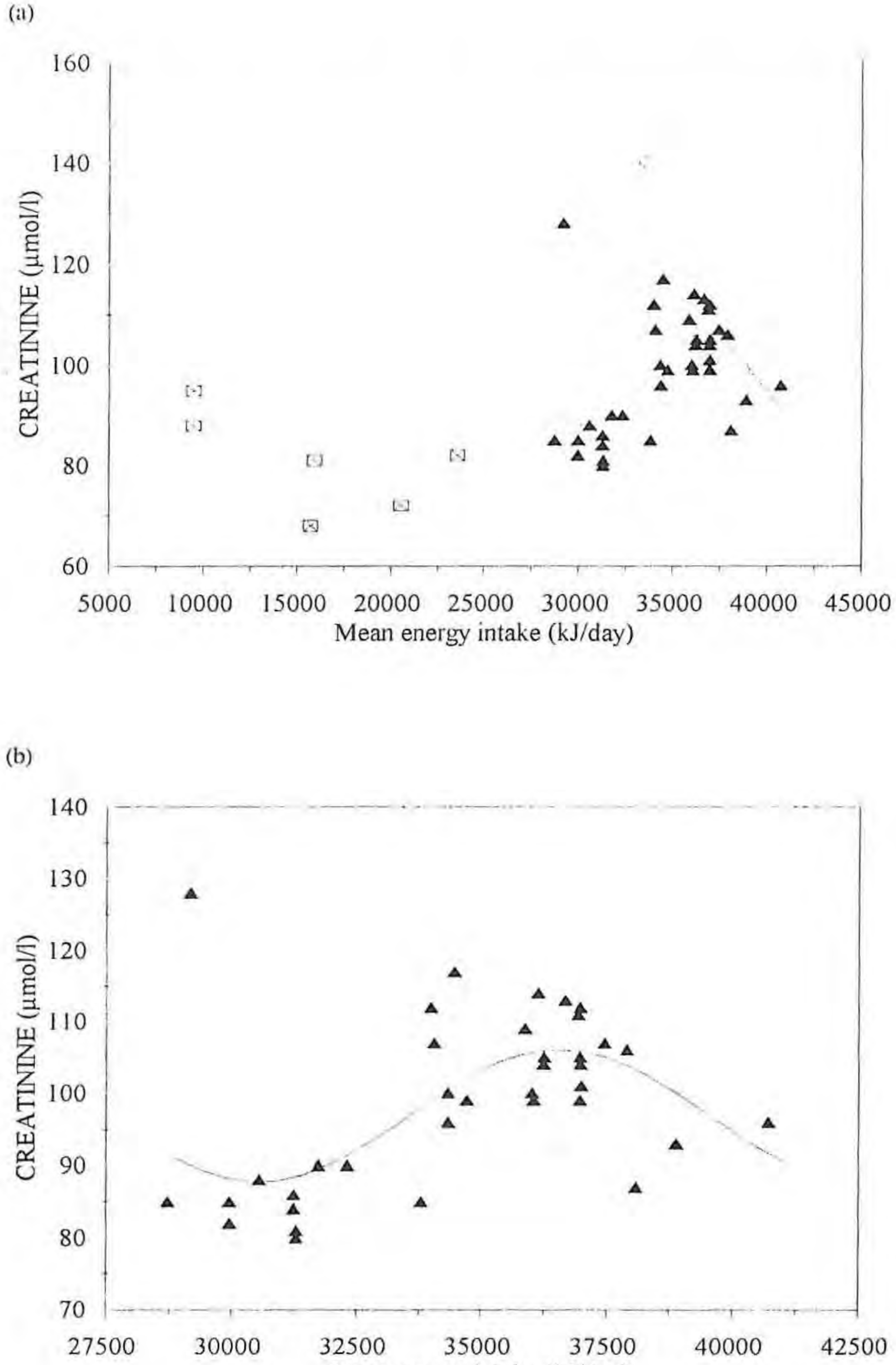
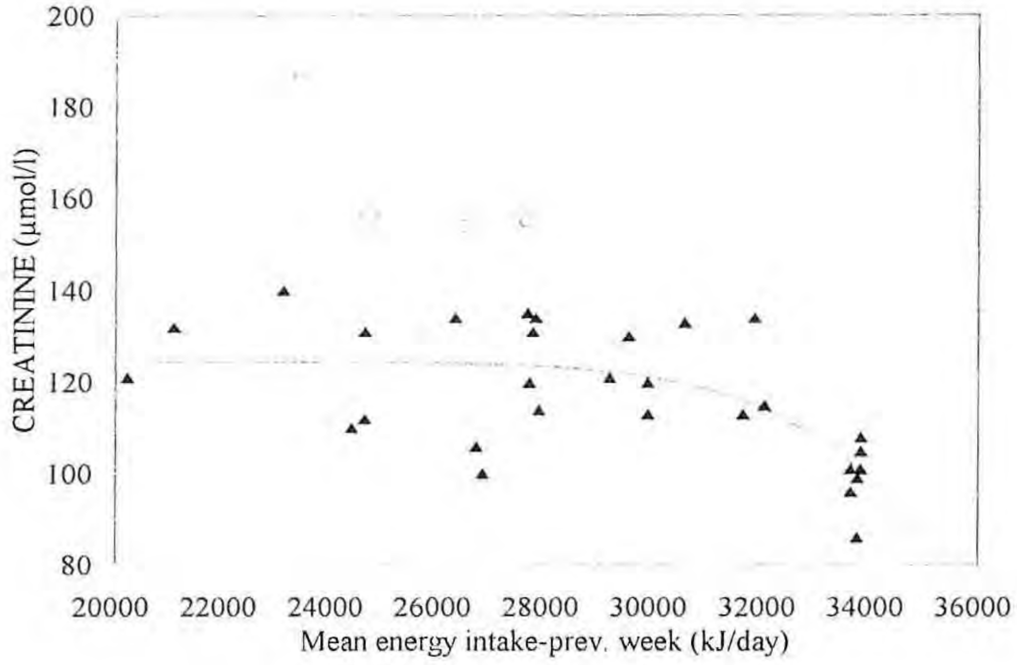


Figure 4.5: Curve of best fit ( ) for Dimple with a significant correlation ($p<0.05$) between serum creatinine and the mean daily energy intake of the week in which blood was sampled. Included in the graphs are all "normal" (see text) data ( ) and statistically determined outliers (x-axis: ; y-axis:).

A.



B.

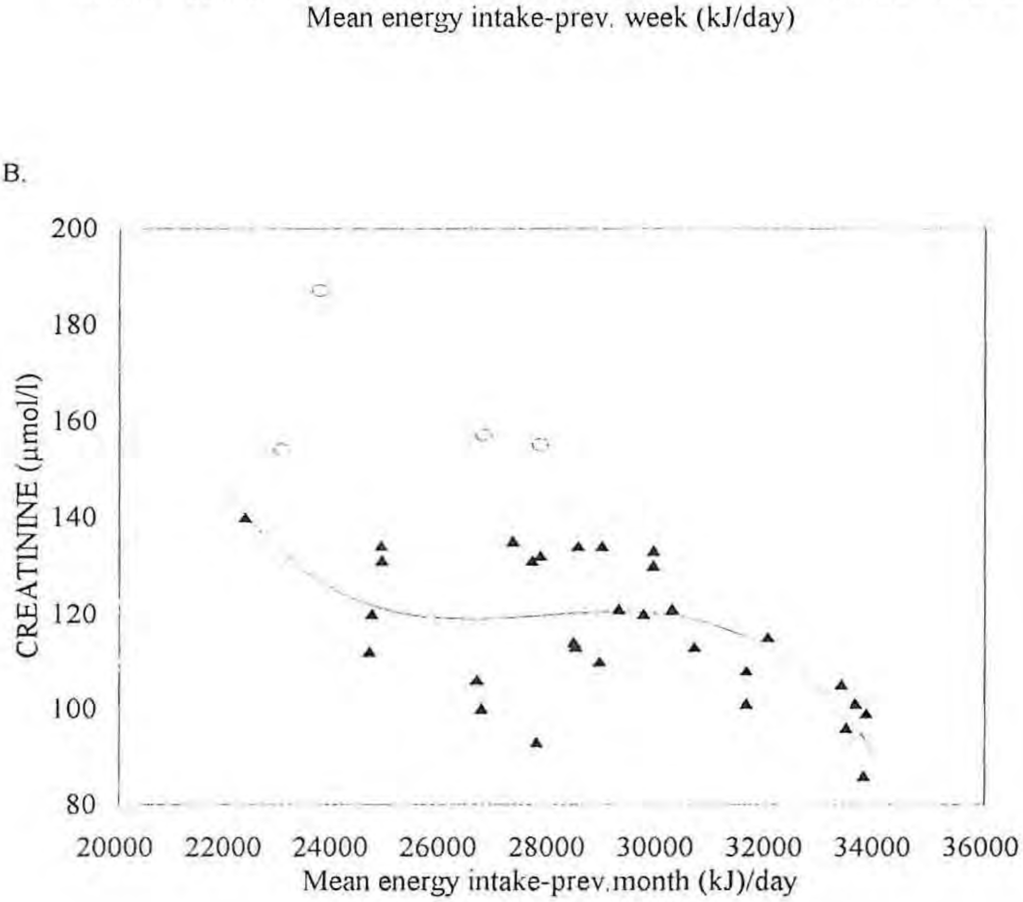


Figure 4.6: Curves of best fit ( ) for Domino with significant correlations ($p<0.05$) between serum creatinine and the mean daily energy intake of the week (A) and month (B) prior to the blood sample. Included in the graphs are all "normal" (see text) data ( ) and statistically determined outliers on the y-axis ( ).

levels corresponding to higher levels of energy intake were generally slightly lower (Fig. 4.6B). Outlying high creatinine levels were all associated with energy intake levels below 28 000 kJ/day.

Seasonal changes in creatinine levels were evident only for Simo (Table 4.2). Data reflected that creatinine levels were slightly reduced in the winter months (June-August). Outlying high creatinine concentrations were usually measured during the summer and autumn months (Fig. 4.7). Creatinine levels were positively correlated with water temperature for Dimple, Simo and Domino (Table 4.2). Dimple and Simo's data reflected an increase in creatinine concentration with increased water temperature, although outlying high creatinine levels were measured throughout the temperature range (Fig. 4.8). Domino's creatinine levels were relatively stable over a wide range of water temperatures, although levels appeared slightly reduced (~80-100 μmol/l) at water temperatures below 16°C (Fig. 4.9). Outlying high creatinine levels for this animal all corresponded with water temperatures below 21°C.

b. **Blood Urea Nitrogen (BUN) / BUN:creatinine**

For all animals, mean levels of BUN for the study were slightly higher than those recorded prior to 1992 (Fig. 4.10). BUN levels for Dimple, Domino and Dolly (except for a single marked increase) remained relatively stable throughout the study. Changes in the BUN:creatinine ratio generally paralleled these trends, although fluctuations were less severe (Fig. 4.11). A dramatic increase in the BUN:creatinine ratio for Simo was not evident in the same animal's BUN data (Fig. 4.10D & 4.11D). Increased variability of these parameters could usually be associated with periods of illness and/or treatment (Fig. 4.10 & 4.11). Outlying high BUN levels were identified in data from Dolly, Thandi and Simo, with outlying low BUN levels only measured in Dimple and Simo (Appendix 3.1B). The range of BUN:creatinine values recorded from all five captive dolphins was similar. Outlying high ratio values were recorded from all five animals but only Dolly's data contained outlying low BUN:creatinine values (Appendix 3.1C).

All animals had significantly different "normal" mean BUN levels (Two sample t-tests, $p<0.05$). However, when significance levels were adjusted (Bonferroni correction, $p=0.005$), mean BUN levels for Thandi (11.06 ± 1.55 mmol/l) and Simo (10.45 ± 1.12 mmol/l) ($t=2.45$; $p=0.02$; 136); and for Dolly and Domino ($t=2.65$; $p=0.01$; $df=79$) were similar (Table 4.1). Thandi and Simo had significantly higher mean BUN values than the other three animals. Those of Dolly (9.21 ± 0.90 mmol/l) and Domino (8.76 ± 0.56 mmol/l) were reduced, with the mean BUN level of Dimple (7.41 ± 0.75 mmol/l) being the lowest (Table 4.1). Two pairs of animals displayed identical mean BUN:creatinine ratios: Dimple and Domino (0.07 ± 0.01) and Thandi and Simo (0.10 ± 0.03). Dolly's BUN:creatinine ratio (0.09 ± 0.01) was not significantly different from that of Simo ($t=-2.46$; $p=0.02$; $df=90$) and Thandi ($t=0.29$; $p=0.78$; $df=132$) (Table 4.1).

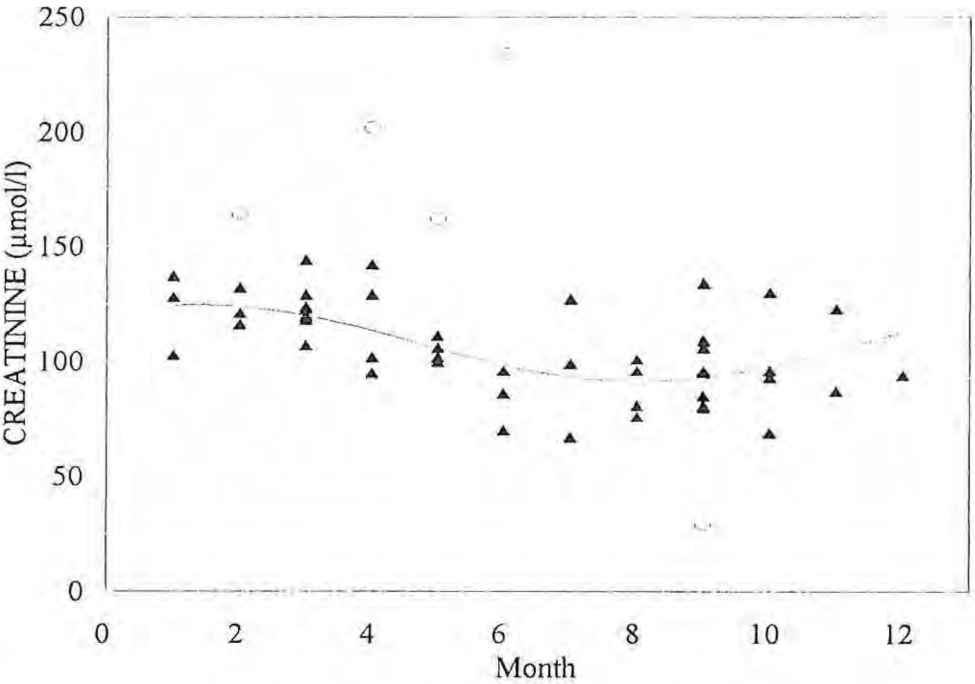


Figure 4.7: Curve of best fit ( ) for Simo with a significant correlation ($p<0.05$) between creatinine and the month of the year. Included in the graph are all "normal" (see text) data ( ) and statistically determined outliers on the y-axis ( ).

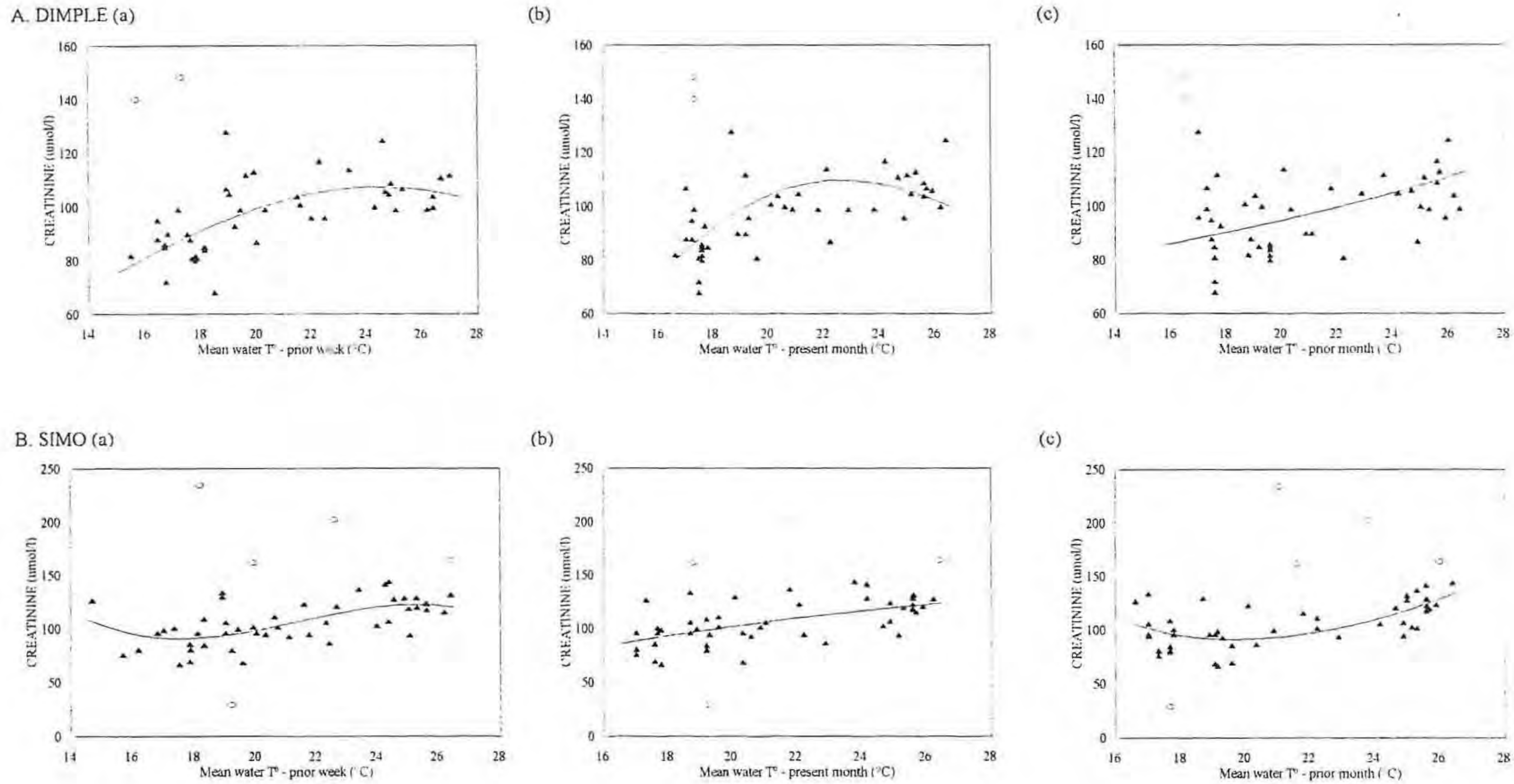


Figure 4.8: Curves of best fit (   ) for animals (A. Dimple; B. Simo) with significant correlations ($p<0.05$) between creatinine and the mean water temperature of the week prior to the blood sample (a); the month in which blood was sampled (b) and the month prior to the blood sample (c). Included in the graphs are all "normal" (see text) data (   ) and statistically determined outliers on the y-axis (   ).

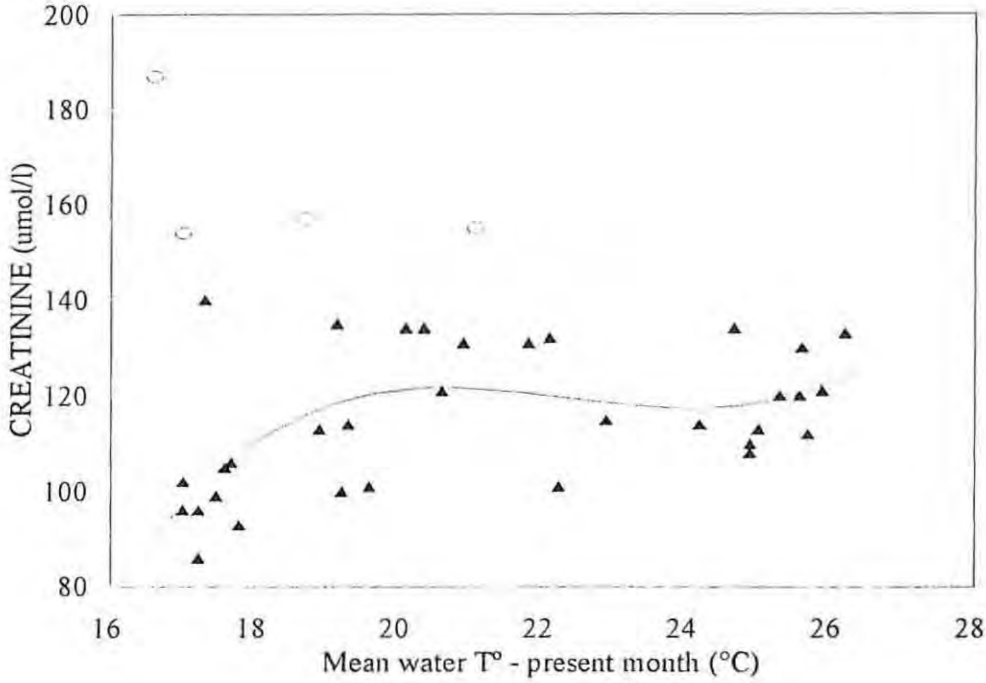


Figure 4.9: Curve of best fit ( ) for Domino with a significant correlation ($p<0.05$) between creatinine and the mean water temperature of the month in which blood was sampled. Included in the graph are all "normal" (see text) data ( ) and statistically determined outliers on the y-axis ( ).

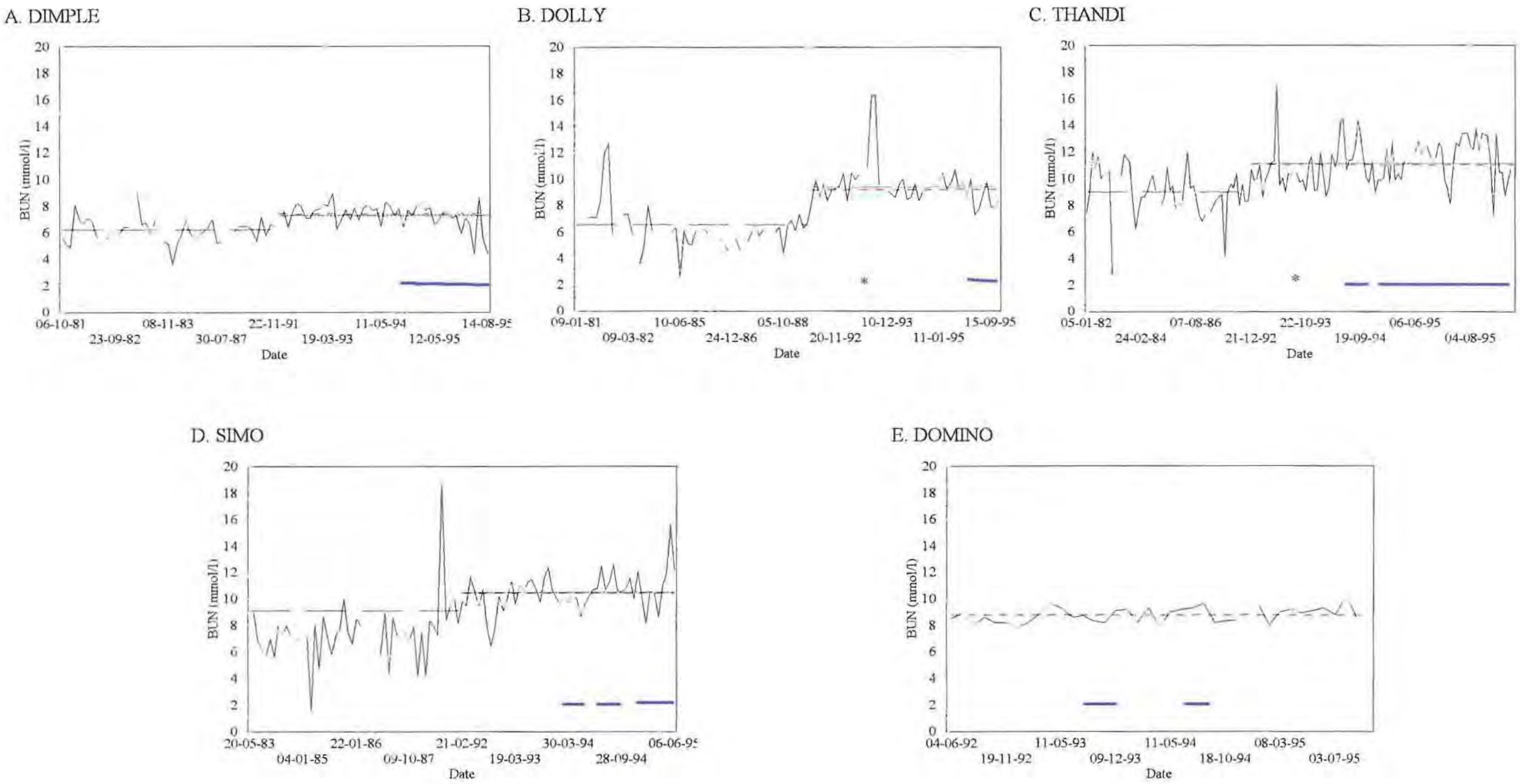


Figure 4.10: Levels of blood urea nitrogen (BUN) (mmol/l) (—) for all five captive animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the overall mean BUN level for all records prior to the study ( ); the overall mean BUN level (—) and the "normal" (see text) mean BUN level (- - -) for the study between 1992 and 1995 (*: parturition; —: extended period of illness and/or treatment or period of aberrant feeding and/or behaviour).

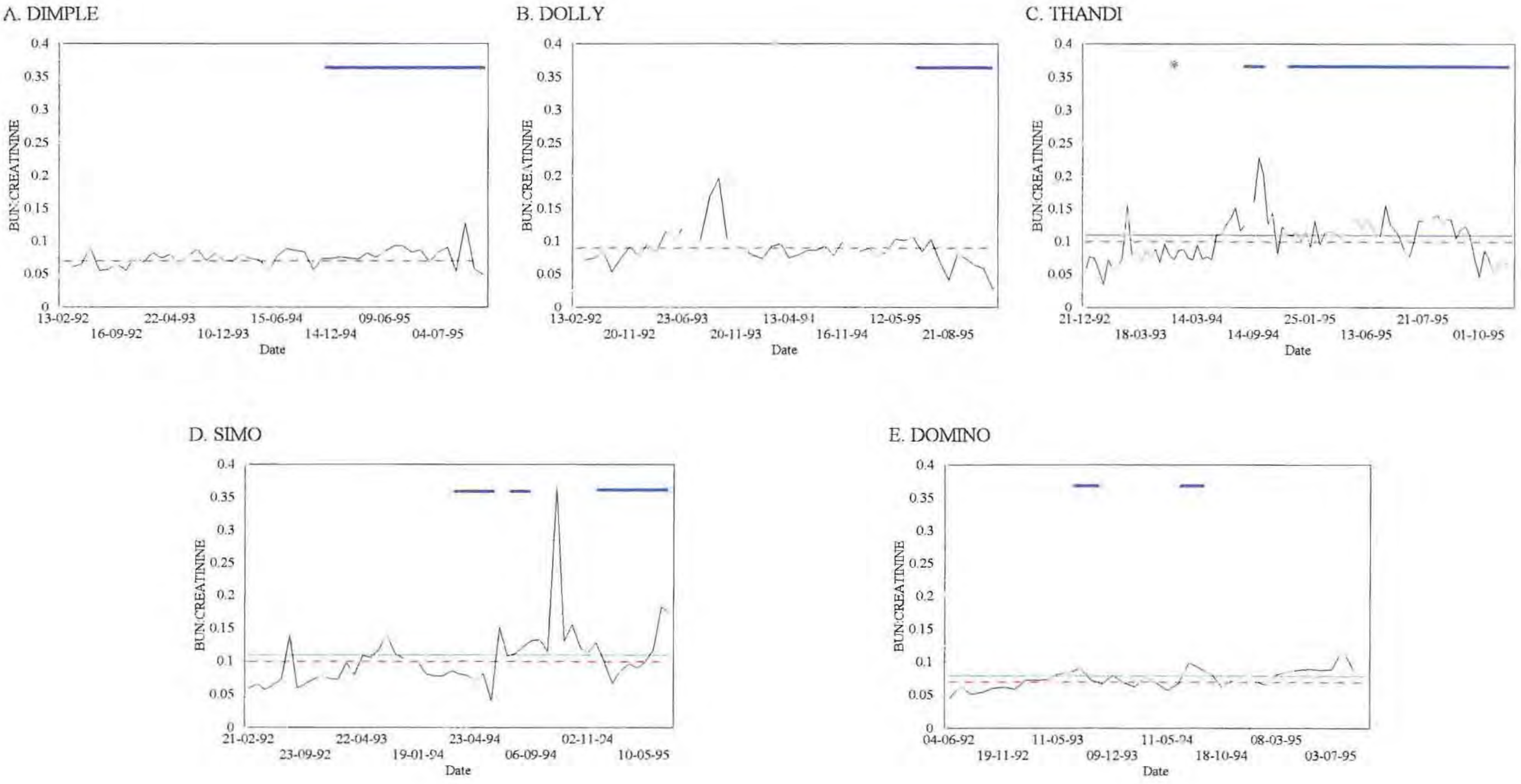


Figure 4.11: BUN:creatinine ratios (—) for all five captive animals (A. Dimple; B. Dolly; C. Thandi, D. Simo; E. Domino). Also shown, for each animal, is the overall mean ratio (—) and the "normal" (see text) mean BUN:creatinine ratio (- - -) for the study between 1992 and 1995 (*: parturition; —— : extended period of illness and/or treatment or period of aberrant feeding and/or behaviour).

Overall, BUN and BUN:creatinine did not appear to be directly related to age. Data from all five animals overlapped considerably, although BUN was lower in Dimple. This reduction was not evident for the BUN:creatinine ratio (Fig. 4.12). During the study, BUN was positively correlated with age only for Domino (Table 4.3; Fig. 4.13A). BUN:creatinine was positively related to age for both Simo and Domino (Table 4.3). This increase was only slight in the case of Simo (Fig. 4.13B), but relatively marked in the case of Domino (Fig. 4.13C). The BUN:creatinine values for Domino increased, particularly between the ages of one and two years, after which variability increased and the rate of increase was reduced.

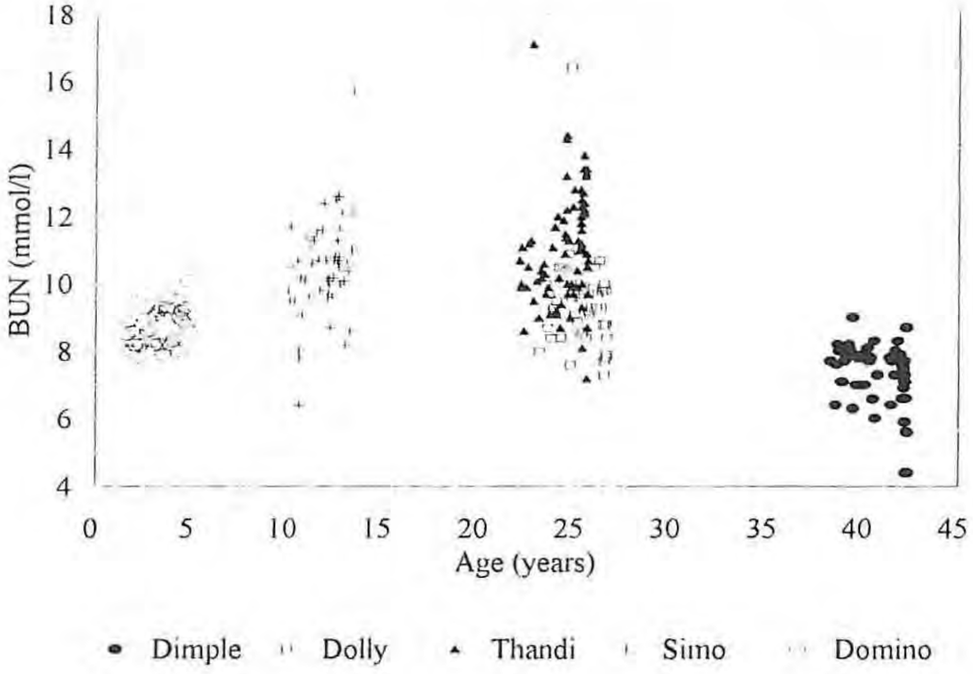
Table 4.3: Correlation coefficients (r), levels of significance (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$) and sample sizes (n) for significant relationships between blood urea nitrogen (BUN) (a) and BUN:creatinine (b) and independent variables for the five captive dolphins (Data are presented as r*(n)) (NS: non-significant ($p>0.05$)).

Variable		Dimple	Dolly	Thandi	Simo	Domino
Age	a	NS	NS	NS	NS	0.37*(37)
	b	NS	NS	NS	0.51***(51)	0.63***(35)
Weight	a	NS	NS	NS	NS	0.39*(35)
	b	NS	NS	NS	NS	0.42**(33)
Blubber thickness	b	NS	NS	NS	NS	0.65*(12)
D1	a	NS	NS	0.57***(68)	NS	0.39*(34)
	b	NS	0.45**(36)	0.64***(64)	NS	0.66***(32)
E1	a	NS	0.51***(38)	0.43***(71)	NS	0.46**(34)
	b	NS	0.41**(37)	0.52***(67)	NS	0.65***(32)
E2	a	NS	0.53***(37)	0.48***(68)	NS	0.39*(34)
	b	NS	0.35*(35)	0.47***(66)	NS	0.70***(32)
E3	a	0.34*(40)	0.47**(39)	0.32**(72)	NS	0.40*(33)
	b	NS	0.36*(37)	0.48***(68)	NS	0.61***(32)
E4	a	NS	NS	NS	NS	0.35*(34)
	b	NS	NS	NS	NS	0.67***(32)
Month	a	-0.29*(45)	NS	NS	NS	NS
	b	NS	NS	NS	0.39**(51)	NS
T1	a	0.41**(45)	NS	-0.32**(85)	NS	NS
	b	NS	NS	-0.26*(81)	-0.46***(51)	NS
T2	a	0.40**(45)	NS	-0.36***(84)	NS	NS
	b	NS	NS	-0.28*(80)	-0.50***(50)	NS
T3	a	0.36*(45)	NS	-0.34**(85)	NS	NS
	b	NS	NS	-0.28**(81)	-0.45***(51)	NS

D1: Total food consumption of week prior to blood sample; E1: Dietary energy intake of day prior to blood sample; E2-E4: Mean daily energy intake of week in which blood was sampled, and week and month prior to blood sample, respectively, T1: Mean water temperature of the week prior to blood sample, T2: Mean water temperature of month in which blood was sampled, T3: Mean water temperature of month prior to blood sample.

Domino was the only animal for which weight was positively related with both BUN and BUN:creatinine (Table 4.3). Although, highly variable data made these relationships difficult to

A.



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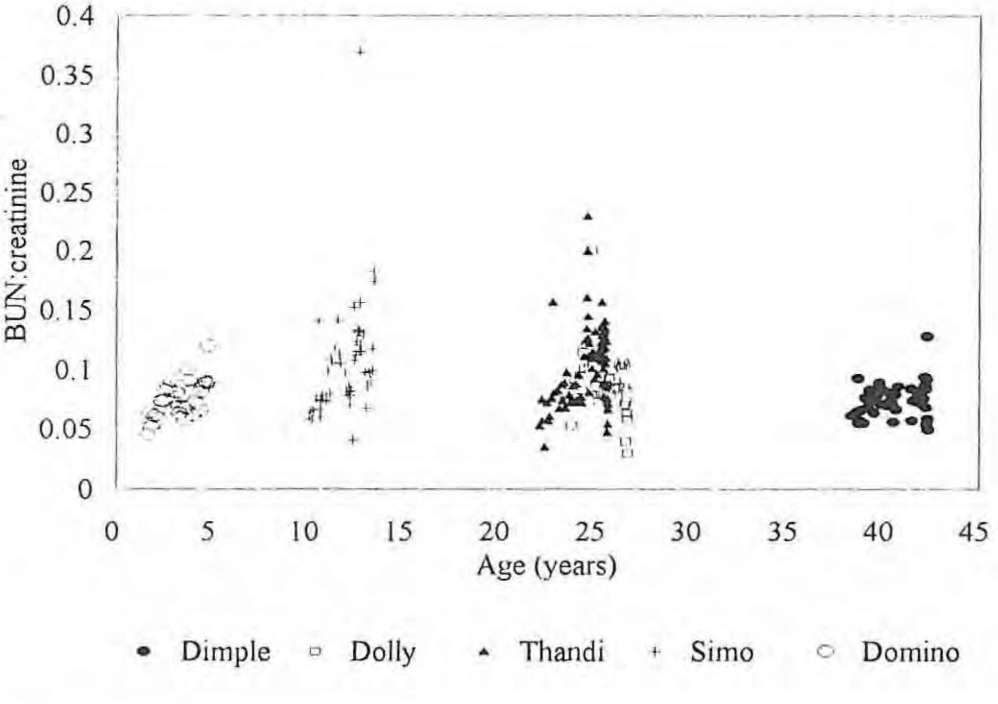
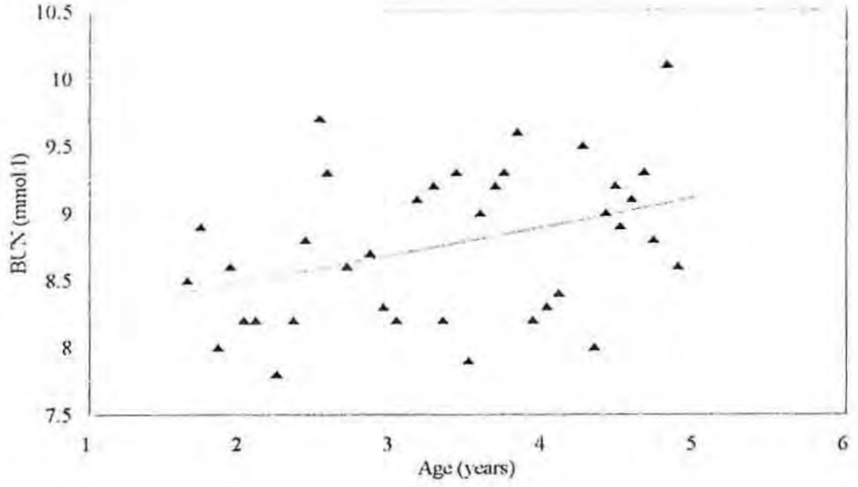
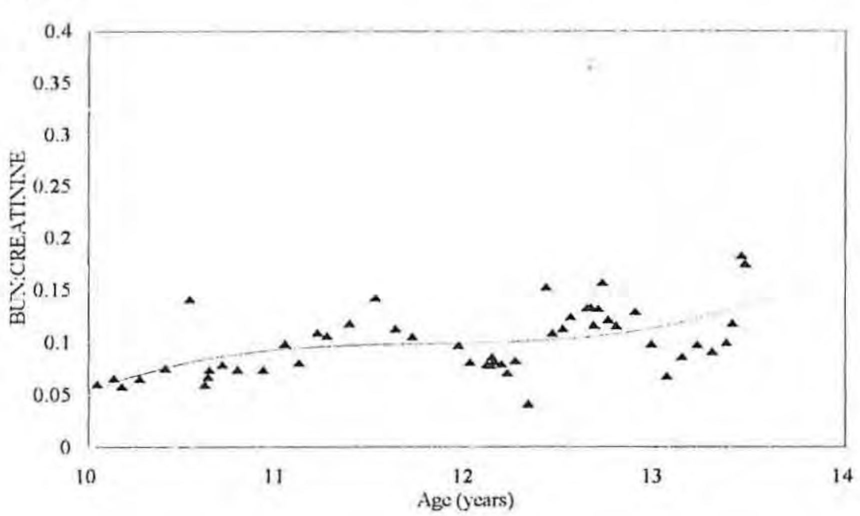


Figure 4.12: Relationship between blood urea nitrogen (BUN) (A) and the BUN:creatinine rat (B) and age for the five captive dolphins, from records obtained between 1992 and 1995.

A. DOMINO



B. SIMO



C. DOMINO

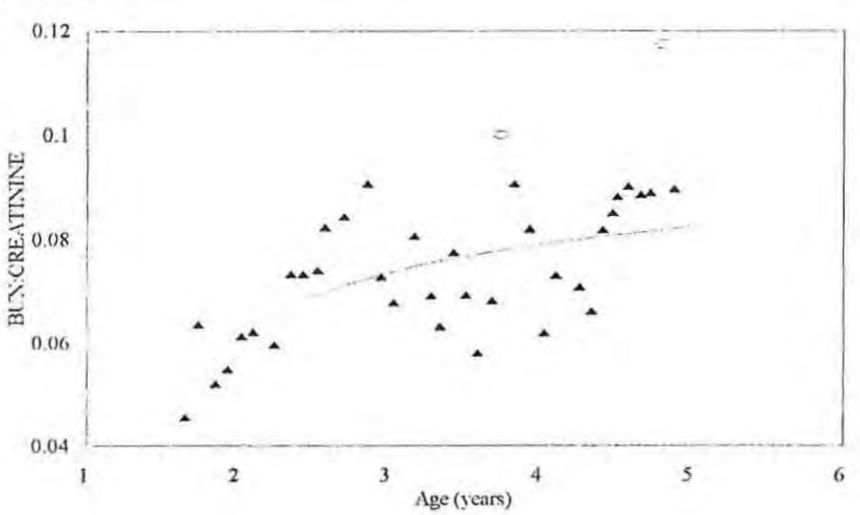


Figure 4.13: Curves of best fit ( ) for animals (A&C. Domino; B. Simo) with significant correlations ($p<0.05$) between blood urea nitrogen (BUN) (A) and the BUN:creatinine ratio (B&C) and age. Included in the graphs are all "normal" (see text) data ( ) and statistically determine outliers on the y-axis ( ).

verify, levels of BUN and BUN:creatinine were predominantly higher when total body weight was increased, particularly above 145 kg (Fig. 4.14A & B). Outlying high BUN:creatinine values were associated with weights greater than 140 kg (Fig. 4.14B). In addition, Domino's BUN:creatinine was positively correlated with blubber thickness (Table 4.3). Data were limited, but this trend was similar to that described between this ratio and total body weight (Fig. 4.14C).

Dolly, Thandi and Domino showed positive correlations between BUN, BUN:creatinine and total food consumption during the week prior to the blood sample (Table 4.3). Although data and respective trends differed between animals, BUN:creatinine increased with increased food consumption (Fig. 4.15). Thandi's BUN:creatinine values appeared relatively stable over a wide range of food consumption levels, but the ratio was reduced at food consumption levels below 55 kg/week (Fig. 4.15C). Overall, outlying BUN and BUN:creatinine data were not associated with aberrant levels of food consumption.

Although the nature of the relationships differed, BUN and BUN:creatinine from Dolly, Thandi and Domino was positively correlated with the dietary energy intake of the day prior to the blood sample (Table 4.3; Fig. 4.16 & 4.17). Highly variable BUN:creatinine values from Dolly made any significant trends difficult to identify (Fig. 4.17A). Data from both Thandi and Domino illustrated that the BUN:creatinine ratio was significantly higher when associated with greater short-term energy intake (Fig. 4.17B & C). Outliers identified in Dolly's BUN and BUN:creatinine data were related to periods of reduced energy intake (Fig. 4.16A).

Blood urea nitrogen was almost identically related to the mean daily energy intake of the week in which blood was sampled as for the previous dietary energy category. In addition, BUN:creatinine from the same three animals was similarly positively correlated with this particular diet parameter (Table 4.3). The BUN:creatinine ratio of both Dolly and Thandi remained relatively stable, as energy intake increased. In contrast, Domino's BUN:creatinine ratio increased steadily as energy intake increased (Fig. 4.18). Although outliers could be associated with significantly higher energy intake, in the case of Thandi, this was not consistent for the other two animals.

Although data varied between animals, BUN from Dimple, Dolly, Thandi and Domino was positively correlated with the mean daily energy intake of week prior to the blood sample (Table 4.3; Fig. 4.19). Outlying low BUN levels from Dimple were associated with significantly reduced energy intake during the prior week (Fig. 4.19A). Data from Dolly, Thandi and Domino also illustrated that BUN:creatinine was positively related to this diet category (Table 4.3). Although these trends were slightly more complex than those described for BUN, higher ratio values were recorded more frequently at increased levels of energy intake. When related to energy intake of the month preceding the blood sample, only Domino's BUN and BUN:creatinine were positively

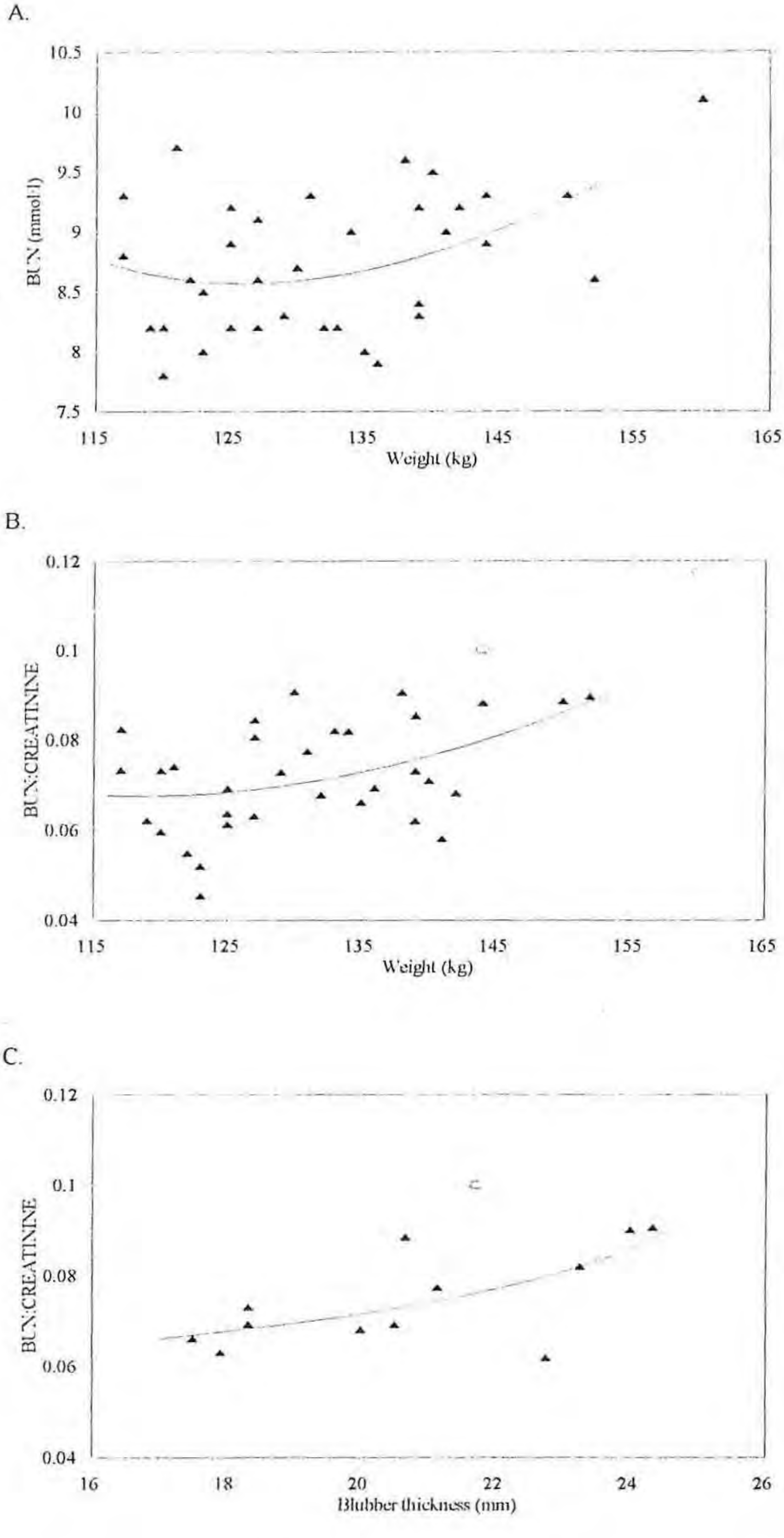


Figure 4.14: Curves of best fit ( ) for Domino with significant correlations ($p<0.05$) between blood urea nitrogen (BUN) (A), BUN: creatinine (B) and total body weight and between BUN:creatinine and blubber thickness (C). Included in the graphs are all "normal" (see text) data ( ) and statistically determined outliers on the y-axis ( ).

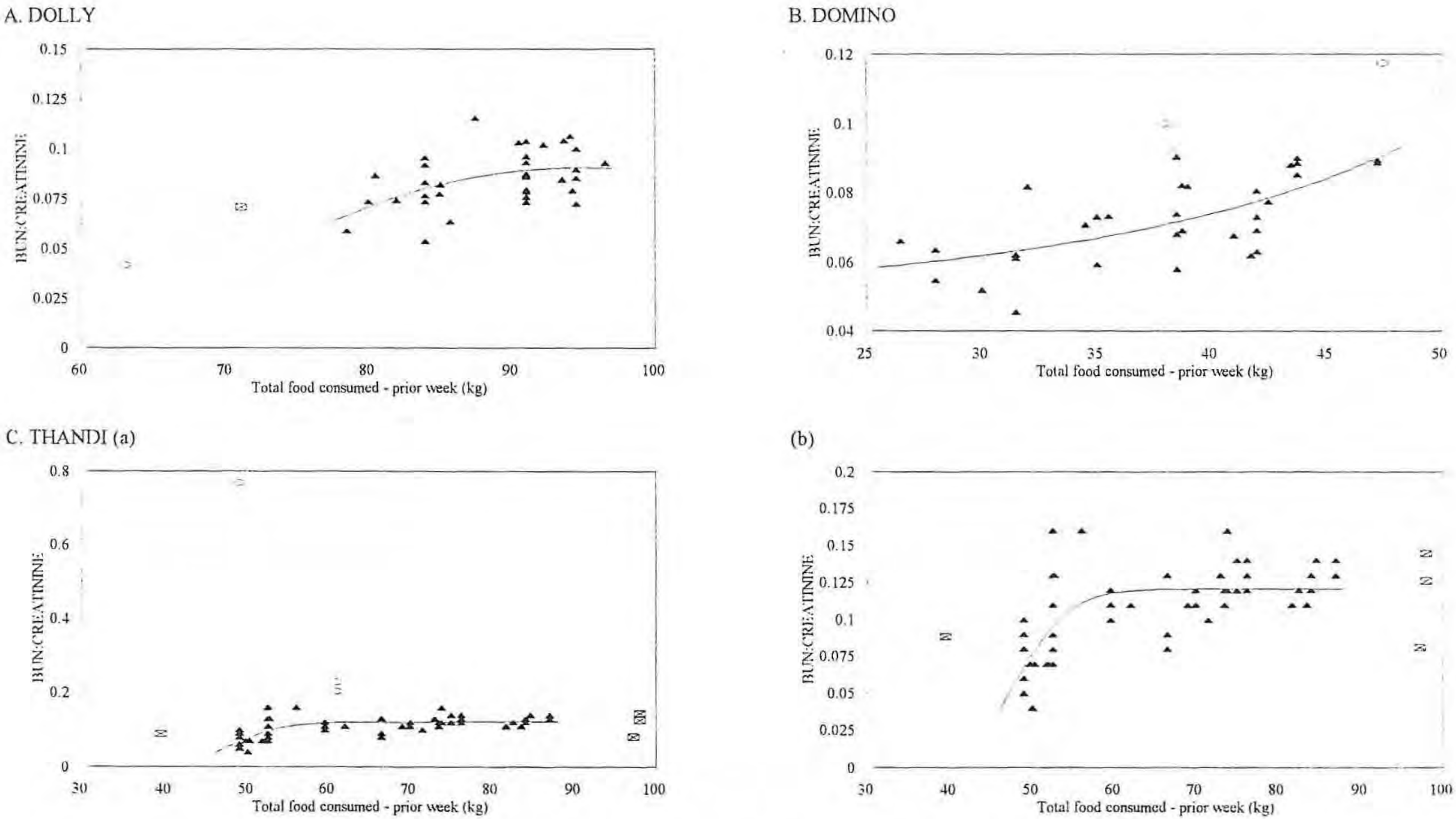
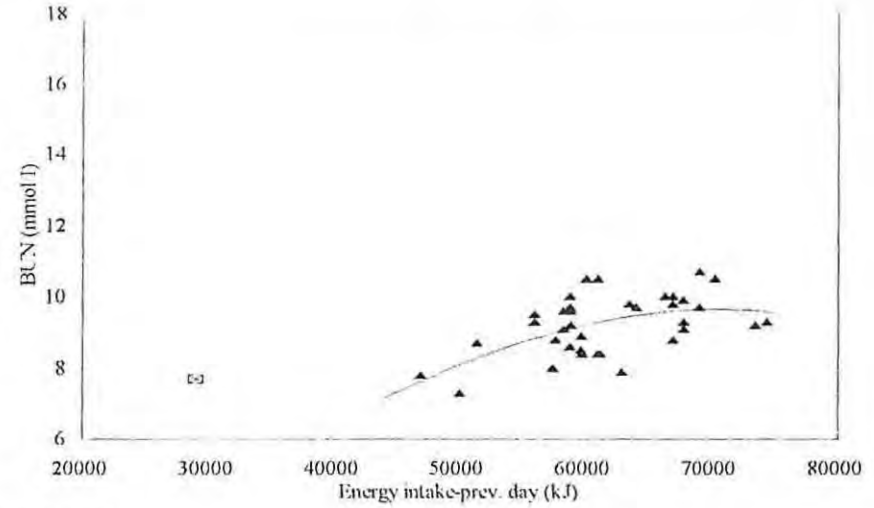
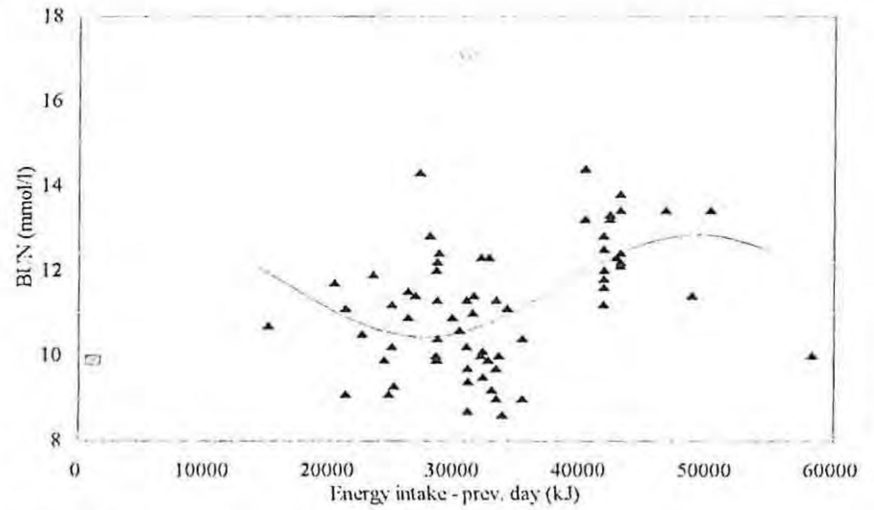


Figure 4.15: Curves of best fit ( ) for animals (A. Dolly; B. Domino; C. Thandi (a & b)) with significant correlations ($p<0.05$) between BUN:creatinine and total food consumed in the week prior to the blood sample. Included in the graphs are all "normal" (see text) data ( ) and statistically determined outliers (x-axis: ; y-axis:).

A. DOLLY



B. THANDI



C. DOMINO

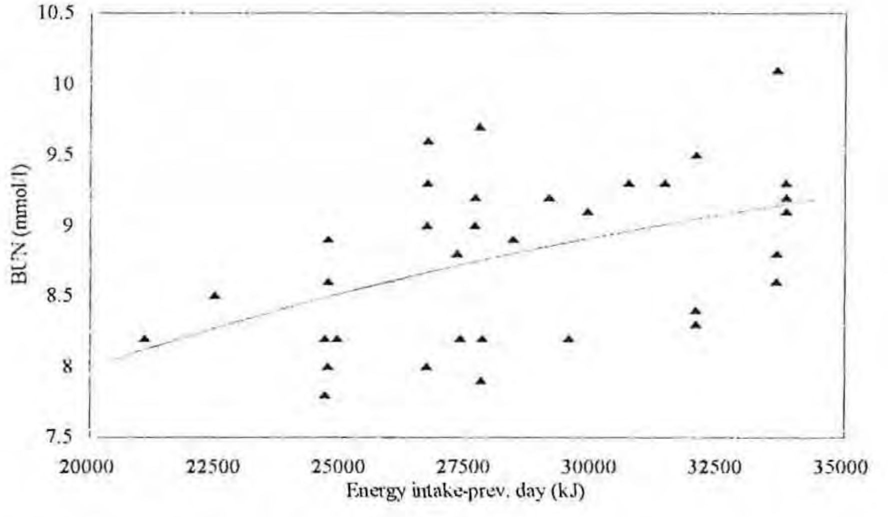
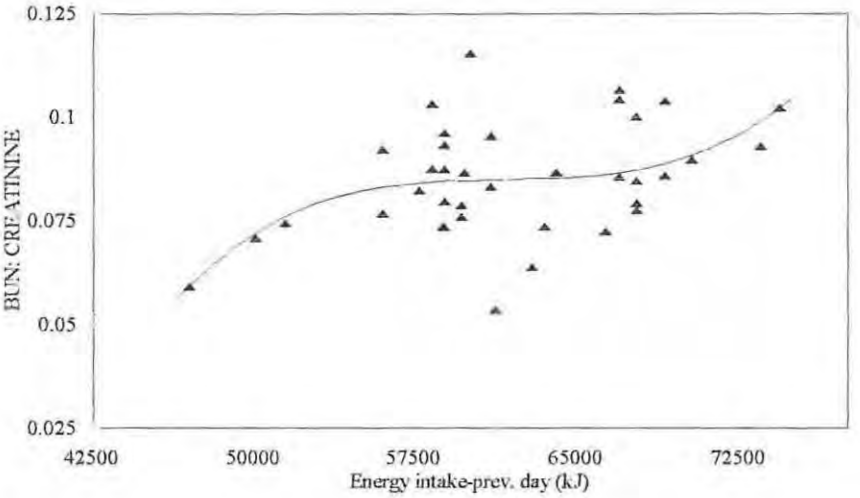
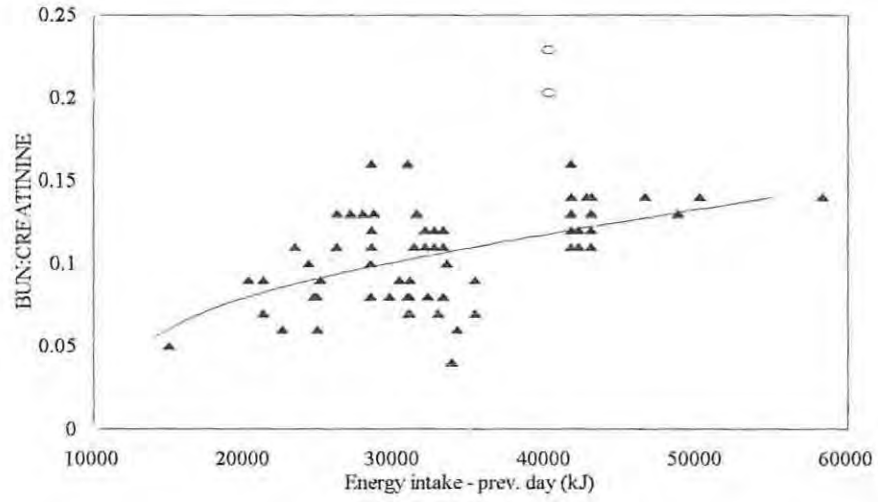


Figure 4.16: Curves of best fit ( ) for animals (A. Dolly; B. Thandi; C. Domino) with significant correlations ($p<0.05$) between blood urea nitrogen (BUN) and the dietary energy intake of the day prior to the blood sample. Included in the graphs are all "normal" (see text) data ( ) and statistically determined outliers (x-axis: ; y-axis:).

A. DOLLY



B THANDI



C. DOMINO

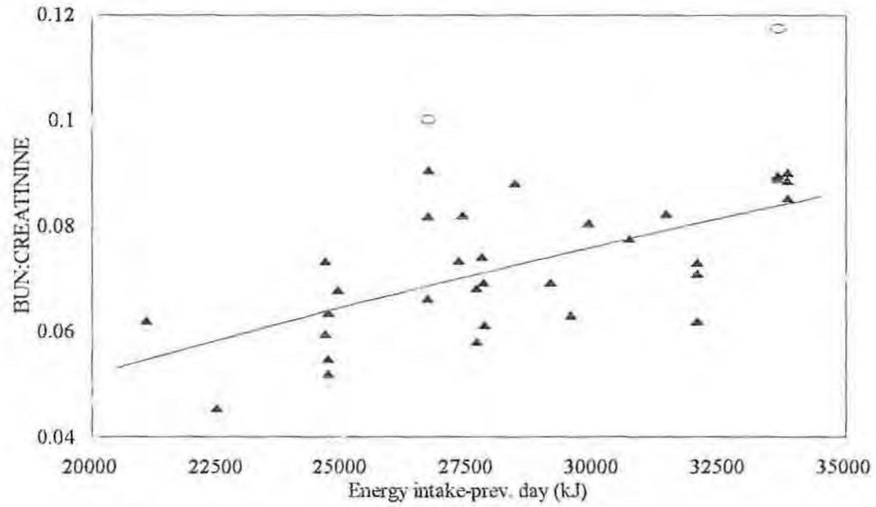


Figure 4.17: Curves of best fit ( ) for animals (A. Dolly; B. Thandi; C. Domino) with significant correlations ($p<0.05$) between BUN:creatinine and the dietary energy intake of the day prior to the blood sample. Included in the graphs are all "normal" (see text) data ( ) and statistically determined outliers on the y-axis ( ).

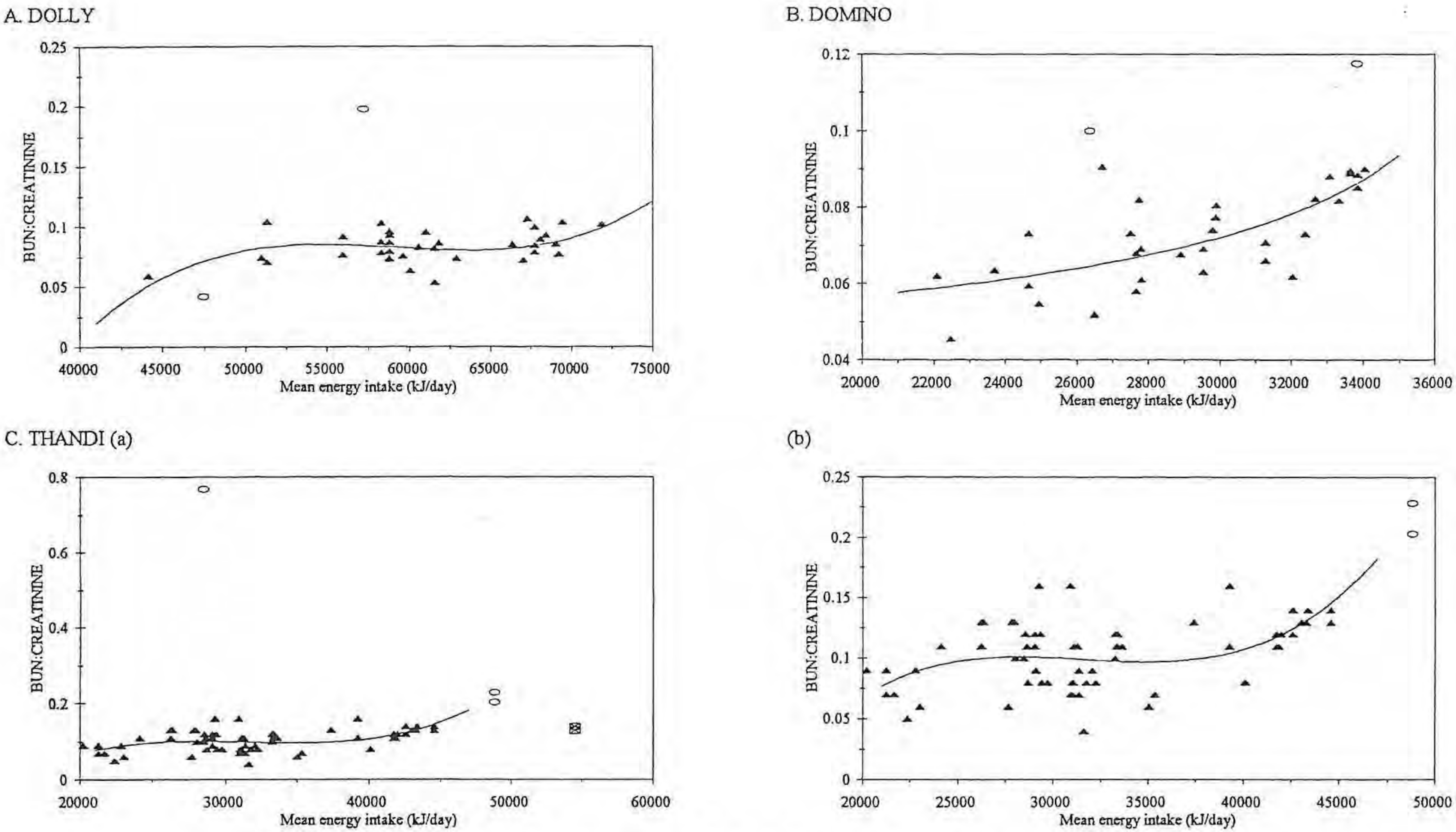


Figure 4.18: Curves of best fit (—) for animals (A. Dolly; B. Domino; C. Thandi (a & b)) with significant correlations ($p<0.05$) between BUN:creatinine and the mean daily dietary energy intake of the week in which blood was sampled. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ⊠ ; y-axis: ○).

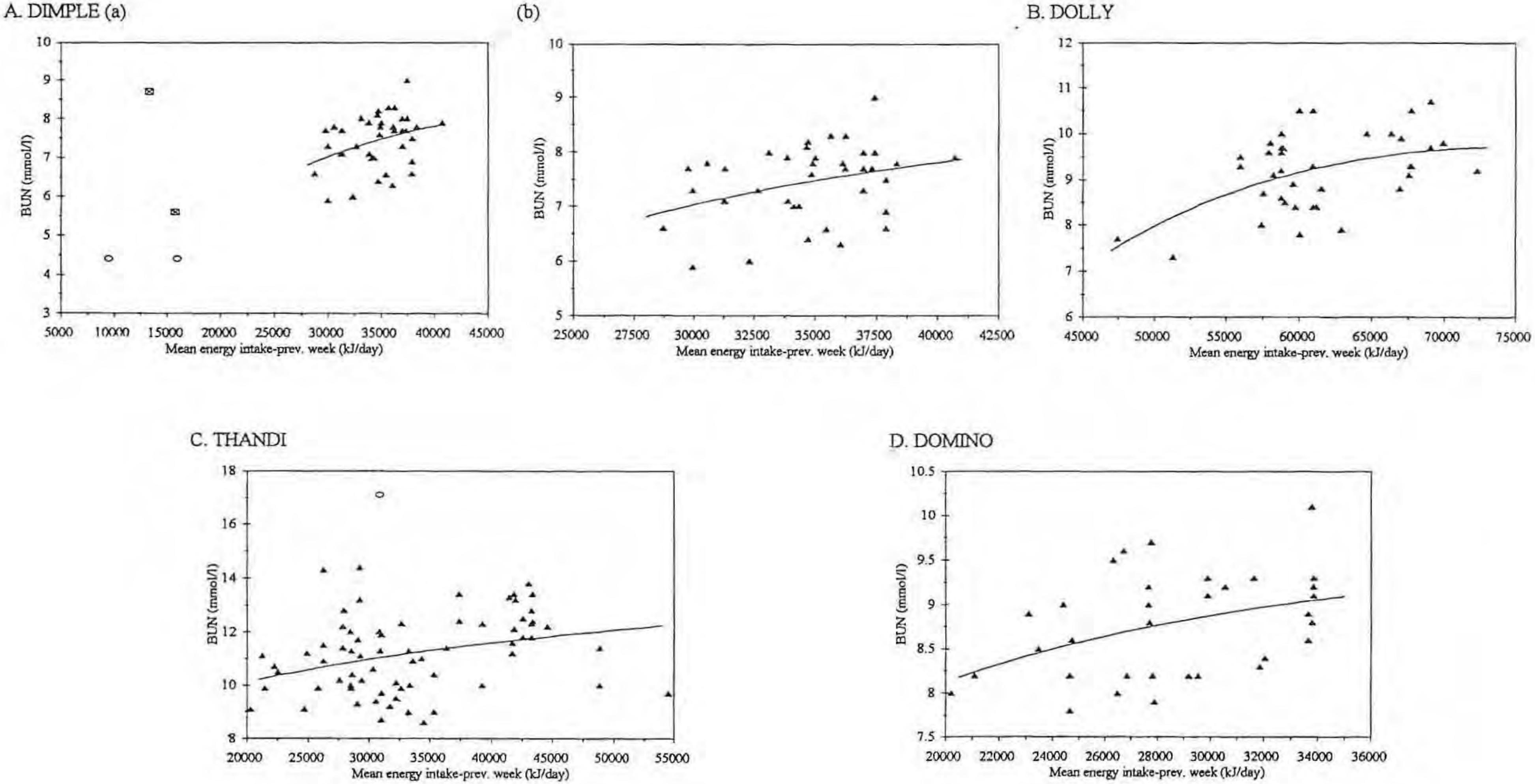


Figure 4.19: Curves of best fit (—) for animals (A. Dimple (a & b); B. Dolly; C. Thandi; D. Domino) with significant correlations ($p<0.05$) between blood urea nitrogen (BUN) and the mean daily energy intake of the week prior to the blood sample. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

correlated (Table 4.3). The trends were similar to other dietary relationships with these blood chemistry parameters.

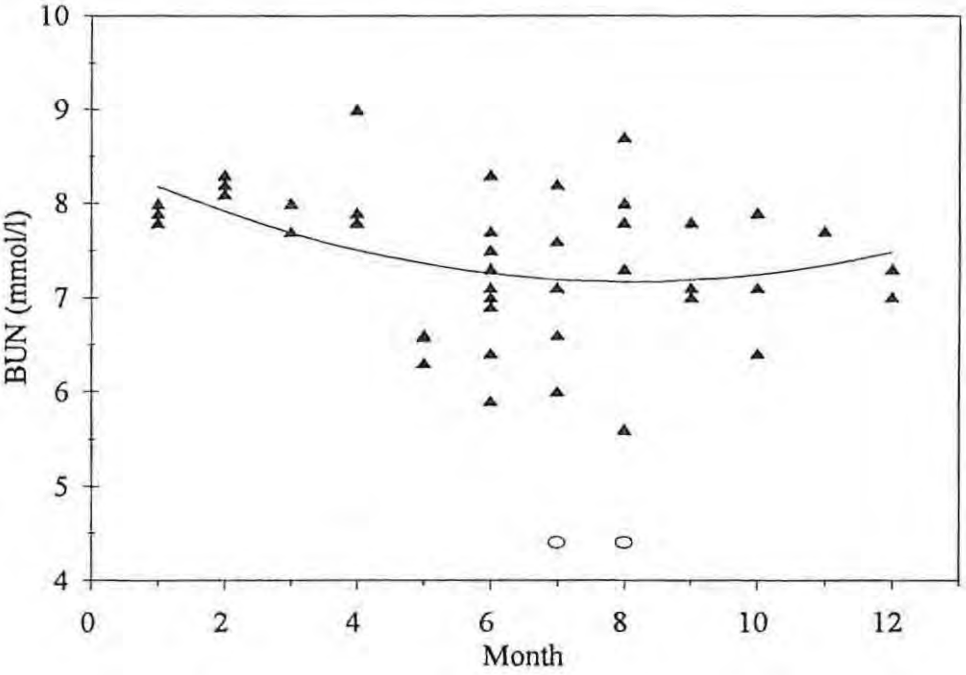
Dimple was the only animal for which BUN was significantly correlated with the month of the year (Table 4.3). Overall BUN levels appeared relatively stable throughout the year, but low BUN was measured only between May and August. In addition, variability was greatest during these months (Fig. 4.20A). Simo's BUN:creatinine ratio was positively related to the month of the year (Table 4.3). This relationship appeared to be the inverse of the trend described for BUN, with BUN:creatinine values being higher in the winter and spring months (Fig. 4.20B).

Dimple and Thandi were the only animals for which BUN was significantly correlated with water temperature (Table 4.3). Data from both animals varied considerably over a wide range of water temperatures. However, Dimple's BUN levels appeared to increase slightly with increased water temperature (Fig. 4.21A). Thandi's relationship was more complex and reflected no distinct trend. Data variability for this animal was highest at water temperatures below 21°C. Outliers were also associated with water temperatures below 21°C (Fig. 4.21). The variability of Thandi's data also made the relationship between BUN:creatinine and water temperature difficult to define (Table 4.3) (Fig. 4.22A). However, the same relationship for Simo was more distinct, reflecting a slight increase in ratio values at water temperatures below 21°C (Fig. 4.22B). Outlying high BUN:creatinine values were recorded only at low water temperatures.

c. Alkaline phosphotase (ALP) / ALP:creatinine

Levels of ALP, from all five animals, fluctuated considerably during the study (Fig. 4.23). Simo was the only animal for which the mean ALP levels calculated during the study were similar to that of prior records (Fig. 4.23D). Marked increases and decreases in ALP levels during the study could, in most cases, be associated with periods of illness and/or treatment (Fig. 4.23). Changes in the ALP:creatinine ratio during the study generally paralleled those of ALP (Fig. 4.24). "Normal" mean ALP levels were generally similar (Table 4.1). Domino was the only animal for which the mean ALP level (958.40 ± 280.20 U/l) was significantly higher than all other animals (Two sample t-tests, $p<0.001$). Dolly (433.00 ± 224.20 U/l) and Simo (304.40 ± 133.30 U/l) also had significantly different mean ALP levels ($t=3.30$; $p=0.001$; $df=87$) (Table 4.1). In this respect, the range of data from Dimple, Simo and Thandi was also similar, with that of Dolly and Domino increasing in variability (Appendix 3.2A). Outlying high ALP levels were recorded only from Thandi and Simo (Appendix 3.2A). Domino's "normal" mean ALP:creatinine ratio (8.16 ± 2.14) was significantly higher than all other animals (Two sample t-test; $p<0.001$). All other animals had similar mean values for this ratio (Two sample t-test, $p>0.005$) (Table 4.1). The respective ranges of ALP:creatinine values for the five captive dolphins were similar to those of

A. DIMPLE



B. SIMO

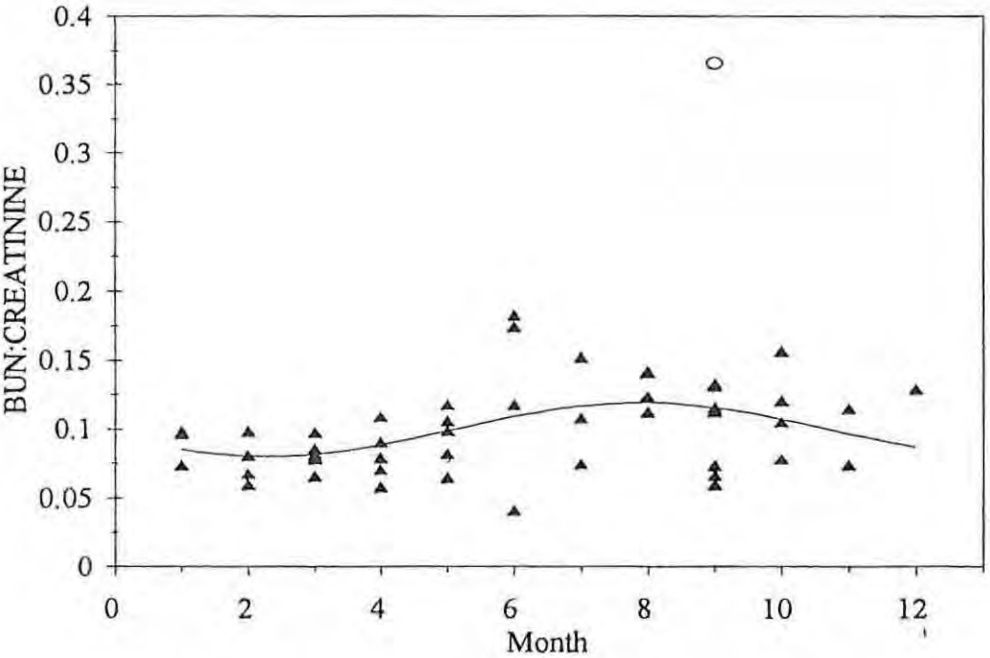


Figure 4.20: Curves of best fit (—) for animals (A. Dimple; B. Simo) with significant correlations ($p<0.05$) between blood urea nitrogen (BUN) (A) and BUN:creatinine (B) and the month of the year. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

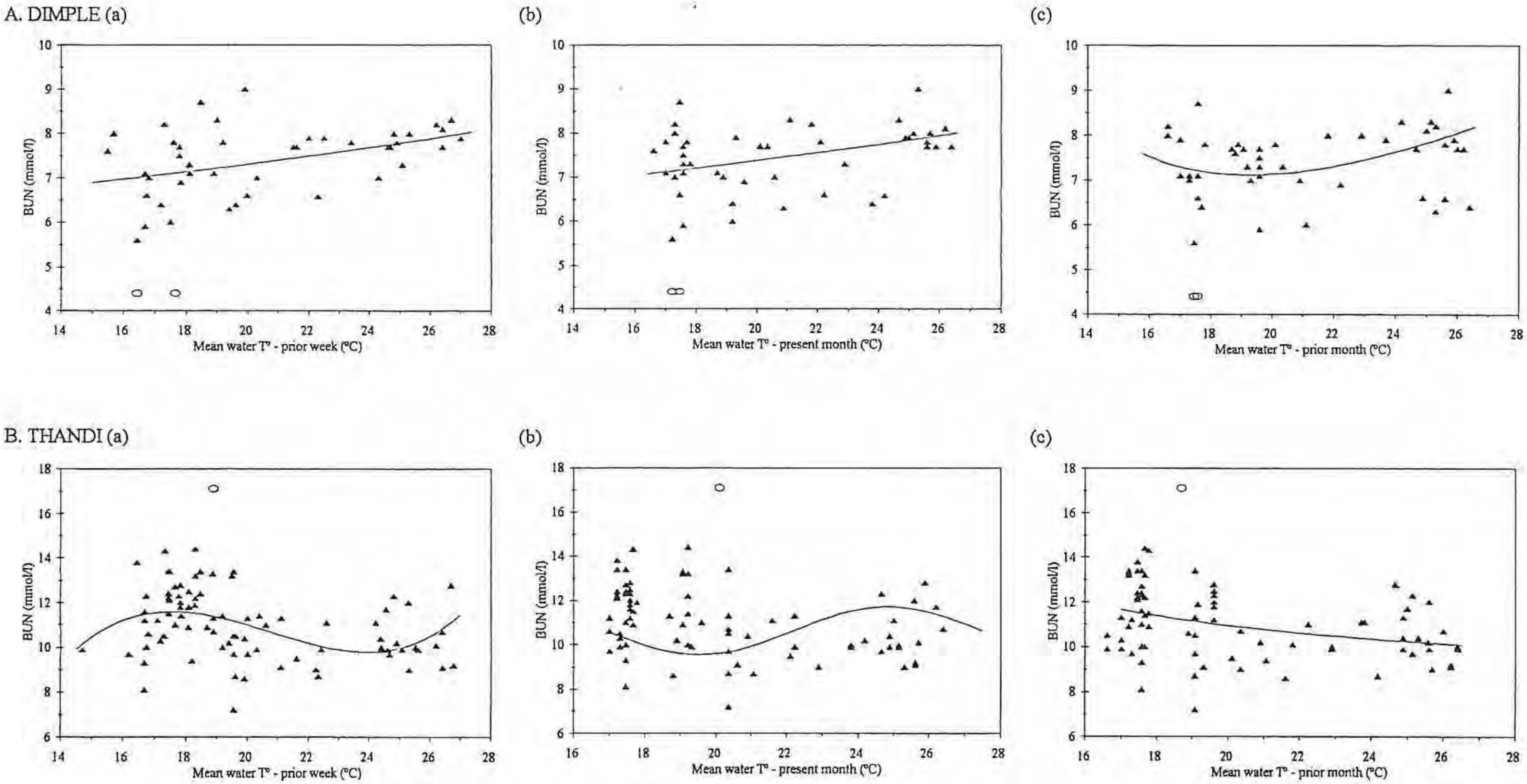


Figure 4.21: Curves of best fit (—) for animals (A. Dimple; B. Thandi) with significant correlations ($p<0.05$) between blood urea nitrogen (BUN) and the mean water temperature of the week prior to the blood sample (a), the mean water temperature of the month in which blood was sampled (b) and the mean water temperature of the month prior to the blood sample (c). Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

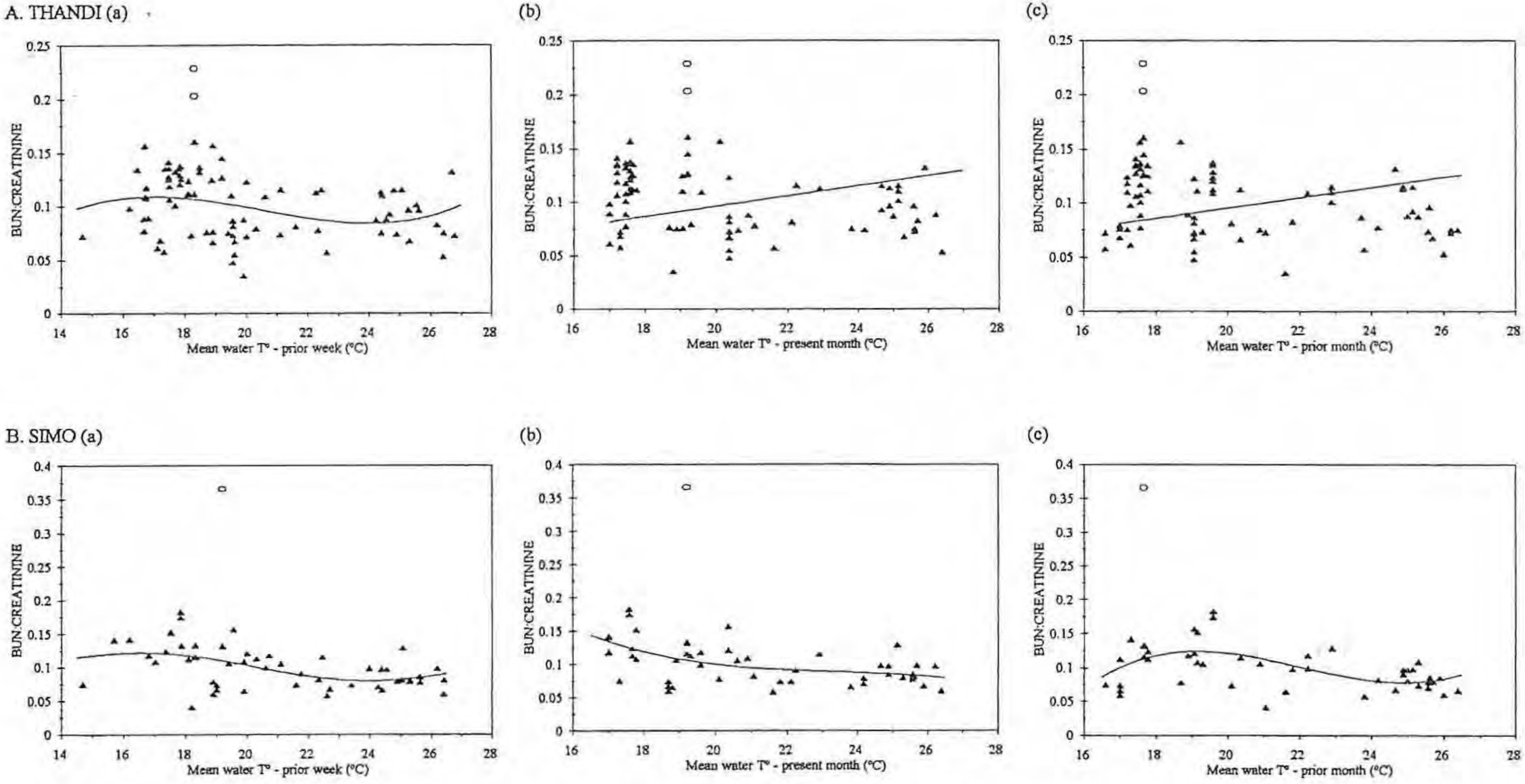


Figure 4.22: Curves of best fit (—) for animals (A. Thandi; B. Simo) with significant correlations ($p<0.05$) between BUN:creatinine and the mean water temperature of the week prior to the blood sample (a), the mean water temperature of the month in which blood was sampled (b) and the mean water temperature of the month prior to the blood sample (c). Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

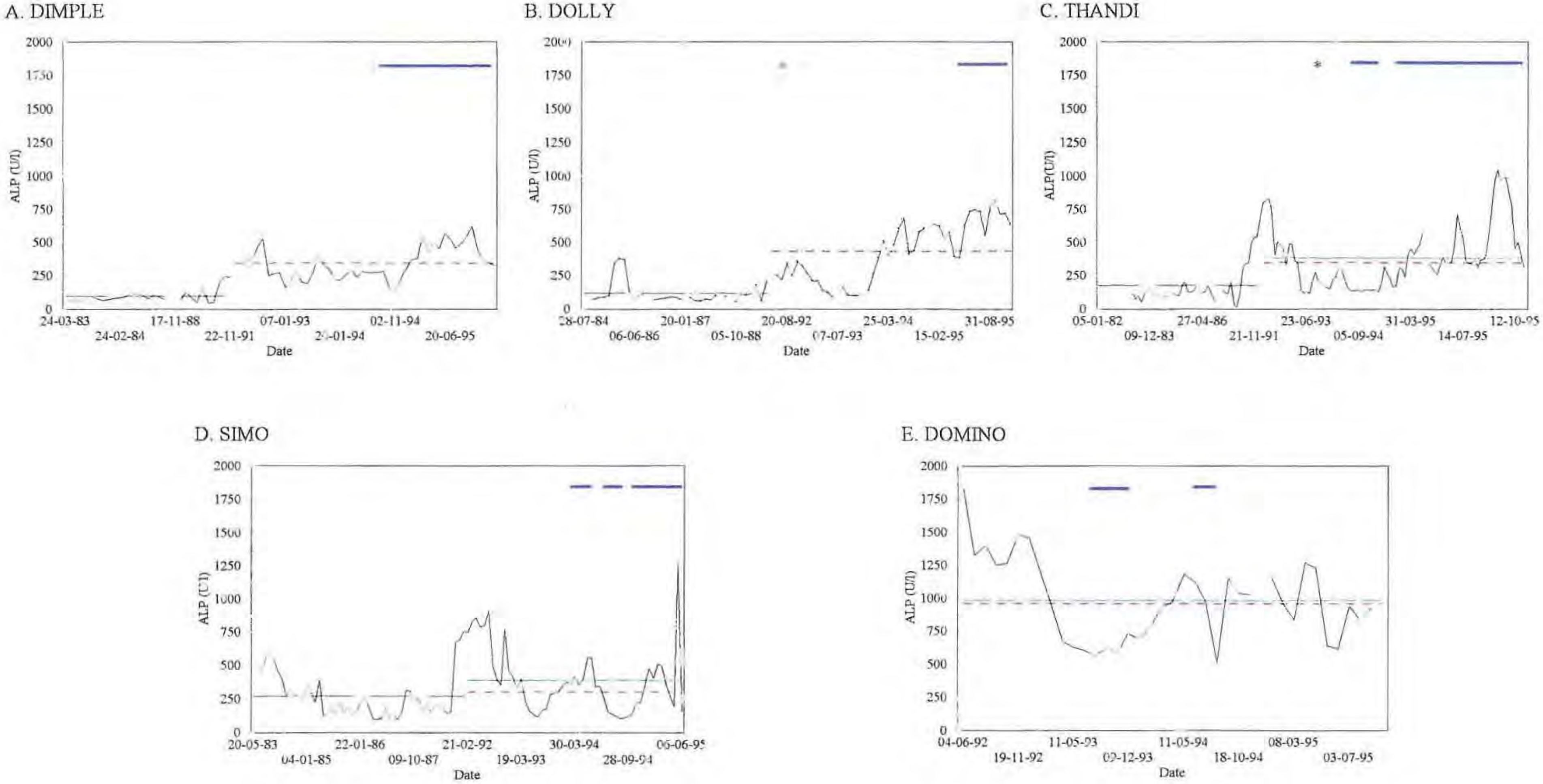
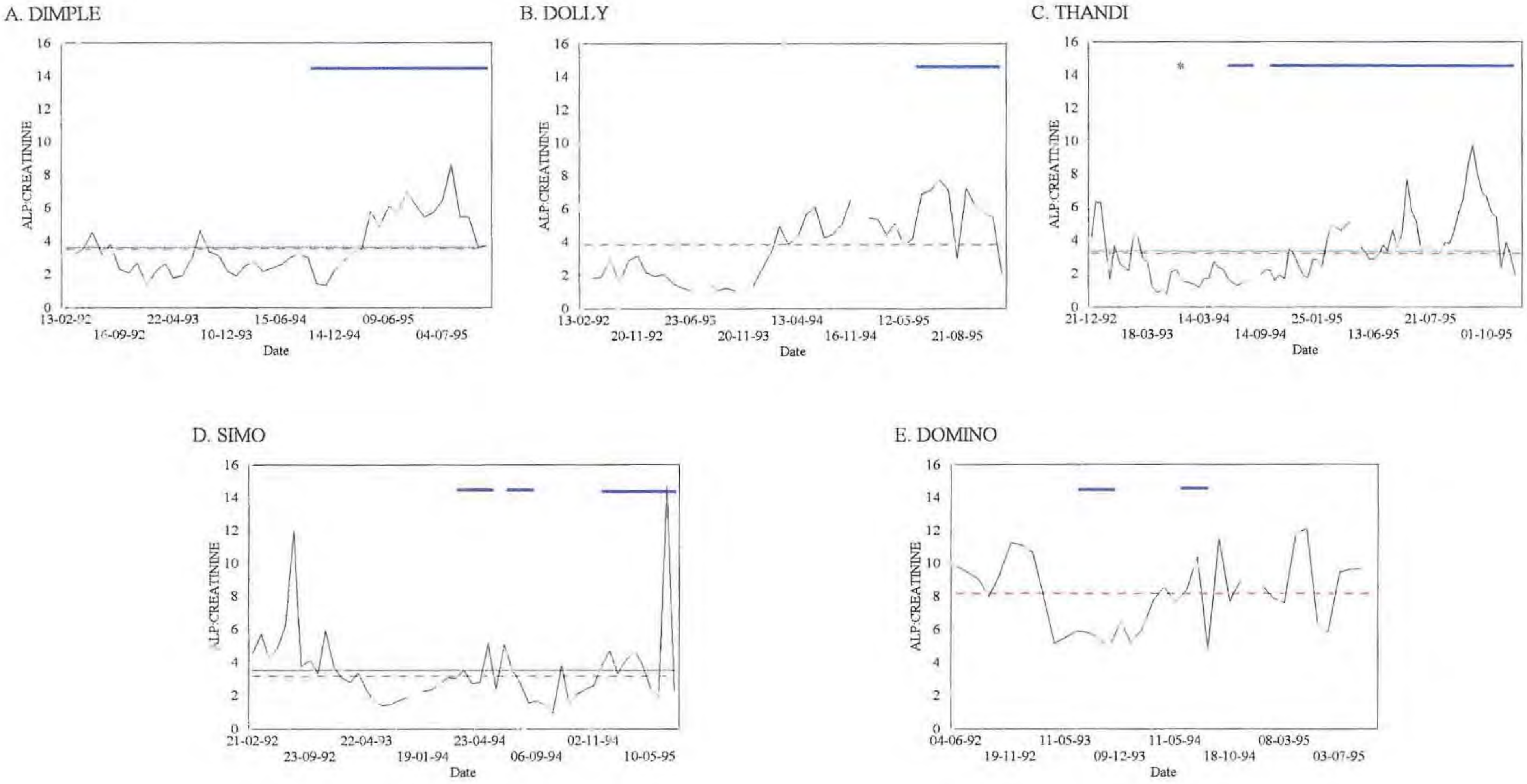


Figure 4.23: Levels of alkaline phosphotase (ALP) (U/l) (—) for all five captive animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the mean ALP level for all records prior to the study (- - -); the overall mean ALP level (—) and the "normal" (see text) mean ALP level (- - -) for the study between 1992 and 1995 (*: parturition; —: extended period of illness and/or treatment or period of aberrant feeding and/or behaviour).


A. DIMPLE

B. DOLLY

C. THANDI

D. SIMO

E. DOMINO

Figure 4.24: ALP:creatinine ratios (—) for all five captive animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the overall mean ratio (—) and the "normal" (see text) mean ALP:creatinine ratio (- - -) for the study between 1992 and 1995 (*: parturition; —: extended period of illness and/or treatment or period of aberrant feeding and/or behaviour).

ALP (Appendix 3.2B). Outlying ALP:creatinine values were recorded for Thandi and Simo (Appendix 3.2B).

Overall, ALP levels and ALP:creatinine were higher in juvenile animals, with data from adolescent and mature animals being relatively similar (Fig. 4.25). During the study, ALP was positively correlated with age only in the two oldest females, Dimple and Dolly (Table 4.4). Curves of best fit for both animals were complex (Fig. 4.26). The four older animals showed a significant correlation between ALP:creatinine and age during the study (Table 4.4). These relationships for Dimple and Dolly were similar to those of ALP and age. Thandi's ALP:creatinine was also positively related to age, but appeared to remain relatively stable throughout the study, with values declining at around the age of 24 years (Fig. 4.27A). Simo's ratio decreased, particularly between the ages of 10 and 11 years, at the beginning of the study (Fig. 4.27B).

Table 4.4: Correlation coefficients (r), levels of significance (*: p<0.05, **: p<0.01, ***: p<0.001) and sample sizes (n) for significant relationships between alkaline phosphotase (ALP) (a) and ALP:creatinine (b) and independent variables for the five captive dolphins (Data are presented as r*(n)) (NS: non-significant (p>0.05)).

Variable		Dimple	Dolly	Thandi	Simo	Domino
Age	a	0.40**(47)	0.80***(44)	NS	NS	NS
	b	0.56***(43)	0.73***(44)	0.31**(82)	-0.37**(50)	NS
Weight	a	0.50***(44)	0.83***(40)	NS	0.42**(38)	NS
	b	0.52***(40)	0.84***(40)	0.39**(55)	NS	NS
D1	a	-0.38*(37)	NS	NS	NS	-0.48**(33)
	b	NS	NS	0.41***(65)	NS	NS
E1	a	NS	NS	NS	NS	-0.43**(33)
	b	NS	NS	NS	0.35*(41)	NS
E2	a	-0.38*(39)	NS	NS	NS	-0.50**(33)
	b	-0.42**(36)	NS	NS	NS	NS
E3	a	-0.38**(40)	NS	NS	NS	-0.42*(32)
	b	NS	0.33*(38)	NS	NS	NS
E4	a	NS	NS	NS	NS	-0.48**(33)
	b	NS	NS	0.42***(80)	NS	NS
Month	a	NS	NS	NS	-0.43**(45)	NS
T1	a	-0.47***(47)	NS	NS	0.51***(45)	NS
	b	-0.48**(43)	NS	NS	NS	NS
T2	a	-0.49***(47)	NS	NS	0.55***(44)	NS
	b	-0.52***(43)	NS	NS	NS	NS
T3	a	-0.34*(47)	NS	NS	0.56***(45)	NS
	b	-0.37*(43)	NS	NS	NS	NS

D1: Total food consumption of week prior to blood sample; E1: Dietary energy intake of day prior to blood sample; E2-E4: Mean daily energy intake of week in which blood was sampled, and week and month prior to blood sample, respectively, T1: Mean water temperature of week prior to blood sample, T2: Mean water temperature of month in which blood was sampled, T3: Mean water temperature of month prior to blood sample.

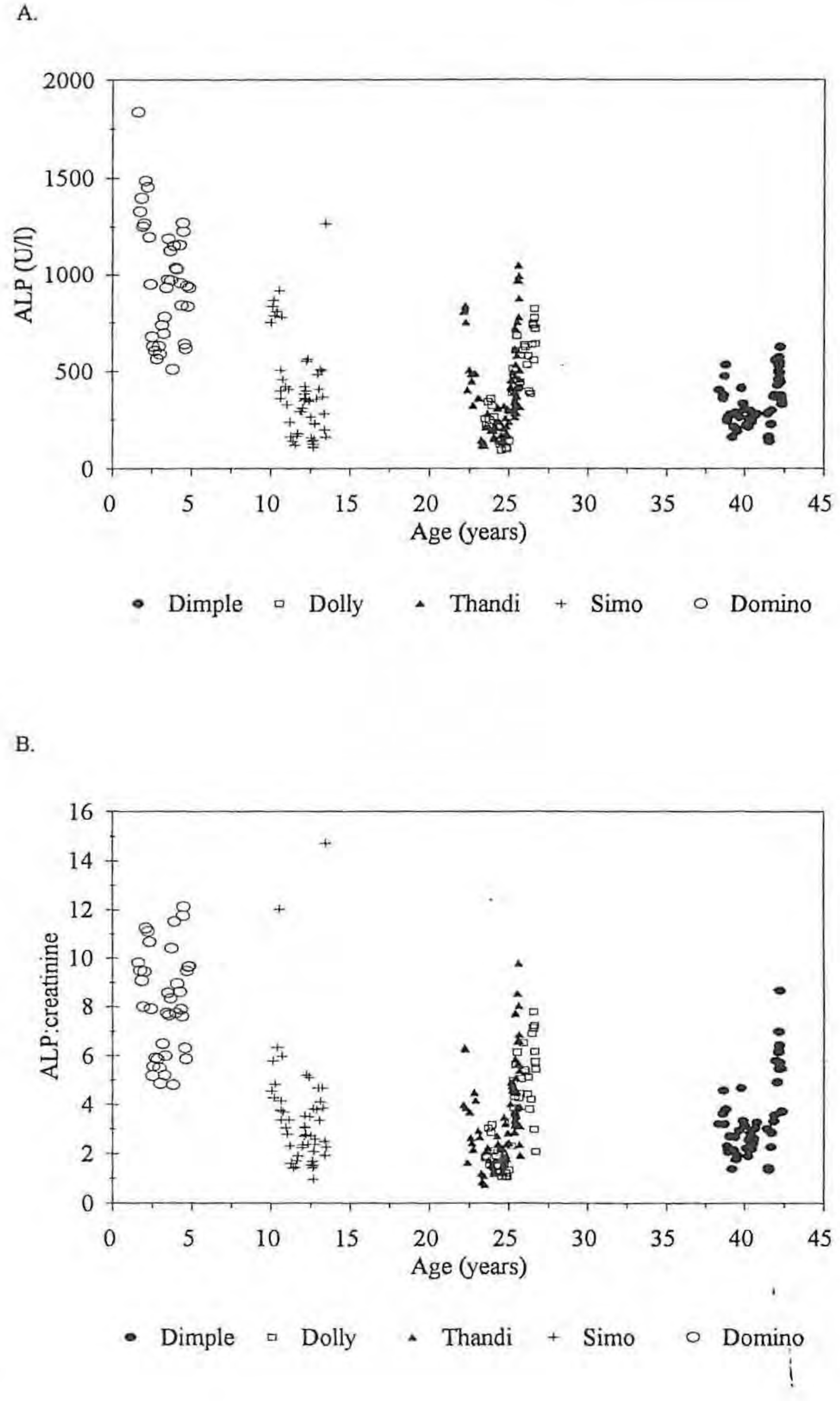
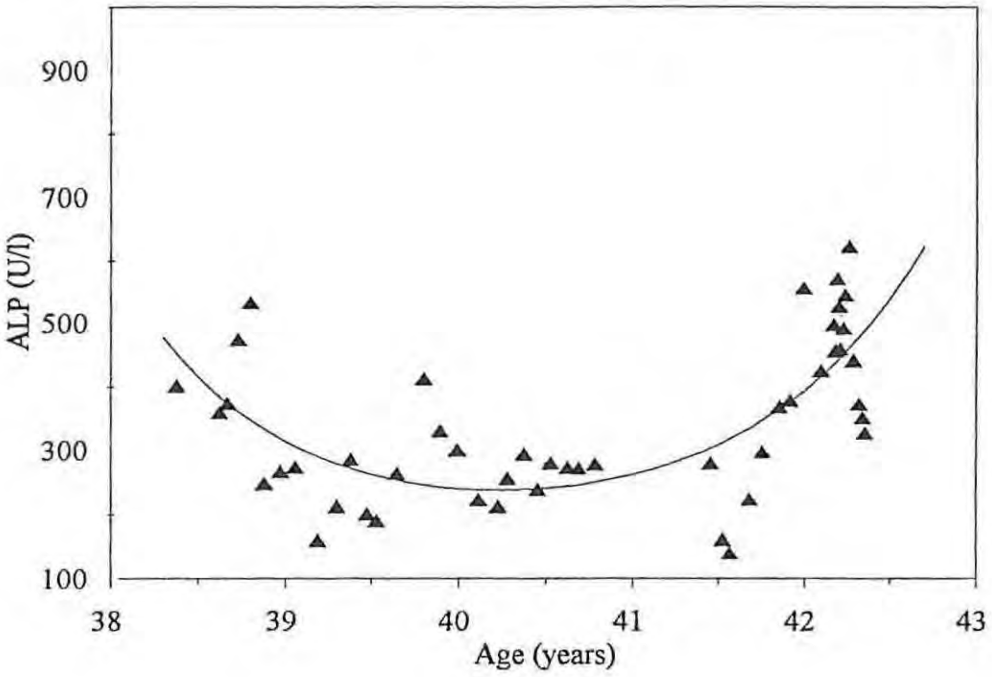


Figure 4.25: Relationship between serum levels of alkaline phosphotase (ALP), the ALP: creatinine ratio and age, for the five captive dolphins, from records obtained between 1992 and 1995.

A. DIMPLE



B. DOLLY

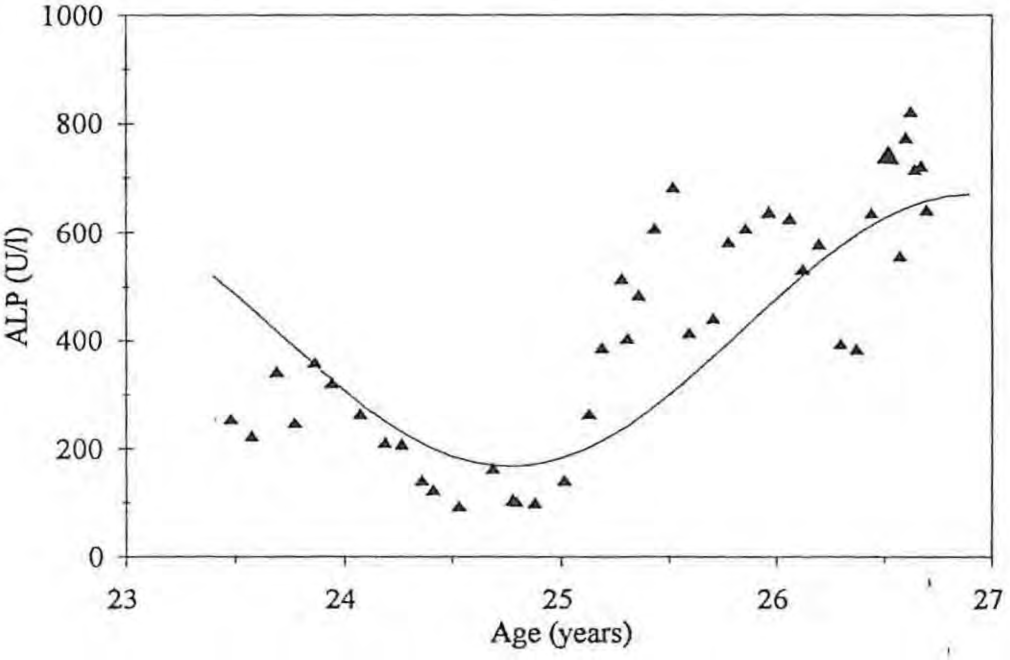
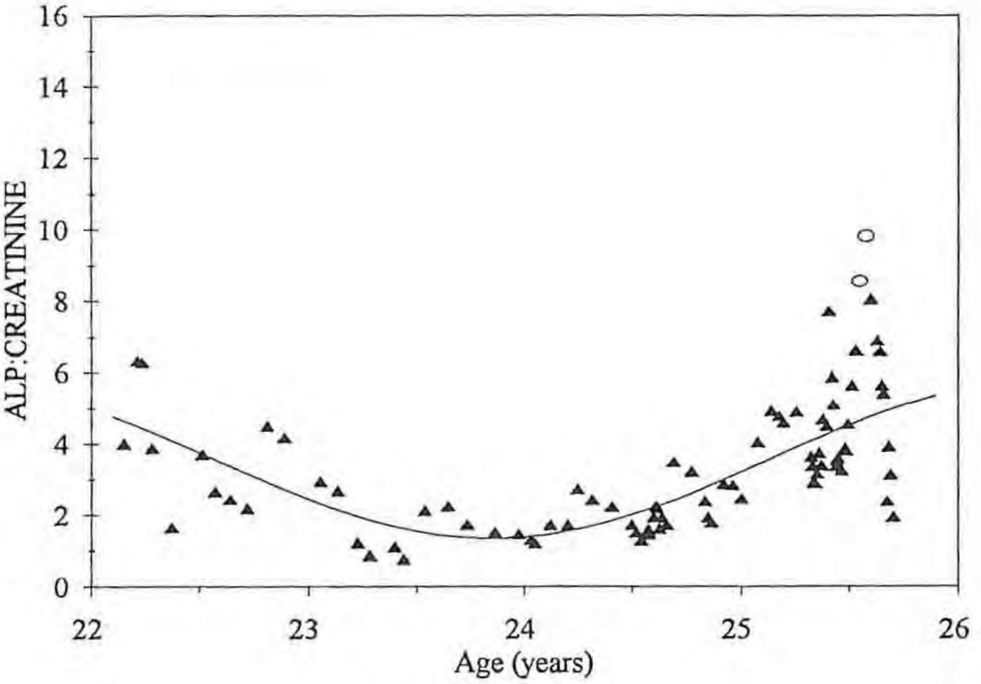


Figure 4.26: Curves of best fit (—) for animals (A. Dimple; B. Dolly) with significant correlations ($p<0.05$) between alkaline phosphotase (ALP) and age. Included in the graphs are all "normal" (see text) data (▲).

A. THANDI



B. SIMO

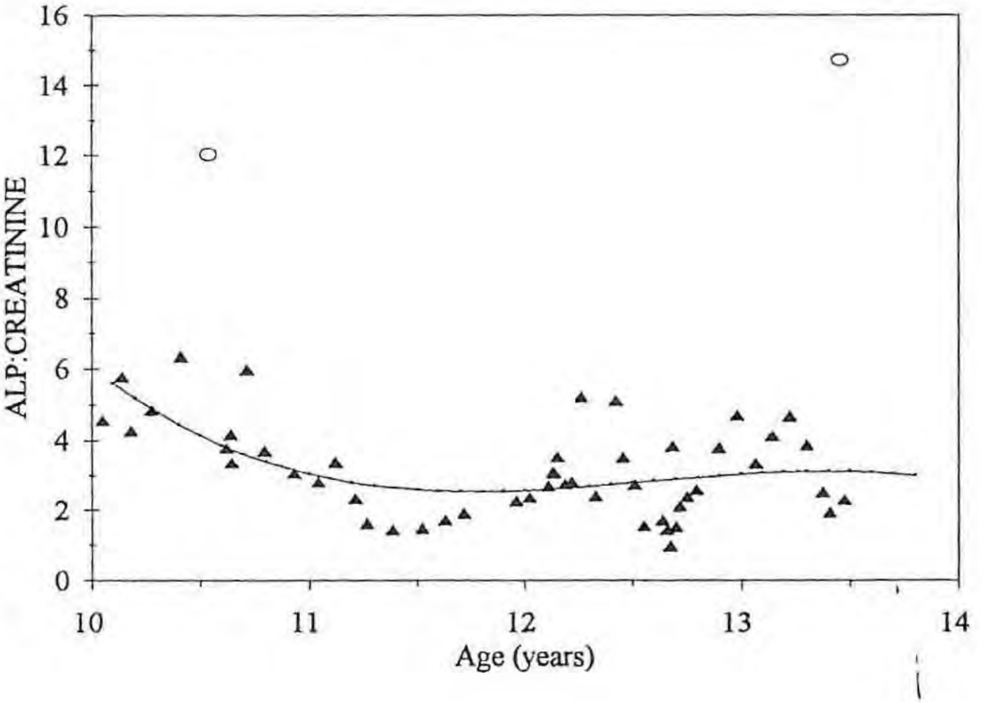


Figure 4.27: Curves of best fit (—) for animals (A. Thandi; B. Simo) with significant correlations ($p<0.05$) between the ALP:creatinine ratio and age. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

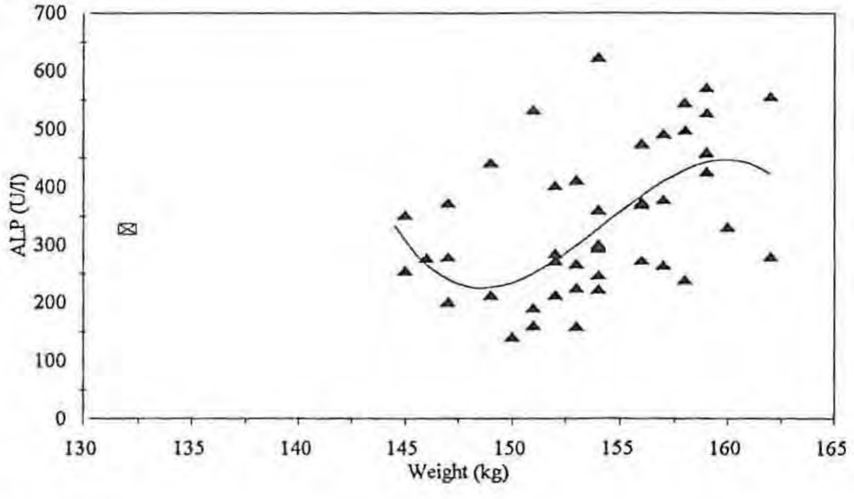
Levels of ALP were positively correlated with total body weight for Dimple, Dolly and Simo (Table 4.4; Fig. 4.28). Dimple's data were highly variable but Simo's ALP levels increased (~100-550 U/l) as weight increased from 165 kg to 185 kg. Reduced ALP levels measured at weights greater than 190 kg (Fig. 4.28C) complicated this particular relationship. The ALP:creatinine ratio was also positively related to weight for Dimple, Dolly and Thandi (Table 4.4). The positive trend was most distinct for Dolly, being almost identical to that described for ALP. Data from Dimple and Thandi were highly variable and although high ALP:creatinine values were measured more frequently at increased weights, these relationships were difficult to define.

Dimple and Domino's ALP levels were significantly related to the total food consumption of the week prior to the blood sample (Table 4.4). Data from both animals (particularly Dimple) were highly variable and aberrant levels of food consumption did not appear to have a marked affect on ALP, all values remaining within the "normal" range during these periods (Fig. 4.29A). Domino's ALP levels appeared to be predominantly higher when food intake was lower (Fig. 4.29B). Thandi was the only animal for which the ALP:creatinine ratio was positively related to food consumption (Table 4.4; Fig. 4.29C).

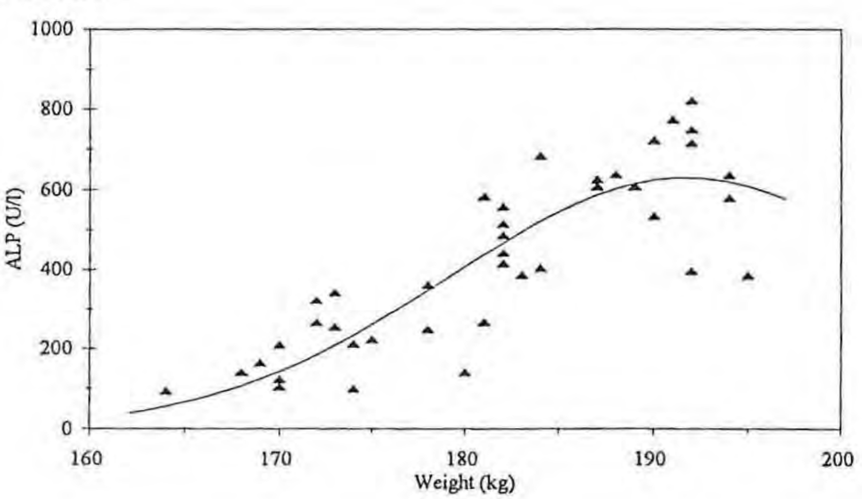
Relationships between ALP and dietary energy intake were difficult to confirm as a result of highly variable data. Domino's ALP levels were negatively related to the energy intake of the day prior to the blood sample and the mean daily energy intake of the week in which blood was sampled (Table 4.4). Both relationships illustrated that ALP was increased above 1 200 U/l when energy intake was below 25 000 kJ/day (Fig. 4.30B). Dimple displayed similar negative relationships between ALP, ALP:creatinine and energy intake during this period (Table 4.4). The trends were more complex, with reduced ALP levels and ratio values corresponding with mean daily energy intake levels around 35 000 kJ/day (Fig. 4.30A). ALP levels measured during periods of aberrant energy intake, for Dimple, were "normal".

When related to the mean daily energy intake of the week prior to the blood sample, ALP and ALP:creatinine were highly variable. The significant relationship between Dimple's ALP levels and this diet category was almost identical to those described for other dietary energy categories (Table 4.4). Domino's ALP levels, in contrast, appeared to decline steadily as energy intake during the prior week increased (Table 4.4) (Fig. 4.31). Although Dolly's ALP:creatinine ratio was positively related to the mean energy intake of the prior week, no clear trend was evident. Although data were variable, Domino's ALP levels were negatively related to the mean energy intake of the month preceding the blood sample (Table 4.4) (Fig. 4.32A). In contrast, Thandi's ALP:creatinine data were positively related to long term energy intake (Table 4.4). Data were highly variable but there was an overall increase in ratio values as energy intake increased (Fig. 4.32B).

A. DIMPLE



B. DOLLY



C. SIMO

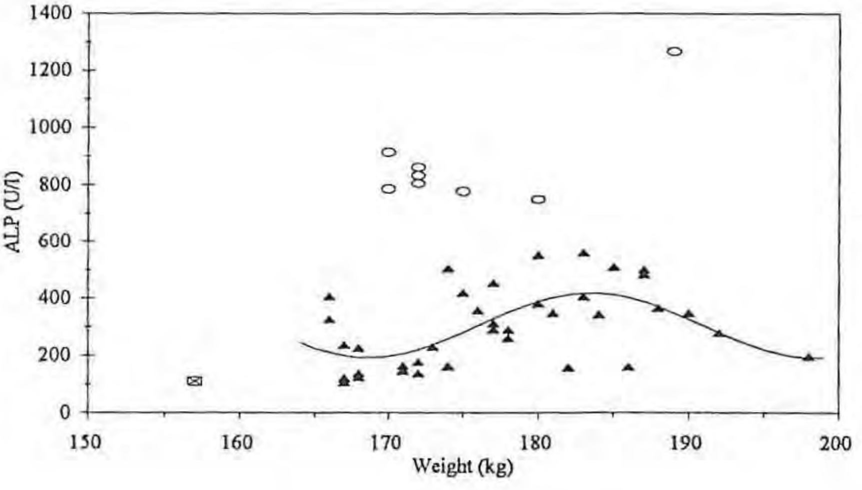
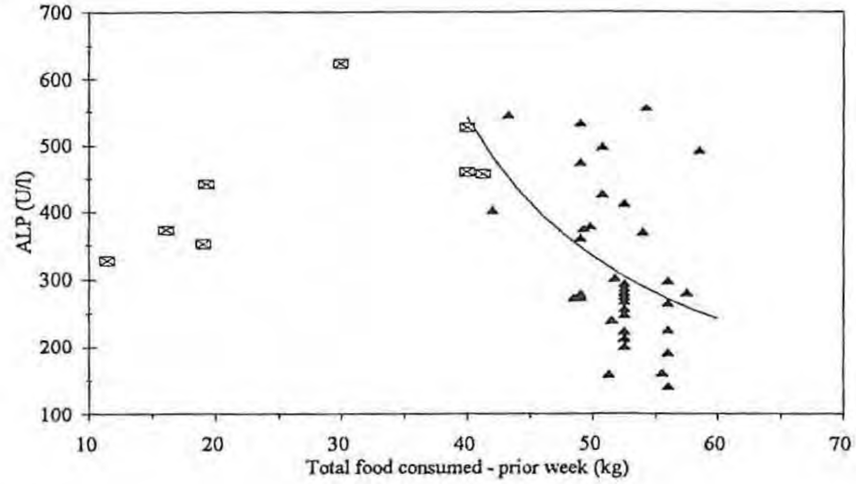
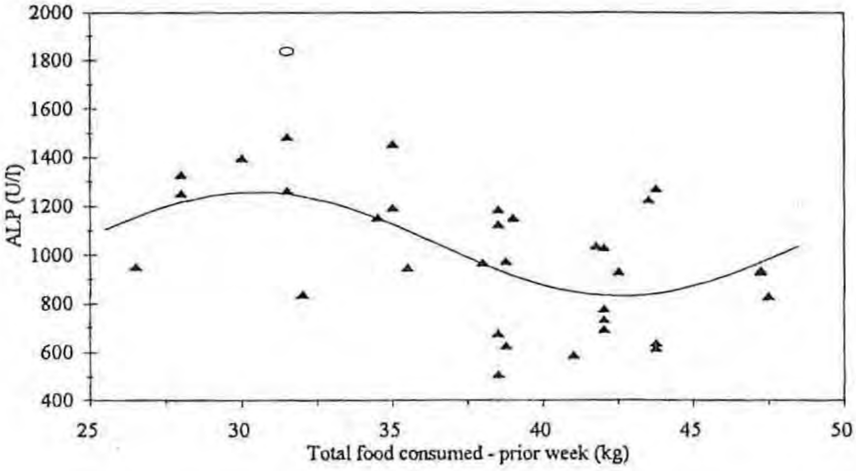


Figure 4.28: Curves of best fit (—) for animals (A. Dimple; B. Dolly; C. Simo) with significant correlations ($p<0.05$) between alkaline phosphotase (ALP) and total body weight. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers. (x-axis: ⊠ ; y-axis: ○).

A. DIMPLE



B. DOMINO



C. THANDI

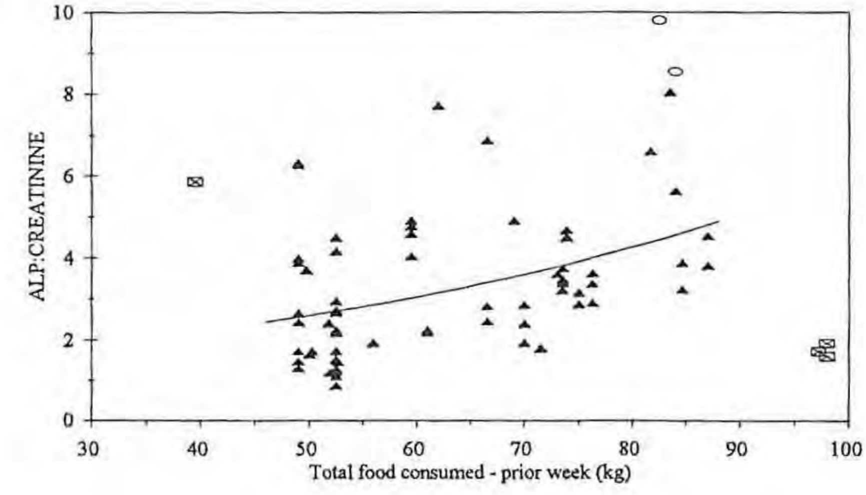
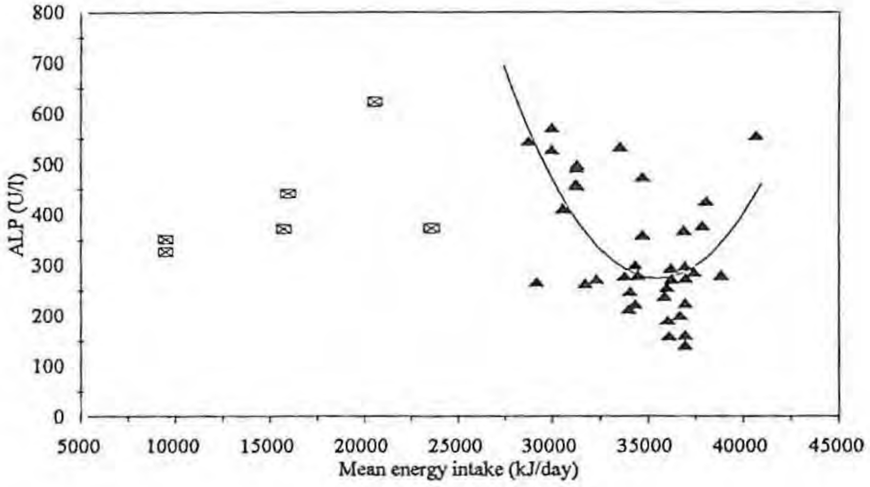
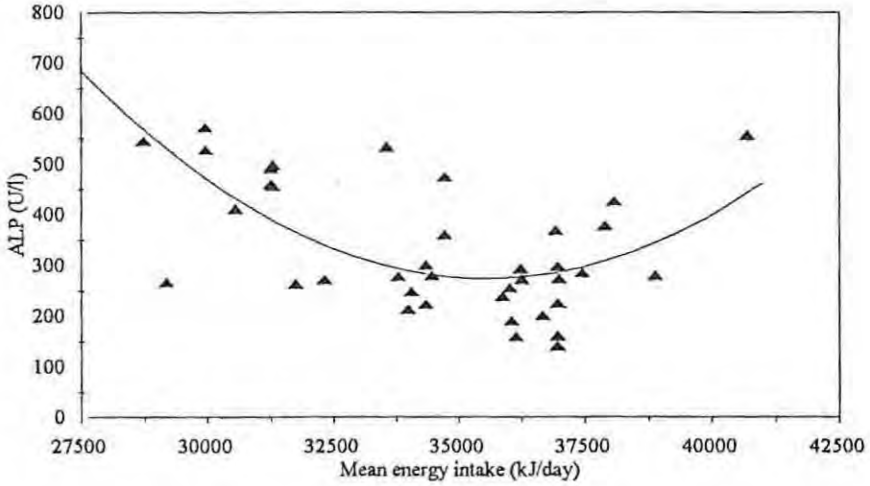


Figure 4.29: Curves of best fit (—) for animals (A. Dimple; B. Domino; C. Thandi) with significant correlations ($p<0.05$) between alkaline phosphotase (ALP) (A&B), ALP:creatinine (C) and total food consumed in the week prior to the blood sample. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒; y-axis: ○).

A. DIMPLE (a)



(b)



B. DOMINO

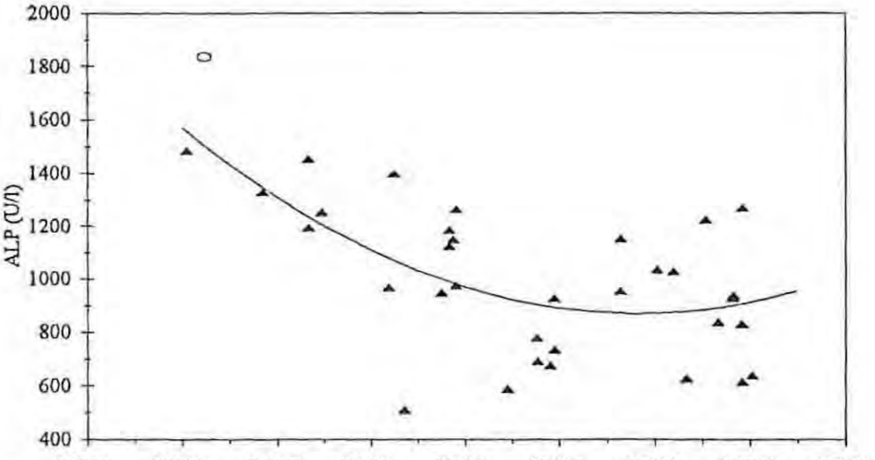
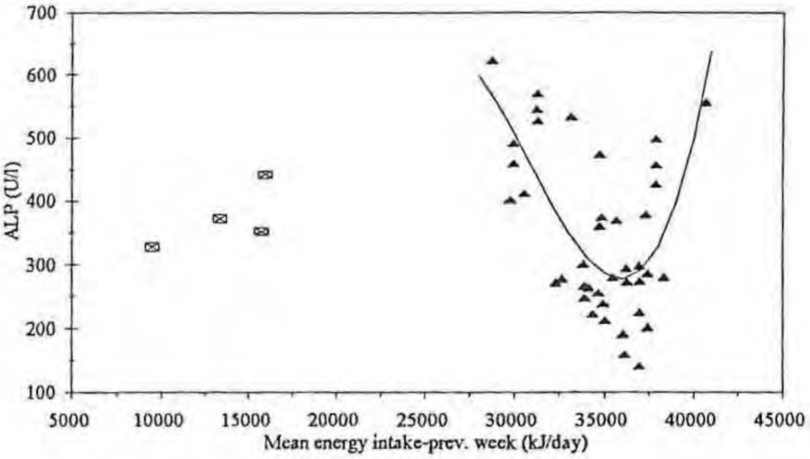


Figure 4.30: Curves of best fit (—) for animals (A. Dimple (a & b); B. Domino) with significant ($p<0.05$) correlations between alkaline phosphotase (ALP) and the mean daily dietary energy intake of the week in which blood was sampled. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

A. DIMPLE (a)



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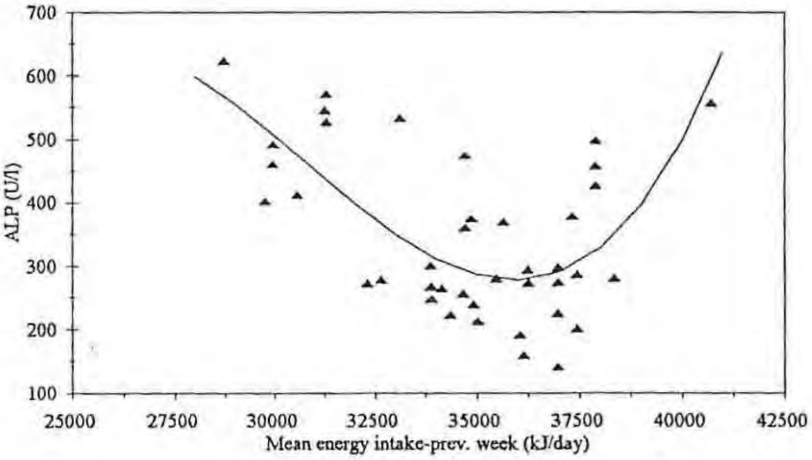


B. DOMINO

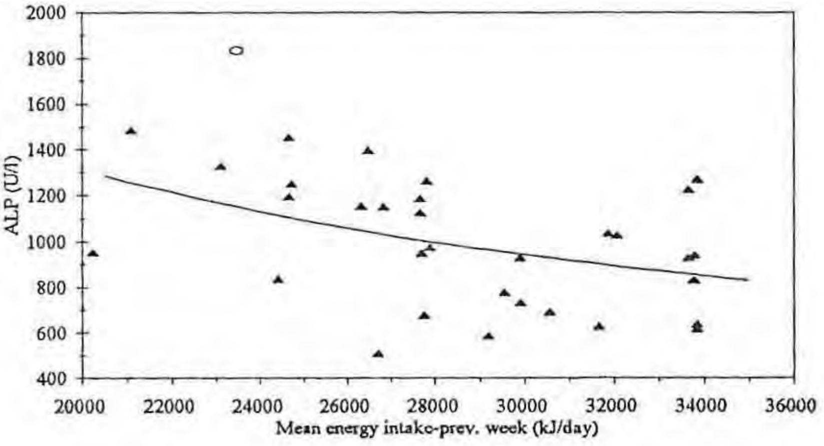
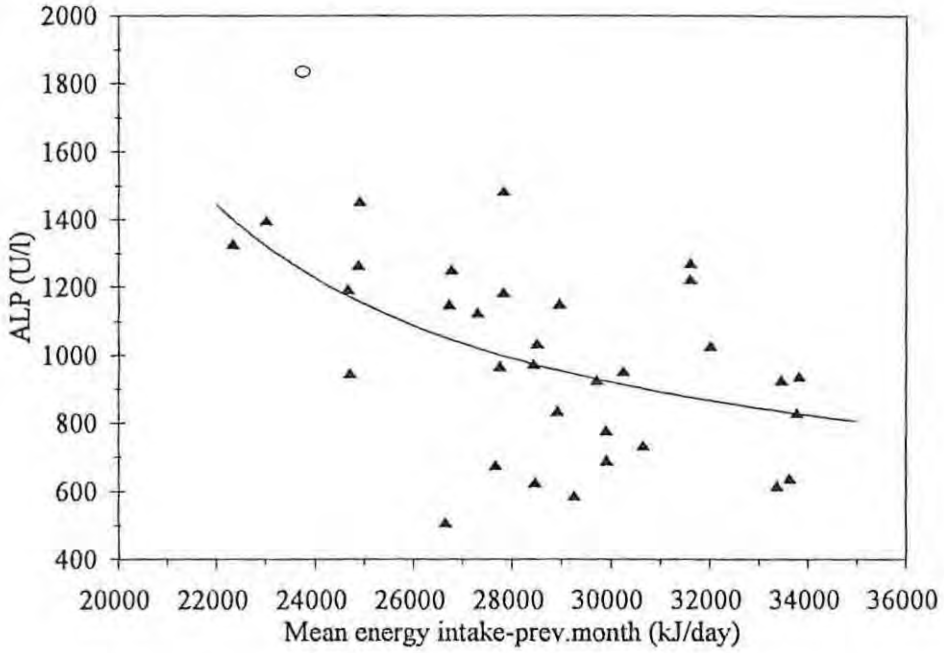


Figure 4.31: Curves of best fit (—) for animals (A. Dimple (a & b); B. Domino) with significant correlations ($p<0.05$) between alkaline phosphotase (ALP) and the mean daily energy intake of the week prior to the blood sample. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

A. DOMINO



B. THANDI

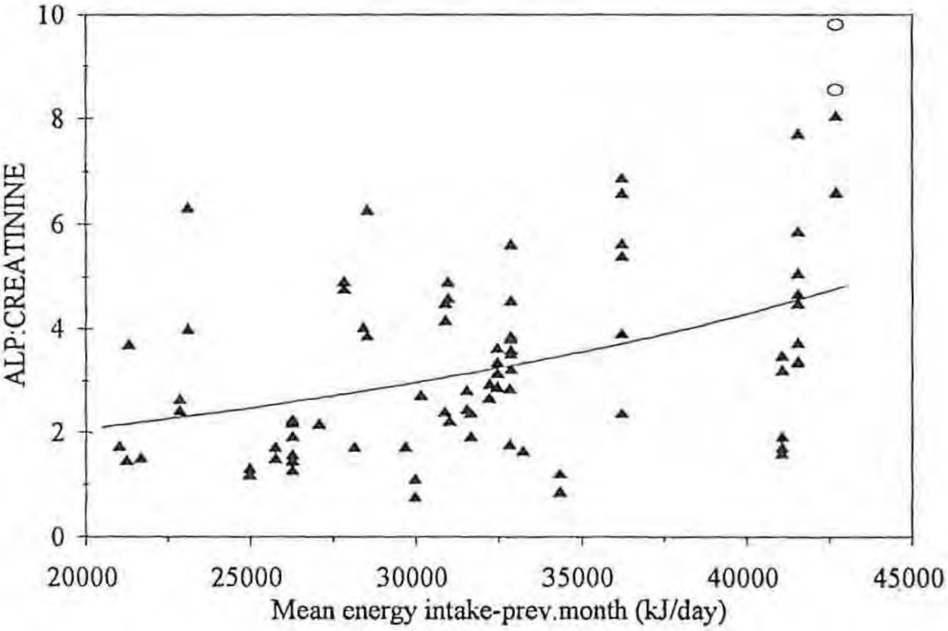


Figure 4.32: Curves of best fit (—) for animals (A. Domino; B. Thandi) with significant correlations ($p<0.05$) between alkaline phosphotase (ALP) (A) and ALP:creatinine (B) and the mean daily energy intake of the month prior to the blood sample. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

ALP levels for Simo were significantly correlated with the month of the year (Table 4.4). This relationship was difficult to define as a result of some divergent data, however, ALP appeared to be slightly increased in the summer months. Outlying high ALP levels were measured throughout the year. Both Dimple and Simo had ALP levels that were significantly related to water temperature, although the relationships displayed opposing trends (Table 4.4; Fig. 4.33). Dimple's ALP levels were higher at water temperatures below 20°C, whereas levels in Simo appeared to be reduced at lower water temperatures. Outlying high ALP levels were measured throughout the temperature range for Simo (Fig. 4.33B). Only Dimple's ALP:creatinine data were negatively correlated with water temperature (Table 4.4). Ratio values appeared to be slightly higher at reduced water temperatures, similar to the trend described for ALP. Outlying high ratio values were associated with water temperatures below 20°C, whereas outlying low ALP:creatinine values were recorded only at water temperatures above 20°C (Fig. 4.34).

d. Aspartate aminotransferase (AST) / Alanine aminotransferase (ALT)

Although levels of AST and ALT were measured in the captive dolphins, logistical problems prevented their analysis in the blood samples from net-caught dolphins (see Chapter 7). For this reason, relationships between these enzymes and age, weight, blubber thickness, diet and water temperature were withdrawn from the current study. Mean values obtained for each animal have been included in Table 4.1 for the sake of information. Additional information pertaining to the range of data and statistical outliers are available in Appendix 3.3.

e. Total bilirubin

For most of the study, total bilirubin levels were extremely stable for all animals (Fig. 4.35). Dimple showed a dramatic increase in total bilirubin, associated with a period of illness, towards the end of the study (Fig. 4.35A). Data from Simo illustrated a dramatic increase in total bilirubin in September 1992, which could not be attributed to recorded periods of illness and/or treatment (Fig. 4.35D). Outlying values were identified from all animals, except Dolly (Appendix 3.3C). The general range of data from Dolly, Thandi, Simo and Domino was similar, with only Dimple having widely varying total bilirubin levels (Appendix 3.3C). Similarly, "normal" mean total bilirubin levels were not significantly different between Dolly (2.40 ± 1.34 μmol/l), Thandi (2.87 ± 1.37 μmol/l), Simo (2.40 ± 1.35 μmol/l) and Domino (2.39 ± 1.22 μmol/l). Dimple's "normal" mean total bilirubin (5.83 ± 5.06μmol/l) was significantly higher (Two sample t-test; $p<0.001$) (Table 4.1).

Overall, total bilirubin was not related to the age of the five captive dolphins (Fig. 4.36A). More specifically, bilirubin values from both Dimple and Dolly were positively correlated with the change in age during the study (Table 4.5). Dolly's data were highly variable, showing no distinct

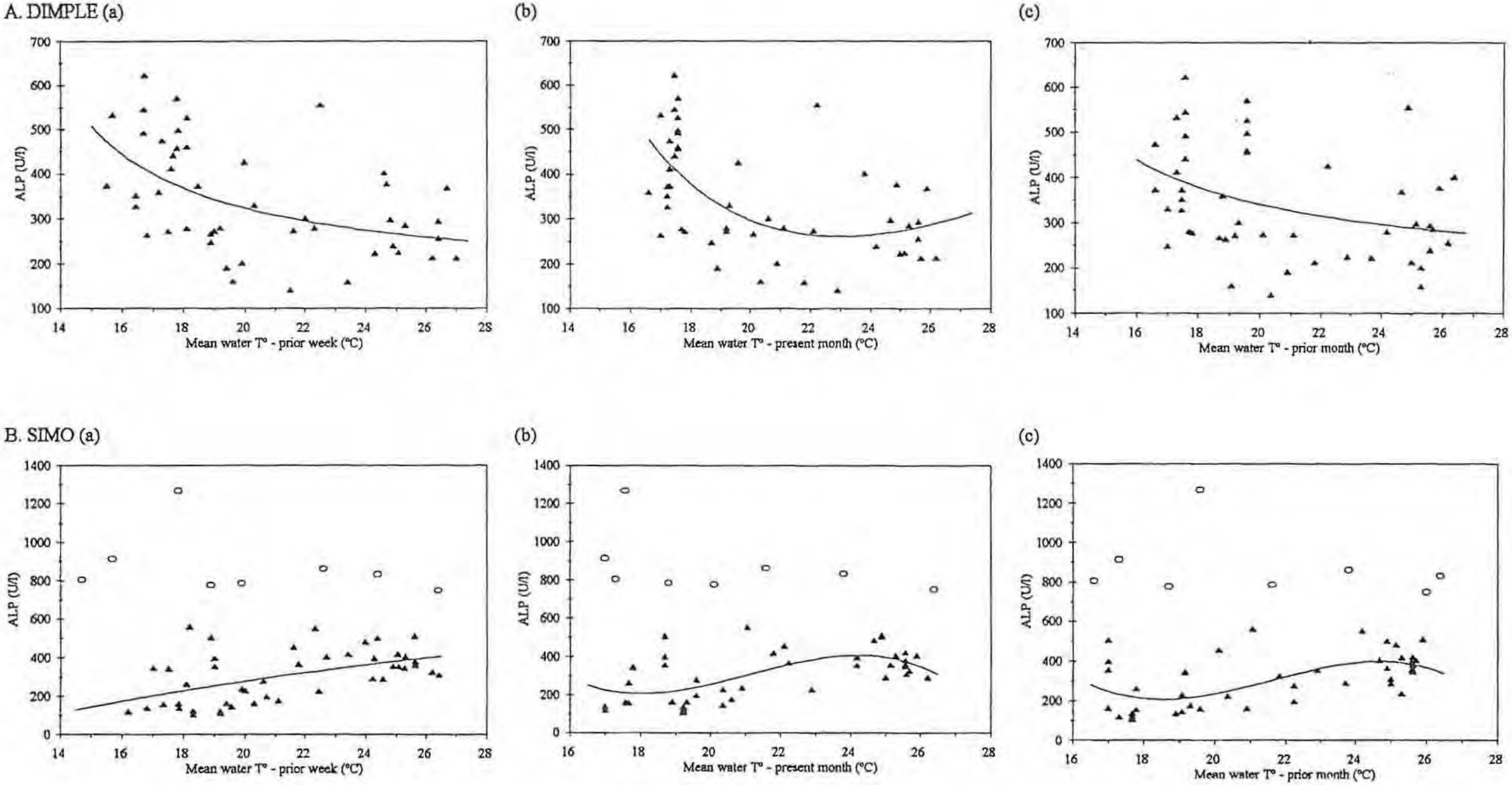


Figure 4.33: Curves of best fit (—) for animals (A. Dimple; B. Simo) with significant correlations ($p<0.05$) between alkaline phosphotase (ALP) and the mean water temperature of the week prior to the blood sample (a), the mean water temperature of the month in which blood was sampled (b) and the mean water temperature of the month prior to the blood sample (c). Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

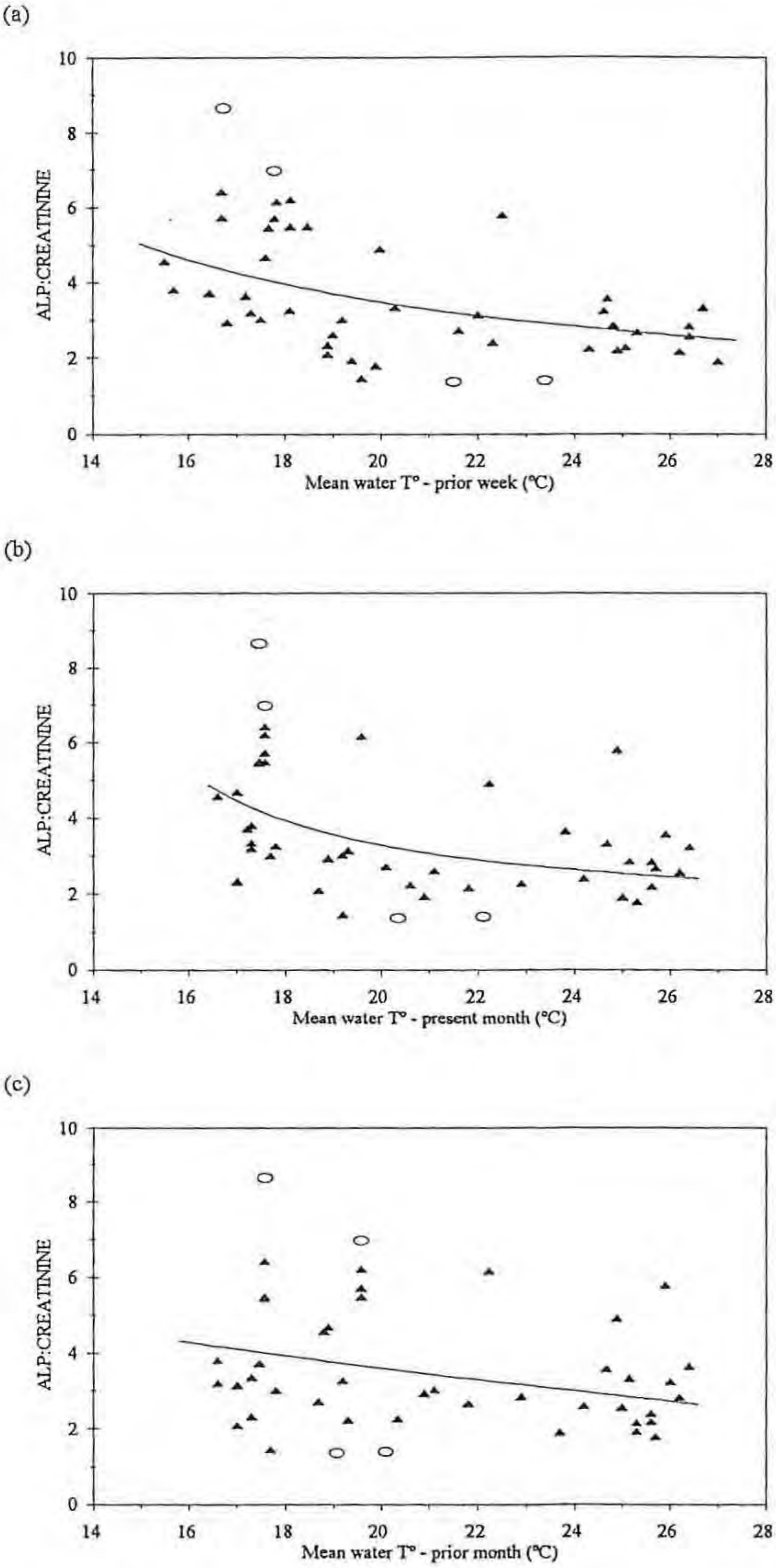


Figure 4.34: Curves of best fit (—) for Dimple with significant correlations ($p<0.0$
between ALP:creatinine and the mean water temperature of the week prior to the blood sample (a), the mean water temperature of the month in which blood was sampled (b) and the mean water temperature of the month prior to the blood sample (c). Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

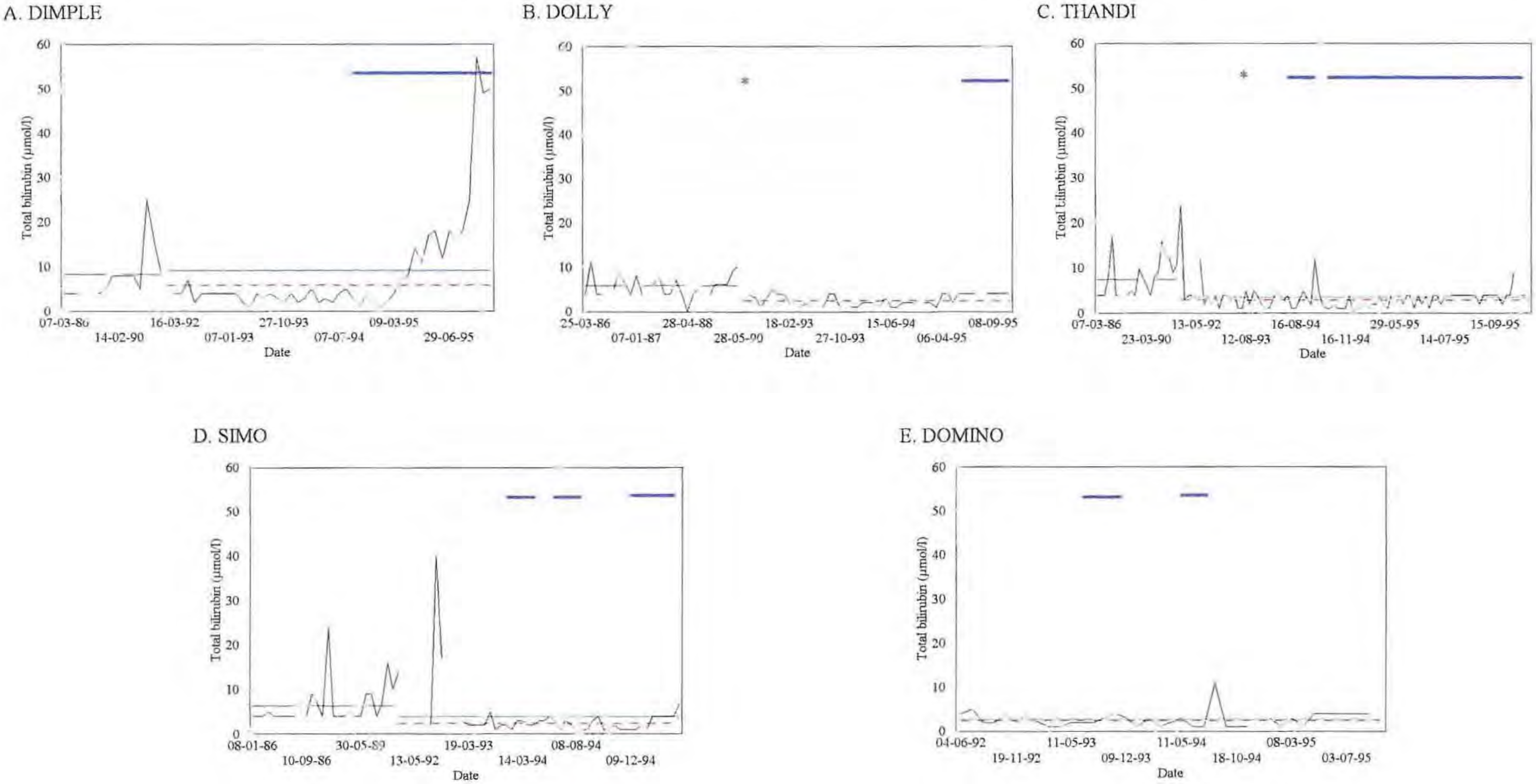
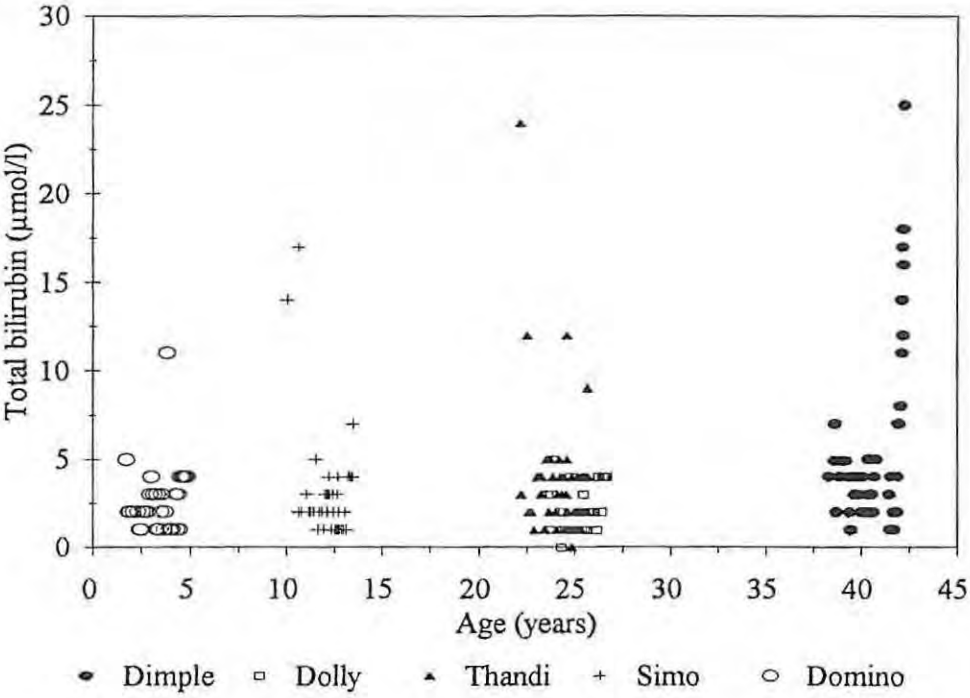


Figure 4.35: Levels of total bilirubin (µmol/l) (——) for all five captive animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the mean total bilirubin level for all records prior to the study (– –); the overall mean bilirubin level (——) and the "normal" (see text) mean total bilirubin level (- - -) for the study between 1992 and 1995 (*: parturition; ——: extended period of illness and/or treatment or period of aberrant feeding and/or behaviour).

A.



B.

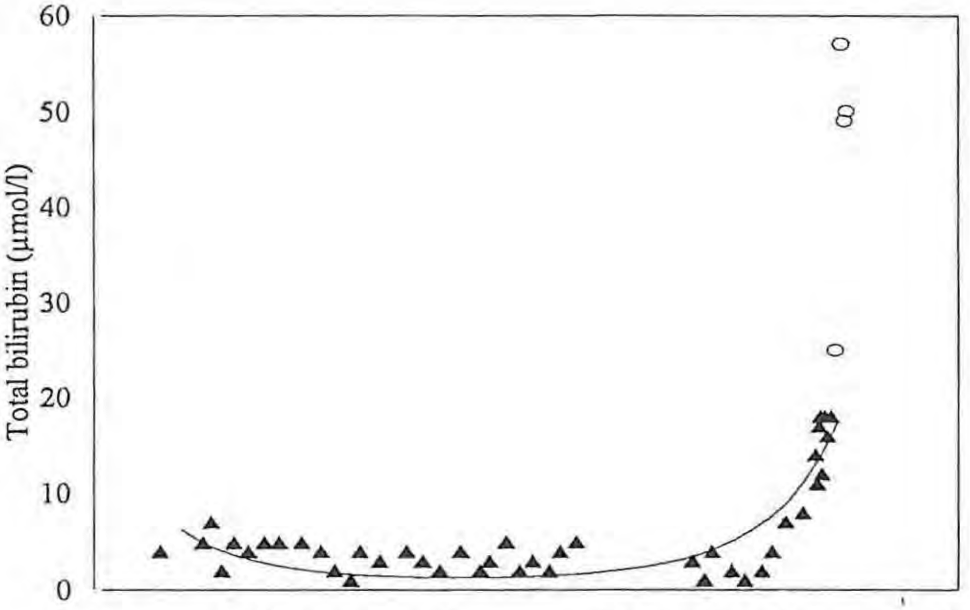


Figure 4.36: Relationship between total bilirubin concentration and age for the five captive dolphins (A) and curve of best fit (—) for Dimple with a significant correlation ($p<0.05$) between total bilirubin and age during the study (B). Included in graph (B) are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

trend. Dimple's bilirubin levels, however, increased significantly after age 42 years, in the final stages of the study (Fig. 4.36B).

Table 4.5: Correlation coefficients (r), levels of significance (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$) and sample sizes (n) for significant relationships between total bilirubin and independent variables for the five captive dolphins (Data are presented as r*(n)) (NS: non-significant ($p>0.05$)).

Variable	Dimple	Dolly	Thandi	Simo	Domino
Age	0.55***(43)	0.45**(40)	NS	NS	NS
Weight	0.43**(43)	0.60***(36)	0.36**(49)	NS	NS
E1	-0.31*(39)	NS	NS	-0.34*(35)	NS
E2	-0.55***(39)	NS	NS	NS	NS
E3	-0.48**(10)	NS	NS	NS	NS
Month	NS	0.31*(40)	NS	NS	NS
T1	-0.49***(43)	-0.38*(40)	-0.25*(76)	NS	NS
T2	-0.50***(43)	-0.38*(40)	-0.26*(75)	NS	-0.38*(33)
T3	-0.37*(43)	-0.44**(40)	NS	NS	NS

E1: Dietary energy intake of day prior to blood sample; E2: Mean daily energy intake of week in which blood was sampled, E3: Mean energy intake of week prior to the blood sample, T1: Mean water temperature of week prior to blood sample, T2: Mean water temperature of month in which blood was sampled, T3: Mean water temperature of month prior to blood sample.

Total bilirubin was positively related to total body weight in Dimple, Dolly, Thandi and Domino (Table 4.5). Although data were variable, bilirubin concentrations from all four animals were predominantly higher at heavier body weights (Fig. 4.37). These trends do, however, need further verification as the relationships were possibly biased by the frequency of bilirubin analyses at different weights. Outliers, measured for Dimple, were associated with low weights (Fig. 4.37A), whereas bilirubin levels at low weights in Thandi were "normal" (Fig. 4.37C).

None of the animals showed total bilirubin to be related to total food consumption of the week prior to the blood sample. Although Dimple and Simo's bilirubin levels were negatively correlated with the energy intake of day preceding the blood sample (Table 4.5), concentrations were highly variable and showed no distinct trend. Dimple's bilirubin levels were also negatively correlated with the mean daily energy intake of the week in which blood was sampled and the week prior to the blood sample (Table 4.5). These trends were identical and relatively clear, with increased bilirubin concentrations corresponding only with mean daily energy intake levels below 32 500 kJ/day (Fig. 4.38). Outlying high bilirubin levels were all associated with extremely low energy intake. Total bilirubin was not related to energy intake in the longer-term (i. e. the month prior to the blood sample).

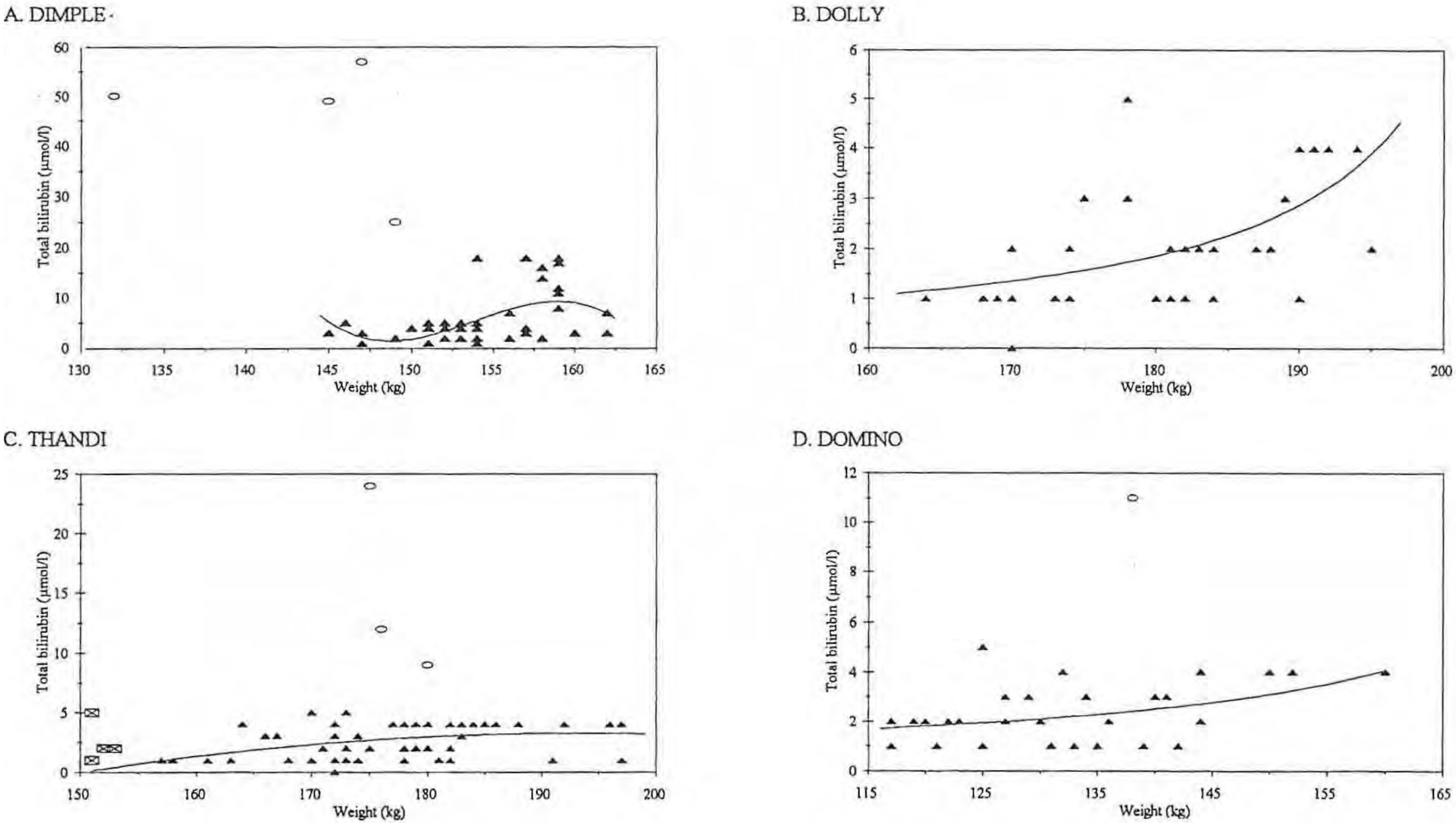
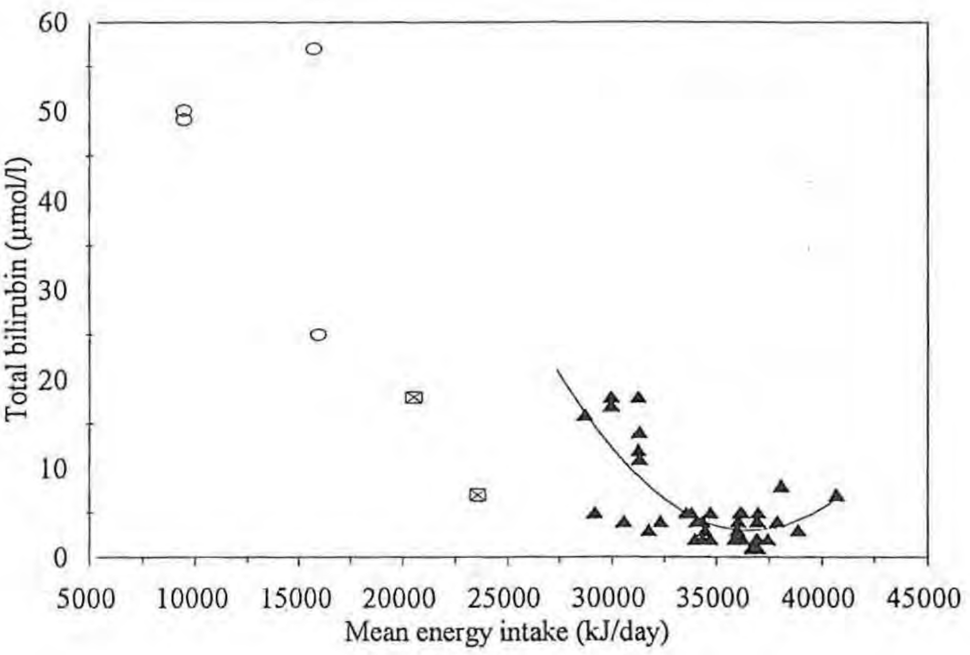


Figure 4.37: Curves of best fit (—) for animals (A. Dimple; B. Dolly; C. Thandi; D. Domino) with significant correlations ($p<0.05$) between total bilirubin and weight. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

(a)



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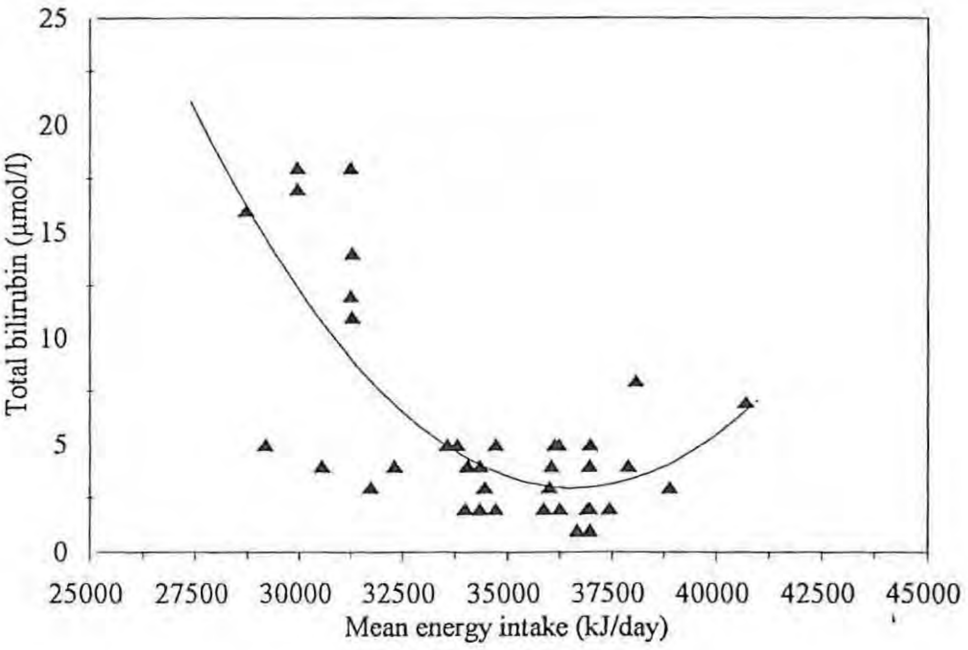


Figure 4.38: Curves of best fit (—) for Dimple with a significant ($p<0.05$) correlation between total bilirubin and the mean daily energy intake of the week in which blood was sampled. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ⊠ ; y-axis: ○).

Total bilirubin levels for Dimple and Dolly were negatively correlated with all categories of water temperature. Domino's total bilirubin levels were negatively correlated with the mean water temperature of the month in which blood was sampled (Table 4.5). Although Thandi's bilirubin levels were also negatively related to certain water temperature categories (Table 4.5), data were highly variable and the declining trend was difficult to confirm. The relationships for Dimple, Dolly and Domino all indicated that total bilirubin levels declined slightly as water temperature increased (Fig. 4.39). The majority of outlying high bilirubin levels were associated with water temperatures below 18°C.

f. Lactate dehydrogenase (LDH) / LDH:creatinine

Levels of LDH in Dimple, Thandi and Simo fluctuated considerably during the study. LDH levels in Dolly and Domino remained relatively stable (Fig. 4.40). Mean LDH levels measured during the study were higher than previous records. Overall, Dimple's LDH levels increased progressively during the study and both Thandi and Simo exhibited dramatic, short-term increases of this enzyme. The majority of these increases could be associated with reported periods of illness and/or treatment, with the exception of a dramatic increase to 9 230 U/l recorded for Simo in September 1992 (Fig. 4.40). These fluctuations generally corresponded with changes in the LDH:creatinine ratio, although changes were not as marked in some cases (Fig. 4.41). The range of LDH values measured during the study was fairly restricted with Dimple and Thandi having the most variable data (Appendix 3.4A). The majority of significant outliers were identified in Simo's data, although outlying high LDH levels were also recorded from Dolly and Thandi. No outlying low LDH levels were recorded. High LDH:creatinine outliers were recorded from all animals, except Dolly and Domino. Dolly did, however, have outlying low ratio values (Appendix 3.5A).

Dimple's mean LDH level (1406.00 ± 482.90 U/l) was significantly higher than the other four animals (Two-sample t-test, $p<0.001$). Dolly (999.00 ± 141.90 U/l), Thandi (1034.00 ± 305.80 U/l), Simo (936.80 ± 229.70 U/l) and Domino (973.00 ± 140.00 U/l) all had similar mean LDH levels (Two sample t-test; $p>0.05$) (Table 4.1). Similarly, Dimple's mean LDH:creatinine ratio (14.52 ± 6.43) was significantly higher than those of the four other animals (Two sample t-tests, $p<0.001$). All other animals had similar LDH:creatinine ratios, with the exception of Dolly (9.31 ± 1.65) and Domino (7.64 ± 1.04) ($t=4.70$; $p<0.001$; $df=68$) (Table 4.1).

As the mean LDH levels and LDH:creatinine ratios reflected, these blood chemistry parameters were relatively similar in juvenile, adolescent and mature animals. Both parameters were increased in the elderly Dimple (Fig. 4.42). However, between 1992 and 1995, LDH concentrations from all

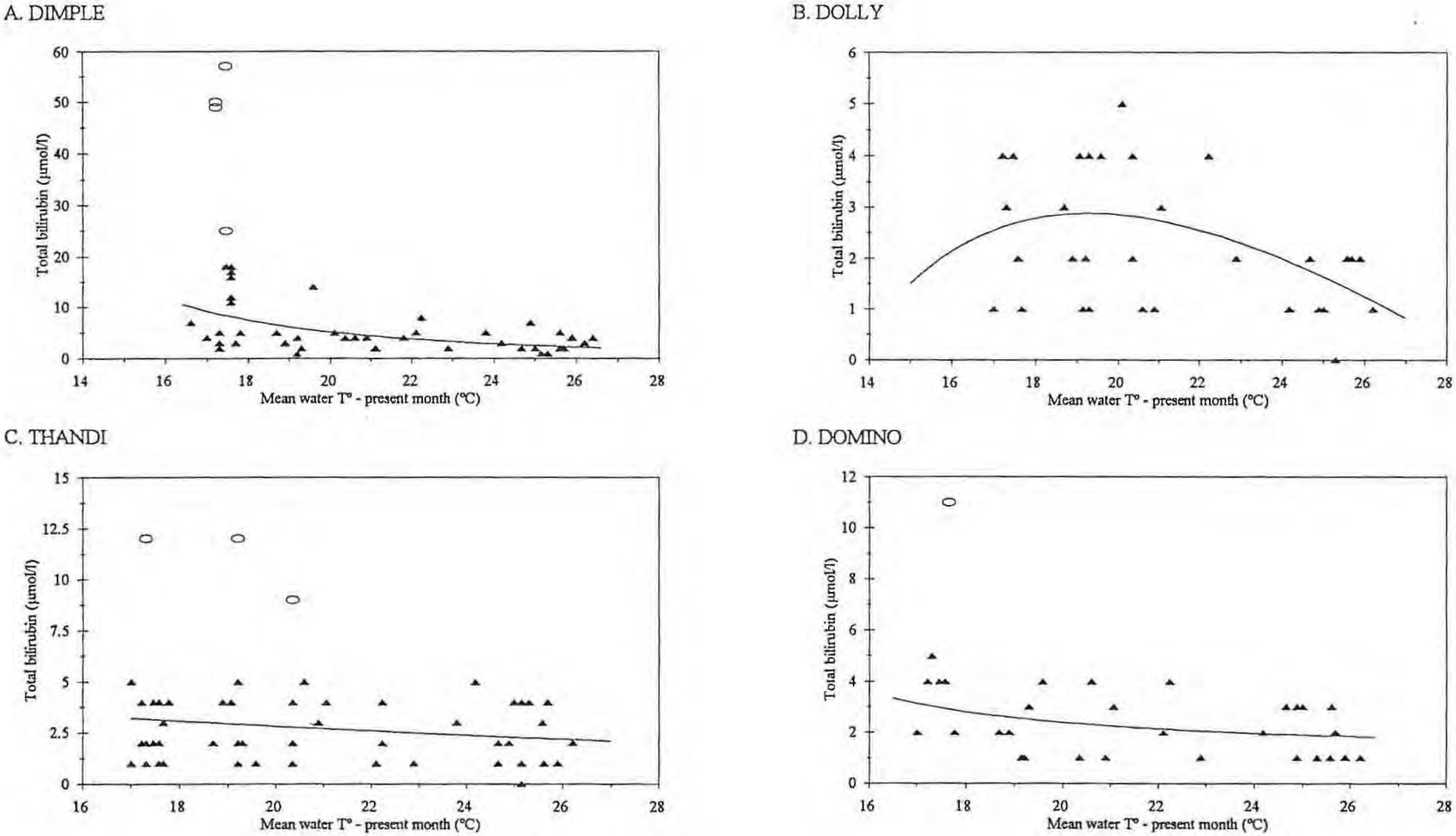


Figure 4.39: Curves of best fit (—) for animals (A. Dimple; B. Dolly; C. Thandi; D. Domino) with significant correlations ($p<0.05$) between total bilirubin and the mean water temperature of the month in which blood was sampled. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

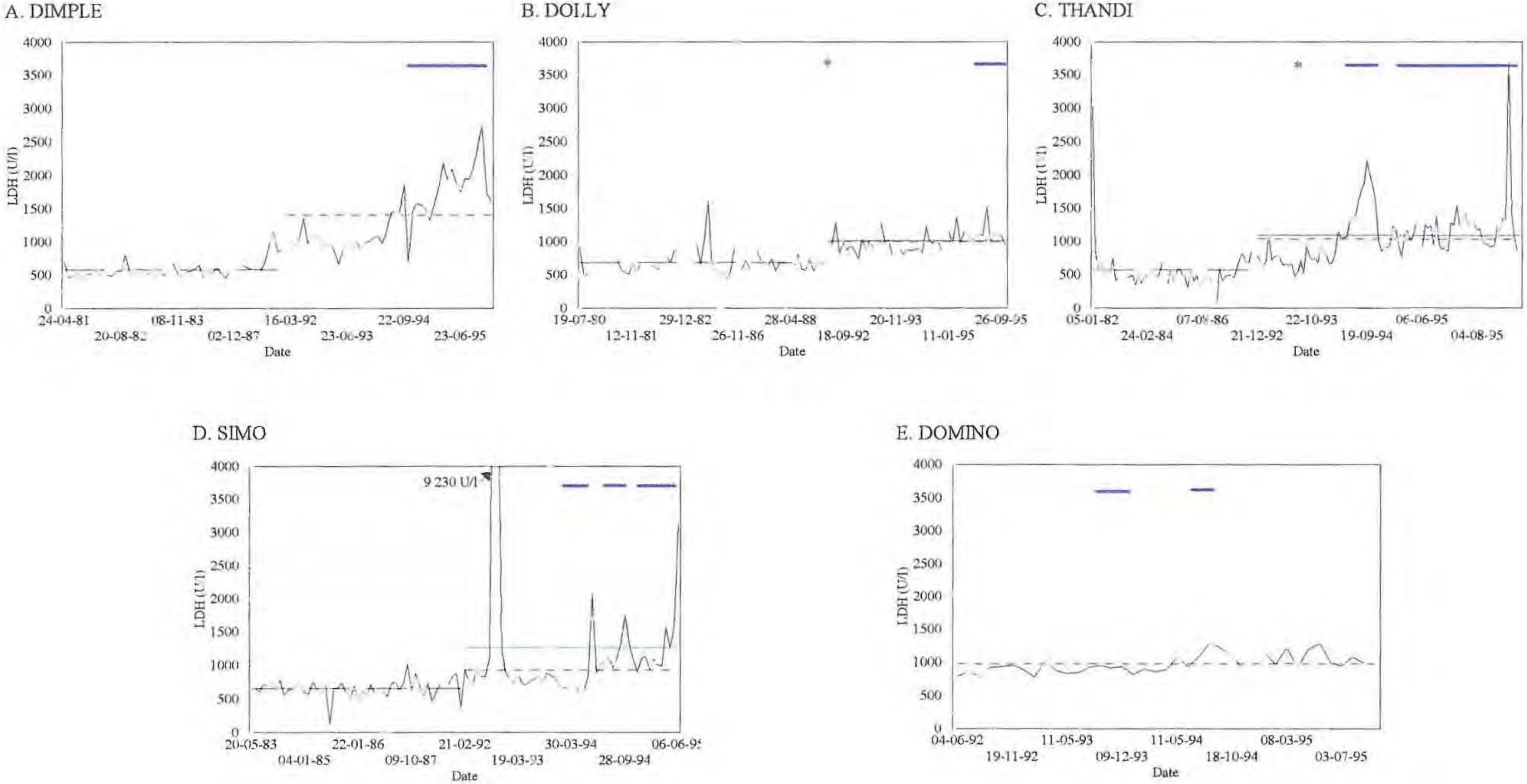


Figure 4.40: Levels of lactate dehydrogenase (LDH) (U/l) (—) for all five captive animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the mean LDH level for all records prior to the study (···); the overall mean LDH level (—) and the "normal" (see text) mean LDH level (- - -) for the study between 1992 and 1995 (*: parturition; ▬: extended period of illness and/or treatment or period of aberrant feeding and/or behaviour).

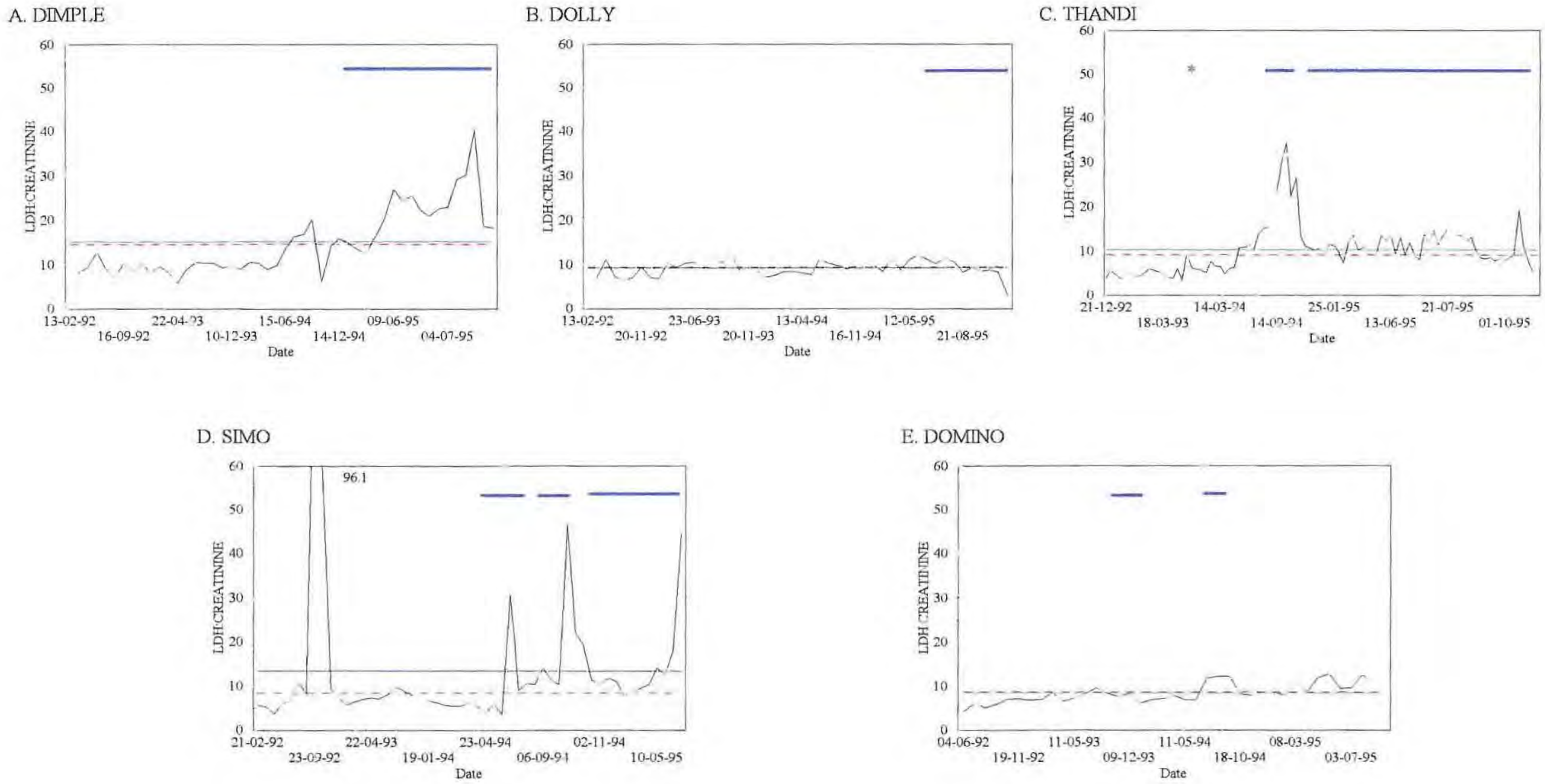


Figure 4.41: LDH:creatinine ratios (—) for all five captive animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the overall mean LDH:creatinine ratio (—) and the "normal" (see text) mean LDH:creatinine ratio (- - -) for the study between 1992 and 1995 (*: parturition; —: extended period of illness and/or treatment or period of aberrant feeding and/or behaviour).

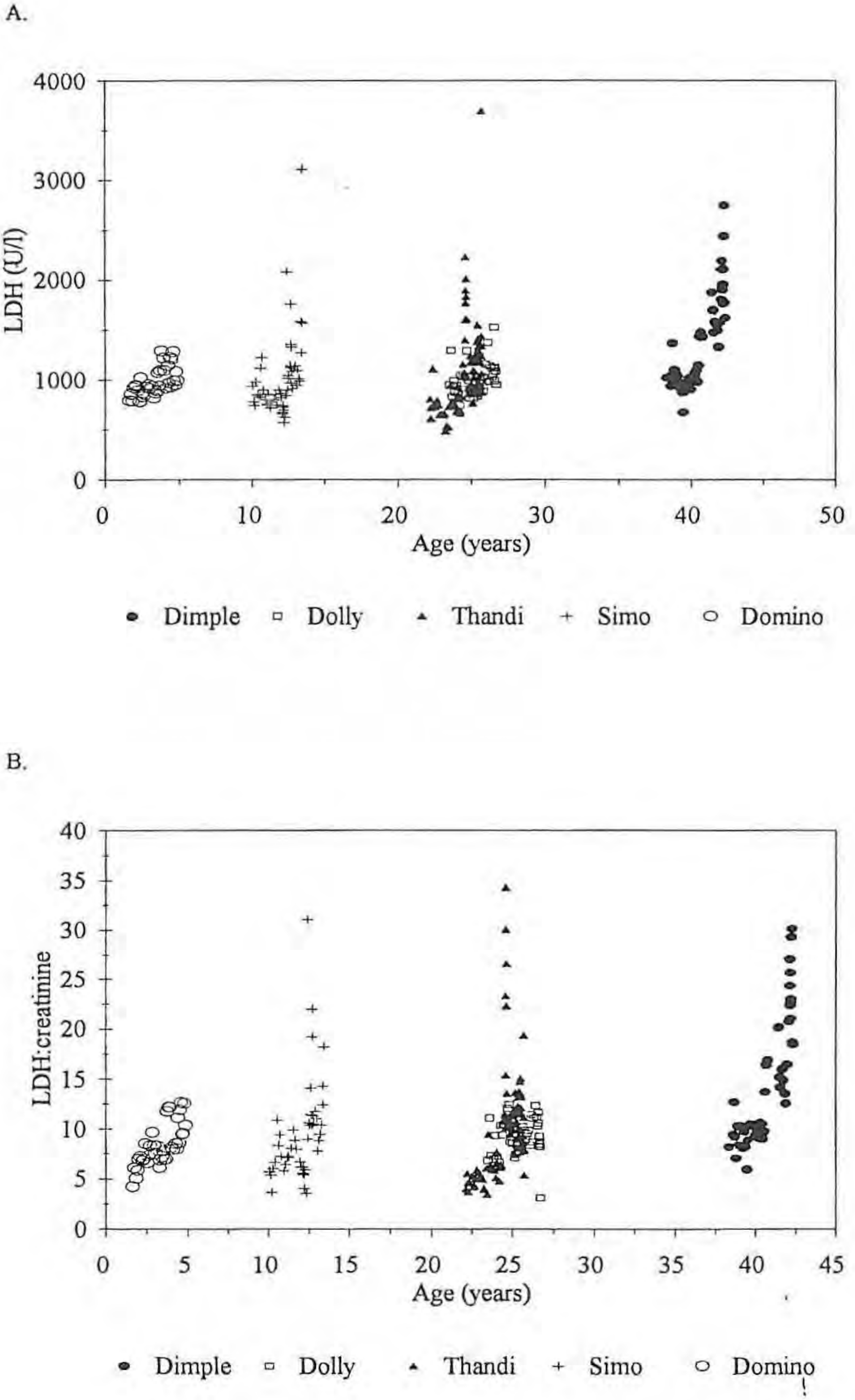


Figure 4.42: Relationship between serum levels of lactate dehydrogenase (LDH) (A), the LDH: creatinine ratio (B) and age for the five captive dolphins, from records obtained between 1992 and 1995.

five captive animals were positively correlated with age (Table 4.6). Dolly's data were highly variable and an increasing trend with age was difficult to confirm (Fig. 4.43B). Levels of LDH from Dimple, Thandi and Simo remained relatively stable during the majority of the study. However, LDH increased with age during the final stages of the study in all three animals (Fig. 4.43A, C, D). Although data were variable, Domino's LDH levels reflected a marked increase after age 3.5 years (Fig. 4.43E).

Table 4.6: Correlation coefficients (r), levels of significance (*: p<0.05, **: p<0.01, ***: p<0.001) and sample sizes (n) for significant relationships between lactate dehydrogenase (LDH) and LDH:creatinine and independent variables for the five captive dolphins (Data are presented as r*(n)) (NS: non-significant (p>0.05)).

Variable		**Dimple**	**Dolly**	**Thandi**	**Simo**	**Domino**
Age	a	0.84***(47)	0.33*(43)	0.57***(83)	0.46***(48)	0.62***(37)
	b	0.83***(46)	0.32*(43)	0.73***(78)	0.36***(45)	0.59***(27)
Weight	a	NS	NS	NS	0.45**(42)	0.55***(35)
	b	0.30*(44)	NS	NS	0.30*(40)	0.45*(26)
Blubber thickness	a	NS	NS	NS	0.81***(13)	NS
D1	a	NS	NS	0.39***(68)	NS	NS
	b	NS	NS	0.63***(64)	NS	0.48*(24)
E1	a	NS	NS	0.37**(70)	NS	NS
	b	NS	NS	0.41***(65)	NS	0.65***(24)
E2	a	NS	NS	0.31**(69)	-0.38*(36)	0.36*(34)
	b	NS	NS	0.43***(66)	NS	0.67***(24)
E3	a	NS	NS	0.41***(71)	NS	NS
	b	NS	0.32*(38)	0.55***(66)	NS	0.50**(24)
E4	a	NS	0.42**(41)	0.26*(81)	NS	NS
	b	NS	0.47**(41)	0.35**(76)	NS	0.64***(24)
Month	a	NS	NS	0.28**(85)	0.32*(48)	NS
	b	NS	NS	NS	0.44**(45)	NS
T1	a	-0.39**(47)	-0.39**(43)	-0.32**(83)	-0.32*(48)	NS
	b	-0.45**(46)	-0.39**(43)	-0.31**(78)	-0.43**(45)	NS
T2	a	-0.41**(47)	-0.34*(43)	-0.33**(82)	-0.33*(47)	NS
	b	-0.45**(46)	-0.35*(43)	-0.32**(77)	-0.47***(44)	NS
T3	a	-0.38**(47)	NS	-0.40***(83)	-0.36*(48)	NS
	b	-0.39**(46)	NS	-0.31**(78)	-0.47***(45)	NS

D1: Total food consumption of week prior to blood sample; E1: Dietary energy intake of day prior to blood sample; E2-E4: Mean daily energy intake of week in which blood was sampled, and week and month prior to blood sample, respectively, T1: Mean water temperature of week prior to blood sample, T2: Mean water temperature of month in which blood was sampled, T3: Mean water temperature of month prior to blood sample.

LDH:creatinine from all five animals was also positively correlated with age during the study (Table 4.6). The increase in LDH:creatinine for Dimple, Thandi and Simo was almost identical to the trend described for LDH. The significant increases in LDH:creatinine with age for Dolly and

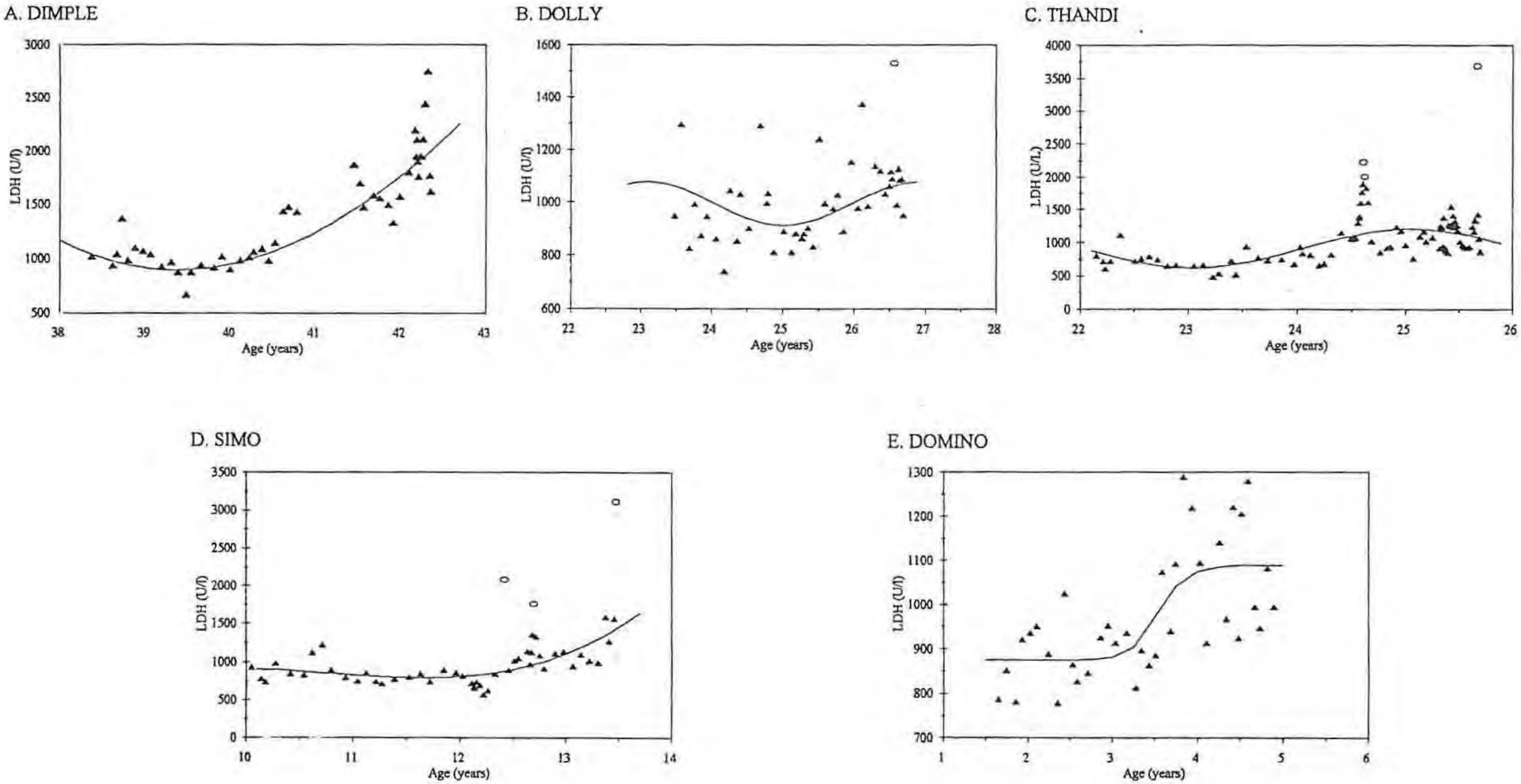


Figure 4.43: Curves of best fit (—) for animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino) with significant correlations ($p<0.05$) between lactate dehydrogenase (LDH) and age. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (o).

Domino were more distinct, with ratio values undergoing an overall increase as the animals increased in age during the study (Fig. 4.44). Outliers identified in Domino's data corresponded with the trend described by the curve of best fit (Fig. 4.44B).

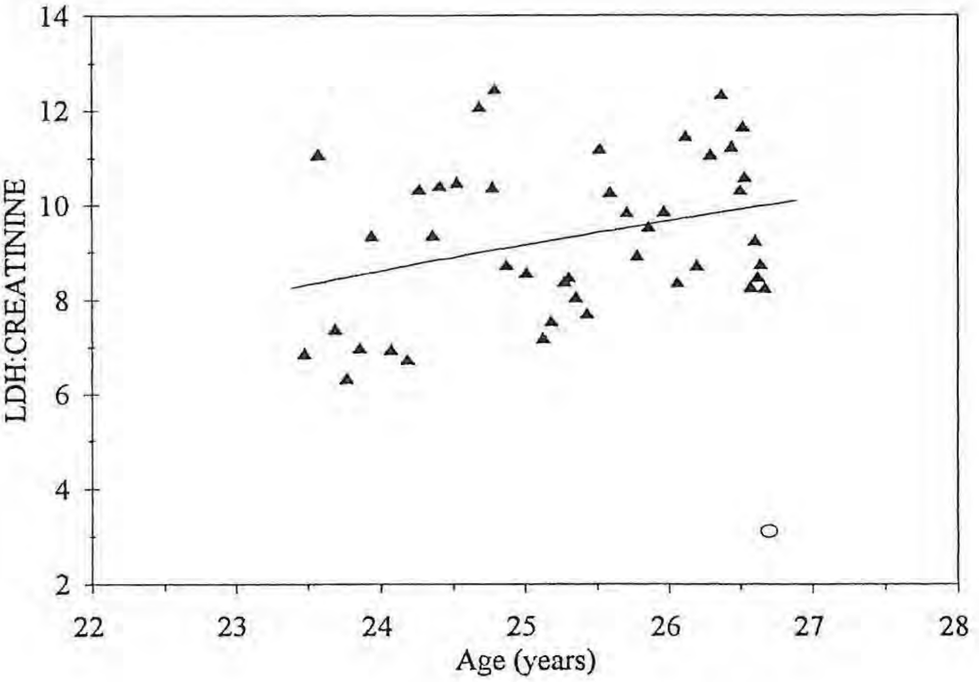
Lactate dehydrogenase was positively related to weight in the two younger, male dolphins only (Table 4.6). Data from both Simo and Domino were variable but LDH levels measured at heavier weights were predominantly higher than those measured at weights below 185 kg and 135 kg, respectively. Several outliers (>1 750 U/l) were measured within Simo's "normal" weight range. Three animals showed significant positive correlations between LDH:creatinine and weight (Table 4.6). Trends for Dimple and Simo were complex and ill defined, but Domino's LDH:creatinine gradually increased with increasing weight. Several extremely high ratios were measured within the "normal" weight range for both Simo and Domino.

Concentrations of LDH were also positively correlated with blubber thickness for Domino (Table 4.6). Although data were limited, LDH levels increased, from approximately 900 U/l to 1 300 U/l, as blubber thickness increased from 17.50 mm to 24.00 mm. This increase was particularly marked at blubber thicknesses greater than 21.00 mm (Fig. 4.45).

Thandi was the only animal for which LDH was significantly related with food consumption (Table 4.6). This relationship was difficult to define, but enzyme levels were more variable; and low LDH levels were measured more frequently; at food consumption levels of less than 60 kg/week (Fig. 4.46A). LDH:creatinine was also positively related to food consumption for Thandi and Domino (Table 4.6). The relationship between these parameters for Thandi was almost identical to that described for LDH (Fig. 4.46B). Domino's LDH levels appeared relatively stable at food consumption levels between 25 and 40 kg/week, increasing only at consumption levels greater than this (Fig. 4.46C). There was no clear association between outlying high LDH:creatinine values and aberrant levels of food consumption.

Concentrations of LDH, in conjunction with LDH:creatinine, from Thandi and Simo were significantly correlated with the dietary energy intake of the day prior to the blood sample. Domino's LDH:creatinine ratio was positively related with this diet category (Table 4.6). Thandi's data were highly variable resulting in an ill-defined relationship between these parameters (Fig. 4.47A). Simo's data were similarly highly variable, but LDH levels and LDH:creatinine values appeared to decline as energy intake of the previous day increased (Fig. 4.47B). This relationship for Domino was similar to the one described between LDH and food consumption for the same animal (Fig. 4.47C).

A. DOLLY



B. DOMINO

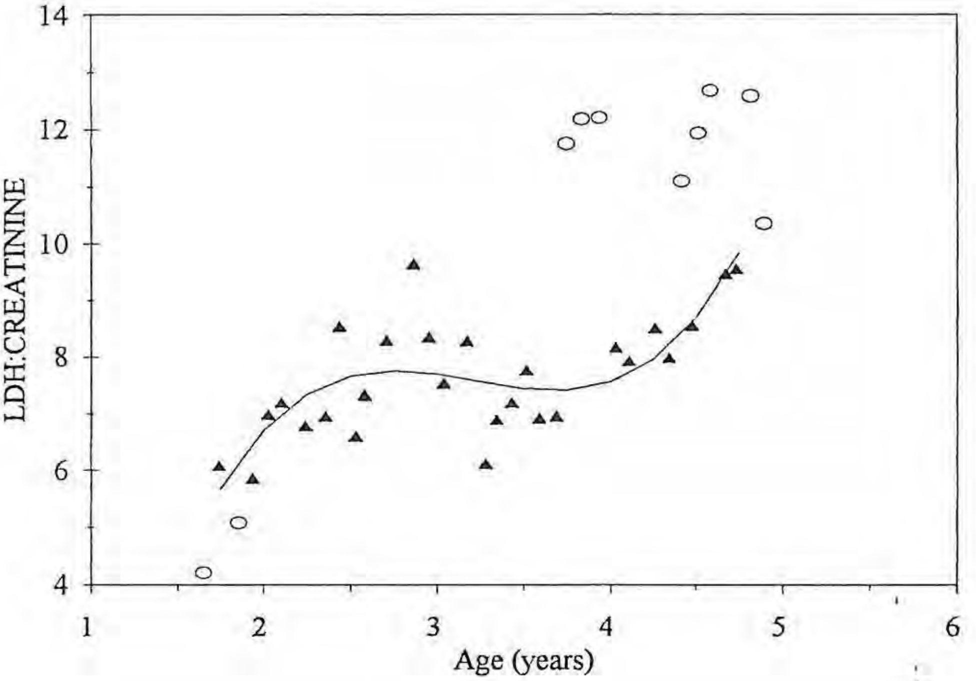


Figure 4.44: Curves of best fit (—) for animals (A. Dolly; B. Domino) with significant correlations ($p<0.05$) between the LDH:creatinine ratio and age. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

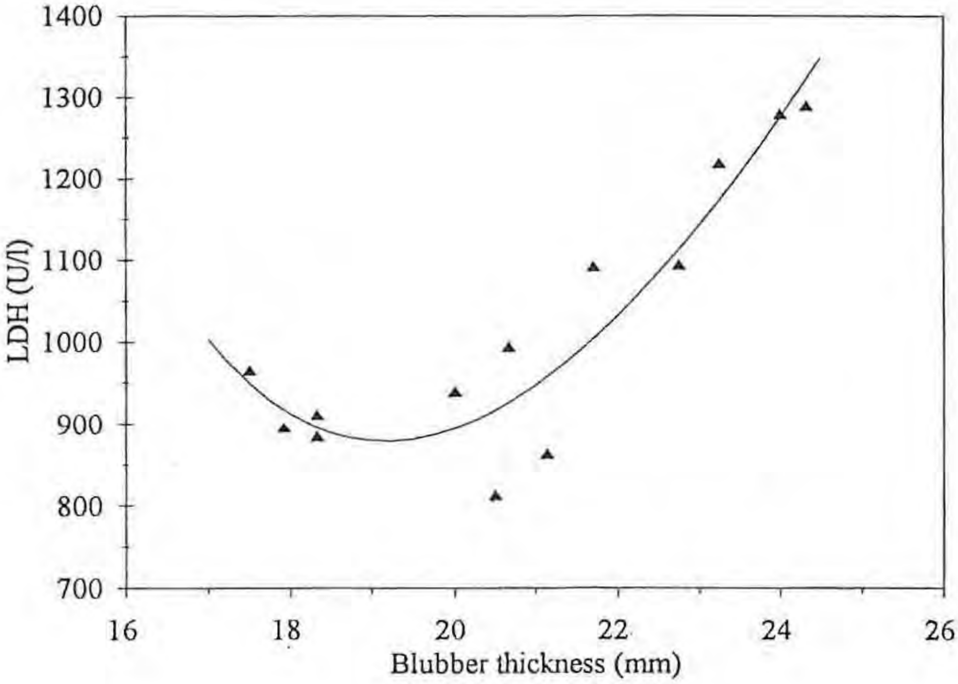


Figure 4.45: Curve of best fit (—) for Domino with a significant correlation ($p<0.05$) between lactate dehydrogenase (LDH) and blubber thickness. Included in the graph are all "normal" (see text) data (▲).

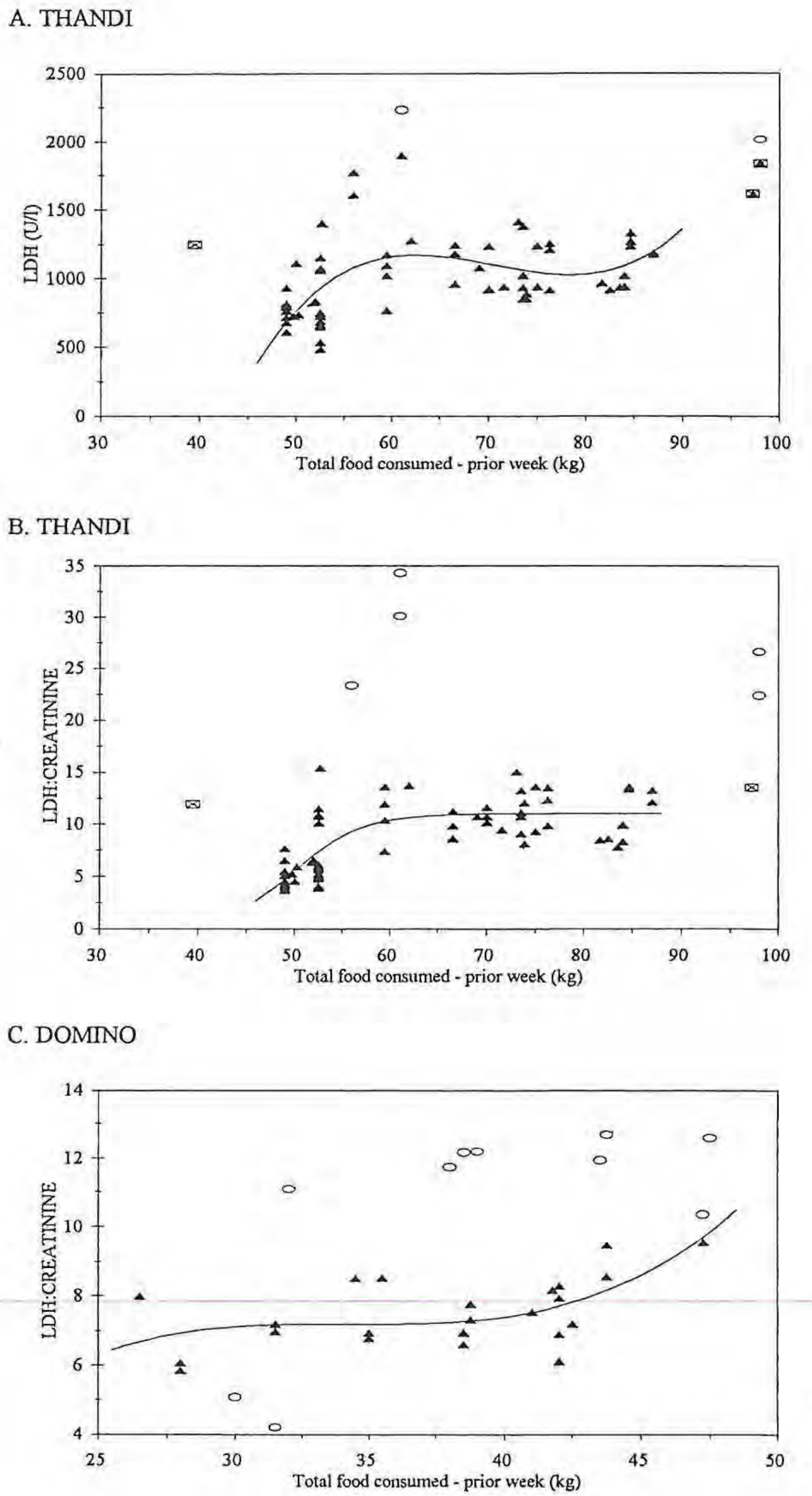
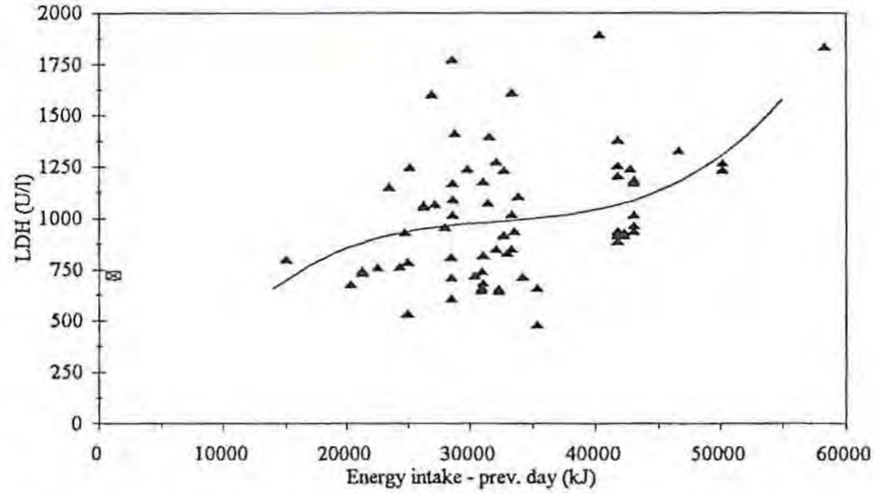
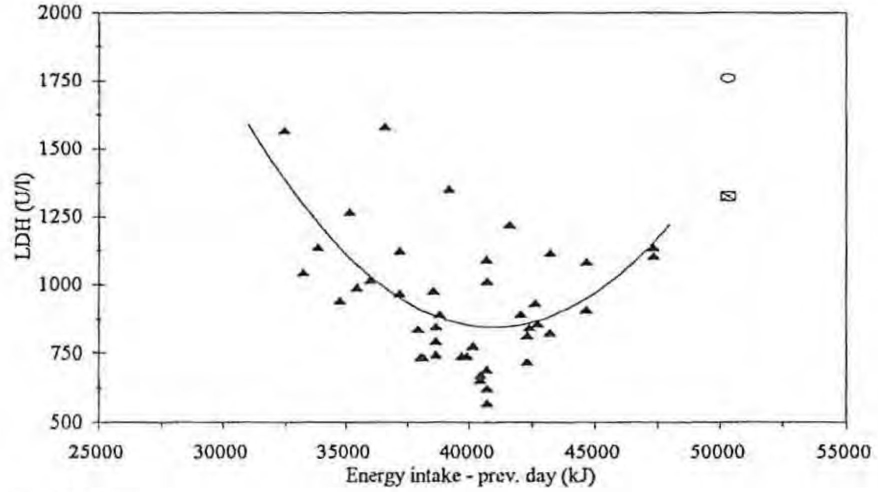


Figure 4.46: Curves of best fit (—) for animals (A&B. Thandi ; B. Domino) with significant correlations ($p<0.05$) between lactate dehydrogenase (LDH) (A), LDH:creatinine (B&C) and total food consumed in the week prior to blood sampling. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

A. THANDI



B. SIMO



C. DOMINO

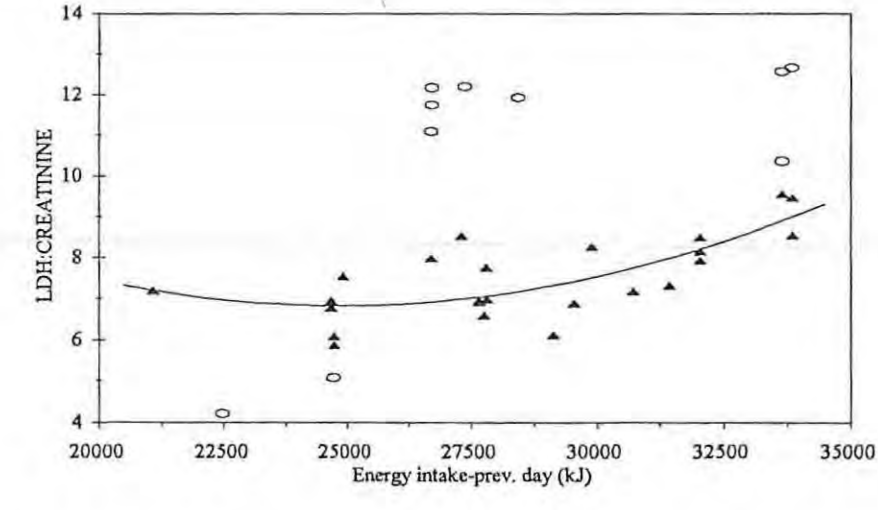


Figure 4.47: Curves of best fit (—) for animals (A. Thandi; B. Simo; C. Domino) with significant correlations ($p<0.05$) between lactate dehydrogenase (LDH) (A&B), LDH:creatinine (C) and the dietary energy intake of the day prior to the blood sample. Included in the graphs are all "normal" (see text) data (▲) and statistically determined (x-axis: ⊠; y-axis: ○).

The LDH levels of Thandi, Simo and Domino were significantly correlated with the mean daily energy intake of the week in which blood was sampled (Table 4.6). Although data were variable, LDH in both Thandi and Domino increased with increased energy intake during this period (Fig. 4.48). This relationship for Simo was complex, with reduced LDH levels measured more frequently at energy intake levels around 40 000 kJ/day (Fig. 4.48 D). Energy intake levels above the "norm" (for Simo) were associated with "normal" LDH levels (Fig. 4.48C). However, outlying high LDH levels, from Thandi, were measured during periods of significantly increased energy intake (Fig. 4.48A). The LDH:creatinine ratio was positively correlated with this diet category for Thandi and Domino (Table 4.6). Thandi's relationship between these parameters was almost identical to that of LDH.

Thandi was the only animal for which LDH was significantly related with the previous week's mean daily energy intake (Table 4.6). This relationship was almost identical to other dietary relationships described for this animal. Data from Dolly, Thandi and Domino implied that LDH:creatinine increased significantly with increased energy intake during the week preceding the blood sample (Table 4.6). However, Dolly's data were widely spread and the positive trend was difficult to define (Fig. 4.49A). The increasing trend was more defined for Thandi and Domino, both exhibiting similar increased LDH:creatinine ratios at higher levels of energy intake (Fig. 4.49B & C).

Data from Dolly and Thandi reflected that LDH levels were positively related to the mean daily energy intake of the month prior to the blood sample (Table 4.6). The relationship for Dolly was complicated by highly variable data, although it was similar to other dietary relationships with LDH described for this animal. LDH:creatinine was similarly positively related to the prior month's mean daily energy intake for Dolly, Thandi and Domino (Table 4.6; Fig. 4.50).

Monthly changes in LDH were evident for Thandi and Simo, the latter animal also showing a significant relationship with LDH:creatinine (Table 4.6). Thandi's LDH levels appeared to be higher between July and October (Fig. 4.51A), with all outliers associated with these months. Simo's relationships indicated a gradual increase in LDH and LDH:creatinine throughout the year (Fig. 4.51B&C). Because of the lack of continuity in this trend, it was assumed that this correlation, although significant, was artifactual.

Levels of LDH were negatively correlated to water temperature for Dimple, Dolly, Thandi and Simo (Table 4.6). Although the exact nature of the correlations and curves of best fit differed slightly between animals, LDH appeared to be higher and more variable below water temperatures of 20°C. In addition, outlying high LDH levels corresponded only with water temperatures below 20°C (Fig. 4.52). The same four animals had negative correlations between LDH:creatinine and

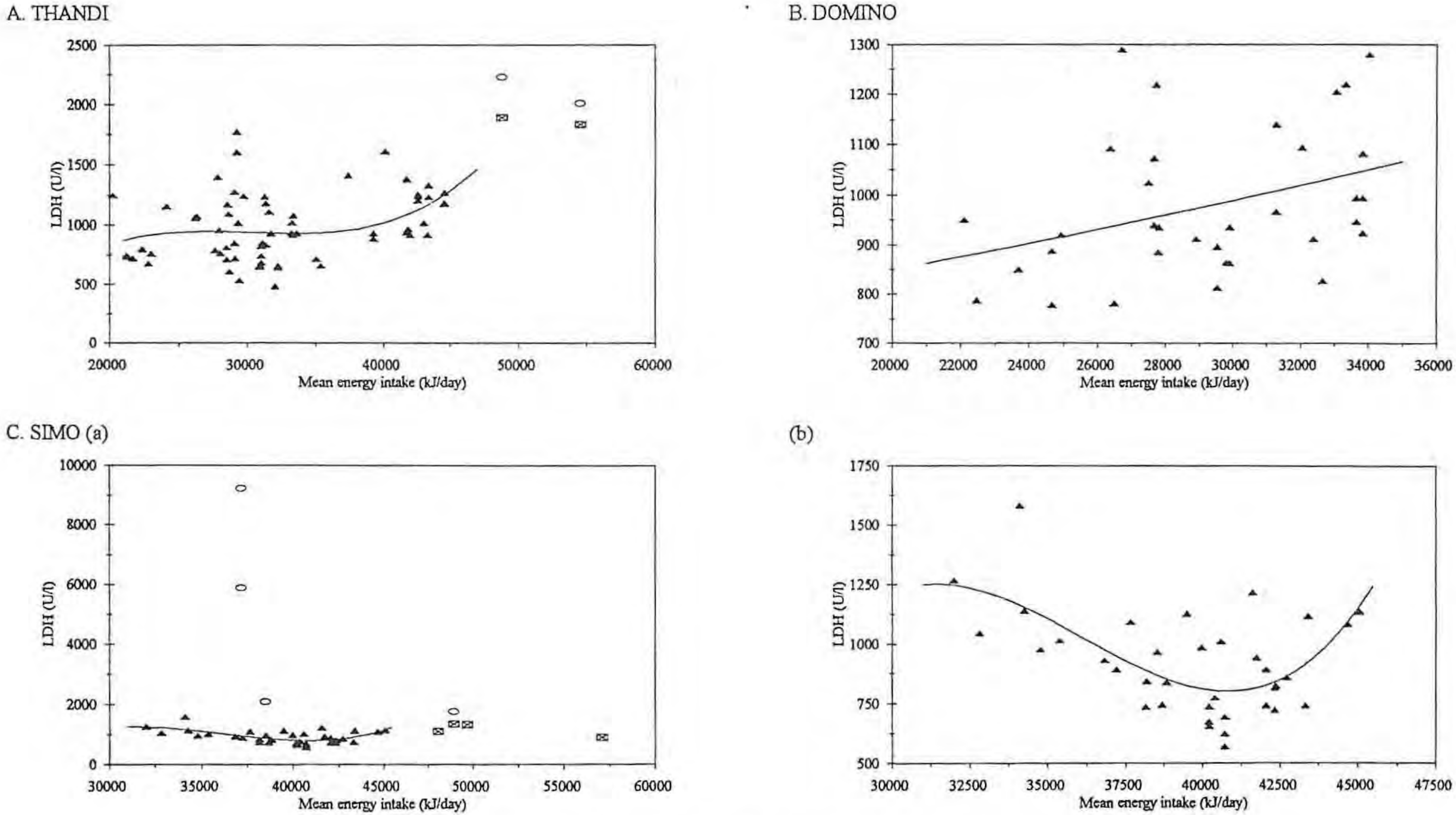
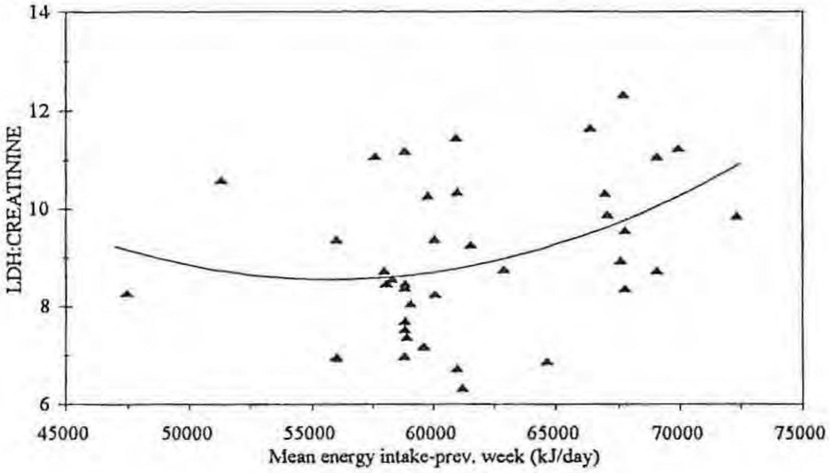
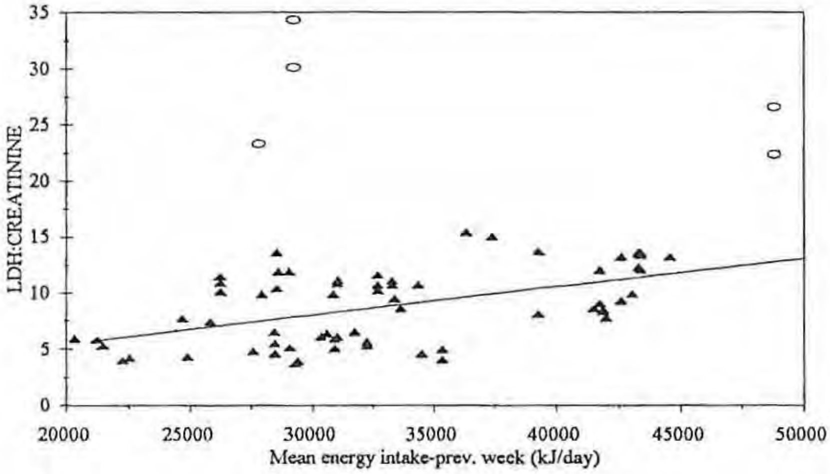


Figure 4.48: Curves of best fit (—) for animals (A. Thandi; B. Domino; C. Simo (a & b)) with significant correlations ($p<0.05$) between lactate dehydrogenase (LDH) and the mean daily dietary energy intake of the week in which blood was sampled. Included in the graphs are all "normal" (see text) data (▲)and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

A. DOLLY



B. THANDI



C. DOMINO

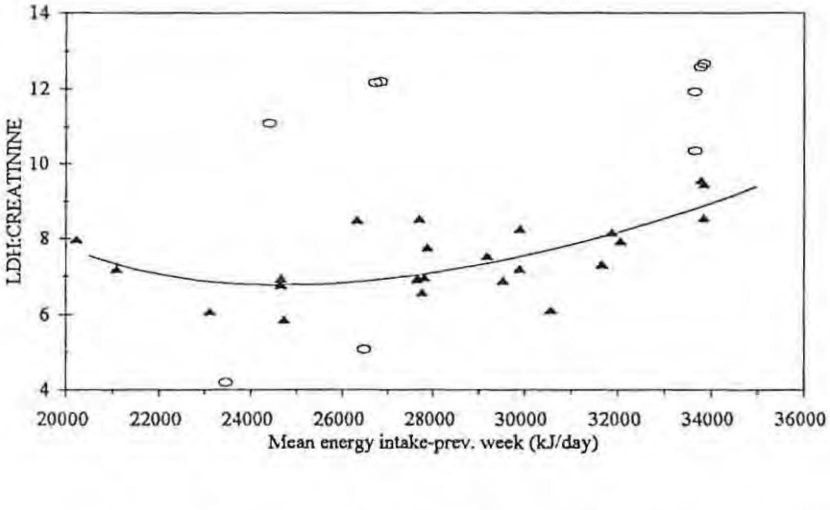
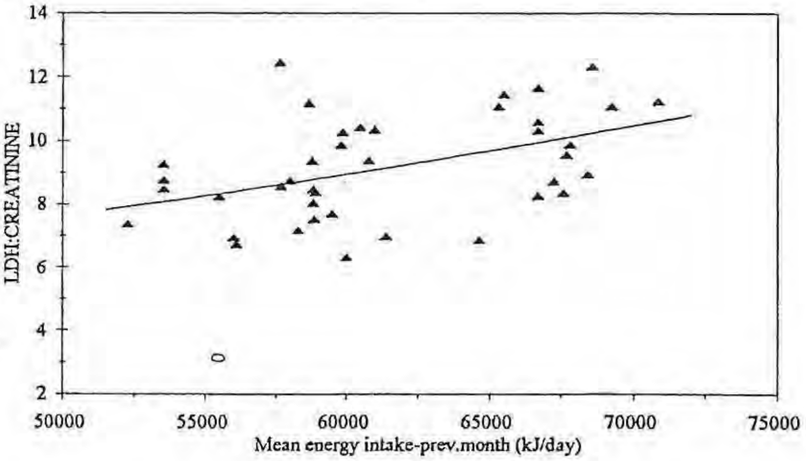
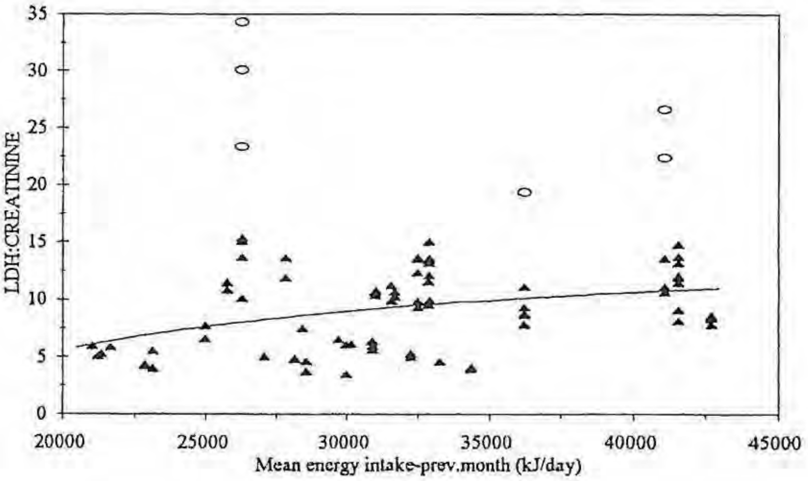


Figure 4.49: Curves of best fit (—) for animals (A. Dolly; B. Thandi; C. Domino) with significant correlations ($p<0.05$) between LDH:creatinine and the mean daily energy intake of the week prior to the blood sample. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

A. DOLLY



B. THANDI



C. DOMINO

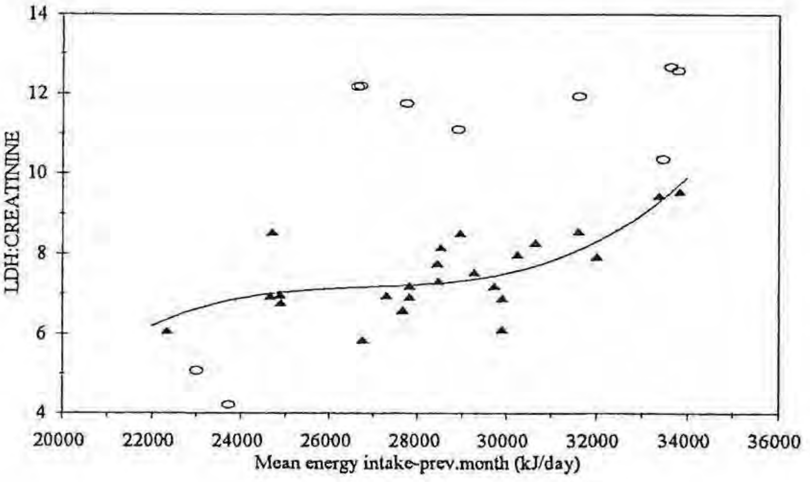
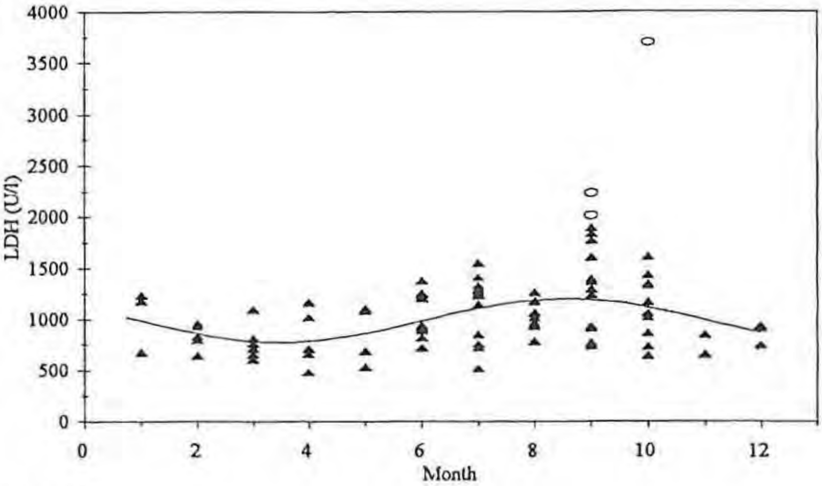
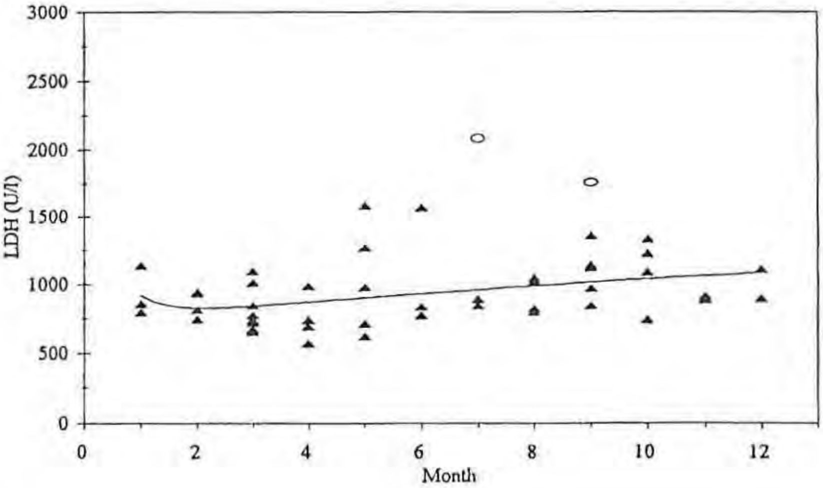


Figure 4.50: Curves of best fit (—) for animals (A. Dolly; B. Thandi; C. Domino) with significant correlations ($p<0.05$) between LDH:creatinine and the mean daily energy intake of the month prior to the blood sample. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

A. THANDI



B. SIMO (a)



(b)

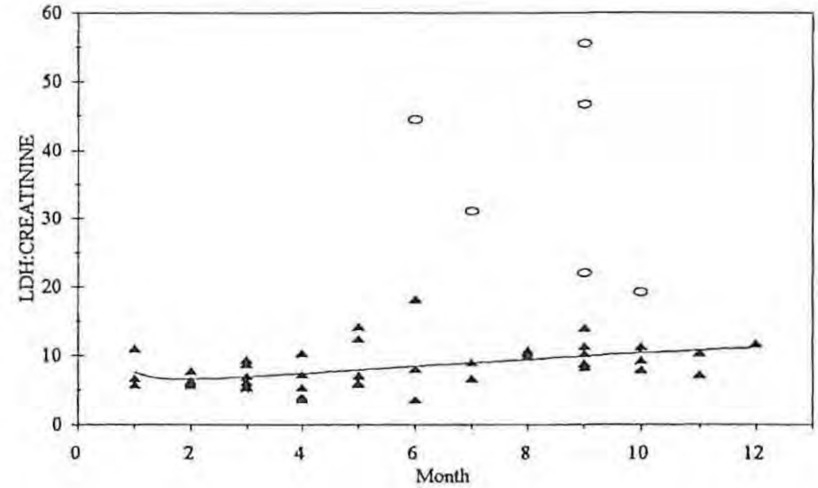


Figure 4.51: Curves of best fit (—) for animals (A. Thandi; B. Simo (a & b)) with significant correlations ($p<0.05$) between lactate dehydrogenase (LDH) (A & B.a) and LDH:creatinine (B.b) and the month of the year. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

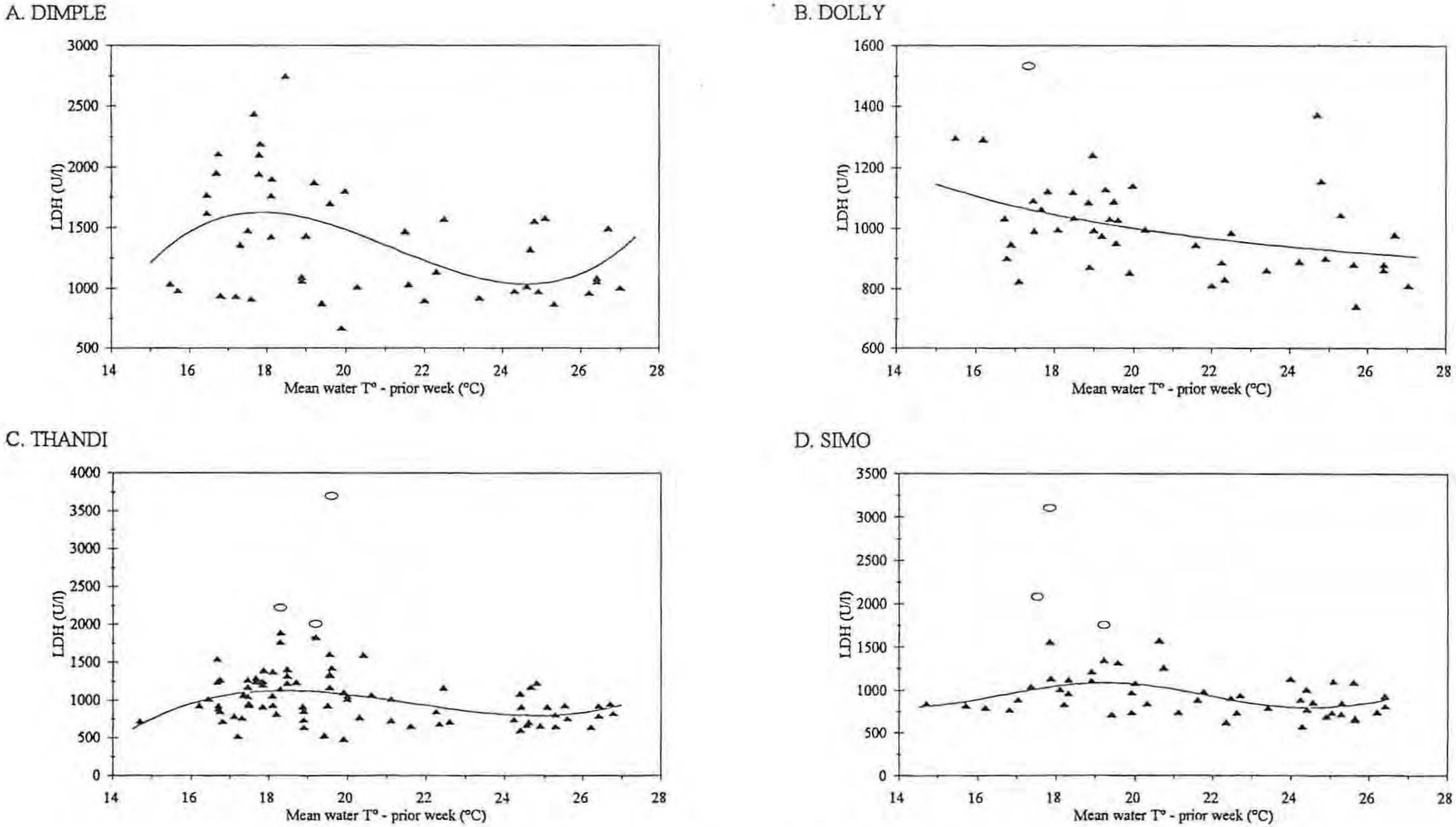


Figure 4.52: Curves of best fit (—) for animals (A. Dimple; B. Dolly; C. Thandi; D. Simo) with significant correlations ($p<0.05$) between lactate dehydrogenase (LDH) and the mean water temperature of the week prior to the blood sample. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

water temperatnre (Table 4.6). Although data were more variable, the relationships also illustrated a predominance of high LDH:creatinine values at low water temperatures.

g. Creatine phosphokinase (CPK) / CPK:creatinine

Levels of CPK in Dolly and Domino were relatively stable, relative to the marked fluctuations evident in data from Thandi and Simo. Dimple's CPK levels increased dramatically during the final stages of the study (Fig. 4.53). The majority of fluctuations could be associated with recorded periods of illness and/or treatment. Thandi's data were highly variable with several significant outliers identified (Appendix 3.4B). Domino was the only animal for which no outlying CPK levels were identified. Marked fluctuations in CPK levels corresponded with changes in CPK:creatinine, although changes in the latter were less marked and/or frequent (Fig. 4.54). The range of CPK:creatinine values from all five animals was similar, with significant outliers identified in data from all animals, except Domino (Appendix 3.5B). Thandi's highly variable data, and the removal of significant outliers, resulted in her "normal" mean CPK level (148.90 ± 58.23 U/l) being substantially, but not significantly, lower than the overall mean (225.80 ± 603.00 U/l) ($Z=0.77$; $p=0.44$; $n=170$). Domino's CPK values were the most stable by comparison and his mean CPK level (228.40 ± 44.29 U/l) was significantly higher than all other animals (Two sample t-test; $p<0.001$). The only other significant difference was between Simo (171.20 ± 51.07 U/l) and Dolly (146.30 ± 18.81 U/l) ($t=-3.00$; $p=0.004$; $df=92$) (Table 4.1). Similar to CPK, Domino had the highest mean CPK:creatinine ratio (1.90 ± 0.55), but it was only significantly higher than those of Dolly (1.36 ± 0.25) ($t=-5.70$; $p<0.001$; $df=77$) and Thandi (1.41 ± 0.74) ($t=-3.63$; $p<0.001$; $df=116$) (Table 4.1). The mean CPK:creatinine ratio's of all other animals were similar (Two sample t-tests, $p>0.005$).

As reflected by the "normal" mean CPK levels, this enzyme was highest in the juvenile animal, decreasing with age in the other four animals (Fig. 4.55A). Although the decline with age was not as clear with CPK:creatinine, younger animals also had higher ratio values (Fig. 4.55B). During the study, there were no significant age-associated changes in CPK and/or CPK:creatinine. In addition, these parameters were not significantly affected by changes in total body weight (Table 4.7). However, CPK and CPK:creatinine were significantly correlated to blubber thickness measurements of Thandi and Domino (Table 4.7). In the case of Thandi, CPK levels and ratio values were higher at lower blubber thickness measurements, decreasing at blubber thicknesses greater than 17.00 mm (Fig. 4.56A). In contrast, Domino's CPK levels and CPK:creatinine increased steadily as blubber thickness increased (Fig. 4.56B).

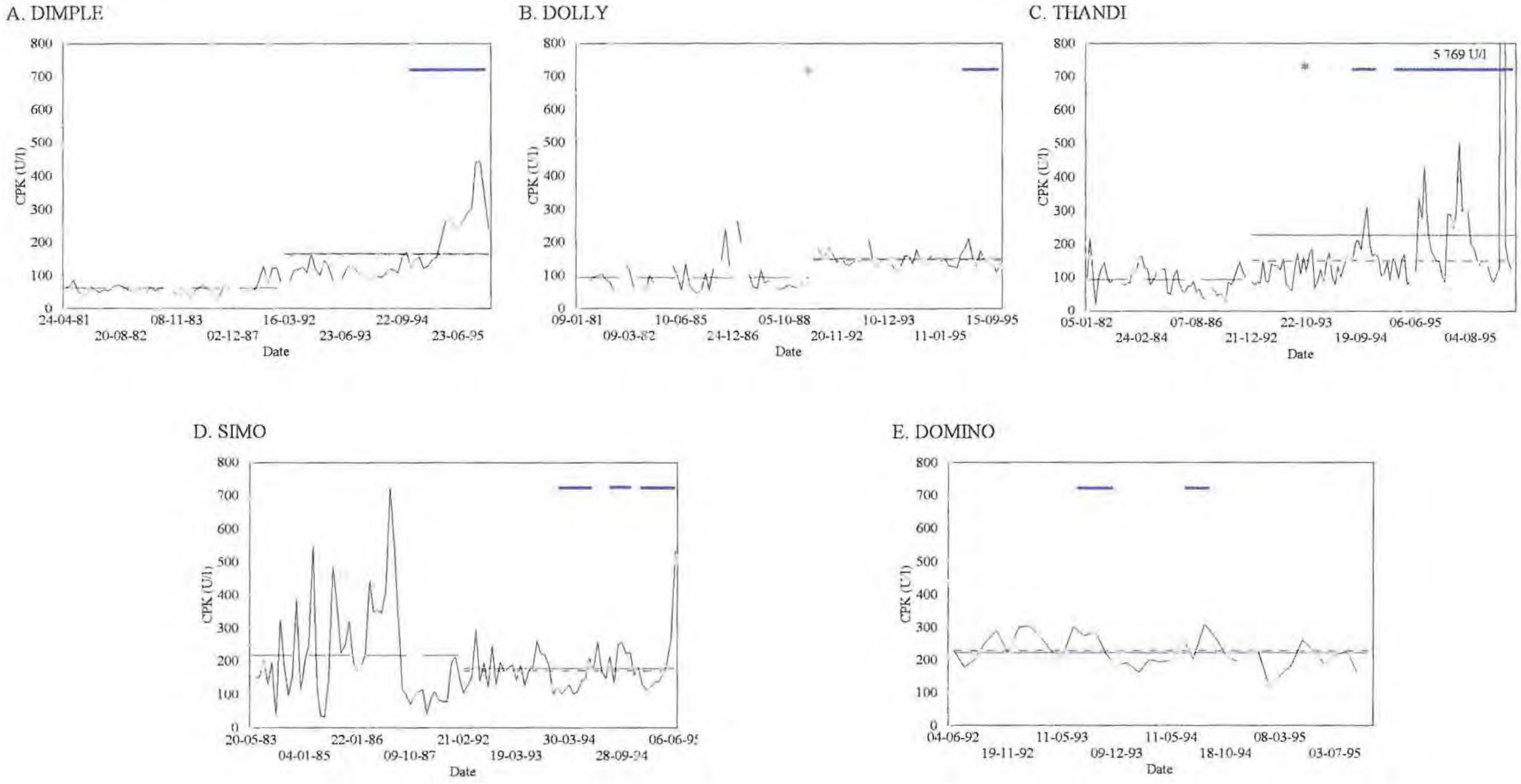


Figure 4.53: Levels of creatine phosphokinase (CPK) (U/l) (—) for all five captive animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the mean CPK level for all records prior to the study (- -); the overall mean CPK level (—) and the "normal" (see text) mean CPK level (- - -) for the study between 1992 and 1995 (*: parturition; —: extended period of illness and/or treatment or period of aberrant feeding and/or behaviour).

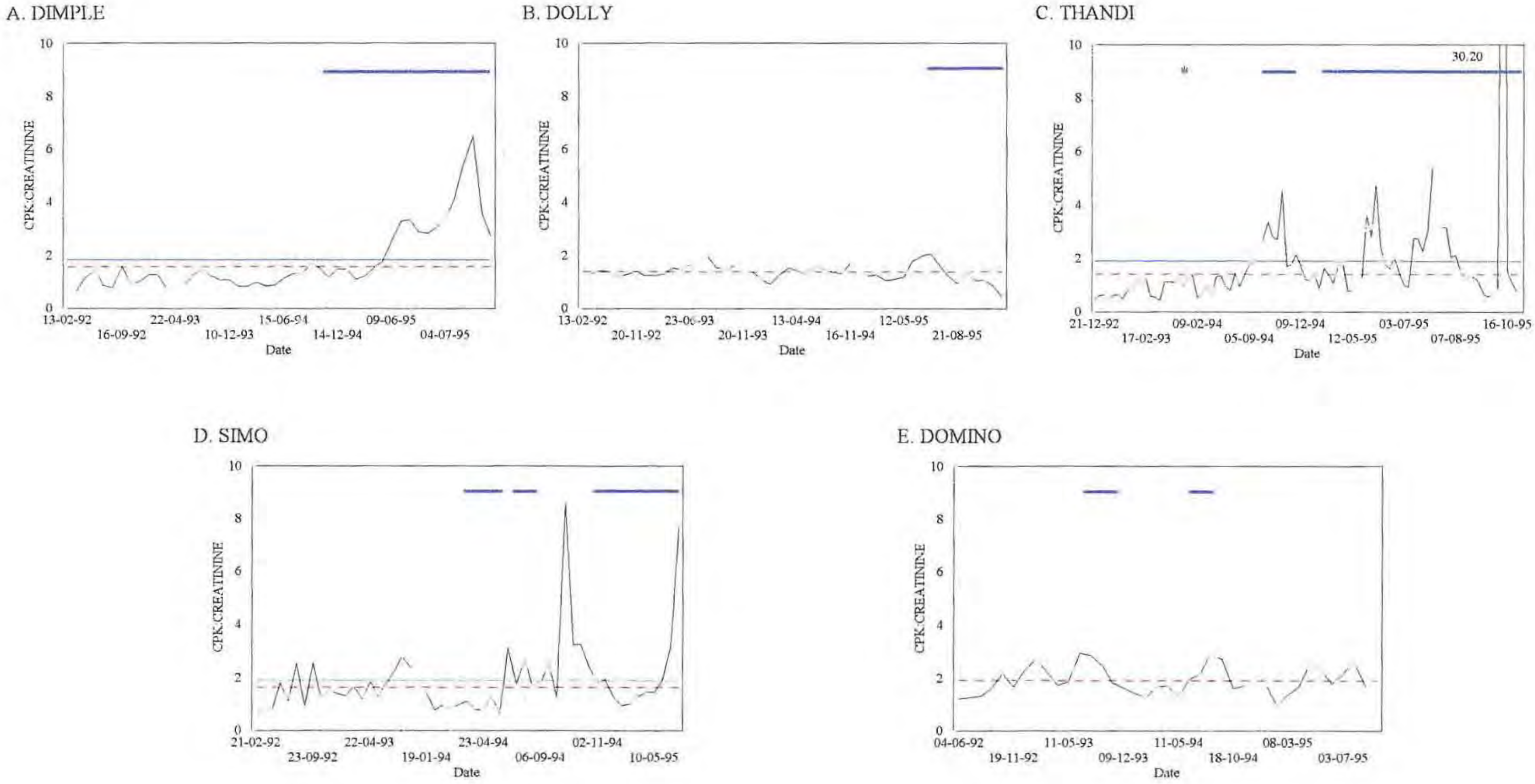


Figure 4.54: CPK:creatinine ratios (—) for all five captive animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the overall mean CPK:creatinine ratio (—) and the "normal" (see text) mean ratio (- - -) for the study between 1992 and 1995 (*: parturition; —— : extended period of illness and/or treatment or period of aberrant feeding and/or behaviour).

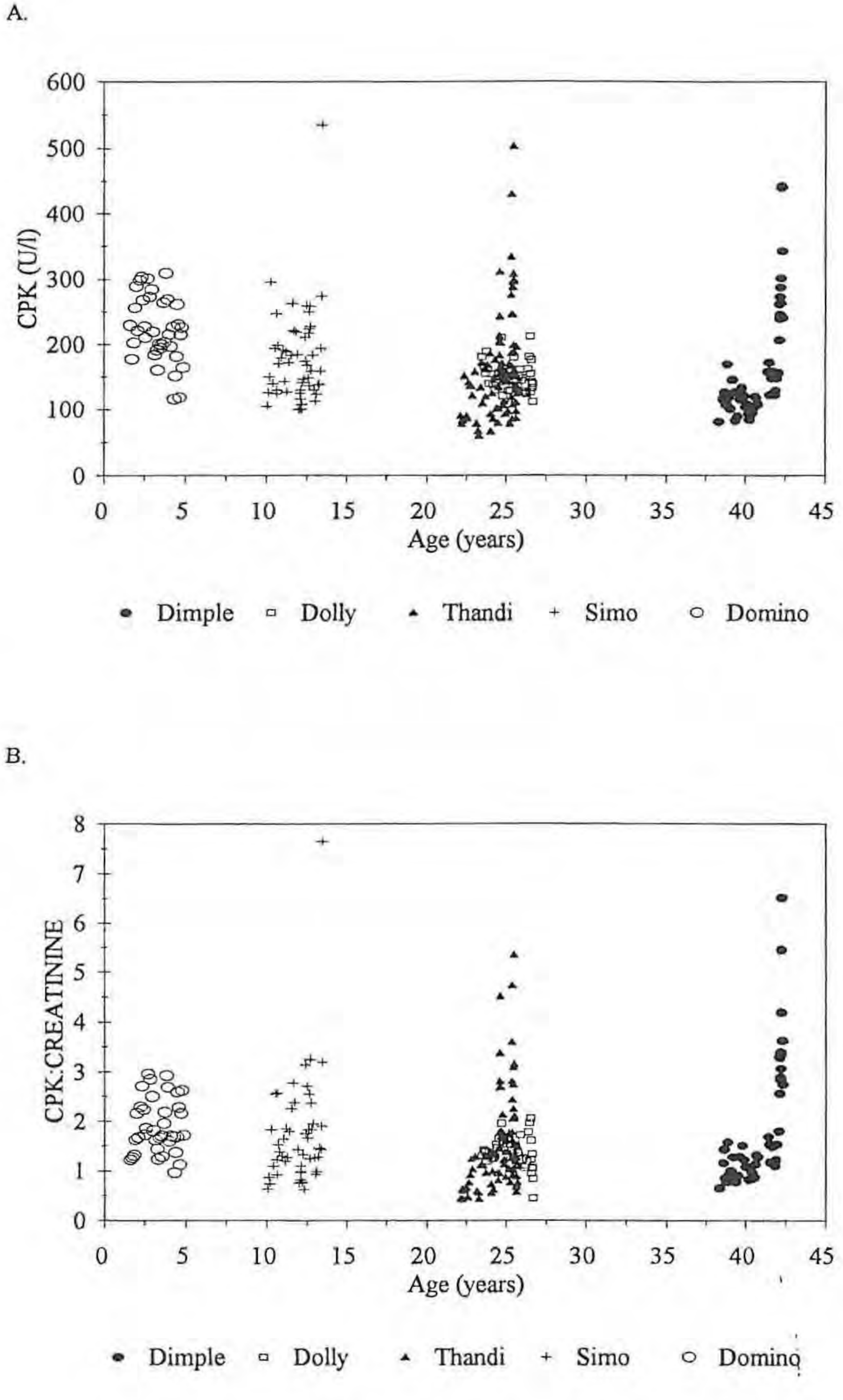


Figure 4.55: Relationship between serum levels of creatine phosphokinase (CPK) (A), the CPK:creatinine ratio (B) and age for the five captive dolphins, from records obtained between 1992 and 1995.

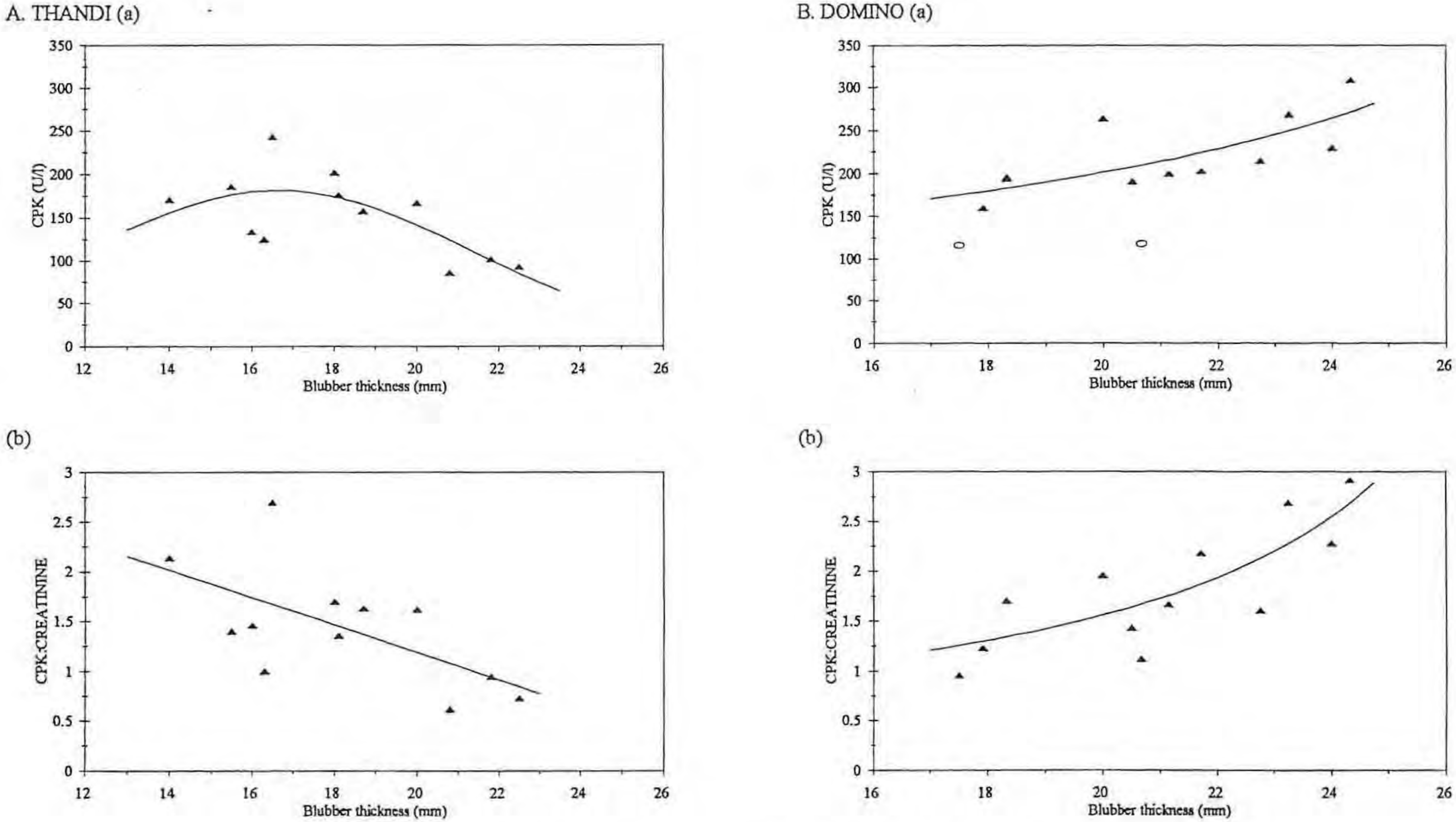


Figure 4.56: Curves of best fit (—) for animals Thandi (A: a&b) and Domino (B: a&b) with significant correlations ($p<0.05$) between creatine phosphokinase (CPK) (a), CPK:creatinine (b) and blubber thickness. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

Creatine phosphokinase was positively related to the total food consumption of the week prior to the blood sample, but only for Thandi (Table 4.7). Although data were variable, enzyme levels appeared to be increased when levels of food intake were greater (Fig. 4.57A). Both Thandi and Dolly showed a positive correlation between CPK:creatinine and food consumption (Table 4.7), however, the increasing trends were difficult to confirm for both animals (Fig. 4.57B&C). Significantly increased or decreased food consumption was associated with CPK levels and ratio values within the "normal" ranges of the respective animals.

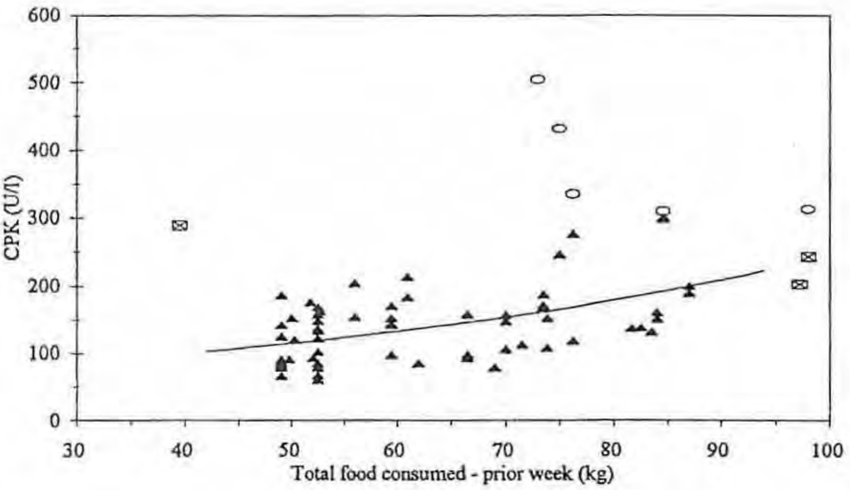
Table 4.7: Correlation coefficients (r), levels of significance (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$) and sample sizes (n) for significant relationships between creatine phosphokinase (CPK) and CPK:creatinine and independent variables for the five captive dolphins (Data are presented as r*(n)) (NS: non-significant ($p>0.05$)).

Variable		Dimple	Dolly	Thandi	Simo	Domino
Blubber thickness	a	NS	NS	-0.60*(12)	NS	0.70*(11)
	b	NS	NS	-0.62*(12)	NS	0.76**(13)
D1	a	NS	NS	0.51***(65)	NS	NS
	b	NS	0.43**(36)	0.54***(64)	NS	NS
E1	a	NS	NS	0.50***(67)	NS	NS
	b	NS	NS	0.54***(66)	NS	NS
E2	a	-0.38*(33)	NS	0.40***(65)	NS	-0.38*(32)
	b	-0.47**(38)	NS	0.44***(64)	NS	NS
E3	a	-0.44**(33)	NS	0.44***(68)	NS	NS
	b	NS	0.32*(37)	0.43***(67)	NS	NS
E4	a	NS	NS	0.29*(78)	NS	-0.39*(32)
	b	NS	0.35*(41)	0.25*(77)	NS	NS
Month	a	NS	NS	NS	0.50***(52)	NS
	b	NS	NS	0.22*(81)	0.57***(50)	NS
T1	a	NS	-0.38*(42)	-0.34**(80)	-0.46***(52)	NS
	b	-0.52***(43)	-0.34*(42)	-0.32**(79)	-0.54***(50)	NS
T2	a	-0.44**(38)	-0.36*(42)	-0.35**(79)	-0.53***(51)	NS
	b	-0.52***(43)	-0.30*(42)	-0.32**(78)	-0.60***(49)	NS
T3	a	-0.32*(38)	NS	-0.39***(80)	-0.55***(52)	NS
	b	-0.42**(43)	NS	-0.37***(79)	-0.62***(50)	-0.32*(37)

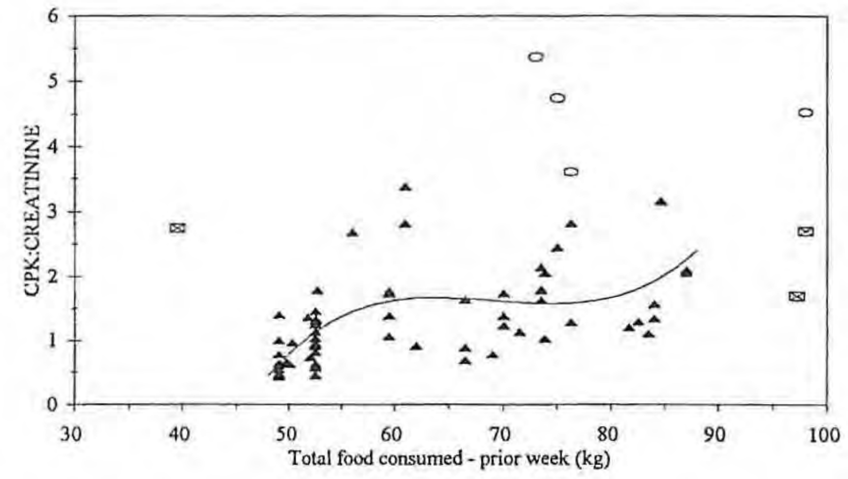
D1: Total food consumption of week prior to blood sample; E1: Dietary energy intake of day prior to blood sample; E2-E4: Mean daily energy intake of week in which blood was sampled, and week and month prior to blood sample, respectively, T1: Mean water temperature of week prior to blood sample, T2: Mean water temperature of month in which blood was sampled, T3: Mean water temperature of month prior to blood sample.

Thandi's CPK levels and CPK:creatinine ratio were also positively correlated with the dietary energy intake of the day prior to the blood sample (Table 4.7). The relationships described by the curves of best fit were similar, if complex, for both parameters. CPK levels and ratio values were

A. THANDI (a)



(b)



B. DOLLY

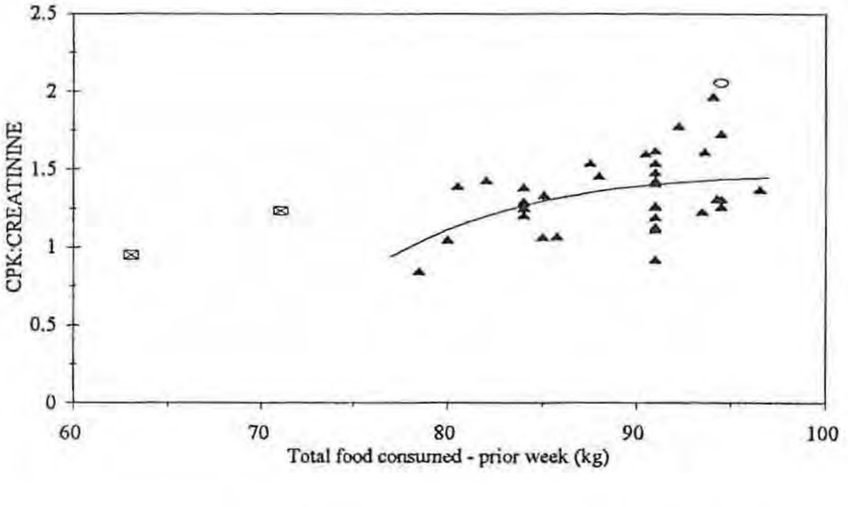


Figure 4.57: Curves of best fit (—) for animals (A. Thandi (a & b); B. Dolly) with significant correlations ($p<0.05$) between creatine phosphokinase (CPK) (A.a) and CPK: creatinine (A.b & B) and total food consumed in the week prior to blood sampling. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

predominantly increased when energy intake was greater than 40 000 kJ/day (Fig. 4.58). CPK concentrations from Dimple, Thandi and Domino were significantly correlated with the mean daily energy intake of the week in which blood was sampled (Table 4.7). Thandi's relationship between these two parameters corresponded with that described for the previous dietary energy category. Although data from Dimple and Domino were variable, CPK levels measured during reduced energy intake were predominantly higher than those measured when energy intake was greater than 32 000 kJ/day and 29 000 kJ/day, respectively (Fig. 4.59). Although some outlying CPK values were associated with significantly reduced energy intake, most were within the "normal" range. CPK:creatinine from Dimple and Thandi were also similarly correlated with this diet category (Table 4.7). The relationships between these parameters, for both animals, were identical to those described for CPK.

Dimple and Thandi's CPK levels were negatively and positively correlated with the mean daily energy intake of the week before the blood sample, respectively (Table 4.7). Similar to other relationships, Dimple's CPK was generally increased at lower energy intake levels, below 32 000 kJ/day (Fig. 4.60A). In contrast, CPK levels in Thandi appeared to increase slightly with increased energy intake (Fig. 4.60B). CPK:creatinine was positively related to this diet category for Dolly and Thandi (Table 4.7). Although ratios were variable, particularly for Dolly, CPK:creatinine tended to be higher at greater levels of energy intake.

Thandi and Domino were the only two animals for which CPK was significantly related to the mean energy intake of the month prior to the blood sample. Additionally, CPK:creatinine values from Dolly and Thandi were positively correlated with this long-term diet category (Table 4.7). Thandi's data reflected a similar relationship to those described previously, a slight and gradual increase in CPK levels and ratio values with increased energy intake. Domino's relationship was complex, with CPK levels highest at energy intake levels between 24 000 kJ/day and 27 000 kJ/day and declining as energy intake increased from 27 000 kJ/day to 32 000 kJ/day. Variable data at energy intake levels below 24 000 kJ/day and above 32 000 kJ/day made this relationship difficult to confirm (Fig. 4.61A). Similarly, the positive correlation between CPK:creatinine and this diet category for Dolly was not distinct (Fig. 4.61B).

Seasonal changes in CPK and CPK:creatinine were similar for Thandi and Simo (Table 4.7). Although the trend for CPK was not particularly clear, concentrations were slightly increased during winter and spring (Fig. 4.62A). A similar relationship was evident for CPK:creatinine, with values (and variability) increasing between June and October. The majority of significant outliers were associated with these months (Fig. 4.62B&C). Water temperature had a significant negative effect on CPK and CPK:creatinine of all five animals (Table 4.7). Although trends differed slightly, relationships between CPK levels, ratio values and all categories of water temperature were almost

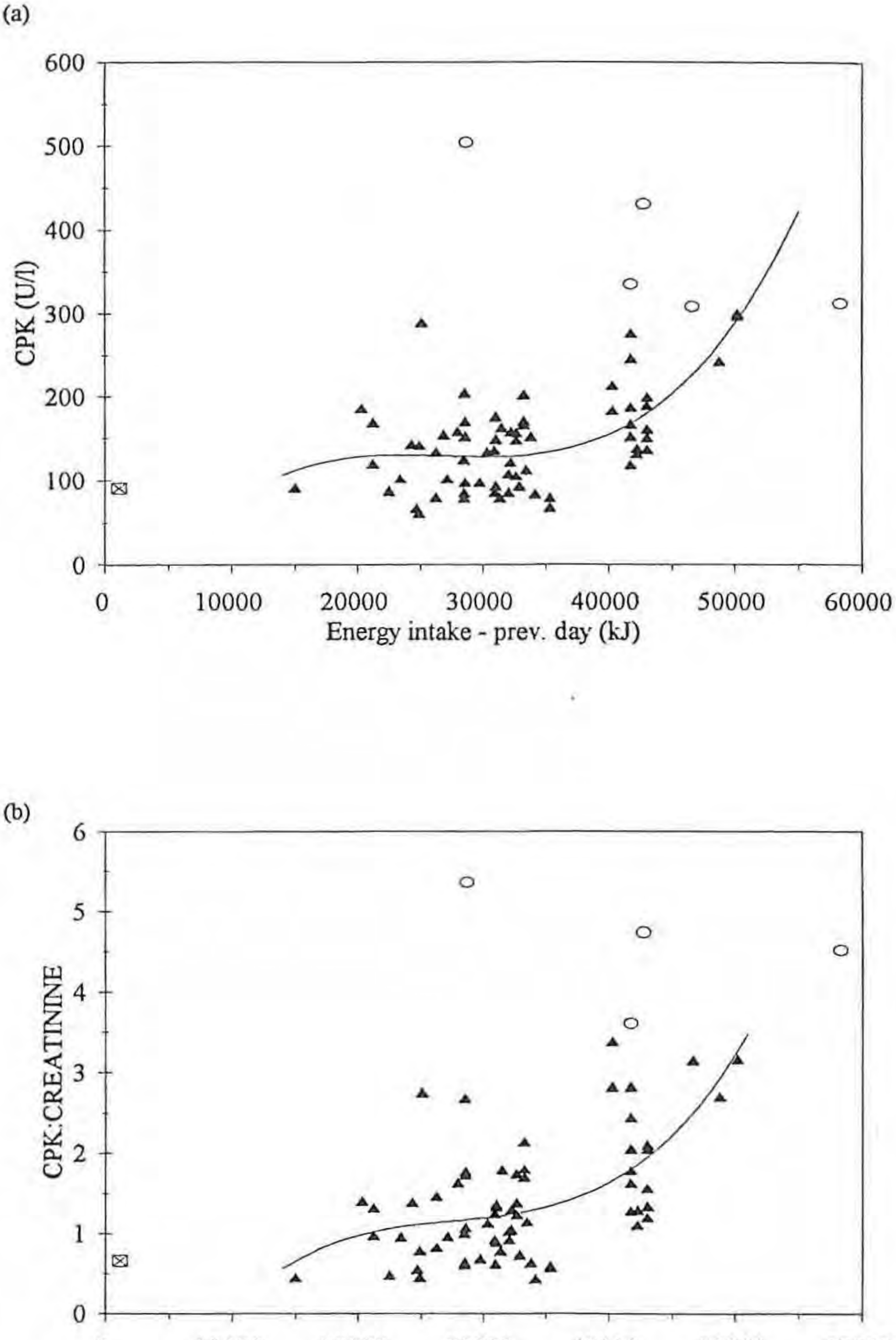
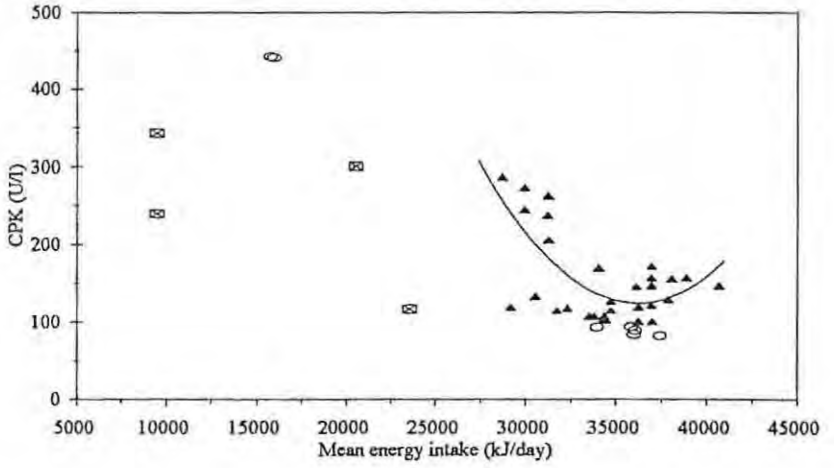
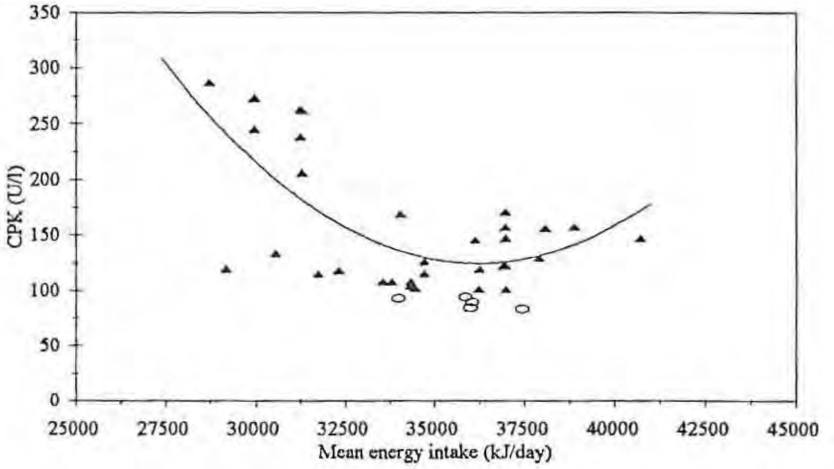


Figure 4.58: Curves of best fit (—) for Thandi with significant correlations between creatine phosphokinase (CPK) (a) and CPK:creatinine (b) and the dietary energy intake of the day prior to the blood sample. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ⊠ ; y-axis: ○).

A. DIMPLE (a)



(b)



B. DOMINO

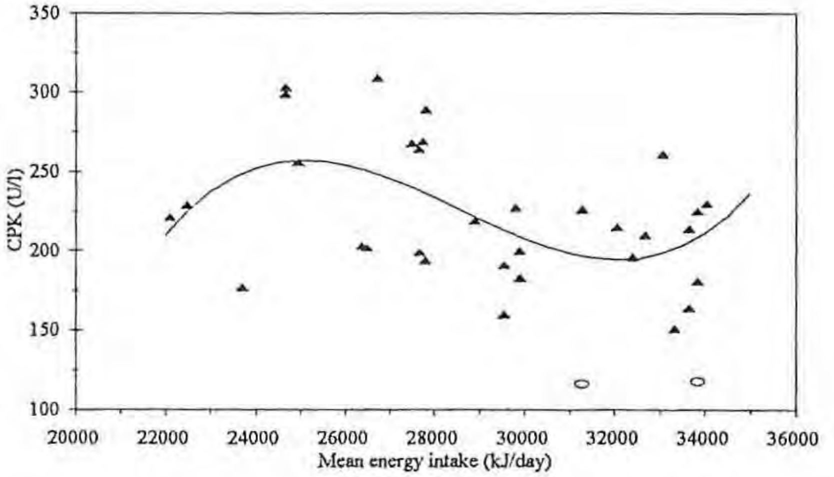
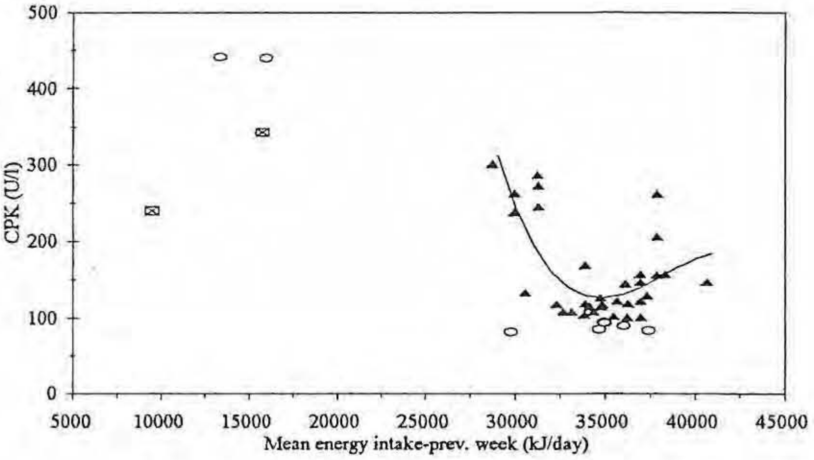
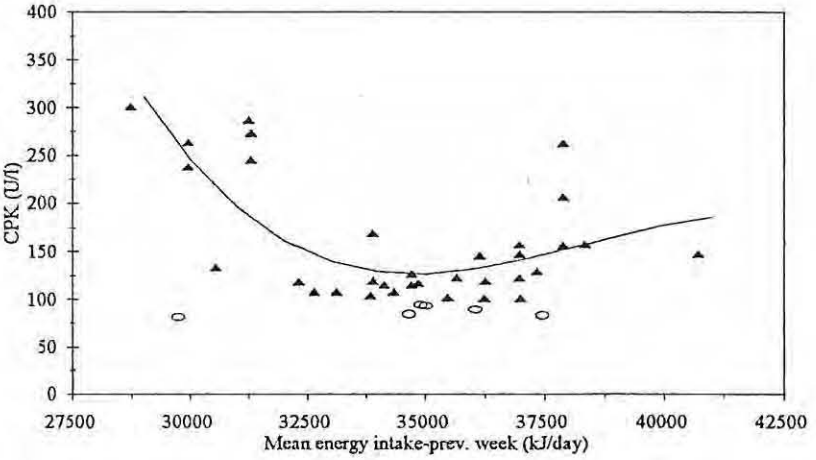


Figure 4.59: Curves of best fit (—) for animals (A. Dimple (a & b); B. Domino) with significant correlations ($p<0.05$) between creatine phosphokinase (CPK) and the mean daily dietary energy intake of the week in which blood was sampled. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ⊠ ; y-axis: ○).

A. DIMPLE (a)



(b)



B. THANDI

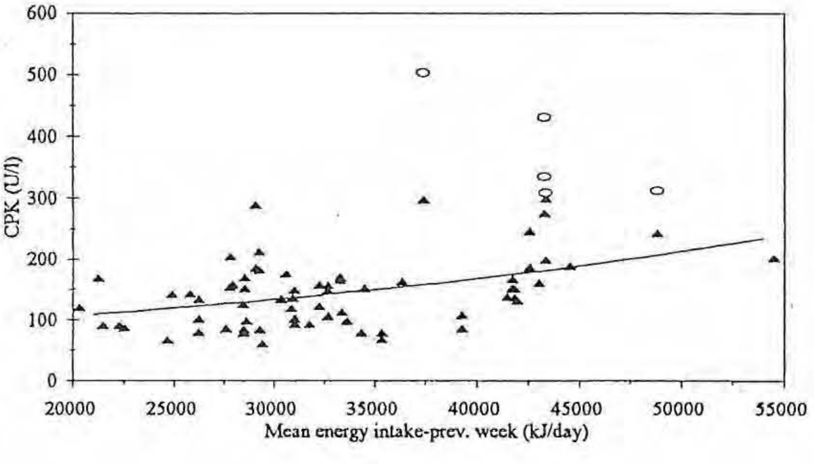


Figure 4.60: Curves of best fit (—) for animals (A. Dimple (a & b): B. Thandi) with significant correlations ($p<0.05$) between creatine phosphokinase (CPK) and the mean daily energy intake of the week prior to the blood sample. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

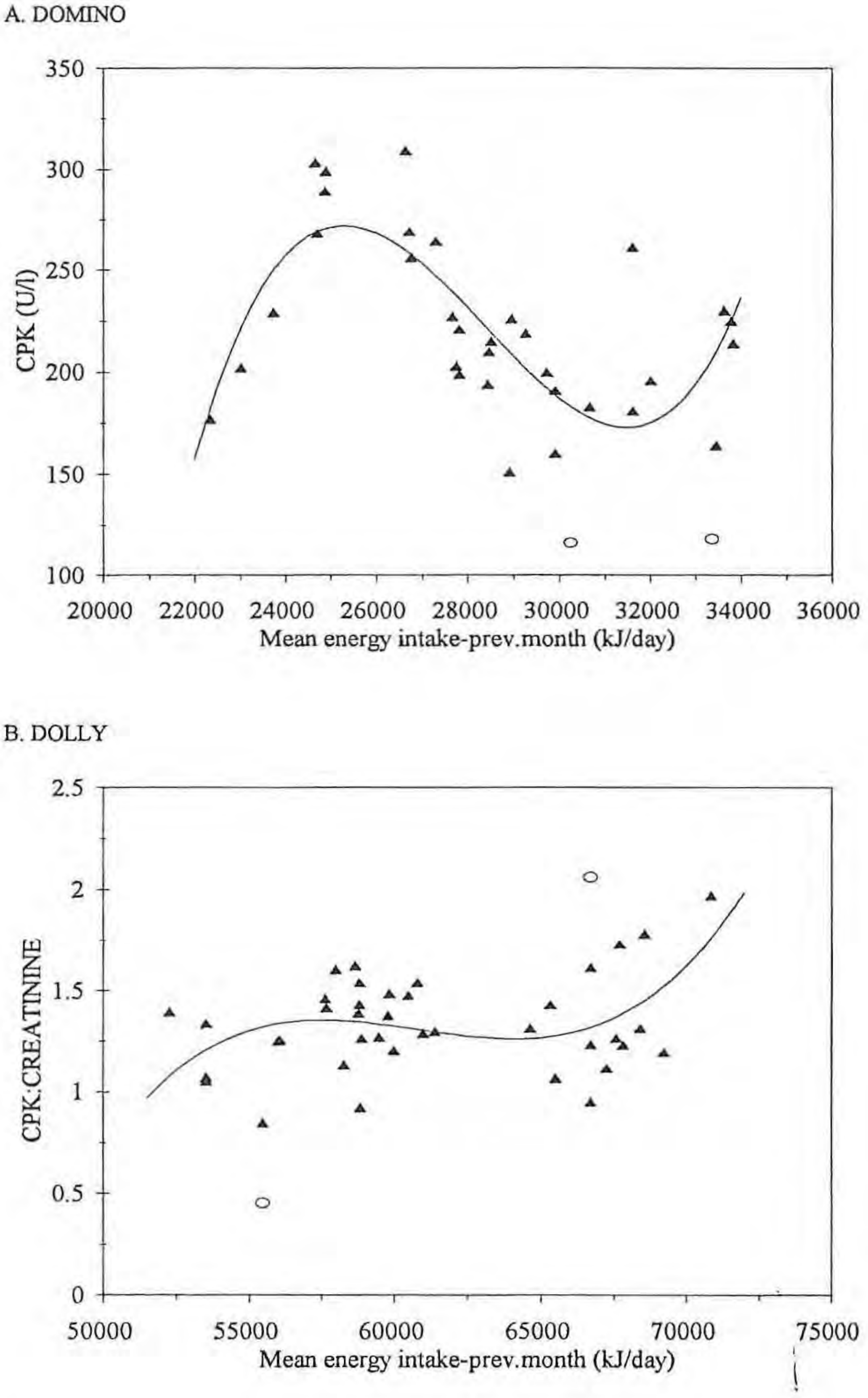
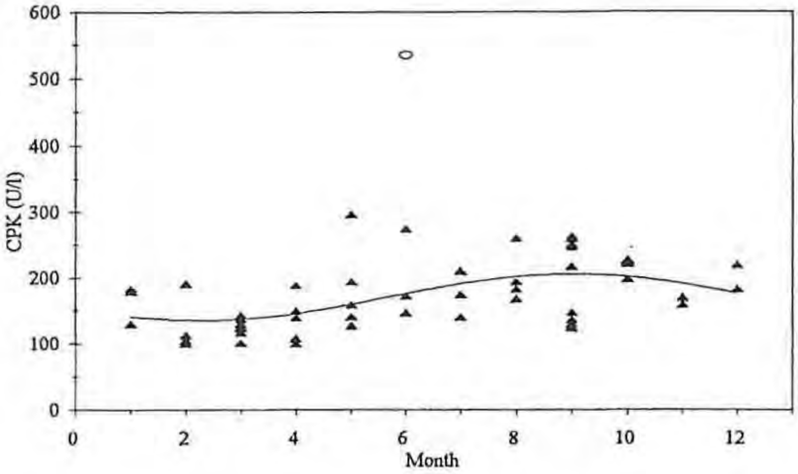
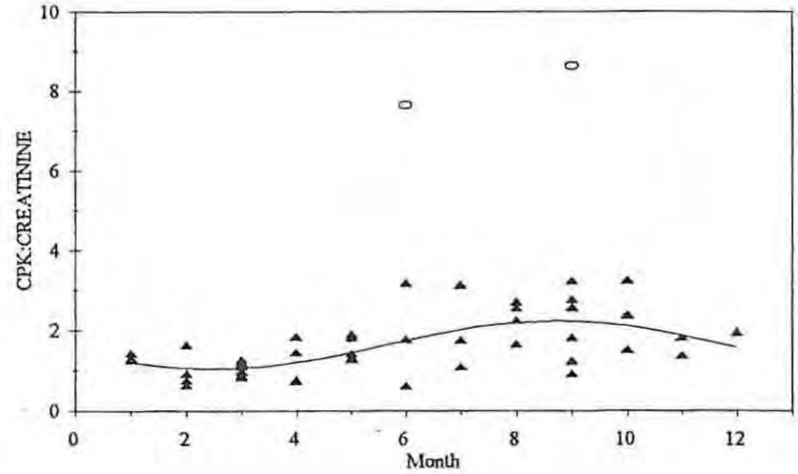


Figure 4.61: Curves of best fit (—) for animals (A. Domino; B. Dolly) with significant correlations ($p<0.05$) between creatine phosphokinase (CPK) (A) and the CPK:creatinine ratio (B) and the mean daily energy intake of the month prior to the blood sample. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

A. SIMO (a)



(b)



B. THANDI

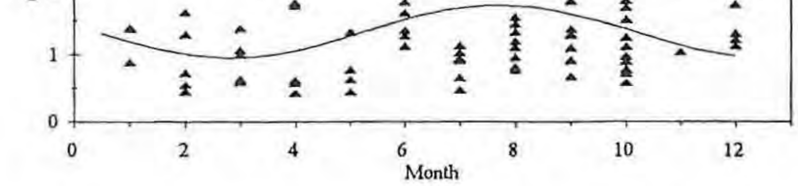


Figure 4.62: Curves of best fit (—) for animals (A. Simo (a & b); B. Thandi) with significant correlations ($p<0.05$) between creatine phosphokinase (CPK) (A.a) and CPK:creatinine (A.b & B) and the month of the year. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

identical for all four animals, reflecting that CPK and CPK:creatinine were slightly higher when water temperatures were below 21°C (for example, Fig. 4.63). Outlying high and low CPK levels were associated with water temperatures below 19°C and above 24°C, respectively (Fig. 4.63A).

h. Sodium / Sodium:creatinine

Sodium levels of all five animals fluctuated considerably during the study. Fluctuations were most frequent for Thandi (Fig. 4.64C). This high variability resulted in the identification of very few outlying values (Appendix 3.6A). It was difficult to associate particular changes in serum sodium with periods of illness and/or treatment (Fig. 4.64). The sodium:creatinine ratio showed relatively little fluctuation (Fig. 4.65). Only Simo showed a single dramatic increase to 5.45 (Fig. 4.65D). The restricted range of sodium:creatinine values resulted in the identification of several outlying data (Appendix 3.6B).

Overall, "normal" mean sodium levels appeared similar, but Simo's mean sodium concentration (157.80 ± 2.24 mmol/l) was significantly higher than that of Dimple (156.00 ± 2.93 mmol/l), Thandi (155.90 ± 3.03 mmol/l) and Domino (156.10 ± 3.18 mmol/l) (Two sample t-tests, $p < 0.005$). Dolly's mean sodium level (157.80 ± 3.50 mmol/l) was significantly higher than Thandi's ($t=3.11$, $p=0.002$, $df=130$). In contrast, Simo's mean sodium:creatinine ratio was similar to mean values from all other animals (Two sample t-tests, $p > 0.005$). Dimple's mean sodium:creatinine (1.60 ± 0.24) was significantly higher than that of Thandi (1.41 ± 0.29) ($t=3.70$; $p<0.001$; $df=126$) and Domino (1.33 ± 0.19) ($t=5.47$; $p<0.001$; $df=79$). The only other significant difference for this ratio was between Dolly (1.48 ± 1.95) and Domino ($t=3.29$; $p=0.002$; $df=75$) (Table 4.1).

Sodium levels were variable for all five of the captive dolphins and the range of data for each of the animals overlapped considerably. There were no apparent age-associated changes in serum sodium and/or sodium:creatinine (Fig. 4.66). Although several animals had data that were positively correlated with the change in age during the study (Table 4.8), the increases were associated more with illness and/or treatment, than the biological change in age. Domino's data were variable but sodium:creatinine values were predominantly higher at age 4.5 years, than the results of initial analyses carried out at age two years (Table 4.8). Sodium and sodium:creatinine were not correlated with total body weight and/or blubber thickness in any of the five captive dolphins (Table 4.8).

Serum sodium was negatively correlated with food consumption for Thandi (Table 4.8). This relationship was complex, reflecting sodium concentrations to be lowest (~150-155 mmol/l) at food consumption levels of between 60 kg/week and 70 kg/week (Fig. 4.67). Sodium:creatinine was significantly related to food consumption for Dolly, Thandi and Domino (Table 4.8). Dolly's data showed that ratio values measured during periods of greater food consumption were generally

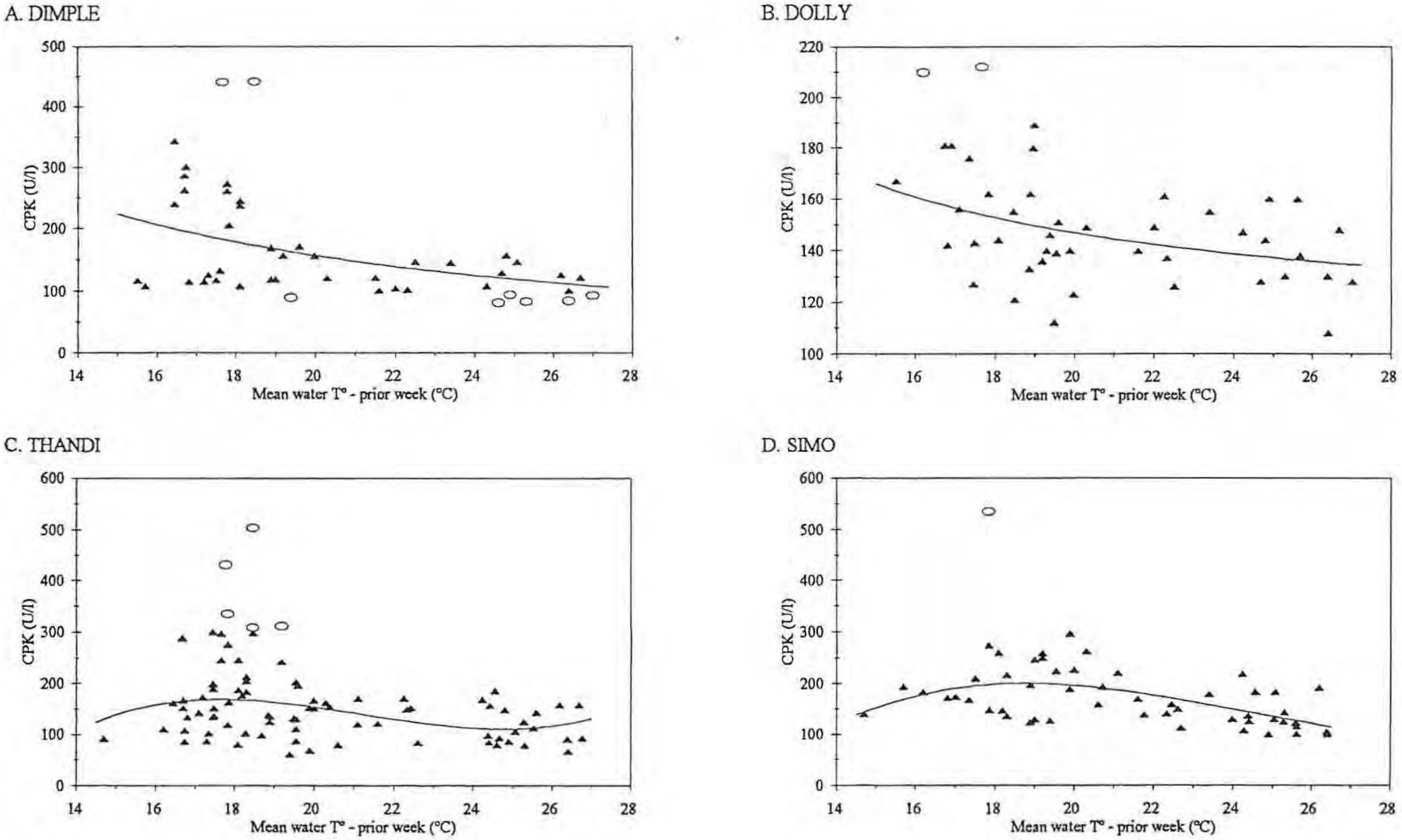


Figure 4.63: Curves of best fit (—) for animals (A. Dimple; B. Dolly; C. Thandi; D. Simo) with significant correlations ($p<0.05$) between creatine phosphokinase (CPK) and the mean water temperature of the week prior to blood sampling. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

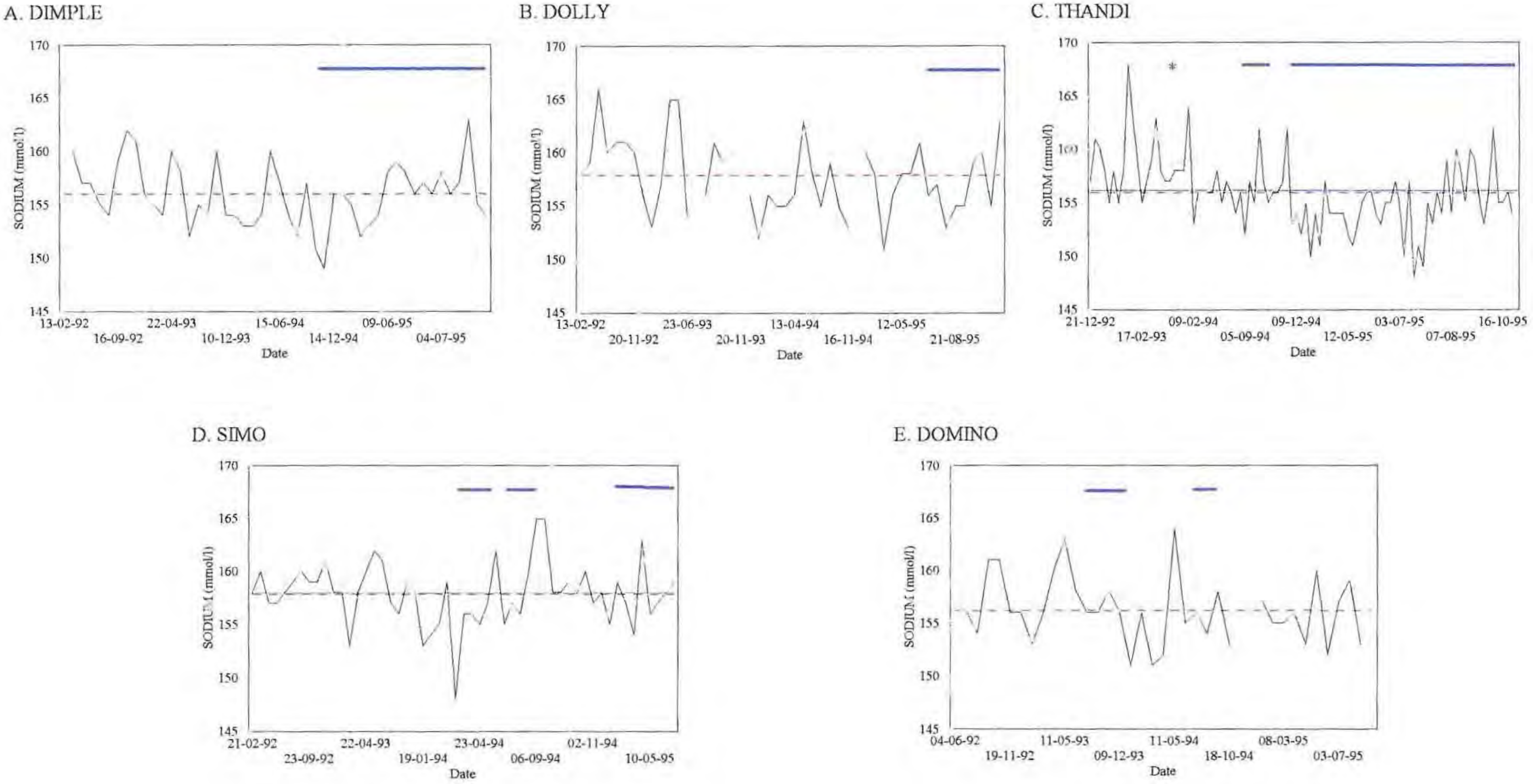


Figure 4.64: Levels of sodium (mmol/l) (—) for all five captive animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the overall mean sodium level (—) and the "normal" (see text) mean sodium level (---) for the study between 1992 and 1995 (*: parturition; —: extended period of illness and/or treatment or period of aberrant feeding and/or behaviour).

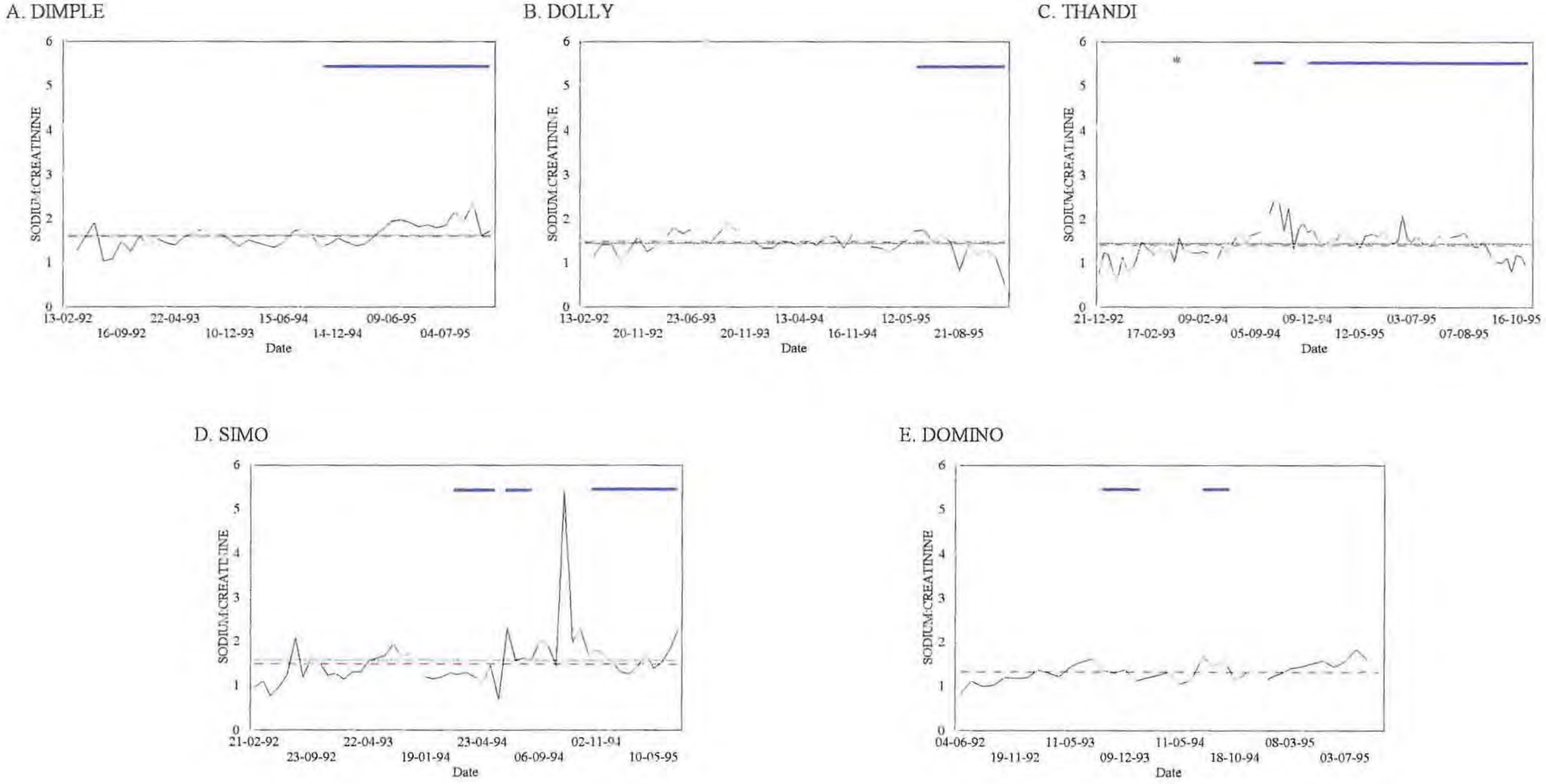


Figure 4.65: Sodium:creatinine ratios (—) for all five captive animals (A. Dimple; B. Dolly; C. Thandi; D. Simo; E. Domino). Also shown, for each animal, is the overall mean ratio (—) and the "normal" (see text) mean ratio (- - -) for the study between 1992 and 1995 (*: parturition; —: extended period of illness and/or treatment or period of aberrant feeding and/or behaviour).

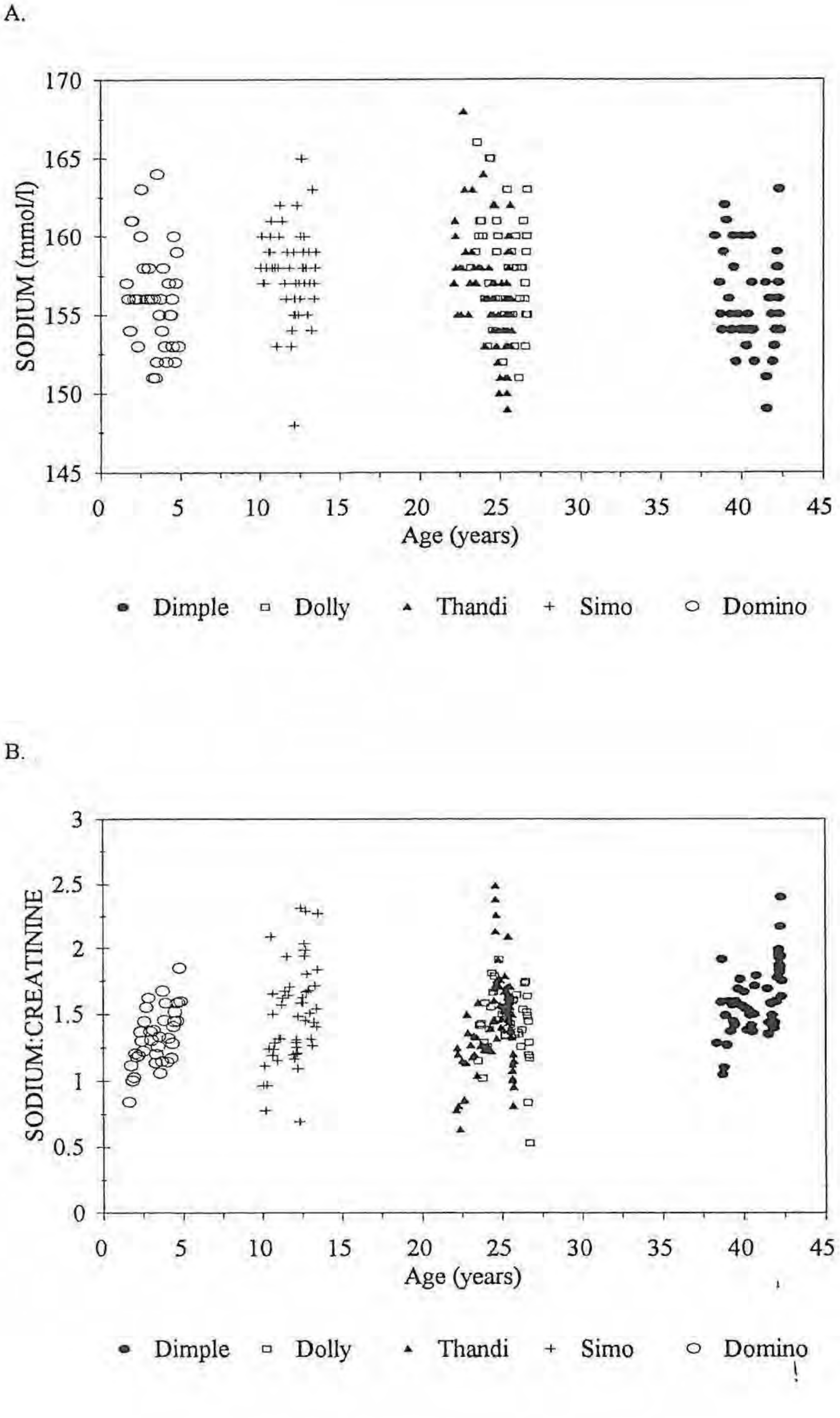


Figure 4.66: Relationship between serum levels of sodium (A), the sodium:creatinine ratio (B) and age for the five captive dolphins, from records obtained between 1992 and 1995.

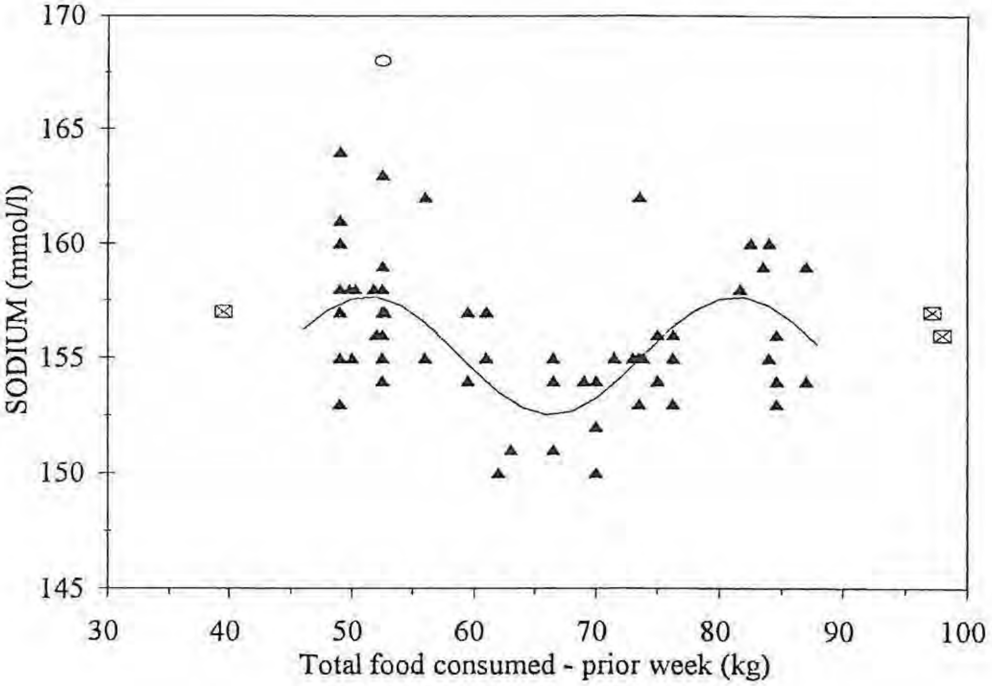


Figure 4.67: Curve of best fit (—) for Thandi with a significant correlation ($p<0.05$) between sodium and total food consumed in the week prior to blood sampling. Included in the graph are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ☒ ; y-axis: ○).

higher (Fig. 4.68A). Thandi's ratio appeared to increase between food consumption of 45-55 kg/week, before stabilising (Fig. 4.68B). Domino's sodium:creatinine ratio increased slightly with increased food consumption (Fig. 4.68C).

Table 4.8: Correlation coefficients (r), levels of significance (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$) and sample sizes (n) for significant relationships between sodium (a) and sodium:creatinine (b) and independent variables for the five captive dolphins (Data are presented as r*(n)) (NS: non-significant ($p>0.05$)).

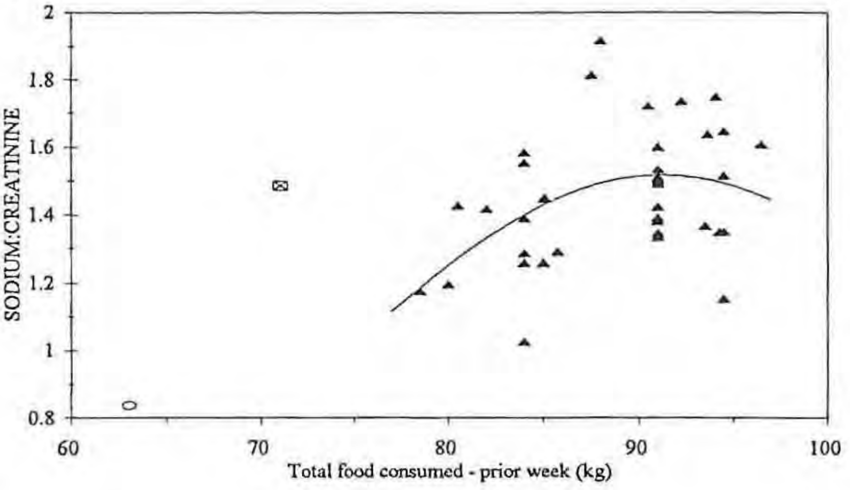
Variable		Dimple	Dolly	Thandi	Simo	Domino
Age	a	NS	-0.30*(45)	-0.42***(85)	NS	NS
	b	0.56***(46)	NS	0.46***(80)	0.41**(51)	0.51**(35)
D1	a	NS	NS	-0.26*(68)	NS	NS
	b	NS	0.36*(37)	0.52***(64)	NS	0.55***(32)
E1	a	-0.38*(40)	NS	NS	NS	NS
	b	NS	NS	0.37**(66)	NS	0.43**(32)
E2	a	-0.31*(39)	NS	NS	NS	NS
	b	-0.48**(39)	NS	0.33**(66)	NS	0.48**(32)
E3	b	NS	NS	0.35**(67)	NS	0.51**(31)
E4	b	NS	NS	NS	NS	0.54**(32)
Month	a	NS	NS	NS	0.33*(50)	NS
	b	NS	NS	NS	0.51***(51)	NS
T1	a	NS	-0.32*(45)	NS	-0.43**(50)	NS
	b	-0.45**(46)	NS	NS	-0.51***(51)	NS
T2	a	NS	-0.29*(45)	NS	-0.39**(49)	NS
	b	-0.48***(46)	NS	NS	-0.55***(50)	NS
T3	b	-0.41**(46)	NS	NS	-0.54***(51)	NS

D1: Total food consumption of week prior to blood sample; E1: Dietary energy intake of day prior to blood sample; E2-E4: Mean daily energy intake of week in which blood was sampled, and week and month prior to blood sample, respectively, T1: Mean water temperature of week prior to blood sample, T2: Mean water temperature of month in which blood was sampled, T3: Mean water temperature of month prior to blood sample (T3).

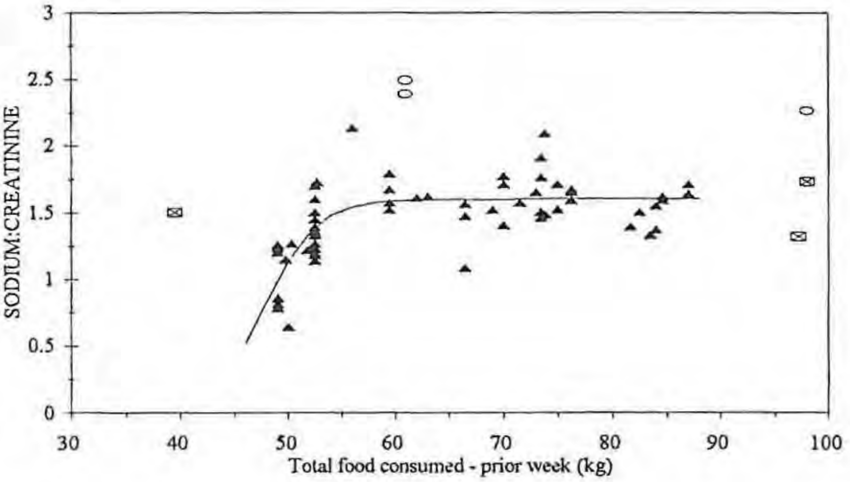
Sodium was negatively related to the dietary energy intake of the day prior to the blood sample for Dimple (Table 4.8). However, data were highly variable and the trend could not be confirmed. Sodium levels measured during periods of zero food intake on the day prior to the blood sample were "normal". Sodium:creatinine was positively related to this diet category for Thandi and Domino (Table 4.8). Only Domino's data reflected a gradual increase in ratio values with increased energy intake, similar to the previous relationship described for this animal.

Dimple's sodium levels were negatively correlated with the mean daily energy intake of the week in which blood was sampled (Table 4.8). The relationships between sodium:creatinine and this diet category for Dimple and Domino were slightly more distinct, but data remained variable. Dimple's

A. DOLLY



B. THANDI



C. DOMINO

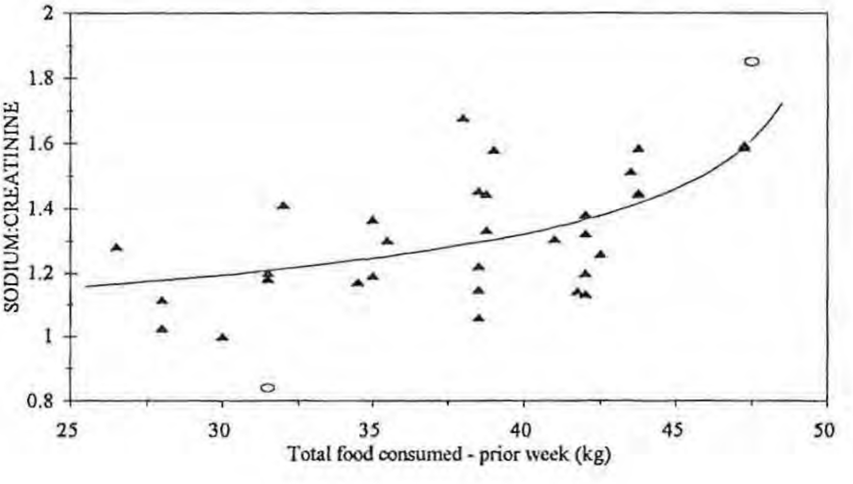


Figure 4.68: Curves of best fit (—) for animals (A. Dolly; B. Thandi; C. Domino) with significant correlations ($p<0.05$) between sodium:creatinine and total food consumed in the week prior to blood sampling. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers (x-axis: ⊠ ; y-axis: ○).

data reflected that sodium:creatinine values were elevated (> 1.70) when mean daily energy intake during the week of the blood sample was less than 32 500 kJ/day (Fig. 4.69A). In contrast, Domino's data implied that increased ratio values (> 1.40) were measured more frequently when mean daily energy intake was above 32 000 kJ/day (Fig. 4.69B).

Thandi and Domino's sodium:creatinine ratios were positively correlated with the mean daily energy intake of the week prior to the blood sample (Table 4.8). Although data from both animals were variable, values tended to be higher at increased levels of energy intake, similar to those trends described in other diet categories. Domino was the only animal for which sodium:creatinine was significantly related to energy intake in the long-term (the month the prior to the blood sample) (Table 4.8). Ratio values were variable, but showed an overall increase as mean daily energy intake during the month preceding the blood sample increased. This trend was almost identical to the relationship described, for this animal, between sodium:creatinine and the mean daily energy intake of the week in which blood was sampled (Fig. 4.69B).

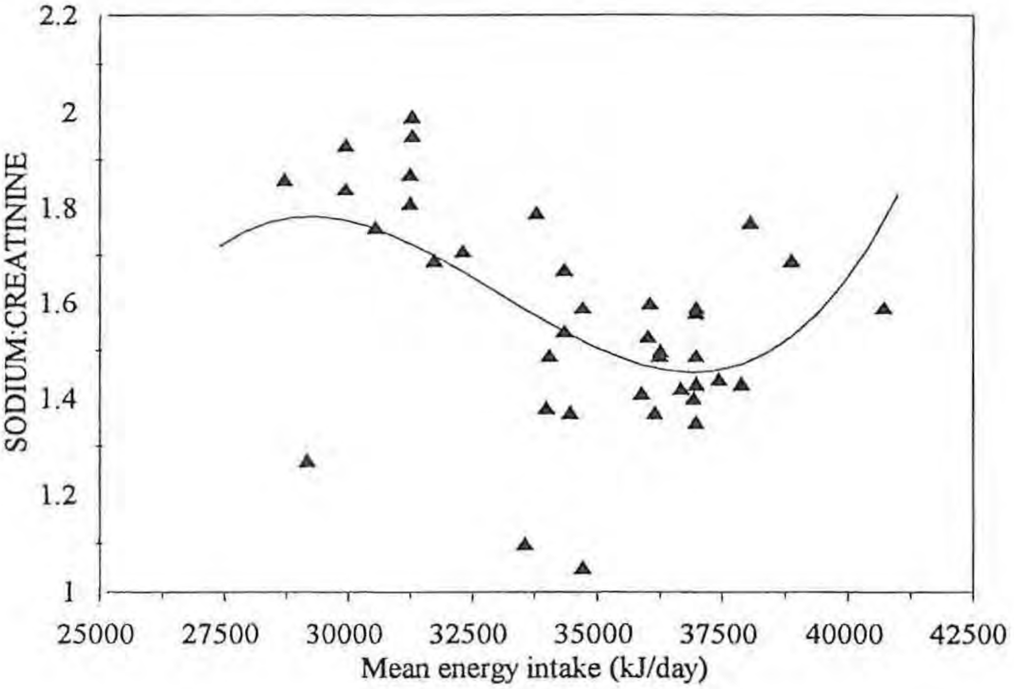
Although Simo's sodium levels were positively correlated with the month of the year (Table 4.8), sodium levels appeared to remain relatively stable throughout the year (Fig. 4.70a). Sodium:creatinine values from the same animal were slightly increased during the winter and spring months (Fig. 4.70b). Sodium levels from both Dolly and Simo were negatively correlated with water temperature (Table 4.8). Simo's sodium levels gradually declined as water temperature increased (Fig. 4.71B). The same relationship, for Dolly, was more complicated, but increased sodium concentrations were measured more frequently at water temperatures below 21°C (Fig. 4.71A). The temperature-related decline in sodium:creatinine values of Dimple and Simo corresponded with the trends for sodium (Table 4.8; Fig. 4.72).

i. Proteins (albumin, globulin, albumin:globulin)

Logistical problems prevented regular analyses of total protein and albumin. Consequently, data for these parameters were limited and any real trends could not be described. Thandi's protein, albumin and globulin levels varied considerably, although values for the albumin:globulin ratio were relatively restricted (Appendix 3.7). In contrast, Simo's protein and albumin levels showed little variation, whereas the albumin:globulin ratio was highly variable (Appendix 3.7). Domino was the only animal for which no protein, albumin, globulin or albumin:globulin values were identified as outlying (Appendix 3.7).

For the purposes of this study, levels of total protein were excluded from analyses based on limited relationships with independent variables. Overall, "normal" mean albumin levels for Dimple (35.50 ± 1.29 g/l) and Dolly (46.45 ± 2.42 g/l) were significantly lower and higher, respectively, than all

A. DIMPLE



B. DOMINO

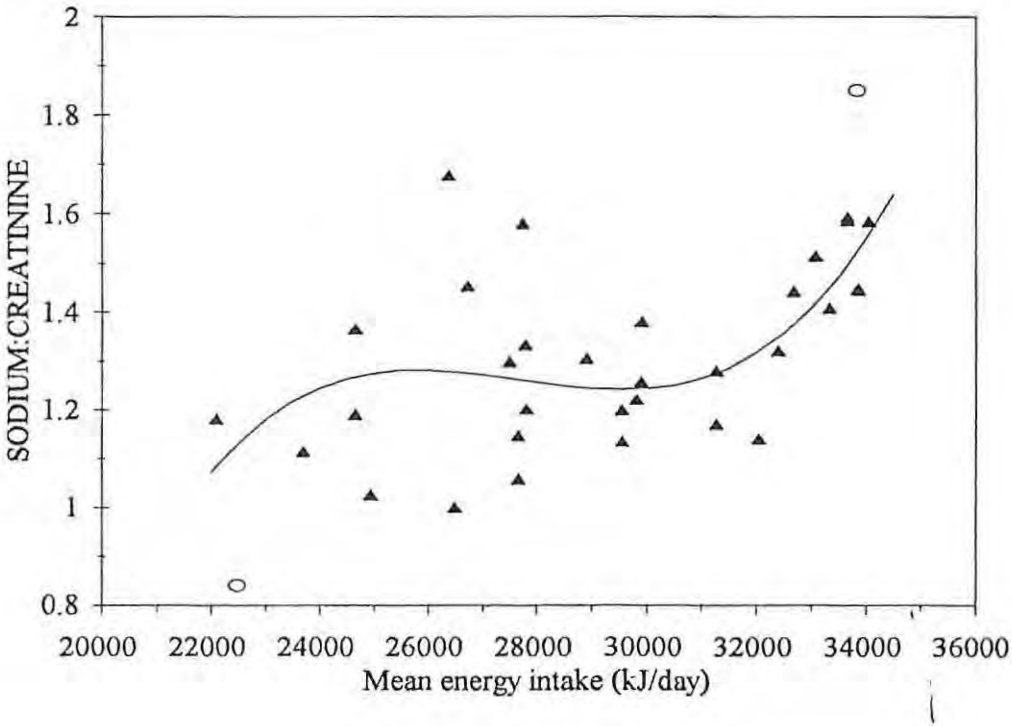
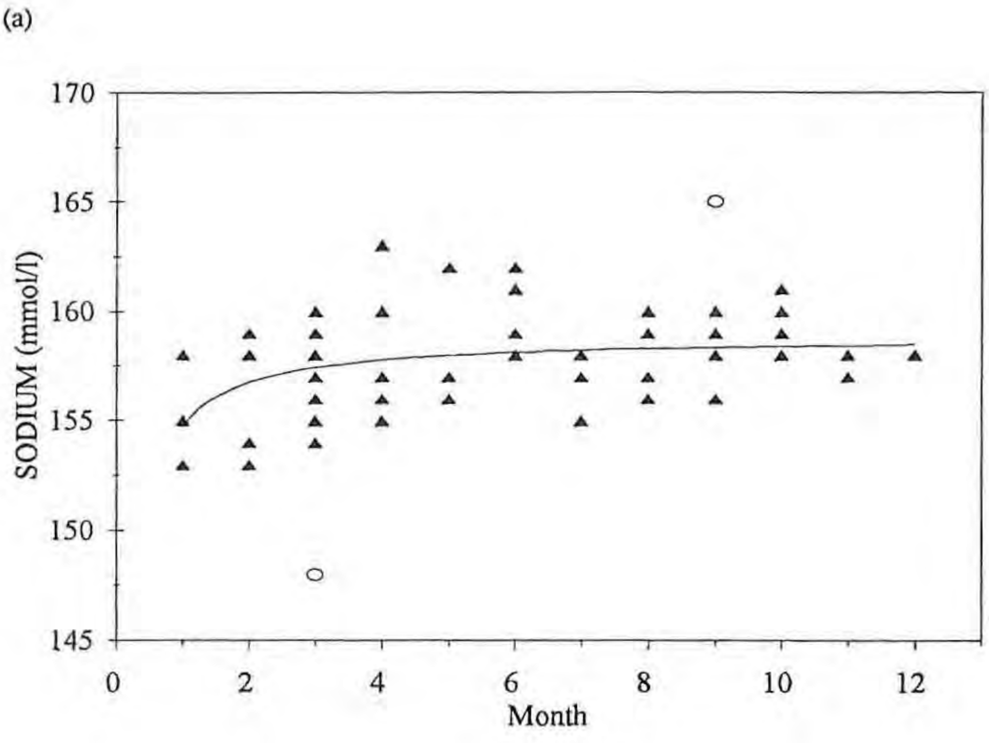


Figure 4.69: Curves of best fit (—) for animals (A. Dimple B. Domino) with significant correlations ($p<0.05$) between sodium:creatinine and the mean daily dietary energy intake of the week in which blood was sampled. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).



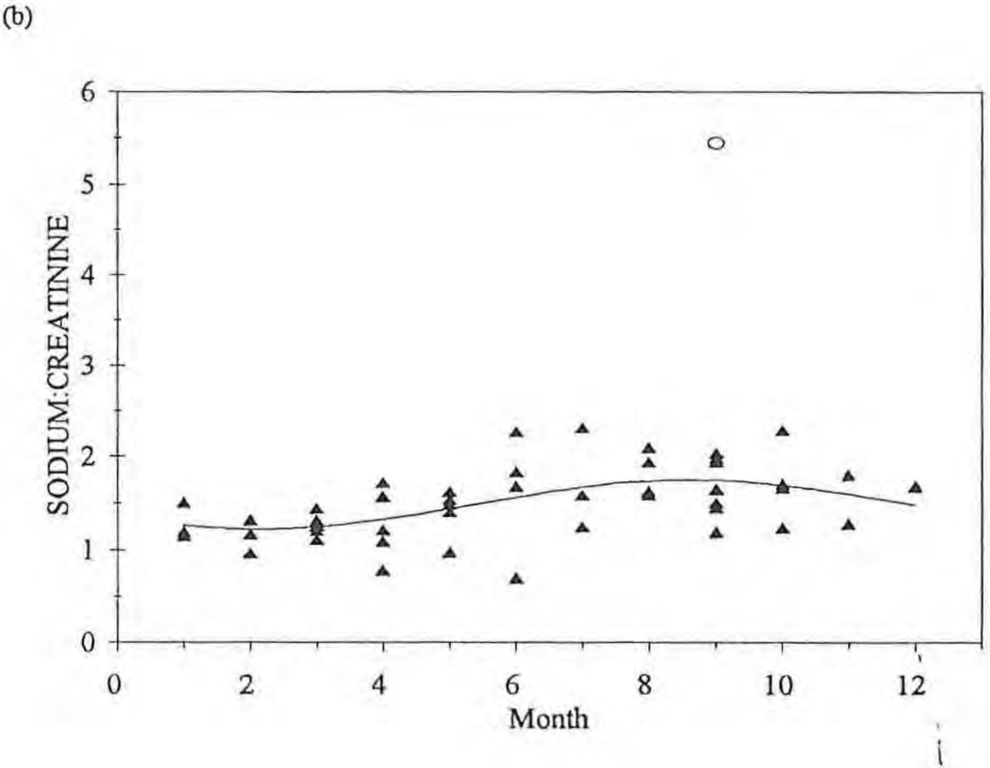
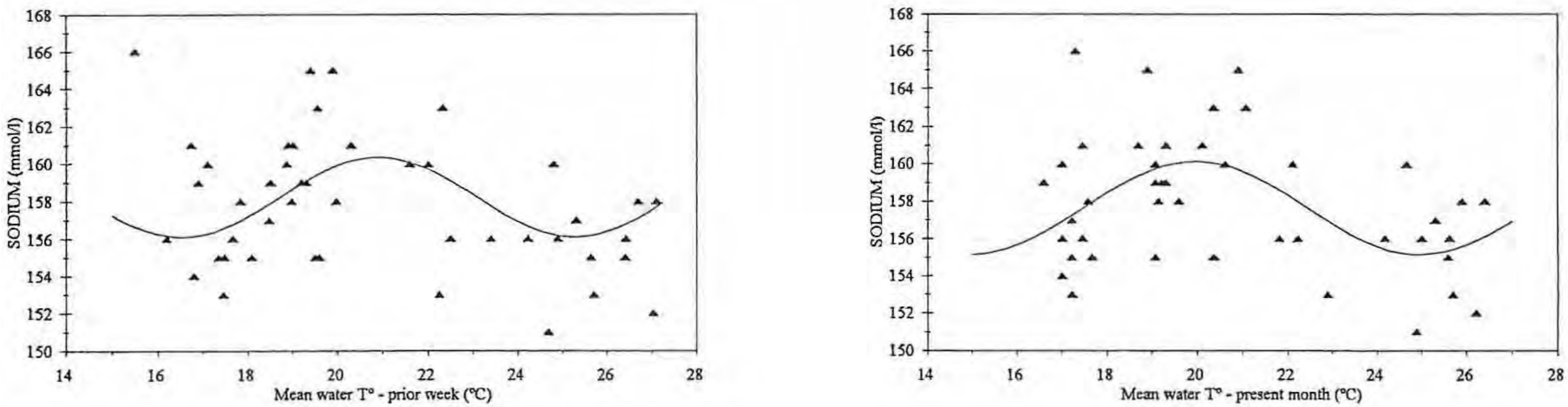


Figure 4.70: Curves of best fit (—) for Simo with significant correlations ($p<0.05$) between sodium (a) and sodium:creatinine (b) and the month of the year. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

A. DOLLY (a) (b)



B. SIMO (a) (b)

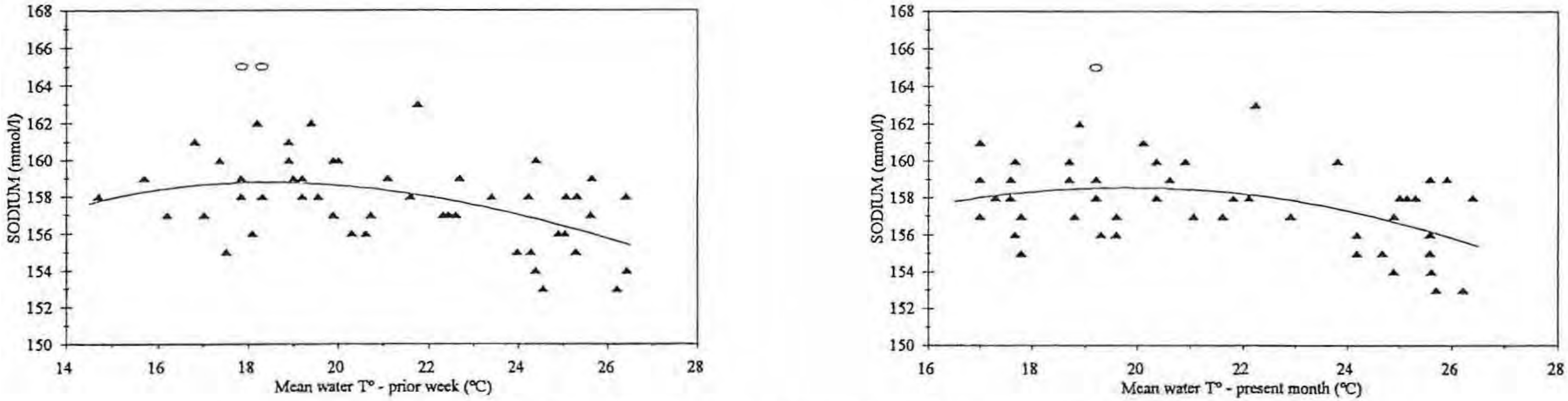


Figure 4.71: Curves of best fit (—) for animals (A. Dolly; B. Simo) with significant correlations ($p<0.05$) between sodium and the mean water temperature of the week prior to the blood sample (a) and the mean water temperature of the month in which blood was sampled (b). Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

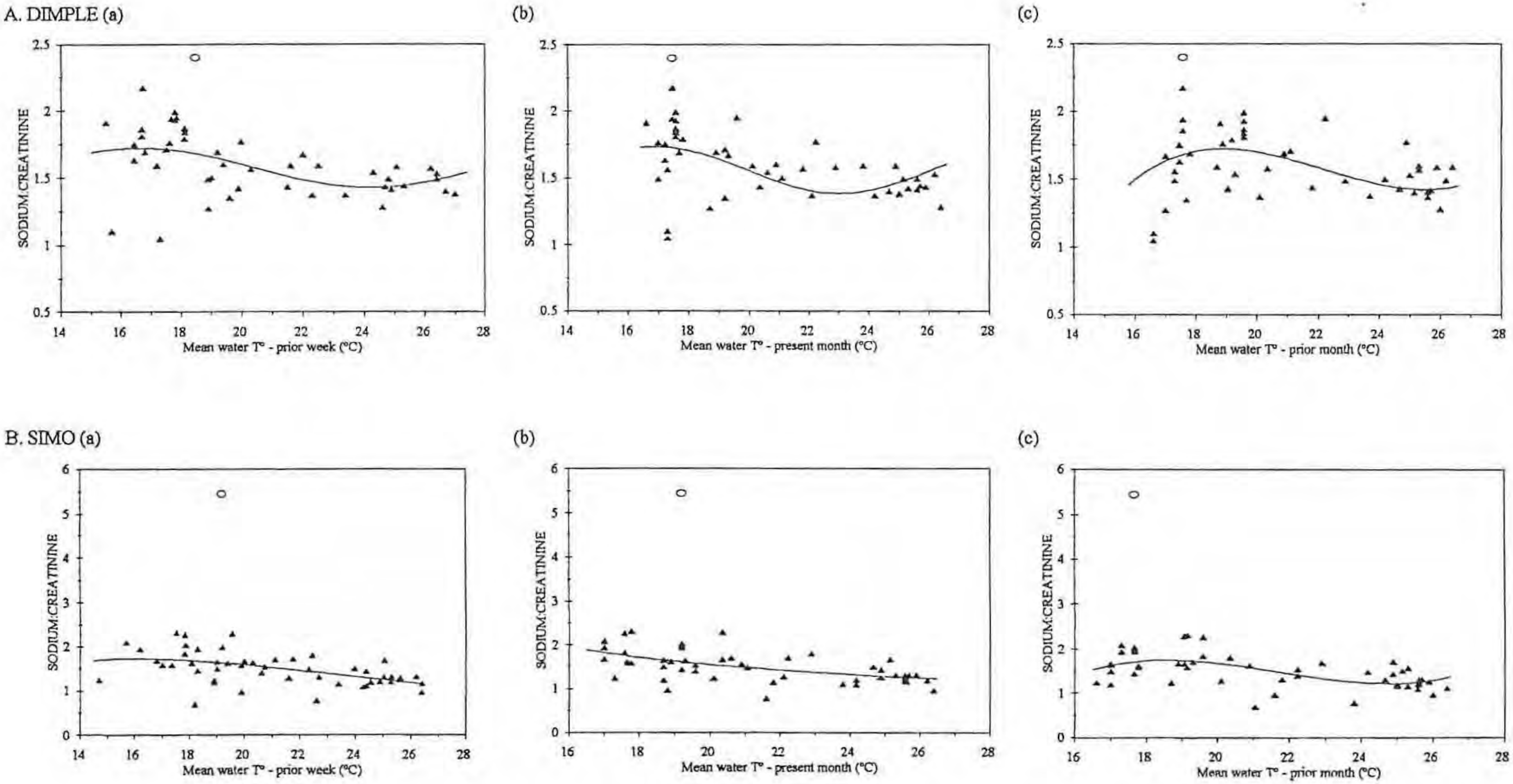


Figure 4.72: Curves of best fit (—) for animals (A. Dimple; B. Simo) with significant correlations ($p<0.05$) between sodium:creatinine and the mean water temperature of the week prior to the blood sample (a), the mean water temperature of the month in which blood was sampled (b) and the mean water temperature of the month prior to the blood sample (c). Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

animals (Two sample t-test, $p<0.005$). The one exception was Dolly and Domino (43.57 ± 2.30 g/l) ($t=2.51$; $p=0.02$; $df=16$). Thandi (40.97 ± 4.01 g/l), Simo (41.33 ± 1.16 g/l) and Domino all had similar mean albumin levels (Two-sample t-test; $p>0.05$) (Table 4.1). Mean globulin levels differed significantly between animals except for Dimple (37.93 ± 2.17 g/l) and Thandi (40.68 ± 5.64 g/l) ($t=-1.75$; $p=0.09$; $df=40$); Dolly (25.36 ± 2.29 g/l) and Simo (19.80 ± 5.89 g/l) ($t=2.79$; $p=0.02$; $df=14$); and Simo and Domino (18.17 ± 2.32 g/l) ($t=-0.63$; $p=0.55$; $df=9$). This pattern of similarity was identical for the albumin:globulin ratio (Table 4.1).

Overall, there was no direct relationship between albumin and the age of the five captive dolphins, although albumin concentrations were markedly reduced in the elderly Dimple (Fig. 4.73A). Globulin levels, however, were lower in the juvenile Domino and adolescent Simo, compared to the older, mature females (Fig. 4.73B). This age-associated increase in globulin levels was paralleled by an apparent decrease in the albumin:globulin ratio with age (Fig. 4.73C). More specifically, globulin was negatively correlated with change in age during the study for Thandi and Simo (Table 4.9). Data for Simo were limited and, although there was a marked decrease in globulin levels between the ages of 12 and 13.5 years, this decline could not be confirmed. Thandi's globulin levels decreased significantly, but the time period was restricted, preventing any conclusions (Fig. 4.74A). Similarly, only Thandi and Simo showed significant positive correlations between albumin:globulin and age (Table 4.9; Fig. 4.74B). However, as stated above, analyses over a short period of time for Thandi, and limited data for Simo, made any interpretation spurious.

Thandi was the only animal for which albumin, globulin and albumin:globulin increased significantly with total body weight during the study (Table 4.9). Although data were limited, there was a definite increase in albumin levels with increasing weight (Fig. 4.75A). This relationship was also reflected in decreasing globulin levels as weight increased from 175 to 200 kg, although some low globulin values were obtained at weights around 180 kg (Fig. 4.75B). In conjunction, albumin:globulin was higher at increased body weights (Fig. 4.75C). Although Dimple's albumin:globulin ratio was negatively correlated with weight (Table 4.9), limited data precluded the identification of a definite trend for this animal.

Relationships between serum proteins and diet were difficult to interpret due to the limited analyses of albumin and globulin. Although particular animals had significantly correlated dietary and serum protein parameters, these relationships were impossible to corroborate. Generally, Domino's albumin and Simo's globulin appeared to be higher at increased levels of food consumption, with an associated decrease in Simo's albumin:globulin ratio (Table 4.9). Similarly, albumin and globulin levels were higher at increased levels of dietary energy intake. These potentially important relationships need further defining with additional data.

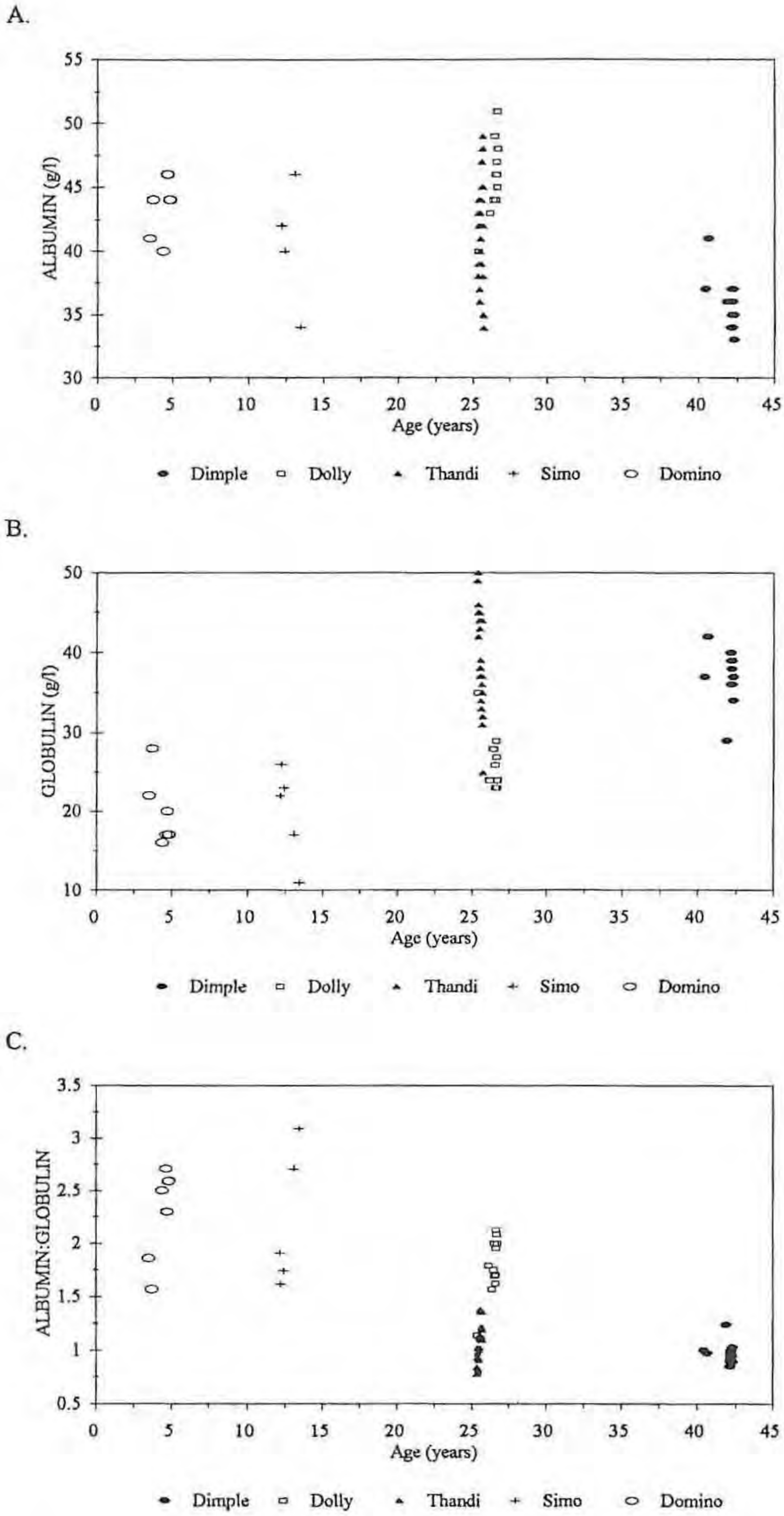
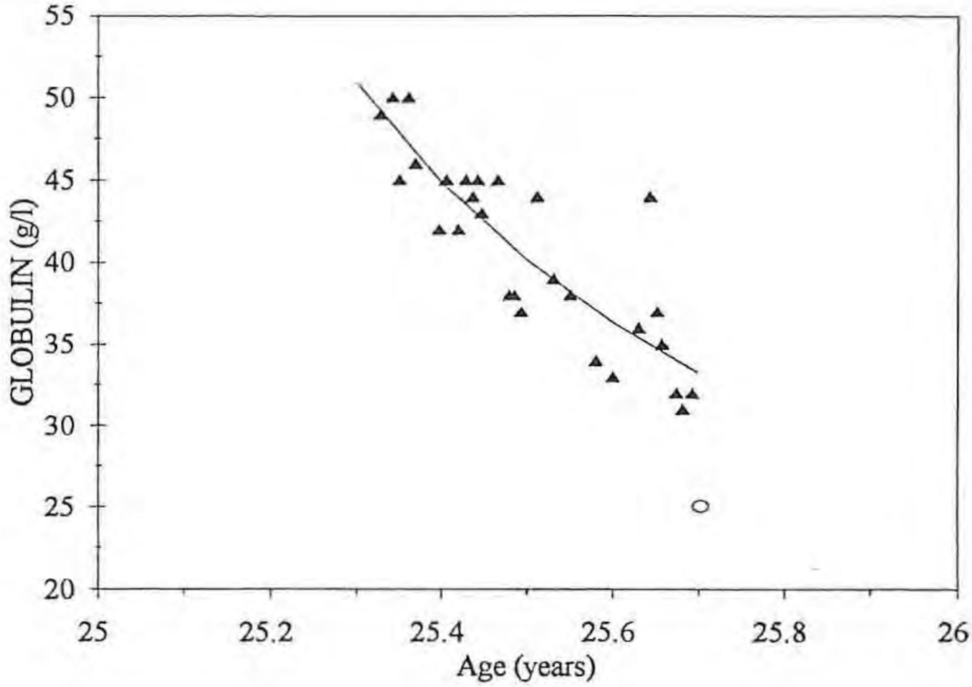


Figure 4.73: Relationship between serum levels of albumin (A), globulin (B) and the albumin:globulin ratio (C) and age for the five captive dolphins, from records obtained between 1992 and 1995.

A.



B.

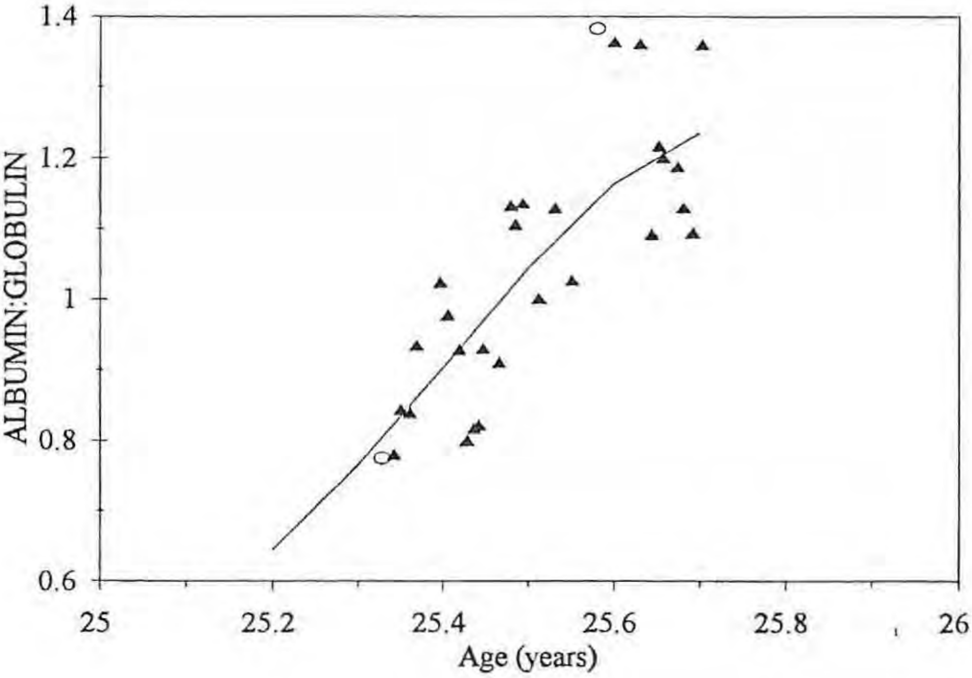


Figure 4.74: Curves of best fit (—) for Thandi with significant correlations ($p<0.05$) between serum levels of globulin (A), the albumin:globulin ratio (B) and age. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

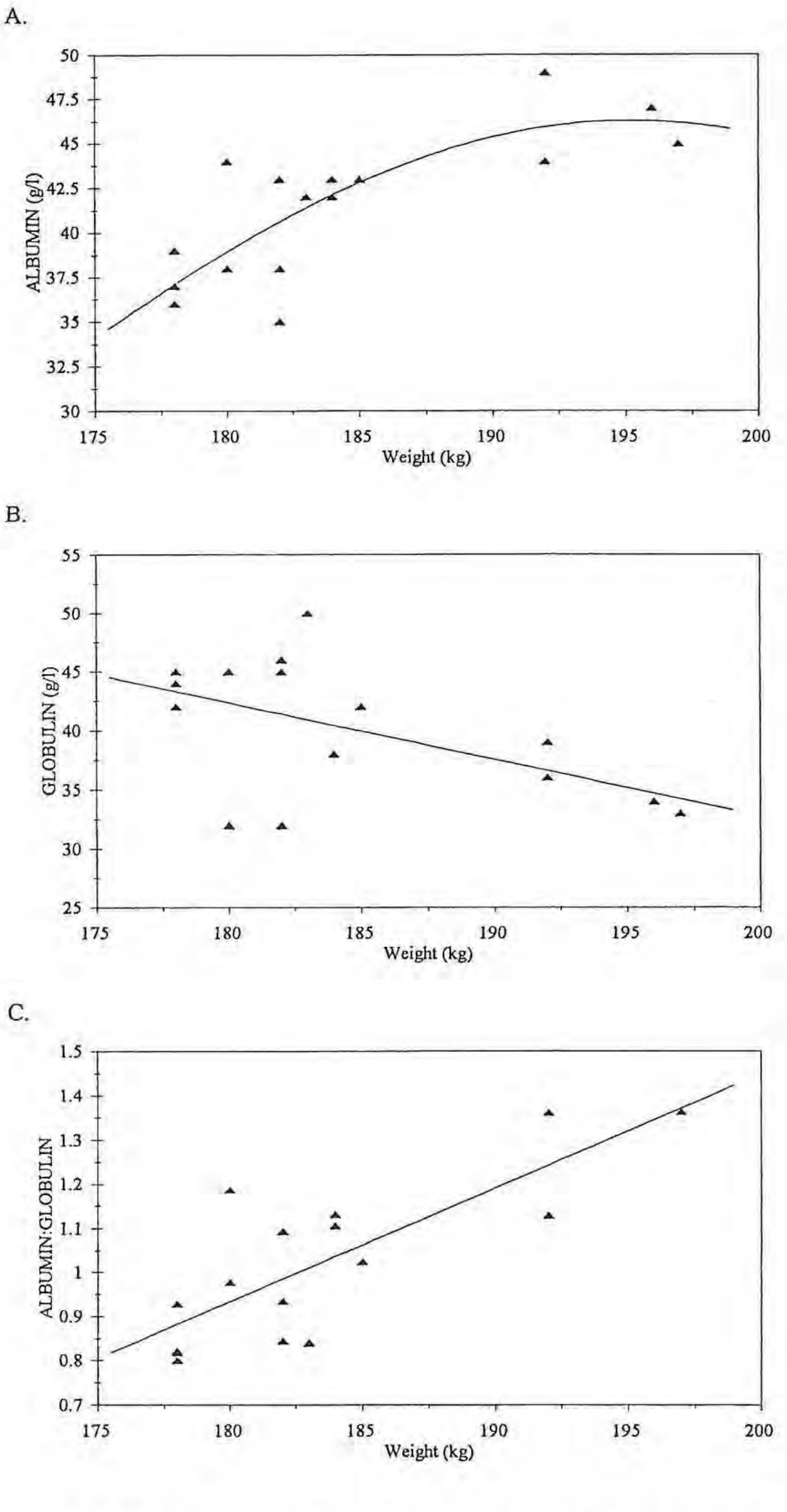


Figure 4.75: Curves of best fit (—) for Thandi with significant correlations ($p<0.05$) between albumin (A), globulin (B), albumin:globulin (C) and total body weight. Included in the graphs are all "normal" (see text) data (▲).

Table 4.9: Correlation coefficients (r), levels of significance (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$) and sample sizes (n) for significant relationships between albumin (a), globulin (b) and albumin:globulin (c) and independent variables for the five captive dolphins (Data are presented as r*(n)) (NS: non-significant ($p>0.05$)).

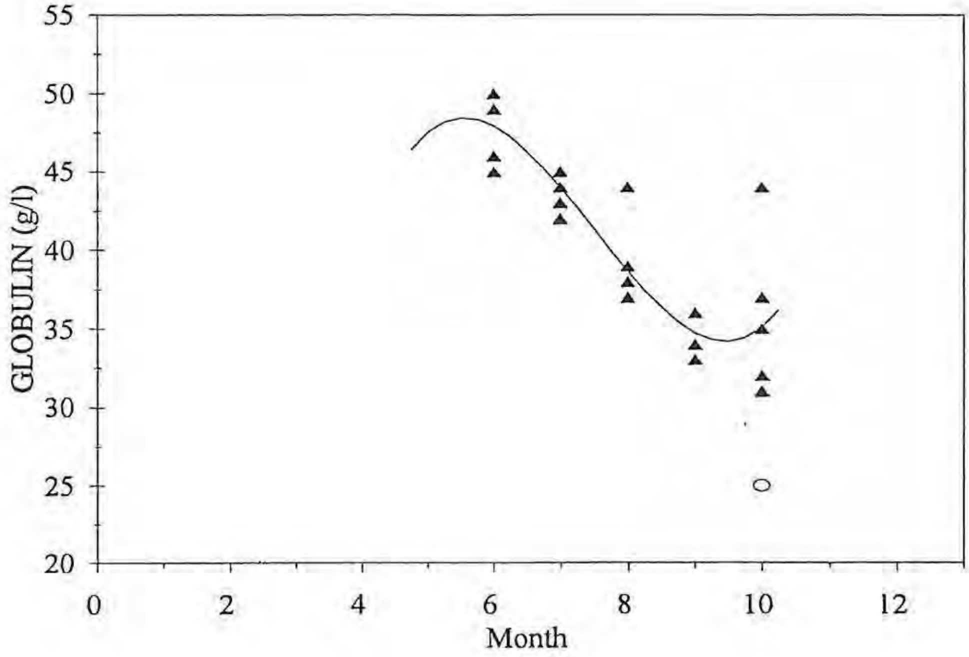
Variable		Dimple	Dolly	Thandi	Simo	Domino
Age	b	NS	NS	-0.85***(28)	-0.95*(5)	NS
	c	NS	NS	0.80***(27)	0.86*(5)	NS
Weight	a	NS	NS	0.73**(10)	NS	NS
	b	NS	NS	-0.81**(11)	NS	NS
	c	-0.64*(11)	NS	0.86**(10)	NS	NS
D1	a	NS	NS	NS	NS	0.77*(7)
E1	a	NS	NS	NS	NS	0.76*(7)
E3	a	NS	NS	NS	NS	0.93**(6)
E4	c	-0.59*(11)	NS	NS	NS	NS
Month	a	NS	0.59*(11)	NS	NS	0.76*(7)
	b	NS	NS	-0.85***(28)	NS	NS
	c	NS	NS	0.81***(27)	NS	NS
T1	a	NS	NS	NS	NS	-0.92**(7)
	b	NS	NS	-0.58**(28)	NS	NS
	c	NS	NS	0.54**(27)	NS	NS
T2	a	NS	NS	NS	NS	-0.91**(7)
	b	NS	NS	-0.63***(28)	NS	NS
	c	NS	NS	0.62***(27)	NS	NS
T3	a	NS	NS	-0.36*(29)	NS	NS

D1: Total food consumption of week prior to blood sample; E1: Dietary energy intake of day prior to blood sample; E3: Mean daily energy intake of week and month (E4) prior to blood sample, T1: Mean water temperature of week prior to blood sample, T2: Mean water temperature of month in which blood was sampled, T3: Mean water temperature of month prior to blood sample.

Albumin was significantly related to the month of the year for Dolly and Domino (Table 4.9). Albumin measurements were not available for all months of the year, which made the definition of any potential patterns impossible. Levels of albumin for Domino appeared to be higher in the winter months. Thandi was the only animal for which globulin and albumin:globulin were significantly correlated with month (Table 4.9). Data were more extensive for this animal, but analyses were not made for all months of the year. Globulin and albumin:globulin appeared to be highest and lowest, respectively, in the winter months (Fig. 4.76). Without additional data for the summer months, this could not be confirmed.

Limited data also made relationships between serum proteins and water temperature impossible to investigate (Table 4.9). Limited data for Domino indicated that albumin concentrations were lower at higher water temperatures. Data for Thandi corresponded only to water temperatures below 21°C and were highly variable, showing no distinct trend. Taking data variability into account, Thandi's

A.



B.

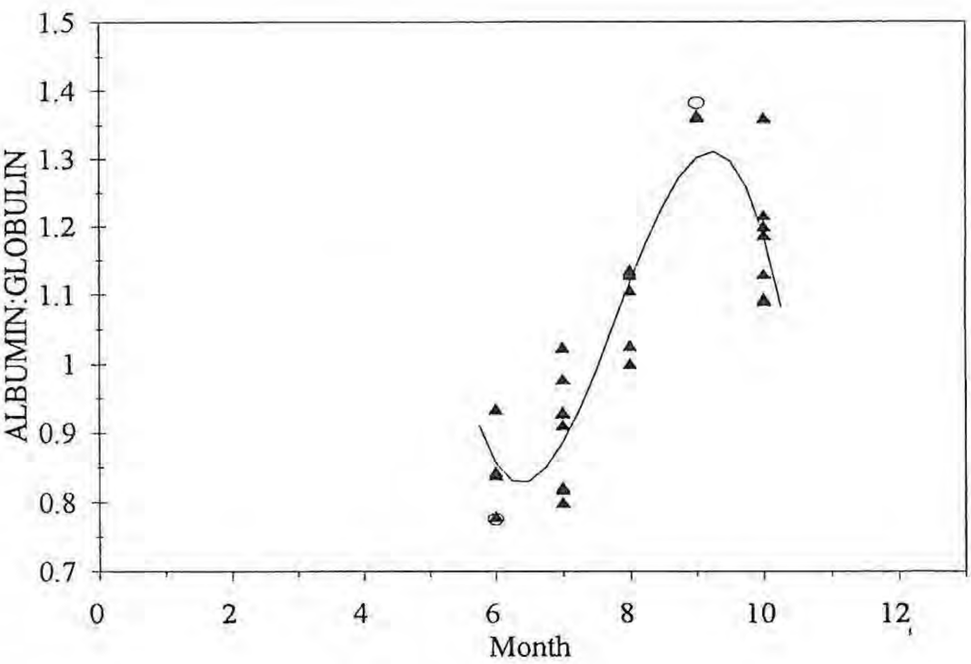


Figure 4.76: Curves of best fit (—) for Thandi with significant correlations (p<0.05) between globulin (A) and albumin:globulin (B) and the month of the year. Included in the graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

globulin levels were generally higher at 17°C than at 20°C, whereas the albumin:globulin ratio was higher at 20°C (Fig. 4.77).

j. Other serum chemistry parameters

A number of other blood chemistry components were analysed during this study. These included serum potassium, calcium, plasma glucose and serum lipids. Investigations into the relationships between these parameters and age, morphology, diet and water temperature were not included in this study for reasons discussed above. However, for the sake of information, mean values and additional data pertaining to them are included in Table 4.1 and Appendices 3.8 and 3.9.

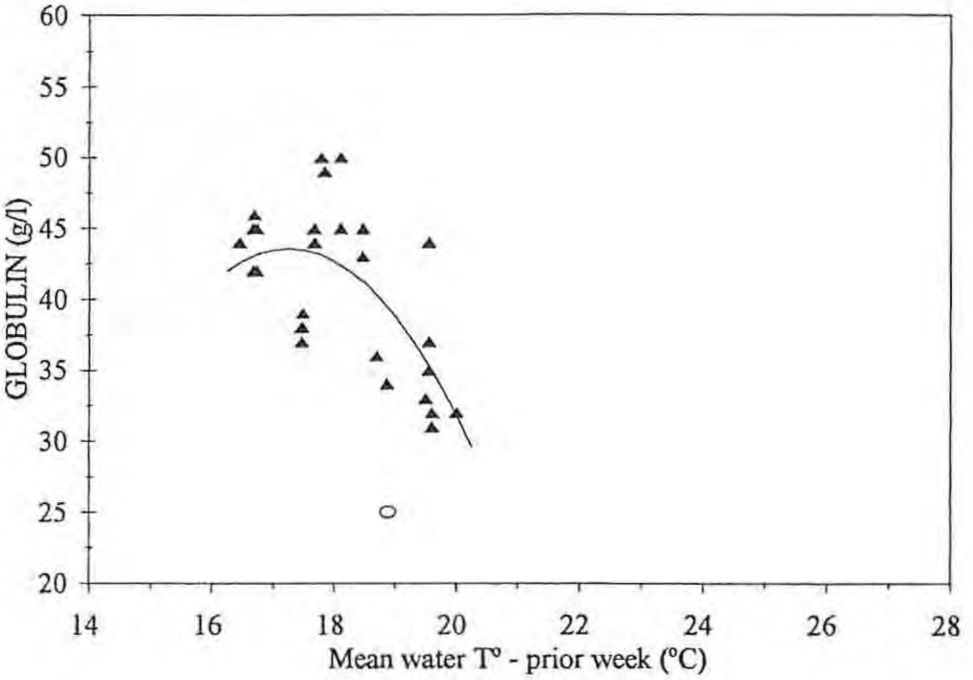
DISCUSSION

The exclusion of certain blood chemistry parameters, based on limited correlations with independent variable, was due primarily to limited and irregular analyses. This was particularly true for serum calcium and lipids and plasma glucose. The potential effectiveness of these parameters as condition indices of captive dolphins should not be ignored without further investigation of the interactions between them and age, morphology, diet and water temperature.

Serum creatinine levels are a function of muscle mass in healthy humans and other mammals (Kaplan and Pesce 1989, Bossart and Dierauf 1990). The amount of creatinine per unit muscle mass is constant and thus the rate of creatinine production is constant. As a result, in the absence of muscle disease, creatinine levels in terrestrial mammals are very stable and are unaffected by diet and changes in the electrolyte balance (Kaplan and Pesce 1989, Bossart and Dierauf 1990). Creatinine increases occur during kidney disease (elevations being proportional to the extent of kidney damage) (Bossart and Dierauf 1990), and during dehydration (Medway *et al.* 1982). As a result, creatinine has been identified as the ideal diagnostic test of renal functioning and kidney condition.

Creatinine levels measured during this study were, in most cases, lower than those recorded for bottlenose dolphins in the literature (Appendix 5), although they were within the range reported by Bossart and Dierauf (1990). Although the oldest and smallest animal (Dimple) had significantly lower creatinine levels, creatinine concentrations were otherwise not related to the age or size of the other four animals. Creatinine production is reportedly a direct reflection of muscle mass and levels are lower (in humans) in females, the elderly and in smaller persons (Kaplan and Pesce 1989). Creatinine was elevated in older, larger male killer whales (Cornell 1983) and female bowhead whales (larger than their male counterparts) had higher

A.



B.

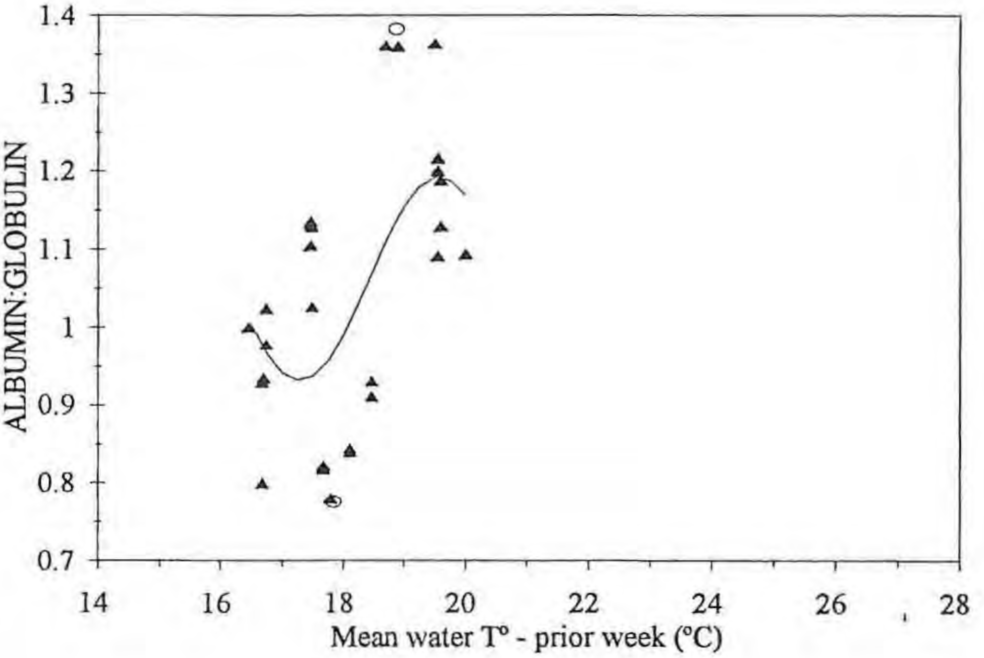


Figure 4.77: Curves of best fit (—) for Thandi with significant correlations ($p<0.05$) between globulin (A), the albumin:globulin ratio (B) and the mean water temperature of the week prior to the blood sample. Included in graphs are all "normal" (see text) data (▲) and statistically determined outliers on the y-axis (○).

creatinine levels (Heidel *et al.* 1996), indicating that it is size (and respective muscle mass), and not age or sex, which influences levels of creatinine. In this context, not only does serum creatinine reflect renal condition but can also be used to provide a valuable indication of the overall morphological, particularly muscular, condition of an animal. The fact that both the male dolphins displayed slight reductions in creatinine as age increased during the study is difficult to explain. This appears to contradict the size trend described previously, as both animals increased in size during the study. In this respect, Domino's creatinine levels also appeared to be slightly reduced at heavier weights. Reasons for this contradictory relationship are unclear.

Previous studies have reported that levels of creatinine are unaffected by diet (Kaplan and Pesce 1989, Bossart and Dierauf 1990). During this study, the relationship between diet and creatinine levels was complicated by variable data but creatinine appeared to be elevated at lower levels of food consumption and dietary energy intake. This parallels the relationship between weight and creatinine, as weight and food consumption and/or energy intake are positively correlated. However, this relationship appears to contradict the fact that higher creatinine levels reflect increased muscle weight. As creatinine concentrations are a direct indication of muscle mass, it would seem reasonable to assume that high levels of creatinine reflect an improved overall condition. Lower levels of creatinine in captive common seals were attributed to a more constant, and less varied, diet than wild animals of the same species (McConnell and Vaughan 1983). Creatinine was lower in harbour seals fed plaice and flounder, compared to those fed on mackerel (Kuiken 1985), indicating that the mackerel diet was possibly higher "quality". However, increased creatinine at low levels of food intake, as seen during this study, probably reveals an increased susceptibility to dehydration (Medway *et al.* 1982). Increased creatinine concentrations in fasted wolves (*Canis lupus*) and red deer (*Cervus elaphus*) were attributed to reduced renal blood flow and glomerular filtration rates associated with reduced protein intake (DelGuidice *et al.* 1987b, Wolkers *et al.* 1994a). Decreased levels of creatinine after long-term feed restriction in red deer reflected a reduction in muscle mass (Wolkers *et al.* 1994a, 1994b).

Seasonal changes in creatinine indicated that creatinine levels were slightly reduced during winter. This concurred with the fact that creatinine concentrations were lower at colder water temperatures, which in turn reflected a reduced muscle mass in colder water temperatures. This may suggest a differential use of energy and metabolites obtained from food during different seasons, although this was difficult to investigate within the realms of the current study. The energy obtained from food during winter may be differentially used to increase the blubber layer in order to maintain body temperature or to cope with the increased demands of a higher level of activity and metabolic rate (Costa *et al.* 1993). In summer, when waters are warmer, elevated levels of creatinine indicate that energy and nutrition from food may be used to build up muscle tissue or alternatively, reflect a relative reduction in the production of blubber and fat. A similar differential use of dietary energy has been described in manatees (*Trichechus manatus*) (Irvine *et al.* 1980). Seasonal changes in mass and fat content of wild harbour (*Phoca vitulina*) and harp (*Pagophilus groenlandicus*) seals were not related to dietary energy consumption, but rather to seasonal changes in metabolic rate and shifting basal requirements (Renouf and Gales 1994). Bottlenose dolphins from Sarasota Bay, Florida also showed seasonal differences in metabolic rates, however, they are generally higher in summer. It has been suggested that this increase is related to increased activity during the summer calving and breeding period, increased "catch per unit effort" feeding regimes and/or increased energy demands for thermoregulation (Costa *et al.* 1993). Winter reductions in metabolic rate have been reported for terrestrial mammals (Silver *et al.* 1969, Seal *et al.* 1978a, 1978b, Wolkers *et al.* 1994a) as a physiological response to changes in climate and food availability resulting in a cessation of growth and fat deposition. This reduction in metabolic rate is associated with a voluntary seasonal restriction of food intake which may have evolved as a physiological adaptation for energy conservation when ambient temperatures are low and food resources are limited (Warren *et al.* 1981, Wolkers *et al.* 1994a).

Although the metabolic rates of the animals used in this study are unknown, the possibility of seasonal changes in metabolic rate and consequent fluctuations in physiological parameters must be kept in mind. The fact that changes in metabolic rate are related to the age, sex and reproductive condition of the animals (Renouf and Gales 1994) emphasises the important influence that these potential seasonal metabolic changes may have on the assessment and monitoring of physiological and morphological condition.

Blood urea nitrogen is an indicator of protein catabolism and the conversion of ammonia to urea by the liver (Kaplan and Pesce 1989). Urea and BUN are increased when more amino acids are metabolised by the liver, such as during high protein diets and tissue breakdown or decreased protein synthesis (Kaplan and Pesce 1989, Bossart and Dierauf 1990). BUN may also increase during dehydration and renal disease (Bossart and Dierauf 1990) due to changes in plasma volume. Liver failure, low protein diets and starvation can depress BUN levels. In other mammals, catabolism, fever, trauma and infection have all been found to affect BUN. Certain drugs, which affect protein metabolism, may also affect BUN levels. The reliability of BUN as a single indicator of renal function is reduced by the influence of these other, non-renal, factors. However, the ratio between BUN and creatinine has been found to provide a valuable, and more reliable, indicator of renal functioning (Anon. 1982, Kaplan and Pesce 1989, Medway and Geraci 1978). This ratio is increased (in humans) whenever diet or steroid therapy increases urea production; and with excessive protein catabolism, which occurs during infections. Other conditions, which may result in an increased ratio, include shock, dehydration and massive gastro-intestinal haemorrhage. A reduced ratio is observed in humans during pregnancy, overhydration, severe liver disease and malnutrition (Anon. 1982). Unfortunately, this ratio has not been used extensively in marine and/or terrestrial mammal studies.

BUN levels in marine mammals are generally higher than terrestrial mammals, particularly in cetaceans (Ridgway 1965, Medway and Geraci 1965, 1978, De Monte and Pilleri 1972, 1977, Geraci *et al.* 1979, Cornell 1983). Mean BUN levels of the animals used in this study were similar to those recorded for other bottlenose dolphins (Appendix 5). Overall, there did not appear to be any relationship between BUN, BUN:creatinine and the age of the animals during the study. However, the data did reflect a relationship between BUN and the size of the animal, with the smaller animals having reduced BUN concentrations. BUN appeared elevated in larger male killer whales (Cornell 1983) but it was unclear whether this was attributable to size of massive daily intake of protein. In this respect, as food intake by captive dolphins is usually calculated as a percentage of weight, the BUN levels of captive dolphins may be linked to food consumption, indirectly related to size. The increasing trend in BUN and BUN:creatinine observed during the study for Domino and Simo were also linked to weight and dietary changes.

An interesting, and potentially valuable, relationship revealed during this study was that of BUN:creatinine and blubber thickness. Although only significant for Domino, the ratio appeared to increase with increased blubber thickness. Elevated levels of BUN are generally associated with an improved nutritional status; and creatinine levels reflect muscle mass (Kaplan and Pesce 1989). Therefore, an increased ratio, in a healthy animal, would indicate improved nutritional condition and/or greater physical condition. This improved nutritional condition is reflected in a greater blubber thickness (Ridgway and Fenner 1982, Ognetov 1990, Lockyer 1986, 1993a, Lockyer *et al.* 1984, 1985). This direct relationship between physiological and morphological condition offers an opportunity to use morphological condition to predict and evaluate physiological condition, state of hydration and nutritional condition.

The distinct relationship between BUN and diet makes this blood parameter an invaluable indicator of nutritional condition. The influence of feeding and diets high in protein and fat (i.e. fish) on BUN has been recorded for several species of cetaceans (Anderson 1968, Ridgway *et al.* 1970, De Monte and Pilleri 1972, 1977, Medway and Geraci 1978, Cornell 1983, Asper *et al.* 1990, Bossart and Dierauf 1990) and seals (Geraci *et al.* 1979, Ronald and Kay 1982, McConnell and Vaughan 1983, Medway and Cornell 1989, Castellini *et al.* 1993) as well as other marine mammals such as polar bears (*Ursus maritimus*) (Lee *et al.* 1977, Derocher *et al.* 1990) and sea otters (*Enhydra lutris*) (Williams and Pulley 1983 in Bossart and Dierauf 1990). Manatee BUN levels are generally lower than that of other marine mammals because of the herbivorous nature of their diet. The influence of diet on BUN has been well documented for terrestrial mammals (Franzmann 1972, Torell *et al.* 1974, deCalesta *et al.* 1975, Kirkpatrick *et al.* 1975, Seal *et al.* 1975, 1978b, Melton and Melton 1982, Warren *et al.* 1982, Lochmiller *et al.* 1985b, 1985d, 1986, DelGuidice *et al.* 1987b, Hellgren *et al.* 1993, Knick *et al.* 1993, Zaugg *et al.* 1993, Wolkers *et al.* 1994a). In deer, urea nitrogen levels decrease when inadequate amounts of protein are consumed, yet are inversely proportional to dietary energy intake (Kirkpatrick *et al.* 1975, Warren *et al.* 1982). BUN increases appear to be proportional to protein increase (Kirkpatrick *et al.* 1975, Franzmann 1972).

Although the relationship between BUN and dietary protein intake was not examined by this study, the relationship between BUN:creatinine and diet was reinforced by significant increases in ratio values with both increased food consumption and greater dietary energy intake. Although BUN:creatinine has not been examined to any great extent for marine mammals, the ratio has been found to be a useful indicator of nutrition in terrestrial mammals (Lochmiller *et al.* 1985b, 1985d, 1986, Wolkers *et al.* 1994a), increasing with greater levels of food intake and when fed diets with higher energy and protein contents. However, authors of terrestrial studies note that initial decreases in BUN:creatinine, which occur during reduced food intake, are followed by an increase in the ratio. This reflects a changeover from fat to protein tissue catabolism for energy during long-term dietary restriction (Lochmiller *et al.* 1985b, 1985d, 1986, Wolkers *et al.* 1994a). The rate of these changes differs between juvenile and adult terrestrial mammals because of proportional differences in fat stores (deCalesta *et al.* 1975).

Results from this study showed that BUN levels were elevated at higher energy intake, which would seem to contradict the findings of terrestrial studies (Kirkpatrick *et al.* 1975, Warren *et al.* 1982). Reasons for this are unclear but it is possible that this contradiction reflects an adaptation to the marine environment. The high level of BUN in marine mammals also helps the kidney to function. Therefore elevated BUN in marine mammals may be due to the necessity of maintaining an osmotic balance in seawater. The plasma electrolyte content of marine mammals is very similar in quality and quantity to that of man, and could not alone maintain osmotic balance for these marine animals (Ridgway 1965, Ridgway *et al.* 1970, De Monte and Pilleri 1972, 1977). In the same context, protein and lipid metabolism of cetaceans is considerably more active than that of terrestrial mammals due, firstly to the energy required by muscles that are continuously in motion, and secondly to the necessity of maintaining body temperature at an adequate level regardless of the thermal condition of the water in which the animal is immersed (Slijper 1962 in De Monte and Pilleri 1972). In this respect, high BUN levels in marine mammals have been related to high protein diets, energy conservation and high metabolic rates (De Monte and Pilleri 1972).

Results from this study indicate that analyses of BUN in captive dolphins provided an ideal opportunity to monitor their nutritional condition. Elevated BUN has been used successfully as an indicator of nutritional status and improved habitat quality for terrestrial mammals (Franzmann

1972, Skeen 1974 in Kirkpatrick *et al.* 1975, Seal *et al.* 1978b, Hellgren *et al.* 1993). However, these studies also emphasise the importance of interpreting BUN values in conjunction with other blood parameters that may aid in the identification of tissue catabolism which increase BUN levels when food intake or diet quality is severely restricted (Ulrey *et al.* 1968, Torell *et al.* 1974, Kirkpatrick *et al.* 1975, DelGuidice *et al.* 1994).

Dimple and Simo both showed positive correlations between water temperature and dietary energy intake. Thandi, in contrast, showed negative correlations between these parameters. This may indirectly explain the different relationships between BUN and water temperature for these animals. Although BUN in Dimple appeared relatively stable throughout the year, low levels were only measured in winter. This concurred with the lower levels of BUN which were measured in colder water temperatures for this animal. Reasons for this are unknown, as energy conservation and higher metabolic rates (De Monte and Pilleri 1972) as well as increased muscular activity (Medway and Geraci 1965, Asper *et al.* 1990) have generally been associated with increased BUN. Thandi, in contrast to Dimple and Simo, generally displayed lower BUN levels at higher temperatures, similar to the negative correlation between diet and temperature. These relationships suggested that BUN was affected more directly by seasonal changes in diet than water temperature. In order to monitor nutritional condition effectively; and seasonal changes thereof, these relationships should be examined in more detail.

Seasonally, BUN:creatinine was slightly increased during winter, but only for Simo. This concurred with the higher BUN:creatinine values recorded at colder water temperatures for the same animal. This, in turn, related to the relationship between blubber thickness and BUN:creatinine. As discussed previously, BUN appeared to be more directly affected by diet than temperature, therefore the increase in BUN:creatinine was more than likely related to the decrease in creatinine during the same period, in conjunction with a supplement in the dietary energy content of food, increased during winter by Oceanarium staff.

Enzymes are normally present in the serum as a result of cell turnover and leakage into circulation. Tissue destruction, whether pathological or physiological, often leads to an increase in circulating enzyme levels (Geraci and St. Aubin 1979). Once the source of the enzyme is known, it can be used to identify the site and extent of tissue damage. Plasma enzymes have been used as diagnostic tests for organ damage in many mammalian species (Kaneko and Cornelius 1970 in Geraci and St. Aubin 1979). Literature on marine mammal enzymes is generally confusing due to the use of several different units in measuring enzyme levels (Appendix 5, 6). The problems are exacerbated by the fact that most of these units are not interconvertible (Medway and Geraci 1978).

The liver is the largest organ in the body and is responsible for producing most of the endogenous energy sources used by the body. It is the principal organ for the metabolism of carbohydrates, proteins and lipids. The liver is also the major storage site for iron, glycogen, lipids and vitamins. It also plays a major role in detoxification and excretion of metabolic end products (Kaplan and Pesce 1989). Increases in the activity of certain hepatic enzymes are common in all kinds of liver disorders. In marine mammals, ALP is liver-specific (Medway and Geraci 1978), although tissue ALP activity in harbour porpoise (*Phocoena phocoena*) was found to be concentrated in the kidney and adrenal gland, with only slight activity in the liver (Geraci and St. Aubin 1979). "Normal" mean ALP concentrations measured for the animals during this study, were generally higher than those of other cetaceans, although the use of different units makes comparisons difficult (Appendix 5). Domino, the youngest animal, had the highest ALP levels during this study, although there were no other age- or sex-related trends amongst the other four animals. Bone growth and associated increased osteoblastic activity (especially in young animals) results in increased ALP. In humans, ALP levels rapidly rise in the first month of life to approximately five or six times higher than normal adult levels, then decrease slowly until puberty, when there is another increase. This is followed by another decrease to adult values at 16-20 years (Kaplan and Pesce 1989). Elevated ALP levels in younger marine mammals have been reported in a number of marine mammal species (Anderson 1968, Lee *et al.* 1977, Geraci *et al.* 1979, MacDonald 1981 in Bossart and Dierauf 1990, McConnell and Vaughan 1983, Asper *et al.* 1990, Bossart and Dierauf 1990, Fothergill *et al.* 1991, Rhinehart *et al.* 1992, Heidel *et al.* 1996) and terrestrial mammals (Seal *et al.* 1975, Melton and Melton 1982, Lochmiller and Grant 1984). For this reason, authors have suggested that ALP may be used as an indicator of physical maturity in marine (Andersen 1968, Fothergill *et al.* 1991) and terrestrial mammals (Seal *et al.* 1975, 1978b, Melton and Melton 1982, Lochmiller and Grant 1984). The elevation in ALP activity in juvenile animals has been ascribed to rapid bone growth and increased metabolism during this period (Seal *et al.* 1978b, Lochmiller *et al.* 1986, 1988, Kaplan and Pesce 1989).

ALP levels above 700 U/l are regarded as normal for Atlantic bottlenose, with values below 200 U/l associated with disease (Thurman and van der Elst 1995). Elevations of ALP in adult marine mammals are assumed to indicate hepatic damage (Medway and Geraci 1978, Geraci and St. Aubin 1979, Thurman and van der Elst 1995) or intrinsic bone pathology (Thurman and van der Elst 1995). ALP levels are increased during liver disease, intestinal inflammation and in the advanced stages of pregnancy. Critically ill bottlenose dolphins have shown dramatic ALP reductions, assumedly because of metabolic changes. Increasing ALP levels in ill bottlenose dolphins are a sign of recovery, indicating that ALP is a useful prognostic indicator in marine mammals (Bossart and Dierauf 1990, Clarke 1990, Fothergill *et al.* 1991). Fothergill *et al.* (1991) reported that the extent of ALP decline was related to the severity of bacterial and viral infections.

Both Dimple and Dolly's ALP levels were significantly correlated with the change in age during the study, but the marked increase evident for Dimple was more than likely associated with clinical changes in hepatic (Medway and Geraci 1978, Geraci and St. Aubin 1979, Thurman and van der Elst 1995), and possibly osteoblastic functioning (Kaplan and Pesce 1989, Thurman and van der Elst 1995), in the period leading up to her death. Changes in the ALP:creatinine ratio for both Dimple and Thandi confirmed this interpretation. The marked increase in Dolly's ALP levels and the ALP:creatinine ratio, at age 25 years, may have been a continuation of a long-term increase in ALP, which was initiated in 1988 (approximately age 20 years). It is possible that this is a physiological change is associated with a change in maturity, similar to that described in humans (Kaplan and Pesce 1989). A marked increase in Simo's ALP levels at approximately age 9 years may also be associated with similar maturity-related changes. Fothergill *et al.* (1991) reported that ALP levels in Atlantic bottlenose dolphins decreased between 17 months and approximately four years, after which they levelled off. However, the authors emphasised the need for further investigation into the changes of ALP with age.

Associations between ALP and weight during this study indicated that ALP activity increased with weight, although these relationships were clearly defined only in the oldest females (Dimple and Dolly). Increasing weight in growing animals is associated with bone growth and consequent increased ALP activity (Thurman and van der Elst 1995). Weight gain or loss has been associated with ALP increases and decreases, respectively in growing cetaceans (Dover *et al.* 1993). However, reasons for this increase in mature animals are unclear. A reduction in weight, in a mature animal, may indicate a decline in overall condition and hepatic functioning, reflected physiologically by reduced ALP levels.

Results from this study, although variable, show that ALP activity was reduced at higher levels of food consumption and dietary energy intake. Certain types of physiological stress have been associated with increased ALP levels (Ronald and Kay 1982) and in this context, this relationship reflected nutritional stress at reduced levels of food consumption. Although enzymes have been found to be useful in several forms of clinical diagnosis, terrestrial mammal studies have shown that they are not reliable indicators of protein-energy status (Lochmiller *et al.* 1985d). ALP, however, does reliably display diet-related changes in several species of terrestrial mammal. Increases in ALP activity, resulting from low quality diets and feed restriction, have been associated with changes in osteoblastic activity, which, in turn, has been related to intestinal malabsorption (Seal *et al.* 1978b, Lochmiller *et al.* 1986, 1988). ALP levels indicate a trend of catabolic versus anabolic states in growing cetaceans, and may be therefore be used to assess whether nutrition is adequate for bone deposition and growth (Dover *et al.* 1993). In fasted harp seal pups ALP levels declined, even while in the growing phase. In pups fasted for a prolonged period, the growth rate was reduced, reflected in decreased ALP levels (Worthy and Lavigne 1982 in Bossart and Dierauf 1990). The diet categories used in this study, although incorporating long- and short-term dietary changes, may not have been extensive enough to monitor changes in ALP with fasting and food deprivation reported by other authors.

Seasonal changes in ALP were difficult to define, although Simo's ALP levels appeared more variable, and possibly slightly lower, in the winter and spring months. This concurred with the trend evident for this animal when ALP levels were considered with water temperature. Increased ALP activity generally occurred at higher water temperatures. As this animal was still growing during the study, this trend may be attributed to the fact that osteoblastic activity, and therefore growth, was maximised during the summer, when water temperatures are warmer. This would seem reasonable when considering that energy stores and metabolites are more available during the summer for growth, as opposed to the need for energy stores and metabolites for basic maintenance and thermoregulatory mechanisms during winter. Seasonal fluctuations in ALP have been recorded for terrestrial (Roussel and Stallcup 1966 in Andersen 1968) and marine mammals (Andersen 1968). Higher ALP concentrations in wild common seals (*Phoca vitulina*) than in captive animals, has been related to higher levels of activity (McConnel and Vaughan 1983). In contrast, Dimple's ALP levels and ALP:creatinine values were higher in winter. Because this animal was mature, and may even be considered geriatric, this may indicate an increased level of physiological stress at colder water temperatures.

With the exception of Dimple, total bilirubin levels measured during the study were slightly lower than those recorded for other populations of bottlenose dolphins (Appendix 6). Dimple's mean total bilirubin (5.83 ± 5.06 µmol/l) was significantly elevated but comparable to a captive female bottlenose dolphin (5.13 ± 3.42 µmol/l) (Ridgway *et al.* 1970) and bottlenose dolphins from Sarasota, Florida (6.33 ± 4.79 µmol/l) (Rhinehart *et al.* 1991). Bilirubin originates from the conversion of the haem portion of HB, following the phagocytosis of senescent and degenerating RBC's (Kaplan and Pesce 1989). Increased production of bilirubin in humans is usually a result of accelerated RBC breakdown (Anon. 1982, Kaplan and Pesce 1989), although it can reflect that bilirubin is not being broken down by the liver (Thurman and van der Elst 1995). Total bilirubin concentrations can be influenced by liver disease and bile duct obstruction, as well as haemorrhaging and fasting, although not all these influences have been documented for marine mammals (Bossart and Dierauf 1990). Elevated bilirubin levels have been reported in clinically ill cetaceans (Medway *et al.* 1966 in Bossart and Dierauf 1990). Liver damage as a result of liver fluke parasitism was responsible for elevated bilirubin levels in wild harbour porpoise (Geraci and St. Aubin 1979, Koopman *et al.* 1995) and a stranded common dolphin (*Delphinus delphis*) (Ridgway *et al.* 1970). As captive animals are regularly treated for parasites, elevated bilirubin levels in captivity may thus reflect a clinical problem (Kastelein *et al.* 1990, Koopman *et al.* 1995).

Overall, total bilirubin levels appeared to increase with age, Domino having the lowest level, Dimple the highest. In humans, bilirubin levels rise after puberty, fall during the third decade and then remain stable (Kaplan and Pesce 1989). Harbour seal neonates (less than seven days old) are reported to have high (>34.20 μmol/l) bilirubin levels (Odell 1980, Gartner 1972 in Bossart and Dierauf 1990). These levels correspond to both human neonates (Kaplan and Pesce 1989) and newborn common seals (87 μmol/l) (McConnell and Vaughan 1983). This elevated level of bilirubin at birth has been attributed to the liver not becoming fully functional until a few days after birth; and by the rapid turnover of foetal HB (McConnell and Vaughan 1983).

Levels of total bilirubin for Dimple increased markedly during the final six months of the study and although the majority of these values were not identified as outliers, the increase was associated with a health problem, which eventually led to this animal's death. It is not clear whether the animal died from natural causes, i.e. extreme old age or from clinical problems. However, the increase in bilirubin levels during this time was indicative of hepatic problems associated with voluntary fasting (Bossart and Dierauf 1990) and/or liver failure (Thurman and van der Elst 1995). This increase artificially increased the "normal" mean bilirubin level of Dimple and realistically, Dimple's mean bilirubin level was probably similar to those of Dolly and Thandi. The fact that Domino had the lowest mean bilirubin level contradicts the findings of other studies, although it is possible that levels recorded for this animal were affected by changes in maturity (as seen in humans, Kaplan and Pesce 1989), not covered by this study.

Although bilirubin levels did vary, increased concentrations were more frequent at heavier body weights for four of the five captive dolphins. This suggested a higher turnover of RBC's at increased weights which coincided with increased RBC counts at heavier weights (see Chapter 3). The possible effect of diet on total bilirubin levels was difficult to clarify. Dimple's results, however, illustrated the interaction between total bilirubin concentrations and reduced food intake during fasting. Bilirubin levels were highest at low levels of energy intake and all significantly increased bilirubin levels for Dimple were associated with significantly reduced food intake. Serum bilirubin was elevated in feed-restricted black-tailed jackrabbits (*Lepus californicus*) (Henke and Demarais 1990), reflecting some liver stress which can be caused by nutritional stress (Iber and Latham 1985 in Henke and Demarais 1990). The fact that only Dimple revealed these relationships indicated that bilirubin was affected only in situations of severe and prolonged dietary restriction.

Although seasonal changes in total bilirubin were unclear, this metabolite was reduced in warmer waters. The increase in bilirubin levels at colder water temperatures coincided with an increase in RBC's (see Chapter 3), concurring with the relationship between the catabolism of RBC's and the production of bilirubin. Higher activity levels in winter, in order to maintain body temperature, may increase the turnover rate of RBC's. There are no data for terrestrial or marine mammals on the effect of ambient temperature on bilirubin. The thermoneutral zone for bottlenose dolphins is approximately 22°C (Ross and Cockcroft 1990, Cockcroft 1991) and elevated bilirubin levels in waters colder than this suggested a change in liver function.

All dolphins used in this study had "normal" mean LDH levels that were markedly higher than those reported for other cetaceans (Appendix 5), although the inconsistent use of units limited the usefulness of any comparisons. Most mean LDH levels reported for bottlenose dolphins are twice or three times lower than those recorded during this study. Reasons for this are unknown. Population differences in LDH levels, which were observed in killer whales from the Pacific and Atlantic (Cornell 1983), may help to explain these differences, although such a marked difference seems unreasonable in terms of population differences. "Normal" values for LDH in marine mammals are typically highly variable and are reportedly of little use diagnostically (Medway and Geraci 1978, Bossart and Dierauf 1990). This variability has been associated with LDH leakage from skeletal muscles as a result of muscle exertion during diving.

In humans, LDH is present in many tissues, but activity is highest in the kidney and heart. LDH is localised in the cytoplasm of cells and is therefore extruded into the serum when cells are damaged (by trauma or disease) or necrotic (Kaplan and Pesce 1989). Measurement of LDH is most useful in humans when a specific organ is known to be damaged. If multiple organs are involved the usefulness of LDH is reduced (Kaplan and Pesce 1989). In marine mammals, an increase in LDH can generally be associated with cell damage or necrosis (Bossart and Dierauf 1990). Increased LDH levels have also been associated with lung inflammation (Cornell 1983), myocardial infarction, liver disease and malignant disease (Thurman and van der Elst 1995).

There were no apparent age-associated changes in LDH and/or LDH:creatinine from the five captive dolphins. The significantly increased LDH levels and LDH:creatinine values from Dimple were attributed to clinical problems arising from voluntary fasting, associated nutritional stress and cellular damage during the final stages of the study. Extensive damage to skeletal muscle, non-specific multi-organ cellular damage, snare-related stress and moulting have all been associated with increases in LDH (Ridgway *et al.* 1970, Lee *et al.* 1977, Ronald and Kay 1982, Bossart and Dierauf 1990). Stress-related increases in LDH have also been reported for terrestrial mammals (Seal *et al.* 1975, Lochmiller and Grant 1984).

The lack of age-related differences in LDH during this study does, however, contrast with other species. LDH levels in humans decrease with age, with levels in children typically 10-15 % higher than adult values (Kaplan and Pesce 1989). Neither were there any apparent sex-related differences in LDH, as described by Ridgway *et al.* (1970) and Asper *et al.* (1990), which both found higher LDH levels in male bottlenose dolphins. Asper *et al.* (1990) recorded higher LDH levels in juvenile bottlenose dolphins, which is similar to results obtained from terrestrial mammals (Seal *et al.* 1978). However, in this context, increases in LDH and LDH:creatinine observed for Simo and Domino during the study were possibly related to changes in maturity. In addition, both male dolphins also displayed a significant increase in LDH with increasing body weight. As discussed above, these changes were probably due to growth and associated increases in metabolic rate during changes in maturity, from adolescent to mature animal in the case of Simo; and from juvenile to adolescent in the case of Domino.

If increased blubber thickness is associated with good overall condition (Ridgway 1965, Ridgway and Fenner 1982, Lockyer *et al.* 1984, Lockyer 1986, 1993, Ognetov 1990, Wells *et al.* 1992, Wells 1993), the positive relationship between LDH and blubber thickness implied that higher LDH levels were indicative of improved morphological (and overall) condition. However, in light of the fact that Domino's blubber thickness measurements were negatively correlated with water temperature, this trend related preferentially to an interaction between water temperature, blubber thickness and activity levels. The increase in LDH levels at greater blubber thicknesses were related to increased muscle and heart activity, associated with a seasonal change in water temperature and activity levels. Excess muscular activity has led to increased LDH in manually restrained pinnipeds (Bossart and Dierauf 1990).

The majority of trends between diet and LDH and/or LDH:creatinine was complex and need further examination. Based on the results of this study, LDH and LDH:creatinine appeared slightly reduced at lower levels of food and dietary energy intake. The fact that Thandi's data showed the highest number of correlations between LDH and diet is cause for "hesitation". Increased food consumption and energy intake were often associated with illness and treatment in this animal; and although LDH levels during these periods were not significantly different, the increases observed at higher levels of food and energy intake may be associated more with internal cell and tissue damage, than diet-related physiological changes. Although Domino's LDH levels tended to increase with increased energy intake, those of Simo appeared to decrease. These contrasting trends emphasise the need for further investigation. Terrestrial mammal studies have shown that LDH may reflect habitat and forage quality (Seal *et al.* 1978a), with increased LDH reflecting a decline in diet quality. LDH levels were slightly increased during winter and spring, which coincided with a similar increase of LDH activity and LDH:creatinine at colder water temperatures. This increase attested to the increased heart and muscle activity during periods of increased activity during winter, as discussed previously.

In humans, CPK is widely distributed in tissues, but concentrations are highest in skeletal muscle. CPK is present in skeletal muscle of marine mammals, as well as the smooth muscle of the myocardium, gastrointestinal tract, uterus, bladder and kidney (Engelhardt 1979, Geraci and St.

Aubin 1979, Bossart and Dierauf 1990). CPK levels measured during this study were generally within the ranges reported in the literature for this species but were all higher than the mean values (Appendix 5). Results from this study showed the youngest dolphin to have the highest levels of CPK (corresponding with the findings of Asper *et al.* (1990)) and the three oldest animals (all females) to have the lowest CPK levels. Although males appeared to have higher CPK levels than females the small sample size prevents any definite conclusions. An adolescent male harbour porpoise showed higher CPK levels than both an adult male and a juvenile female of the same species (Kastelein *et al.* 1990). In humans, CPK values are lower in females (Kaplan and Pesce 1989). Additionally, age, sex and race-related differences have been found for different human sub-populations. In female humans, median CPK levels decrease during the first 20 years of life, after which they stabilise until after 55 years of age. In males, a fairly constant mean level of serum CPK seems to exist, except for a peak at approximately 15-18 years, which may be related to increased activity (Kaplan and Pesce 1989). Simo's CPK levels were higher and more variable prior to age five or six years, after which they were significantly reduced. This may be similar to the peak described in male humans (Kaplan and Pesce 1989). The fact that muscle mass reportedly affects CPK levels also contributes to males generally have higher CPK levels (Kaplan and Pesce 1989). However, this fact appears inconsistent, as some adult male terrestrial mammals have lower CPK levels (Seal *et al.* 1978a).

None of the five animals had CPK levels or CPK:creatinine ratios significantly related to their total body weight. This is extraordinary in view of the fact that CPK is reportedly affected by muscle mass (in humans) (Kaplan and Pesce 1989). Reasons for the lack of any relationship are unknown. However, CPK and CPK:creatinine were related to blubber thickness for both Thandi and Domino. Opposing trends for these animals, however, made this relationship difficult to confirm. Domino's CPK levels and CPK:creatinine values were higher when blubber thickness was greater, indicating an increase in muscular activity. Blubber is typically thicker in colder waters (Ridgway and Fenner 1982, Ognetov 1990, Lockyer 1993a, Wells 1993); and activity levels are generally higher; which indirectly results in the relationship evident for Domino during the current study. The decrease in CPK levels and ratio values seen at greater blubber thickness measurements for Thandi suggested that the blubber of this animal was enough to maintain body temperature without having to increase activity levels. The extremely thick blubber layer of beluga whales limits the need for increased metabolic rates under cold conditions (Slonim 1971 in Ognetov 1990) and it is possible that the opposing trends evident during this study may be a size-related (surface to volume ratio) difference in metabolic responses to cold temperatures.

As described for LDH, the relationship between CPK and diet was complex and no other data on marine mammal CPK and diet exists. Thandi displayed higher CPK levels and higher CPK:creatinine values at increased levels of energy intake, however, it is suspected that these values were associated with increased food intake during illness and treatment, as was the case between LDH and diet. Handling and frequent blood sampling, as well as a small amount of tissue damage, may have caused CPK to increase. The increase in CPK:creatinine with increased dietary energy intake for Dolly, who was generally healthy throughout the study, suggested greater muscular activity when the quality of the diet was improved. This relationship contrasted with those of Dimple and Domino, for which CPK levels were higher at lower levels of energy intake. Reasons for this contradictory relationship may be attributed to the high levels of stress, associated with voluntary fasting by Dimple, although these trends need further investigation. The only documented diet-related change in marine mammal CPK levels was an increase which occurred at the onset of feeding in fasted harp seal pups (Worthy and Lavigne 1982 in Bossart and Dierauf 1990).

The seasonal relationship between CPK, CPK:creatinine and water temperature was similar in nature to that described for LDH. Increased activity during winter, when water temperatures were reduced below the thermoneutral zone (Ross and Cockcroft 1990, Cockcroft 1991), resulted in increased CPK concentrations. The fact that outlying high CPK levels were only recorded at colder water temperatures reflected that extremely low water temperatures may act as an environmental stress and result in these animals being more susceptible to muscle overexertion during winter. CPK elevations in marine mammals has characteristically been associated with cardiac or skeletal muscle damage, strenuous activity and handling or exercise stress (Seal *et al.* 1975, Geraci and Medway 1973, Geraci and St. Aubin 1979, De Monte and Pilleri 1983, DelGuidice *et al.* 1987a, Bossart and Dierauf 1990, Thurman and van der Elst 1995, Heidel *et al.* 1996). As a result, CPK has been used as an indicator of muscular activity in dolphins (Geraci and Medway 1973).

Sodium is the major cation in the extracellular fluid with total body sodium (in humans) being a reflection of the balance between sodium input (dietary intake) and output (renal excretion) (Kaplan and Pesce 1989). Sodium intake depends on the quantity and type of food ingested. Sodium accumulates in the human body when sodium intake exceeds sodium output because of some abnormality of sodium homeostatic mechanisms (Kaplan and Pesce 1989). Sodium levels measured during this study were similar to almost all other values recorded for cetaceans (Appendix 7). There were no age or sex associations for this parameter. Additionally, there were no definite trends between sodium or sodium:creatinine and the change in age or weight during the study. The increase in sodium:creatinine values at the end of the study for Dimple was associated with electrolyte and kidney problems resulting from severely reduced food intake and dehydration. In humans, sodium is increased during cardiac failure, liver and renal diseases and during pregnancy. Similar causes have been recorded for marine mammals (Bossart and Dierauf 1990). Dehydration has resulted in elevated levels of sodium for several species of cetacean (Boice and

Roberts 1966, Medway and Geraci 1978, Clarke 1990, Thurman and van der Elst 1995). Kidney shutdown is reflected in severe cases of sodium elevation (Clarke 1990).

The increase in sodium:creatinine for Domino during the study was similar to that of other chemistry parameters and may be related to growth, feeding and associated changes in the electrolyte balance. In a similar context, positive relationships between sodium, sodium:creatinine and diet reflected that increased values for these parameters were indicative of improved nutritional condition. Sodium levels were higher in ringed seal pups (*Phoca hispida*) in good condition (based on weight, coat condition and behaviour) (Geraci *et al.* 1979). A diet comprising predominantly of squid, which is high in water and low in sodium, was found to reduce the sodium levels of a Gray whale calf (Medway and Cornell 1989). The relationship between diet and serum sodium revealed in the results of this study concurred with those of Geraci (1977), which found that diets of poor quality fish also result in reduced serum sodium. Low-energy prey species fed to the dolphins in this study were generally high in water (for example, hake and squid) and this may account for reduced sodium levels at lower energy intake. However, it must be noted that prolonged fasting can eventually lead to haemoconcentration and increases in serum sodium (Medway and Geraci 1965, 1978), as was evident for Dimple at the end of the study. Similar diet-related changes in serum sodium have been noted for terrestrial mammals. Reduced food intake resulted in low sodium levels in collared peccaries (Lochmiller *et al.* 1985d), which was attributed to low salt intake. A similar decrease, in white-tailed deer, was suggested to be a result of partial osmotic adjustment to increased urea levels (Seal *et al.* 1978b). Evidence obtained from terrestrial mammals suggests that mineral deficiencies arising from malnutrition result in decreased immunity and increased susceptibility to infection (Chandra and Dayton 1982 in Lochmiller *et al.* 1985d).

Sodium concentrations appeared to be reduced at water temperatures above 24°C, although this trend was not consistent and needs further definition. Low sodium has been recorded as a result of non-specific stress (Geraci 1972) and it is possible that high water temperatures represent a form of environmental stress. Sodium:creatinine values also appeared slightly higher in the winter and spring months, in colder water temperatures. Many stress-related responses in marine mammals are characterised by an acute decrease in sodium values (Geraci 1972, Geraci *et al.* 1979, Engelhardt 1979). Sodium was found to be reduced in ringed seals exposed to the stress of oil exposure and transport (Geraci and Smith 1975, Geraci *et al.* 1979) and bowhead whales subjected to the stress of harpooning (Heidel *et al.* 1996). Thin, lethargic ringed seals, experiencing the stress of moulting also exhibited severely reduced sodium levels (Geraci *et al.* 1979, Ronald and Kay 1982).

Most serum proteins are synthesised by the liver, which has the ability to increase serum protein output approximately two-fold during diseases associated with protein loss. For this reason, total serum protein (in humans) is not changed until liver function has been extensively impaired

(Kaplan and Pesce 1989). Measurement of total protein has been found to be of limited diagnostic value for marine mammals (Thurman and van der Elst 1995) as it may be altered by changes in plasma volume without altering the albumin:globulin ratio. Albumin is one of the most important serum proteins produced by the liver (Kaplan and Pesce 1989). In humans, albumin represents 50-60 % by weight of all plasma proteins. Functions of human albumin include nutrition, maintenance of osmotic pressure and serum transport of calcium unconjugated bilirubin, free fatty acids, drugs and steroids. Because of this multifunctional role, albumin is an important analyte in the monitoring of liver condition in humans (Kaplan and Pesce 1989) and marine mammals (De Monte and Pilleri 1982). However, albumin has a short half-life and this reduces its usefulness as an index of acute liver disease (Berkow 1982).

Globulin increases are generally associated with liver disease and the stimulation of the immune system. Globulin reductions occur during immunodeficiencies (Bossart and Dierauf 1990). In harbour seal neonates, globulins decrease when animals are deprived of colostrum, although the degree of immunoglobulin transfer between mother and pup is unknown (Bossart and Dierauf 1990). In marine mammals, it appears that the globulin distribution is species specific and albumin:globulin ratios are lower in seals than in cetaceans (Ridgway 1972, Engelhardt 1979, Bossart and Dierauf 1990).

Albumin levels and albumin:globulin ratios are somewhat higher in cetaceans than in domestic species (Medway and Geraci 1978). Albumin levels measured during this study were similar to those recorded for other populations of bottlenose dolphins and other cetaceans (Appendix 7). Besides the oldest animal having the lowest concentration of albumin, there were no other age- or sex-related associations for this protein. Levels of globulin and the albumin:globulin ratio were, however, significantly lower and higher, respectively, in younger animals, implying that younger animals were not as immunologically active as older animals (Bossart and Dierauf 1990). Lower albumin concentrations in older animals have been reported for chacma baboons (Melton and Melton 1982). Total protein was found to be lower in younger captive killer whales (Cornell 1983) and common seal pups (McConnell and Vaughan 1983), although the proportion of albumin was not measured. Total protein and albumin:globulin was lower and higher, respectively in juvenile collared peccaries (Lochmiller and Grant 1984).

Overall, it appeared that those animals with fewer periods of illness and abnormal behaviour and more regular eating habits had higher mean concentrations of albumin. Albumin has been found to be a valuable guide to nutrition in marine mammals (Medway and Cornell 1989, Thurman and van der Elst 1995), reductions occurring during lack of adequate diet and malnutrition as well as during liver and kidney disease, protein losing enteropathy (prolonged fever and trauma) and as a result of gastrointestinal parasites (Bossart and Dierauf 1990). Manatees suffering from malnutrition and starvation exhibit reduced albumin levels. Albumin levels reportedly decline during infectious process in marine mammals (Ridgway 1972). This decline parallels an increase in globulins. Albumin increases during dehydration and shock in marine mammals, but these increases are relative, as a true overproduction of albumin is probably non-existent (Bossart and Dierauf 1990, Thurman and van der Elst 1995).

Thandi was the only animal for which albumin, globulin and albumin:globulin were all significantly related to weight, although this was probably due to the fact that serum protein analyses were most numerous for this animal. Higher albumin, lower globulin levels and increased albumin:globulin values at heavier weights implied an interaction between elevated serum protein concentrations and increased food intake, improved nutritional status and overall condition. In the same context, reduced globulins at increased body weights reflected a reduced susceptibility to infection when overall body condition was improved (Asper *et al.* 1990). Similar trends have reflected good protein metabolism and liver function in a Gray whale calf (Medway and Cornell 1989). However, these relationships must be treated with caution as Thandi was generally heavier and consuming a greater amount of food during periods of illness and treatment. In this respect, although increased albumin and reduced globulin may reflect improved nutritional condition, based on dietary intake, they may not necessarily be a reliable indication of overall condition.

Although dietary relationships with serum proteins were difficult to characterise, there was a tendency for albumin levels to be higher at increased levels of food consumption and dietary energy intake. The visceral compartment of an animal is of particular importance in protein synthesis and the immune system. The ability to assess nutritional status by measuring serum levels of various protein fractions and thus provide important insight into visceral function (and hence animal condition) has long been recognised for terrestrial mammals (Shenkin and Steele 1978). Total protein and albumin are reportedly resilient to dietary restriction in some species of terrestrial mammal (Seal *et al.* 1978b, Warren *et al.* 1981, 1982, Lochmiller *et al.* 1984, 1985b, 1985d, 1986). However, albumin has been shown to decrease during protein restriction in other terrestrial mammal species (Lowrey *et al.* 1962 and Yen *et al.* 1982 in Lochmiller *et al.* 1986, Hyvarinen *et al.* 1975). Reduced food intake also resulted in declining albumin concentrations in red deer, reflecting a reduced protein or nutritional status (Wolkers *et al.* 1994a). Haemoconcentration may counteract eventual decreasing plasma protein levels (DelGuidice *et al.* 1987b), reducing the reliability of plasma proteins as an indicator of nutritional status.

Lack of data prevented the definition of any potential seasonal trends in serum proteins. Thandi's globulin concentrations decrease markedly between June and October, which concurred with a decrease in globulin levels, as water temperature increased from 16°C to 21°C. This may reflect a reduced susceptibility to infection during summer, and in warmer waters, although more data is needed to expand this relationship. Changes in albumin and globulin values have been associated with changes in immune system activity by Asper *et al.* (1990). The albumin:globulin ratio displayed an increase in the summer months which suggests an improved nutritional status (Medway *et al.* 1982, Asper *et al.* 1990) and condition, which may aid in reducing the chances of infection. Seasonal changes in albumin and globulin levels in reindeer have been linked to seasonal changes in diet quality (Hyvarinen *et al.* 1975).

The exclusion of any analyses and/or discussion concerning serum lipids is due only to logistical and funding limitations. These blood components are recognised as potentially valuable tools in the assessment of dolphin condition. The relationship between the diets of different marine mammal species and their respective serum lipid profiles has already been recognised (De Monte and Pilleri 1982) and, in this respect, the analysis of serum lipids, in conjunction with diet and blubber composition analyses may provide an essential insight into the most important aspects of dolphin condition.

Summary

Based on conclusions and summaries of all data obtained during this study, an "index of condition" using blood chemistry components was established for captive bottlenose dolphins (Table 4.10). This index provides an indication of physiological condition and the nature of physiological changes, which may occur with respect to fluctuations in the animal's condition.

In summary, results of this study showed that certain blood chemistry parameters are valuable indicators of morphological and nutritional condition and provide an insight into seasonal physiological changes, with respect to diet, temperature and overall condition. Blood parameters respond acutely to dietary changes in terrestrial mammals (Lochmiller *et al.* 1986, Dinkines *et al.* 1991) and thus, offer information on short-term and recent metabolic function. Results from this study indicate that several blood chemistry components from marine mammals can offer the same perspective. Parameters found to be most useful in the monitoring of terrestrial mammal metabolism and nutritional condition include changes in weight and the serum chemistry components of BUN, BUN:creatinine, ALP (Franzmann 1972, Seal *et al.* 1978b, Lochmiller *et al.* 1986, Dinkines *et al.* 1991, Wolkers *et al.* 1994a) and albumin:globulin (Lochmiller *et al.* 1985b). Creatinine, BUN:creatinine and ALP are reportedly more effective in the short-term, although BUN and BUN:creatinine are valuable indicators of long-term undernutrition (Wolkers *et al.* 1994a).

Based on the results from the present study, creatinine, BUN, BUN:creatinine and albumin provided the best indication of nutritional condition and diet quality in captive dolphins. In this respect, these parameters are potentially valuable in the assessment and monitoring of habitat quality, in terms of food resources. In addition, creatinine and BUN:creatinine reflected changes in morphological condition. Reduced ALP implied nutritional stress but this enzyme was more effectively used as an indication of growth activity and metabolism, particularly in growing animals. However, in light o the important relationship between growth and nutrition, ALP may be an important analyte in the assessment of nutritional status, relative to age. The enzymes LDH and CPK were typically variable and fluctuated according to activity levels, limiting their use as condition indices. Levels of serum sodium were an important indicator of diet quality and hydration status. Many of these parameters effectively illustrated that physiological condition was compromised outside of the thermoneutral zone of the species. The relationships evident in the results of this study highlighted the important interactions between physiological condition and morphological, nutritional and environmental variables. These base-line relationships are a prerequisite for comparative condition studies of free-ranging dolphins and set vital standards for the assessment and monitoring of wild dolphin condition and the status of the marine environment as a whole.

Table 4.10: Blood chemistry condition index for captive bottlenose dolphins. (A: optimal condition, B: precautionary, C: less than ideal condition).

		"Condition factor"		
Parameter		**A**	**B**	**C**
Creatinine (μmol/l)	Juvenile	113-127	130-160	>170
	Mature	95-125	130-160	>180
	Elderly	94-103	105-130	>140
BUN (mmol/l)	Juvenile	8.5-9.0	<7.8, >10.0	
	Mature	9.0-10.8	<7.5, >13.0	
	Elderly	7.0-7.6	<5.0, >8.0	
BUN:creatinine		0.07-0.10	0.12-0.20	>0.25
ALP (U/l)	Juvenile	700-1 000	<600	
	Mature	200-500	<200, >500	<100, >700
ALP:creatinine	Juvenile	6.0-10.0	<6.0	
	Mature	2.1-4.5	<2.0, >6.0	
Total bilirubin (μmol/l)		1.9-5.0	5.0-11.0	>15.0
LDH (U/l)		770-1 200	1 400-1 600	>2 000
LDH:creatinine		6.5-9.7	10.0-20.14	>30.0
CPK (U/l)	Juvenile	190-240	240-300	>300
	Mature	130-200	200-300	>300
CPK:creatinine	Juvenile	1.7-2.2	2.3-3.0	>3.0
	Mature	1.2-1.7	2.0-4.0	>4.0
Sodium (mmol/l)		155-159	<151, >160	<148, >164
Sodium:creatinine		1.3-1.7	<1.0, >1.8	<0.5, >2.0
Albumin (g/l)	Juvenile	41-45	<40	
	Mature	44-47	<42	
	Elderly	34-36	<32	
Globulin (g/l)	Juv./Adolescent	17-22	23-27	>28
	Mature	23-28	30-34	>35
Albumin:globulin	Juv./Adolescent	1.7-2.5	<1.6	
	Mature	1.6-2.0	<1.4	

CHAPTER 5:

MORPHOLOGICAL INDICATORS OF CONDITION IN NET-CAUGHT BOTTLENOSE (*Tursiops truncatus*) AND COMMON (*Delphinus delphis*) DOLPHINS.

INTRODUCTION

Morphological parameters have been used extensively in the assessment of condition in both terrestrial and marine mammals. The majority of early studies relied on total body weight to reflect overall condition. However, as condition assessment and monitoring became more valuable, additional parameters were found to provide valuable insights into morphological and overall body condition. Traditionally, morphological parameters used in the assessment of body condition have included age-specific body size (Eberhardt and Sniff 1977, Fowler and Sniff 1992), skeletal size (Krebs and Singleton 1993), kidney and bone marrow fat, deposited fat reserves, adrenocortical hypertrophy and other aspects of body growth (Hanks 1981). However, the majority of these traditional measures of body condition was destructive and could only be carried out on dead animals. The establishment of non-destructive indices of condition has become vital for the purposes of wildlife management and conservation.

Morphological indices such as weight, length, girth and blubber thickness have been used extensively in marine mammal research (Ridgway and Fenner 1982, Kastelein and van Battum 1990, Castellini and Calkins 1993). Although body weight is a basic condition index, it remains useful in the marine environment. Condition assessments, using changes in weight, have been used to investigate changes in population growth rates of endangered Stellar sea lions (*Eumetopias jubatus*) (Merrick *et al.* 1995) and southern elephant seals (*Mirounga leonina*) (Hindell *et al.* 1994). Total body weight, sometimes in conjunction with indices of nutritional status, has been used to assess differences in reproductive success; and growth and development of offspring in several seal species (Boyd 1984, Arnbom *et al.* 1993, Iverson *et al.* 1993, Muelbert and Bowen 1993, Walker and Bowen 1993). Some of the most important indices of marine mammals morphological condition include measures of body fat or blubber (Ridgway and Fenner 1982, Lockyer 1986, 1993a, Lockyer *et al.* 1984, 1985, Ognetov 1990). Modern technology has allowed for the development of a number of non-invasive indices of blubber condition. Ultrasound and/or isotopic evaluation of blubber thickness and/or composition (Slip 1992, Wells *et al.* 1992, Costa *et al.* 1993, Wells 1993, Rosen and Renouf 1997), in conjunction with girth measurements, have been used to calculate blubber volume (Slip 1992, Worthy *et al.* 1992, Renouf *et al.* 1993, Rosen and Renouf 1997). In addition, alternative non-invasive methods of condition assessment have included the photography and investigation of zones of discontinuity in the eye lens (Dawson *et al.* 1992) and bioelectrical impedance (Gales *et al.* 1994, Arnould 1995).

The accessibility of seals during seasonal haul out periods has resulted in the majority of studies on marine mammal body condition and/or composition concentrating on otarids and pinnipeds (for example (Slip 1992, Worthy *et al.* 1992, Gales *et al.* 1994, Arnould 1995, Rosen and Renouf 1997). Cetaceans are less accessible and as a result, condition assessments of healthy, wild cetaceans are limited (Rhinehart *et al.* 1991, 1992, Wells 1993). Several studies have made use of specimens caught during whaling operations (Lockyer *et al.* 1984, 1985, Lockyer 1986, Kvadsheim *et al.* 1996) and other types of fisheries (Lockyer 1993a), which have provided insights into the morphological condition of free-ranging large whales. These include interesting relationships between body fat condition, food abundance and fecundity. Stranded and incidentally caught specimens also afford an opportunistic insight into morphological condition (Kastelein *et al.* 1990).

The relationship between certain morphological and/or physiological measures often allows for generalisations about relative changes in morphology and/or physiological processes over a wide range of body sizes (Ridgway and Fenner 1982, Ridgway and Kohin 1995). The usefulness of these relationships is apparent when applied to free-ranging populations, where extensive physiological measurements are not readily available. These relationships can also be interpreted with respect to age and habitat type (Ridgway and Kohin 1995). The influence of age, reproductive status and other factors, such as diet and water temperature, on morphological, particularly blubber, condition of marine mammals is well documented (Ridgway and Fenner 1982, Ross 1984, Lockyer *et al.* 1984, 1985, Lockyer 1986, 1993a, Ognetov 1990, Wells 1993). However, it remains unclear how physiological, morphological and environmental variables interact.

Terrestrial studies have proved there to be valuable relationships within and between physiology, morphology and the environment, which reflect nutritional condition of animals, as well as overall habitat quality. Terrestrial species that have been most extensively examined include collared peccaries (*Tayassu tajacu*) (Lochmiller *et al.* 1985b, 1985d, 1989, Sams *et al.* 1998) and white-tailed deer (*Odocoileus virgianus*) (Seal *et al.* 1978a, 1978b, Warren *et al.* 1981, 1982, Dinkines *et al.* 1991). However, studies on a wide variety of other terrestrial species have reflected important interactive relationships between morphology, physiology, food supply, diet quality and the environment (Bailey 1968, Smith 1970, Franzmann, 1972, Hyvarinen *et al.* 1975, Seal *et al.* 1975, Hanks *et al.* 1976, Brooks 1978, Robinson 1979, Melton and Melton 1982, DelGuidice *et al.* 1987, Henke and Demarais 1990, Grant *et al.* 1992, Altmann *et al.* 1993, Choquenot 1993, Crete *et al.* 1993, Hellgren *et al.* 1993, Knick *et al.* 1993, Van Rooyen 1993, Rietkerk *et al.* 1994, Wolkers *et al.* 1994b, Gallivan *et al.* 1995, Huot *et al.* 1995, Clode *et al.* 1995). Studies have also placed emphasis on the interconnected relationships between body condition, nutrition, fertility and reproductive success (Robinette *et al.* 1973, Allaye Chan-McLeod *et al.* 1994, 1995, White *et al.* 1997). Recently, interesting physiological and morphological condition indices have been shown to be density-dependent (Blumstein and Foggin 1997, Sams *et al.* 1998).

Monitoring the condition of wild animals can provide valuable information about the potential survival, reproductive success and "well-being" of a population (Kirkpatrick 1980, Hanks 1981). Body condition can reflect the nutritional state of animals and can provide insights into factors influencing animal-habitat interactions (Hanks 1981, Virgl and Messier 1993). In this regard, age-specific body size of marine mammals has been suggested as an index of population status or habitat quality (Eberhardt and Sniff 1977, Fowler and Sniff 1992). Long-term monitoring of morphological condition in free-ranging marine mammals is, currently, almost impossible. An exceptional, isolated study in Sarasota, Florida has successfully monitored the morphology and physiology of a local population of bottlenose dolphins for over two decades (Rhinehart *et al.* 1991, 1992, Wells 1993). There are no similar studies ongoing, particularly in the Southern Hemisphere. This study comprises the first examination of morphological condition of coastal dolphins along the south-east coast of Southern Africa.

The south-east coast of South Africa coast supports 37 species of marine mammal (Ross 1984) however; knowledge of the condition of any of these species is lacking. In light of increasing coastal development and the associated increase of factors such as marine pollution and depleted food resources, it is vital to assess the condition of marine mammals in the region. Although the environmental conditions and geography of the area prohibit regular capture and sampling of free-ranging animals, annual incidental catches of dolphins in anti-shark nets along the Kwazulu-Natal coast provide a representative sample of the local dolphin population (Cockcroft 1990, Cockcroft and Ross 1989, 1991, Young and Cockcroft 1994). These animals provide a valuable opportunity to investigate the potential use of morphological measures of condition for the monitoring of wild animal populations.

In this context, by using incidentally caught dolphins, this study aimed to achieve the following objectives:

- To obtain morphological data from dolphins incidentally-caught in anti-shark nets
- To identify and assess the effect of species, age, sex and reproductive state on the morphology of net-caught dolphins.
- To identify and appraise the relationship between morphological condition and environmental variables such as diet and water temperature.

- To investigate the potential use of morphology in assessing the overall condition of net-caught dolphins.

- To investigate the potential use of the morphology of net-caught dolphins to assess and monitor the condition of local dolphin populations and the regional marine environment.

MATERIALS AND METHODS

1. MORPHOLOGICAL PARAMETERS

Incidentally caught specimens of bottlenose (*Tursiops truncatus*) and common (*Delphinus delphis*) dolphins were used during this study. This sample of net-caught animals was a sub-sample of all dolphins caught in shark nets along the Kwazulu-Natal coast, South Africa between 1993 and 1996 (Fig. 5.1). Shark nets were checked daily (Monday to Friday and weather permitting) by Natal Sharks Board staff and all animals retrieved from the nets were transported back to the Sharks Board base in Umhlanga Rocks, Kwazulu-Natal, South Africa.

All animals were weighed (to the nearest kilogram), directly after which blood samples were taken (see Chapters 6 and 7). Whenever possible, animals were dissected immediately after blood sampling. If not dissected immediately, specimens were frozen at -20°C until dissection was possible. External morphological measurements included total body weight (measured on arrival); total body length (from tip of lower jaw to notch in flukes); girth (at axilla, maximum and anus) and blubber thickness (mid-dorsal, mid-lateral and mid-ventral). Blubber thicknesses were measured midway between the pectoral flippers and umbilicus. Measurements were made to the nearest millimetre, with the exception of blubber thickness, which was measured to the nearest tenth of a millimetre using Vernier calipers.

Upon the completion of external measurements, one side of blubber (between the neck and caudal peduncle) was removed and weighed. This weight was doubled to indicate total blubber weight. Total muscle weight comprised of a doubling of the weight of one side of dorsal and abdominal muscle. Both blubber and muscle were weighed to the nearest kilogram. The oesophagus and stomach were removed and weighed (*sensu* Cockcroft and Ross 1990b) before being frozen for later examination.

All data and samples obtained from these animals are stored in the Marine Mammal Collection of the Port Elizabeth Museum, South Africa.

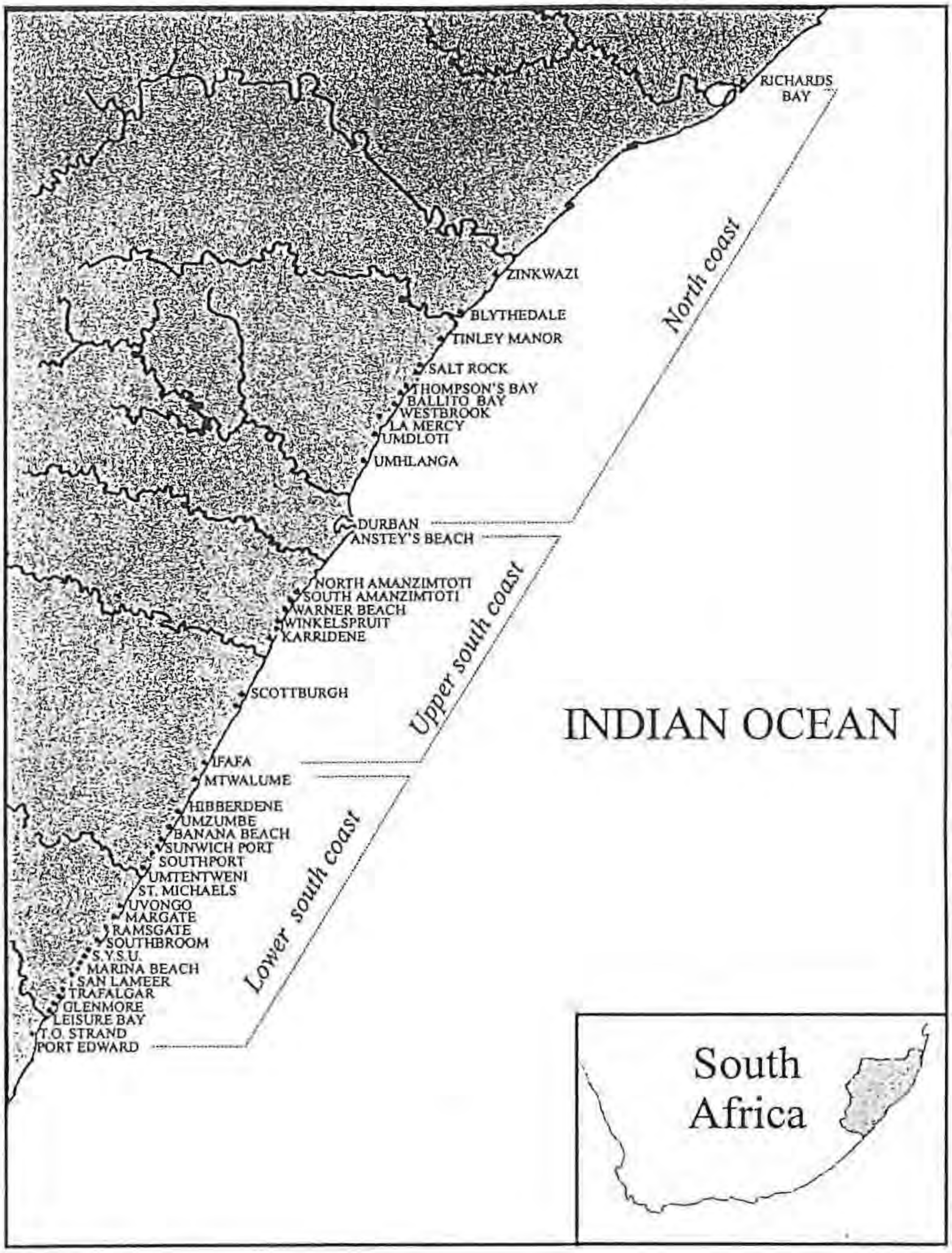


Figure 5.1: Map of Kwazulu-Natal coast, illustrating the localities at which dolphins are incidentally caught in anti-shark nets.

2. AGE AND GROWTH

Samples of teeth were obtained, wherever possible, from animals for which blood was sampled (see Chapters 6 and 7) and used to estimate the age of the animal. Techniques used in the mounting, sectioning and reading of teeth are described in (Cockcroft and Ross 1989). Age estimates were not carried out for all animals.

3. REPRODUCTION

Reproductive organs were examined for signs of sexual maturity and/or activity. The presence of sperm in the epididymis was recorded as an indication of male sexual activity. Lactating females were identified through the presence of milk in the mammary glands. All foetuses from pregnant animals were weighed and measured.

Animals were divided into age and sex classes according to growth layer group (GLG) counts, total body weight and reproductive condition. Weights and ages used to determine the different classes of maturity for bottlenose dolphins are described in Cockcroft and Ross (1990b) and for common dolphins in Mendolia (1990).

4. DIET AND FEEDING

Frozen excised stomachs were thawed and weighed. Stomach contents were removed, washed and separated *sensu* Cockcroft and Ross (1990b). All fish otoliths and cephalopod beaks were identified using the reference collection of the Port Elizabeth Museum. Otolith diameters and beak measurements were used in established regression calculations to determine reconstituted prey length and weight (Smale *et al.* 1995). The energetic (calorific) content of stomach contents was assessed using calorific estimates for the same or similar prey species in the literature (Table 3.1).

5. ENVIRONMENTAL MEASURES

Daily water temperatures were recorded from all 44 netted beaches by Natal Sharks Board staff. These records were used to establish mean water temperatures for all beaches. Temperature categories used in this study included the "local" water temperature of the beach at which the animal was caught, on the day of capture, the mean local water temperature for the month of capture and month prior to capture; and the mean "regional" water temperature of the month of capture and month prior to capture. The term "regional" pertains to the coastal region (north coast, upper south coast, lower south coast) in which the beach is located (Cockcroft 1990) (Fig. 5.1).

Statistical analyses were undertaken using several commercially available statistical computer packages. For all data sets, outliers were determined using box-whisker plots. Data were analysed including and excluding these outliers to determine their influence, if any, on mean values.

Relationships between morphological parameters, diet and water temperature were examined using multiple correlation analysis.

RESULTS

Morphological measurements were obtained from 96 animals (58 common dolphins; 38 bottlenose dolphins) between 1993 and 1996. The annual composition of samples comprised 33 animals in 1993, 58 in 1994 and four in 1995. Morphological data were obtained from a single bottlenose dolphin caught in January of 1996 (Fig. 5.2). Overall, captures were generally more frequent in June, July, and August. Twenty-three common dolphins were caught in September 1994. Common dolphins were caught predominantly during the winter months. Catches of bottlenose dolphins were most frequent in June, but were less seasonal than common dolphin catches. Common dolphin catches, although occurring along almost the entire Natal coast, were most frequent at the beaches of the lower north and upper south Kwazulu-Natal coasts. Captures of bottlenose dolphins occurred predominantly on the lower south coast of Kwazulu-Natal (Fig. 5.3).

The majority of common dolphins were adult males (38 %), although lactating females (16 %) and calves (21 %) also made up a large portion of the common dolphin catch (Fig. 5.4). Samples taken from bottlenose dolphins were more evenly distributed, with the exception of juvenile females. Thirty four percent of the bottlenose dolphin data were from calves (Fig. 5.4).

Parameters (dependent and independent) that were significantly different between species were considered separately. Where data for the two dolphin species were not significantly different, data were pooled and re-analysed. There were no significant sex-differences between morphological, dietary and environmental (water temperature) variables between male and female animals of the same species (Two sample t-test, $p<0.05$). Therefore males and females of the same age group were pooled. Some physiological parameters were significantly different between males and females of the same age group. These are discussed separately.

1. MORPHOLOGICAL PARAMETERS

a. Common and bottlenose dolphins (pooled data)

The size and estimated age (GLG counts) composition of the two species were not significantly different (ANOVA, $p>0.05$), although more larger, older bottlenose dolphins were caught (Table 5.1). Several morphological relationships were related to obvious changes in size with age. These included positive relationships between total body weight ($r=0.83$, $p<0.001$, $n=58$), length ($r=0.86$, $p<0.001$, $n=58$) and age (Fig. 5.5). Girth (axilla, maximum, anus) measurements were significantly

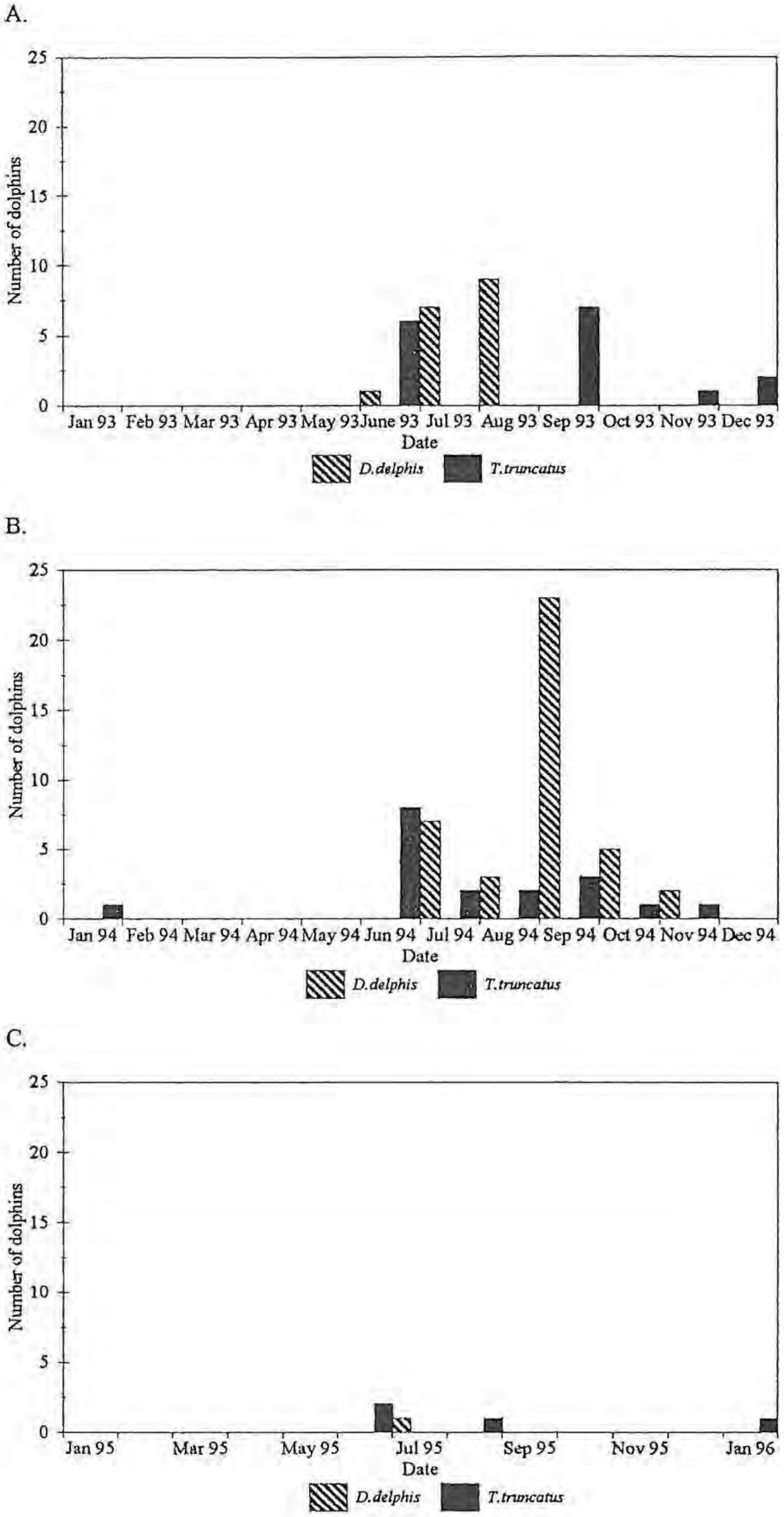


Figure 5.2: Frequency of common (D. delphis) and bottlenose (T. truncatus) dolphin captures in shark nets for 1993 (A), 1994 (B) 1995 and 1996 (C).

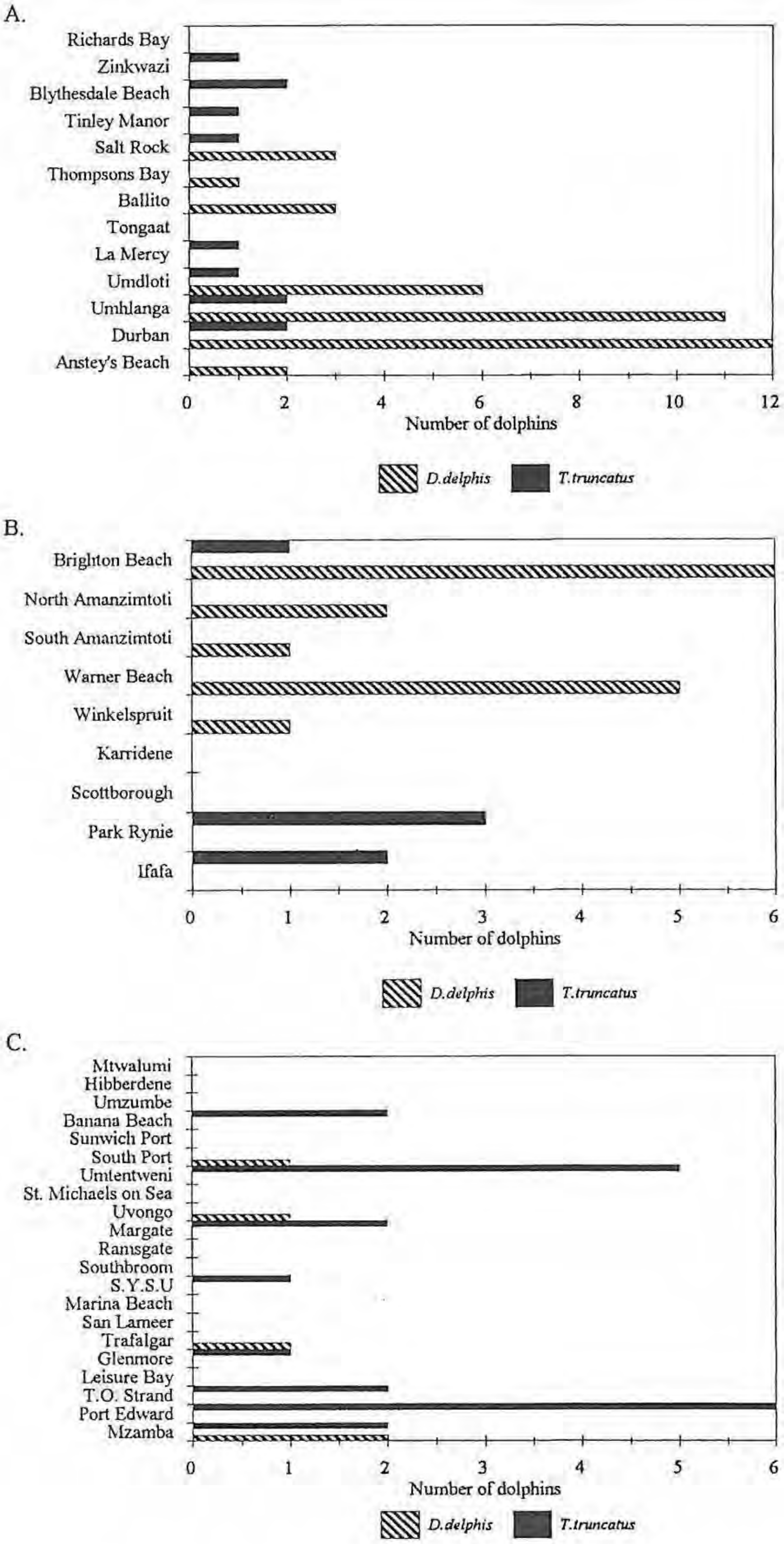


Figure 5.3: Regional catch composition of dolphins caught in shark nets along the Kwazulu Natal coast (A. north coast; B. upper south coast; C. lower south coast) (The y-axes denote beaches with shark nets, in order, from north to south).

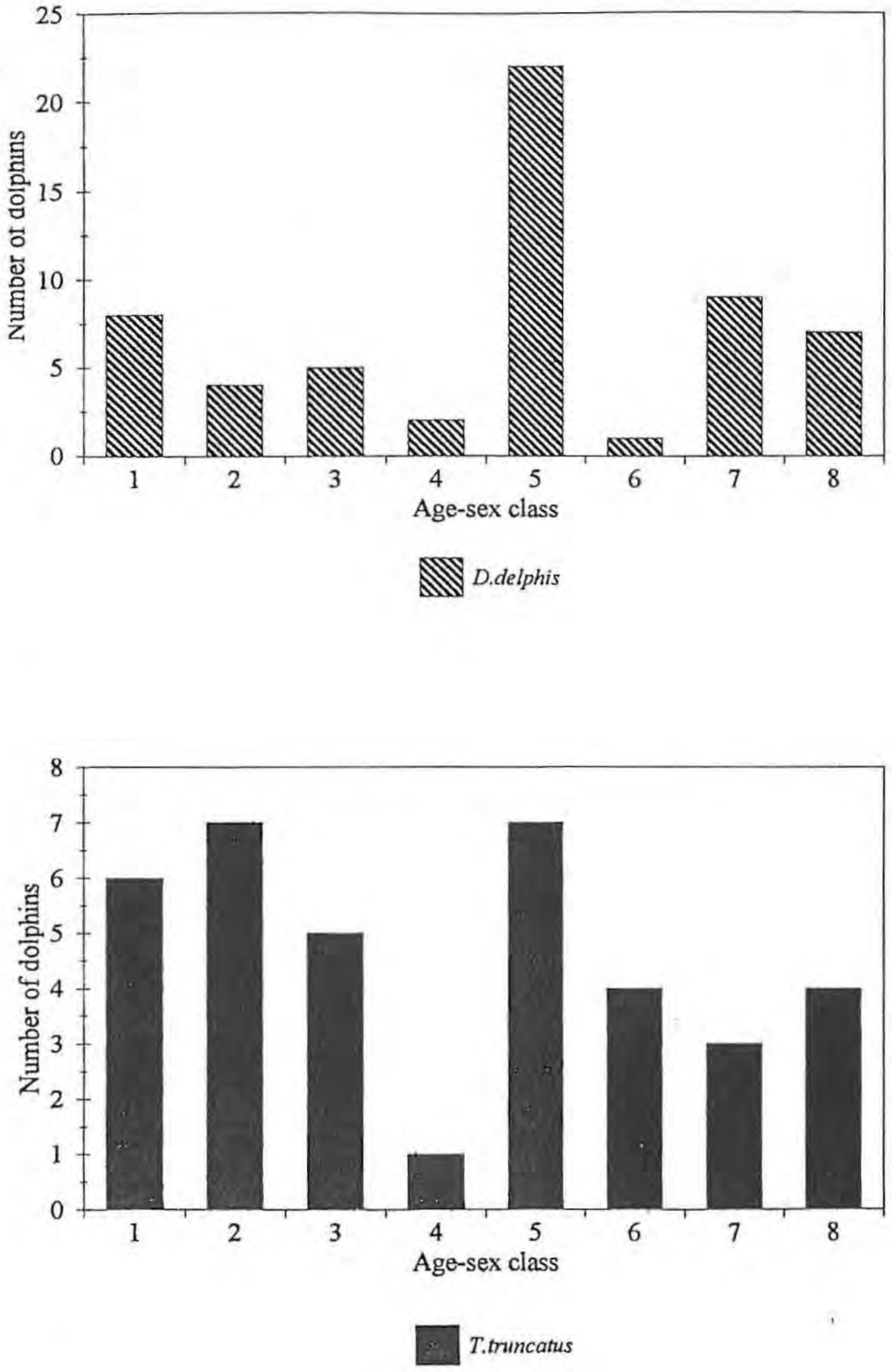


Figure 5.4: Age and sex composition of common (D. delphis) and bottlenose (T.truncatus) dolphins caught in shark nets (1. male calves, 2. female calves, 3. juvenile males, 4. juvenile females; 5. adult males; 6. resting adult females; 7. lactating females; 8. pregnant females).

Table 5.1: Mean values (± standard deviations) for morphological parameters from common and bottlenose dolphins caught in shark nets between 1992 and 1996 (C: calf, J: juvenile, A: adult, FL: lactating females, FP: pregnant females; FPL: simultaneously pregnant and lactating females)

Morphological measure	Unit		Common dolphin	n	Bottlenose dolphin	n	Combined Species	N
Age	(years)		8.87 ± 6.16	34	11.88 ± 7.40	25		
Total body length	(cm)		213.84 ± 53.27	58	212.42 ± 40.60	36		
Total body weight	(kg)		108.06 ± 43.71	58	124.67 ± 44.32	36		
		FL	110.2 ± 18.98	8			124.86 ± 27.47	10
		FP	126.75 ± 9.19	6				
Girth	(cm)							
Axilla^			105.99 ± 16.16	49	113.42 ± 24.08	26		
		C					83.49 ± 9.66	22
		J					104.17 ± 12.86	12
		A					102.04 ± 9.82	47
Maximum^			116.09 ± 20.68	49	121.04 ± 26.48	26		
		C					87.98 ± 11.35	22
		J					110.2 ± 14.55	12
		A					132.27 ± 10.07	47
Anus^			67.62 ± 15.65	46	69.85 ± 17.61	26		
		C					48.68 ± 5.93	21
		J					63.58 ± 7.65	11
		A					78.09 ± 10.62	46
Blubber thickness	(mm)							
mid-dorsal^"			15.00 ± 5.16	49	16.58 ± 2.98	31		
		C					13.96 ± 2.28	25
		J					17.27 ± 3.30	11
		A					16.48 ± 5.20	51
		FL					14.05 ± 4.41	10
mid-lateral			12.19 ± 5.04	47	11.56 ± 2.05	31		
mid-ventral*^"			13.00 ± 2.74	48	14.94 ± 3.42	31		
		C			12.51 ± 2.84	13		
		J			14.53 ± 1.50	3		
		A			17.12 ± 2.84	15		
Blubber weight*^"	(kg)		14.94 ± 5.45	50	18.82 ± 5.45	31		
		C	8.09 ± 2.78	11	9.31 ± 2.28	13		
		J	13.23 ± 4.61	6	19.15 ± 2.36	4		
		A	17.52 ± 4.03	33	27.56 ± 6.06	14		
		FP			31.25 ± 4.85	3		
		FL	15.26 ± 2.57	8				
Proportional blubber weigl	(%)		15.26 ± 3.90	50	17.19 ± 3.43	31		
		C	20.05 ± 2.44	11	19.59 ± 2.82	13		
		J	17.38 ± 2.85	6	16.83 ± 2.94	4		
		A	13.28 ± 2.72	33	15.07 ± 2.67	14		
Muscle weight^	(kg)		36.25 ± 16.11	51	36.82 ± 22.10	32		
		C					13.43 ± 5.50	24
		J					25.34 ± 8.42	14
		A					48.49 ± 10.60	53
Proportional muscle weigh	(%)		33.77 ± 6.45	51	29.99 ± 3.99	32		
		C			27.57 ± 3.01	13		
		J			29.84 ± 5.37	4		
		A			32.13 ± 3.31	15		

*: significantly different between species (Two-sample t-test, $p<0.05$)

^ significantly different between age groups (ANOVA, $p<0.05$)

": significant differences between reproductively active females and resting females (Two-sample t-test, $p<0.05$).

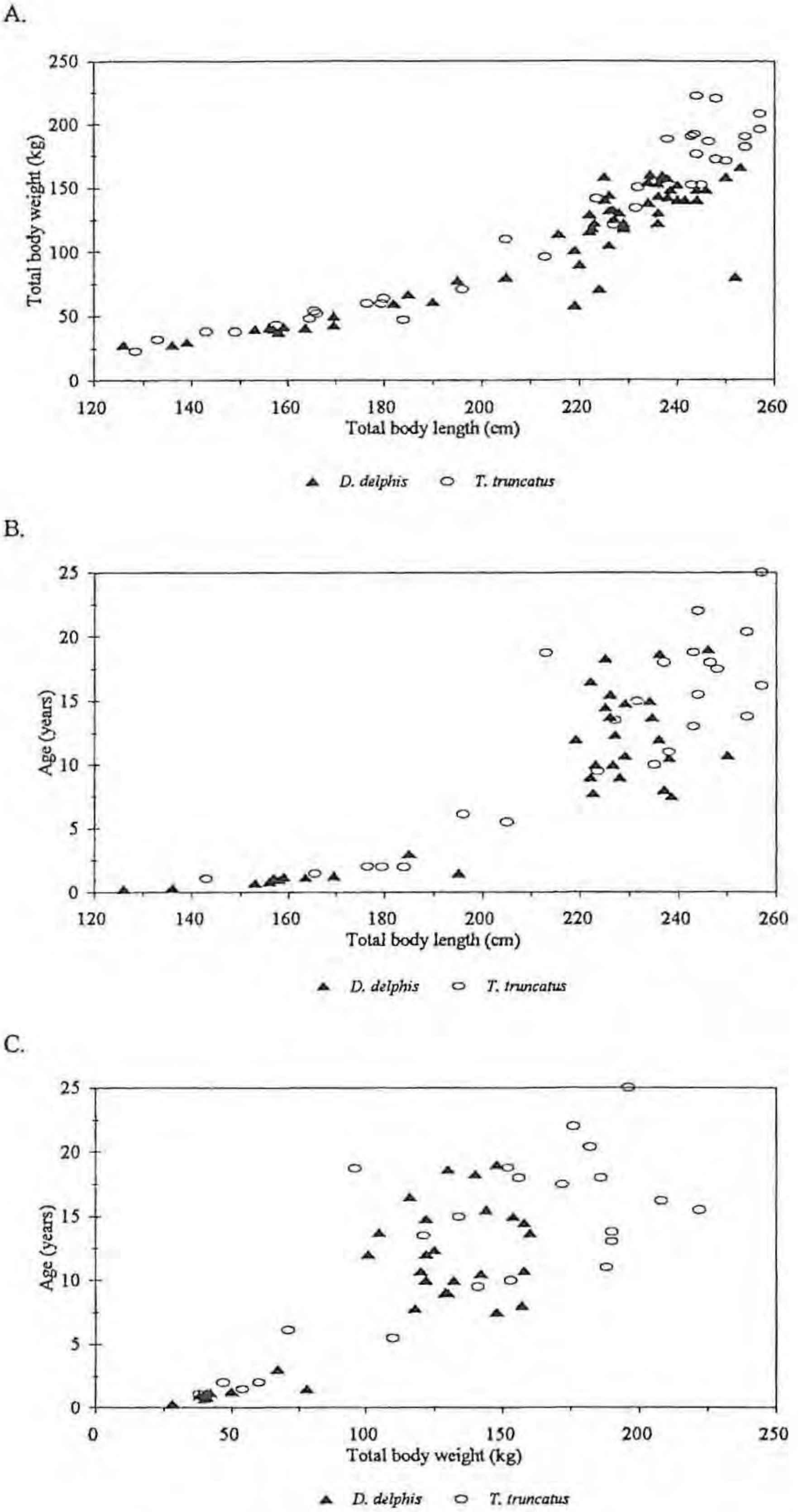


Figure 5.5 : Significant correlations between total body weight and total body length (A), and between age and total body length (B) and total body weight (C) for common (D. delphis) and bottlenose (T. truncatus) dolphins caught in shark nets between 1992 and 1996.

different between age groups (ANOVA, $p<0.05$) (Table 5.1) and increased significantly with increased total body length and weight (Fig. 5.6).

Stepwise multiple regression showed that total body weight could be predicted from girth and length measurements in the following manner:

Common dolphins: $W = -146.990 + (0.7 \times Gmax) + (0.607 \times L) + (0.649 \times Gan)$

Bottlenose dolphins: $W = -182.455 + (1.314 \times L) + (2.908 \times Gan) - (1.445 \times Gmax)$

where W is total body weight,
L is total body length,
Gmax is maximum girth
Gan is the girth at the anus.

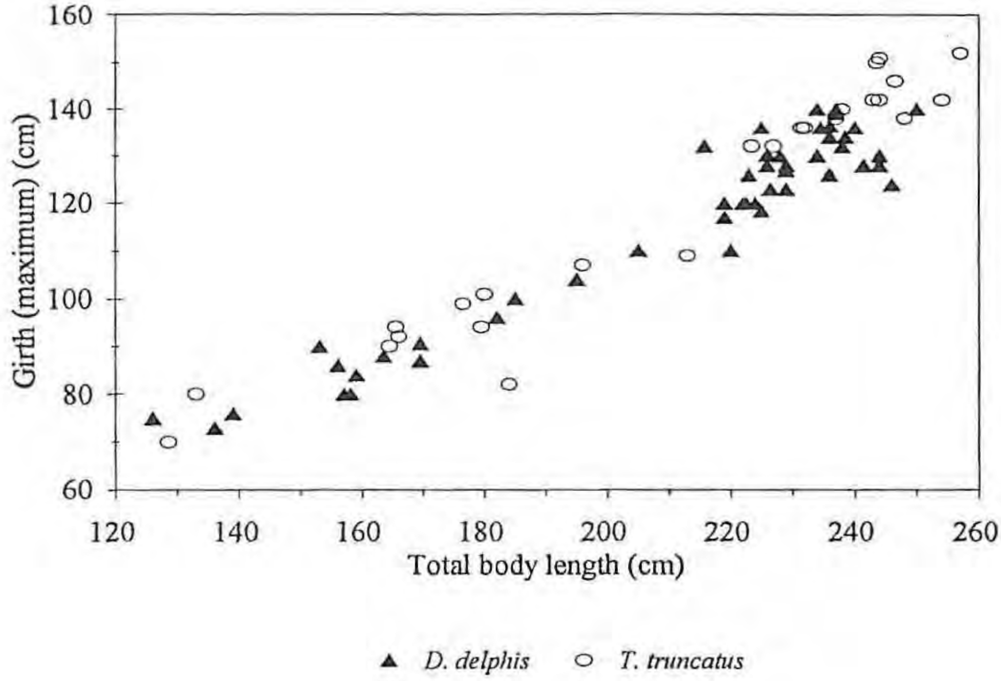
Pregnant females, from both species, were not morphologically different from other adults and/or resting adult females (Two-sample t-test, $p>0.05$). However, lactating bottlenose and common dolphins weighed significantly less than other adult female dolphins ($t=-2.25$, $p=0.03$, $df=27$).

Blubber thickness measurements of common and bottlenose dolphins were similar, with the exception of common dolphins having significantly thinner blubber at the mid-ventral site (Table 5.1). Blubber thickness at the dorsal site differed significantly between age groups ($F=3.51$, $p=0.03$, $df=84$) (Table 5.1). Lactating females of both species had significantly reduced dorsal blubber (14.05 ± 4.41 mm) when compared to that of resting females (17.42 ± 5.13 mm) ($t=-2.21$, $p=0.04$, $df=23$).

The mean blubber weight of bottlenose dolphins (18.82 ± 9.67 kg) was significantly higher than that of common dolphins (14.94 ± 5.45 kg) ($t=-2.31$, $p=0.02$, $df=79$). In conjunction, the mean proportional weight of blubber to total body weight (%B) of common dolphins (15.26 ± 3.98 %) was significantly lower than that of bottlenose dolphins (17.19 % ± 3.43 %) ($t=-2.27$, $p=0.03$, $df=79$). These species differences were apparent for all age groups (Table 5.1). Blubber weight ($F=25.23$; $p<0.001$; $df=47$) and %B ($F=28.67$; $p<0.001$; $df=47$) differed significantly between calves, juveniles and adults (Table 5.1).

The age-associated differences were also evident in positive correlations between blubber weight and total body weight ($r=0.86$, $p<0.001$, $n=89$) and negative correlations between %B and age ($r=-0.52$, $p<0.001$, $n=53$), total body length ($r=-0.61$, $p<0.001$, $n=89$) and total body weight ($r=-0.59$, $p<0.001$, $n=89$) (Fig. 5.7). Similar trends in blubber weight ($r=0.83$, $p<0.001$, $n=76$) and %B ($r=-0.58$, $p<0.001$, $n=76$) were seen with increased girth (Fig. 5.8). The decline in %B with increased girth, within specific age groups, was difficult to characterise but increased girth in bottlenose dolphin calves was associated with reduced %B (Fig. 5.9).

A.



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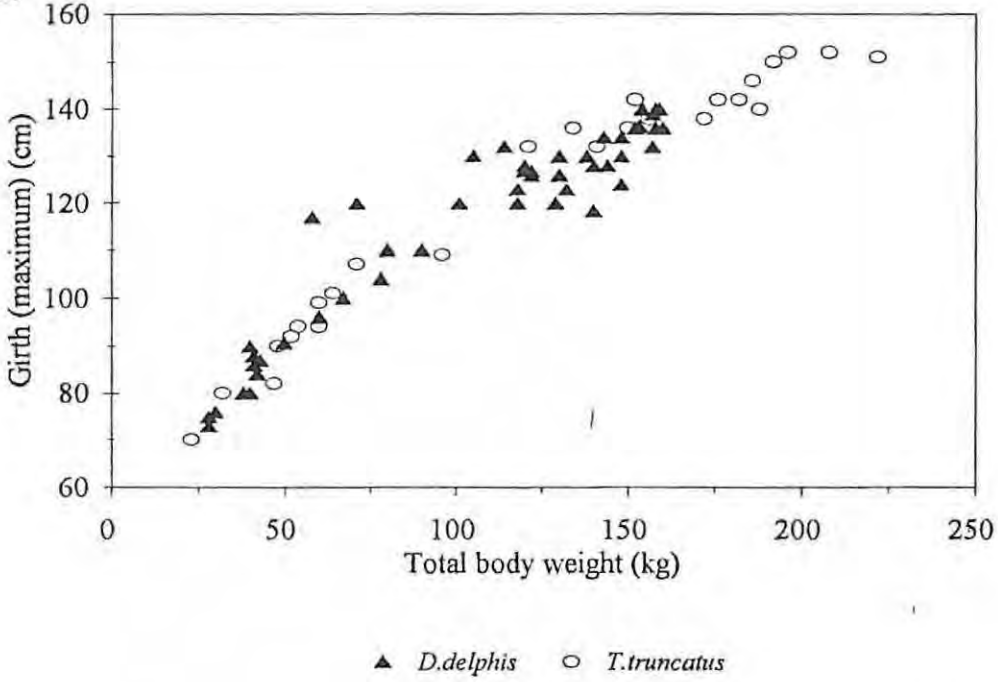


Figure 5.6: Significant correlations between girth (maximum) and total body length (A) and total body weight (B) for common (D. delphis) and bottlenose (T. truncatus) dolphins caught in shark nets between 1992 and 1996.

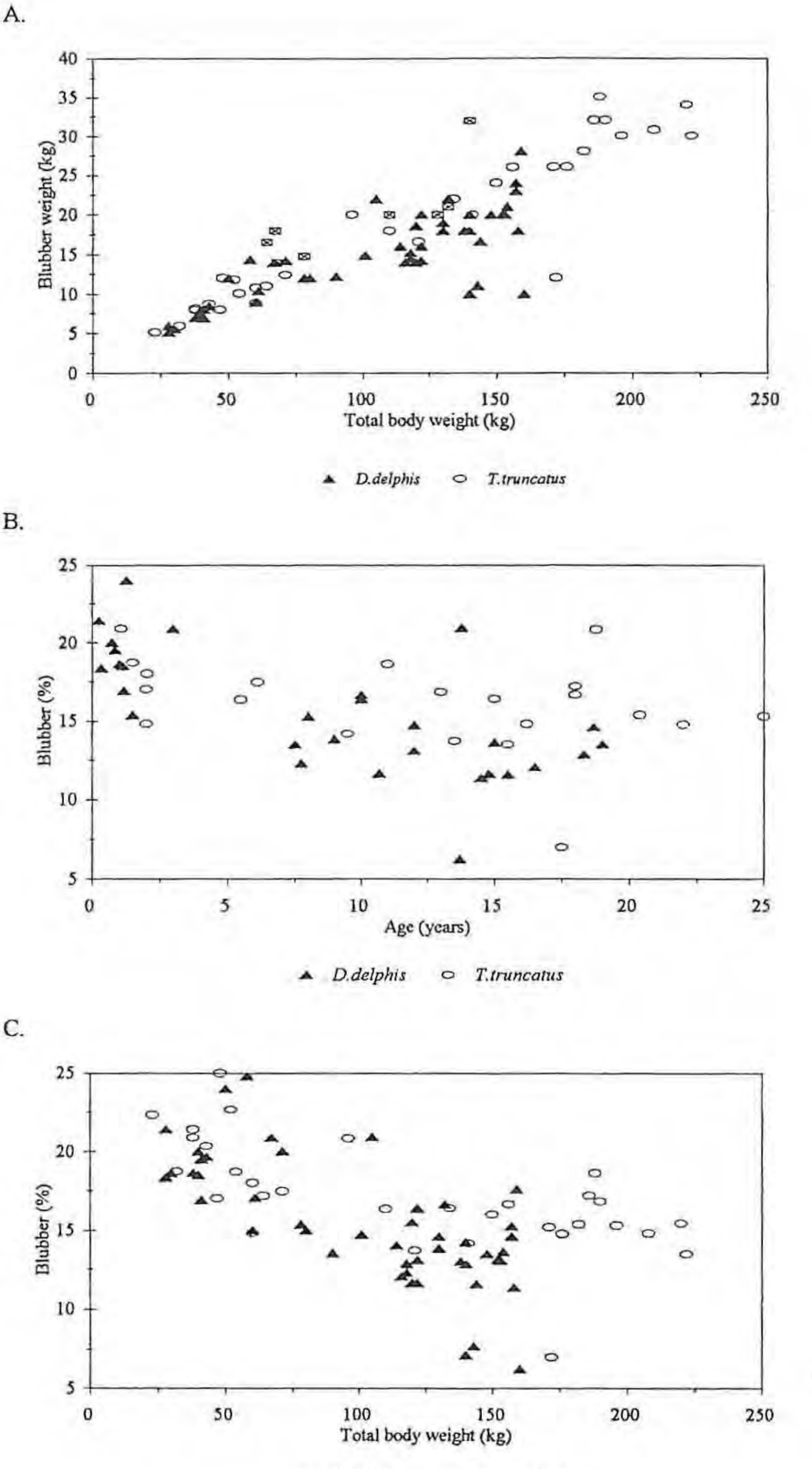


Figure 5.7: Significant correlations ($p<0.05$) between total blubber weight and total body weight (A); between proportional blubber weight and age (B) and total body weight (C) for common (D. delphis) and bottlenose (T. truncatus) dolphins caught in shark nets between 1992 and 1996.

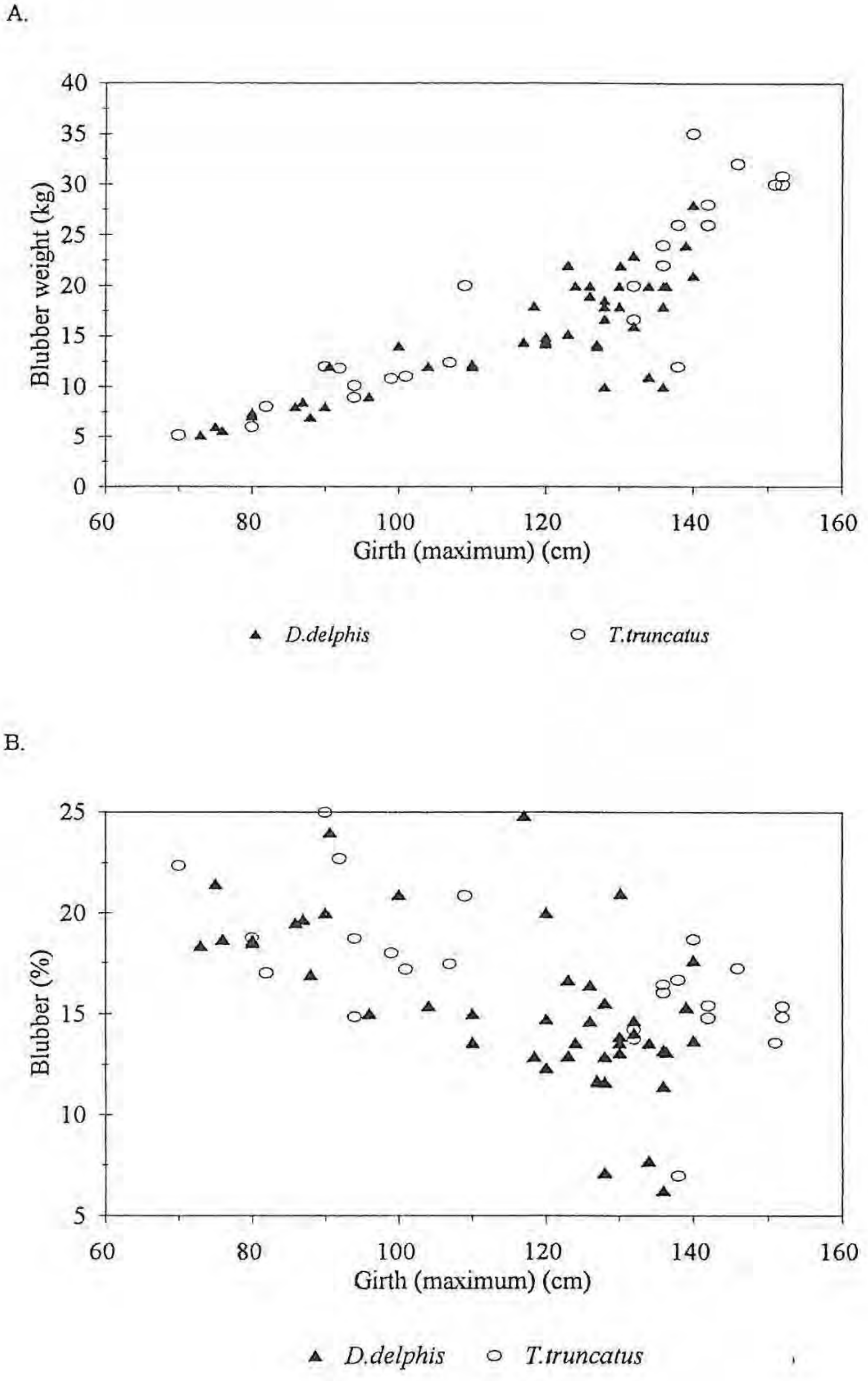
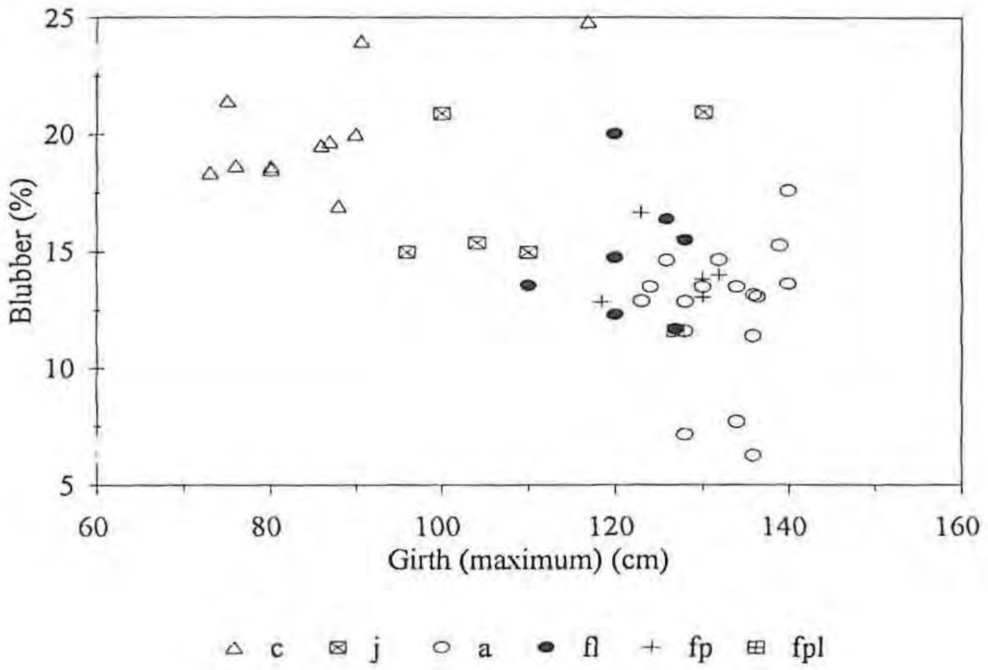


Figure 5.8: Significant correlations ($p<0.05$) between blubber weight (A) and proportional blubber weight (B) and maximum girth for common (D. delphis) and bottlenose (T. truncatus) dolphins caught in shark nets between 1992 and 1996.

A.



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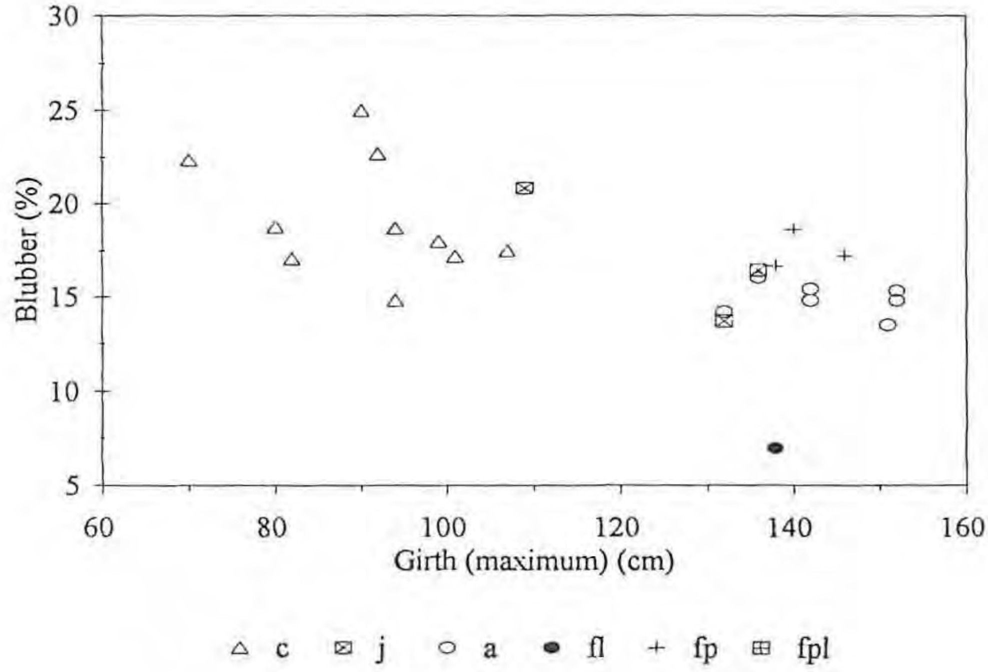


Figure 5.9: The relationship between proportional blubber weight and maximum girth for the age and reproductive groups of common (D. delphis) (A) and bottlenose (T. truncatus) (B) dolphins caught in shark nets between 1992 and 1996 (c. calves, j. juveniles,a. adults, fl. lactating females, fp. pregnant females, fpl. simultaneously pregnant and lactating females).

Stepwise multiple regression showed that % B could best be estimated using external morphological measurements in the following equations:

Common dolphins: % B = 19.07515 - (0.5461 x W) + (0.16032 x Bv)

Bottlenose dolphins: % B = 25.30548 - (0.04616 x L) + (0.62631 x Bd) - (0.04789 x Gmax) - (0.19458 x Bv)

Where W is total body weight,
L is total body length,
Bv is ventral blubber thickness,
Bd is dorsal blubber thickness,
Gax is axillary girth
Gmax is maximum girth.

Mean total muscle weights of the two species were almost identical (t=-0.14, p=0.89, df=81) (Table 5.1). However, mean proportional weight of muscle to total body weight (%M) of common dolphins (33.77 ± 6.45 %) was significantly higher than that of bottlenose dolphins (29.99 ± 3.99 % muscle) (t=2.97, p=0.004, df=81). Data from both species reflected a significant, almost linear, increase in total muscle weight with increased body weight (r=0.95, p<0.001, n=84).

The %M of both species was positively related to total body length (r=0.33; p=0.002; n=84). However, data were variable and no marked trend could be identified. The %M was best estimated by the following equations:

Common dolphins: % M = 4.885917 + (0.201339 x L) - (0.131117 x W)

Bottlenose dolphins: % M = 24.45909 + (0.00562 x W) + (0.20619 x Gan) - (0.12290 x Gax) + (0.02001 x L)

Where W is total body weight,
L is total body length,
Gax is axillary girth,
Gmax is maximum girth
Gan is the girth at the anus.

a. Common dolphins

Proportional blubber weights of this species were negatively correlated with total muscle weight (r=-0.59, p<0.001, n=48), reflecting reduced %B with increased growth and muscle weight. This trend was not evident within any of the age and/or reproductive classes. Total blubber weights of lactating common dolphins were significantly lower than those of other adult females (t=-2.15; p=0.05; df=14) (Table 5.1), although %B was similar for the two groups.

b. Bottlenose dolphins

Dorsal blubber thickness measurements from bottlenose dolphins were positively correlated with age (r=0.42, p=0.001, n=21), total body weight (r=0.32, p=0.002, n=38) and length (r=0.28, p=0.008, n=38) (Fig. 5.10).

Although the number of reproductively active bottlenose dolphins was limited, data reflected that pregnancy had a significant effect on blubber weight and %B in this species. Pregnant bottlenose dolphins had significantly higher blubber weights (31.25 ± 4.85 kg) than other female dolphins (21.60 ± 5.23 kg) (t=2.82; p=0.03; df=7). Similarly, %B was significantly higher in pregnant females (17.33 %) than both adult male and resting adult female dolphins of the same species (14.16 %) (t=2.32; p=0.04; df=12).

Bottlenose dolphins were the only animals for which total blubber weight was positively correlated with both dorsal and ventral blubber thickness (p<0.05) (Fig. 5.11 A&B). Proportional blubber weight (%B) was negatively related to ventral blubber thickness (r=-0.40; p=0.03; n=29) (Fig. 5.11C). These relationships were generally associated with the specific age groups but trends were evident within these groups. One lactating female had a significantly low %B associated with a relatively high blubber thickness (Fig. 5.11C).

Bottlenose dolphin %M data was positively correlated with total body weight (r=0.47; p=0.006; n=32). This relationship concurred with significantly different mean muscle weights and %M in calves, juveniles and adults (Table 5.1). In conjunction with relationships between blubber weights and %B described previously, muscle weights and %M data from this species were positively correlated with blubber thickness (r=0.59, p<0.001, n=30) (Fig. 5.12 A&B). In addition, %M was significantly correlated with blubber weight (r=0.43, p=0.02, n=31). However, within each age group, %M appeared to decrease as blubber weight increased (Fig. 5.12C).

2. DIET AND FEEDING

a. Common and bottlenose dolphins (pooled data)

The only dietary parameter that varied significantly between species was the mean dietary energy value of stomach contents, with common dolphins consuming prey with a significantly higher mean energy content (Table 5.2). The size of prey varied significantly between animals of different ages (ANOVA, p<0.05). Juvenile dolphins of both species fed on significantly smaller prey items than calves and adults (Table 5.2). In this context, prey size (length (r=0.35; p=0.005; n=65) and weight (r=0.27; p=0.03; n=65)) was positively correlated with dolphin size.

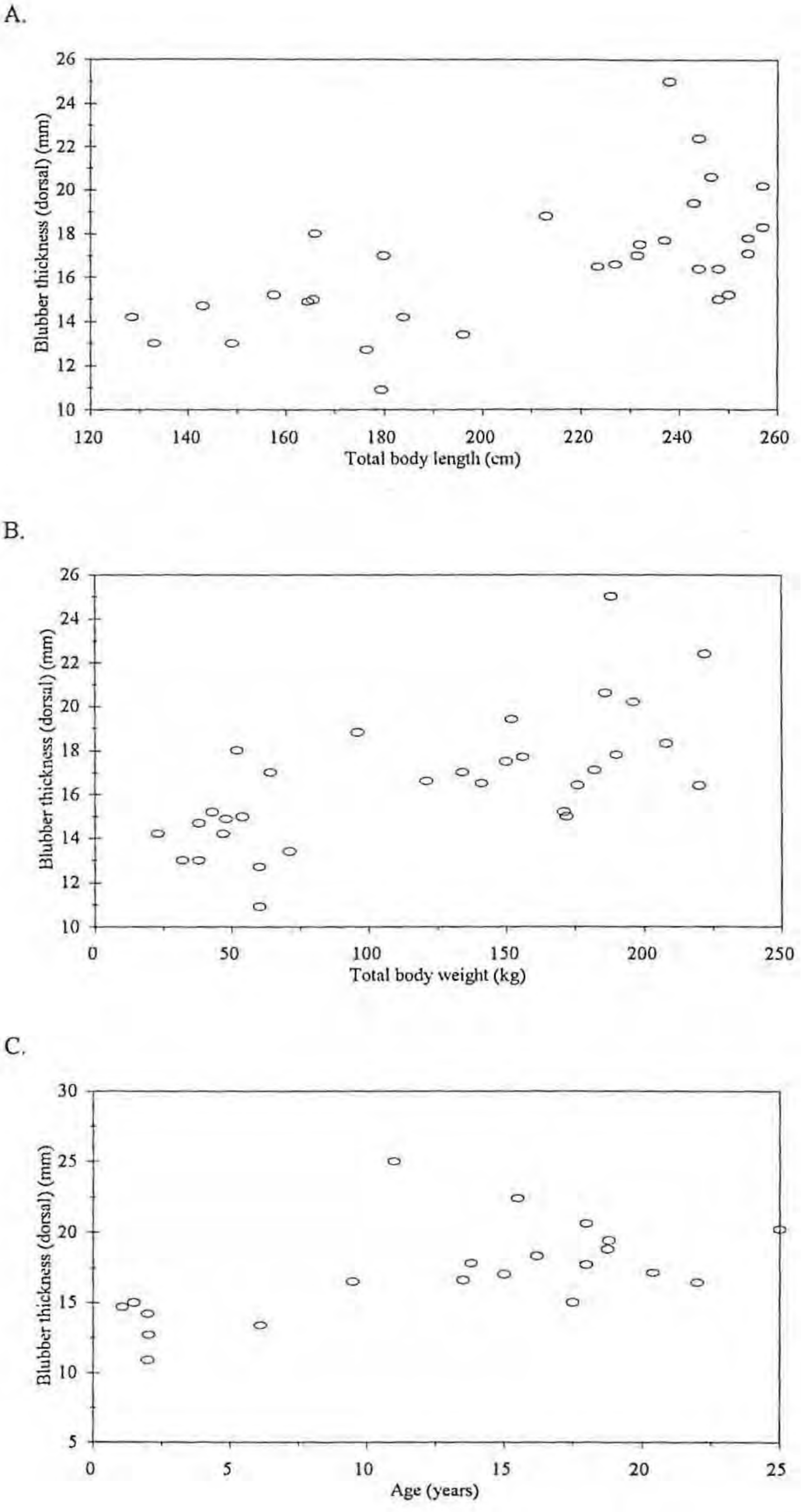


Figure 5.10: Significant correlations ($p<0.05$) between dorsal blubber thickness an total body length (A), weight (B) and age (C) for bottlenose dolphins (T. truncatus) caught in shark nets between 1992 and 1996.

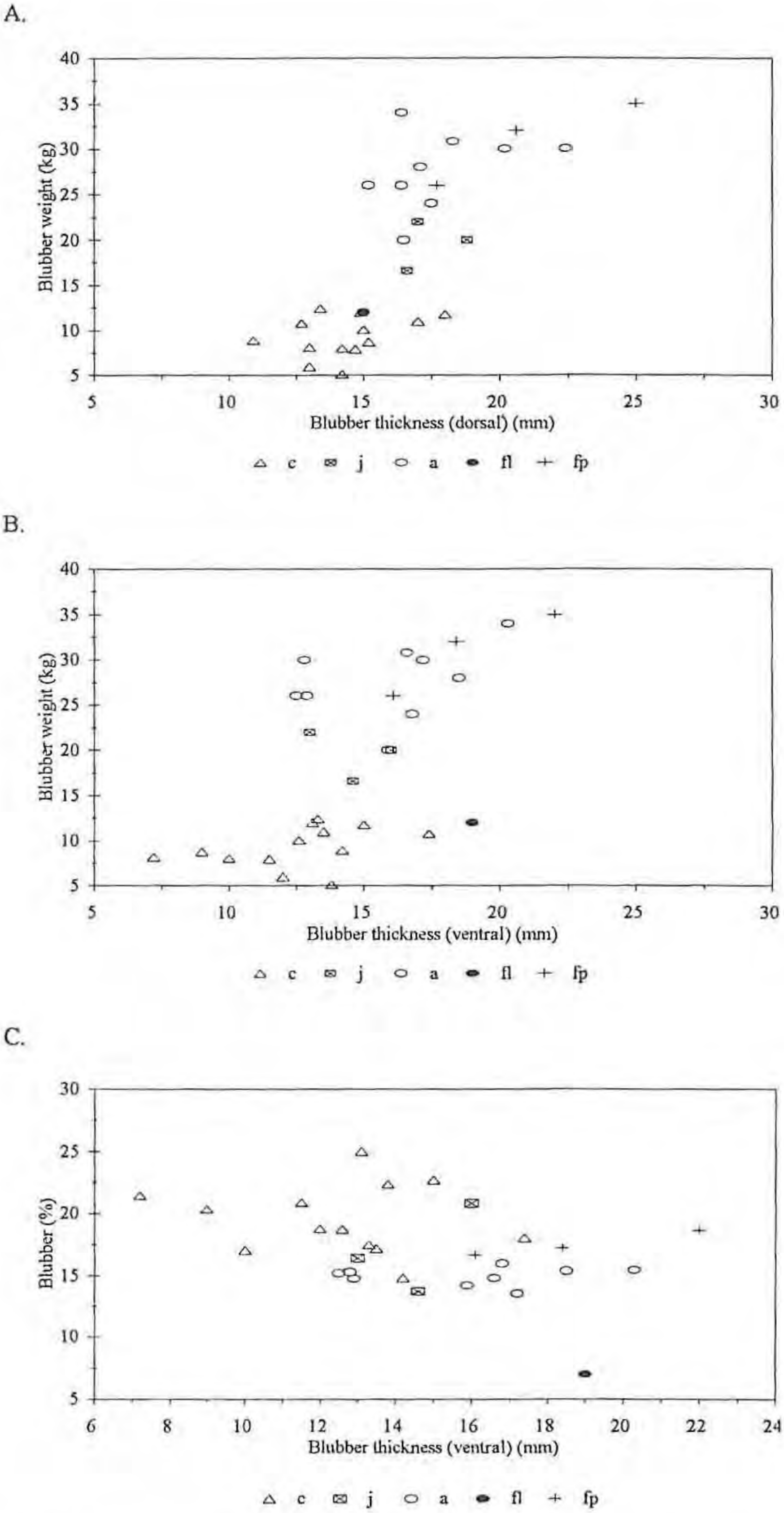


Figure 5.11: Significant correlations between blubber thickness and total blubber weight (A&B) and between blubber thickness and proportional blubber weight (C) for the age and reproductive groups of bottlenose dolphins caught in shark nets between 1992 and 1996 (c: calves, j: juveniles, a: adults, fl: lactating females, fp: pregnant females).

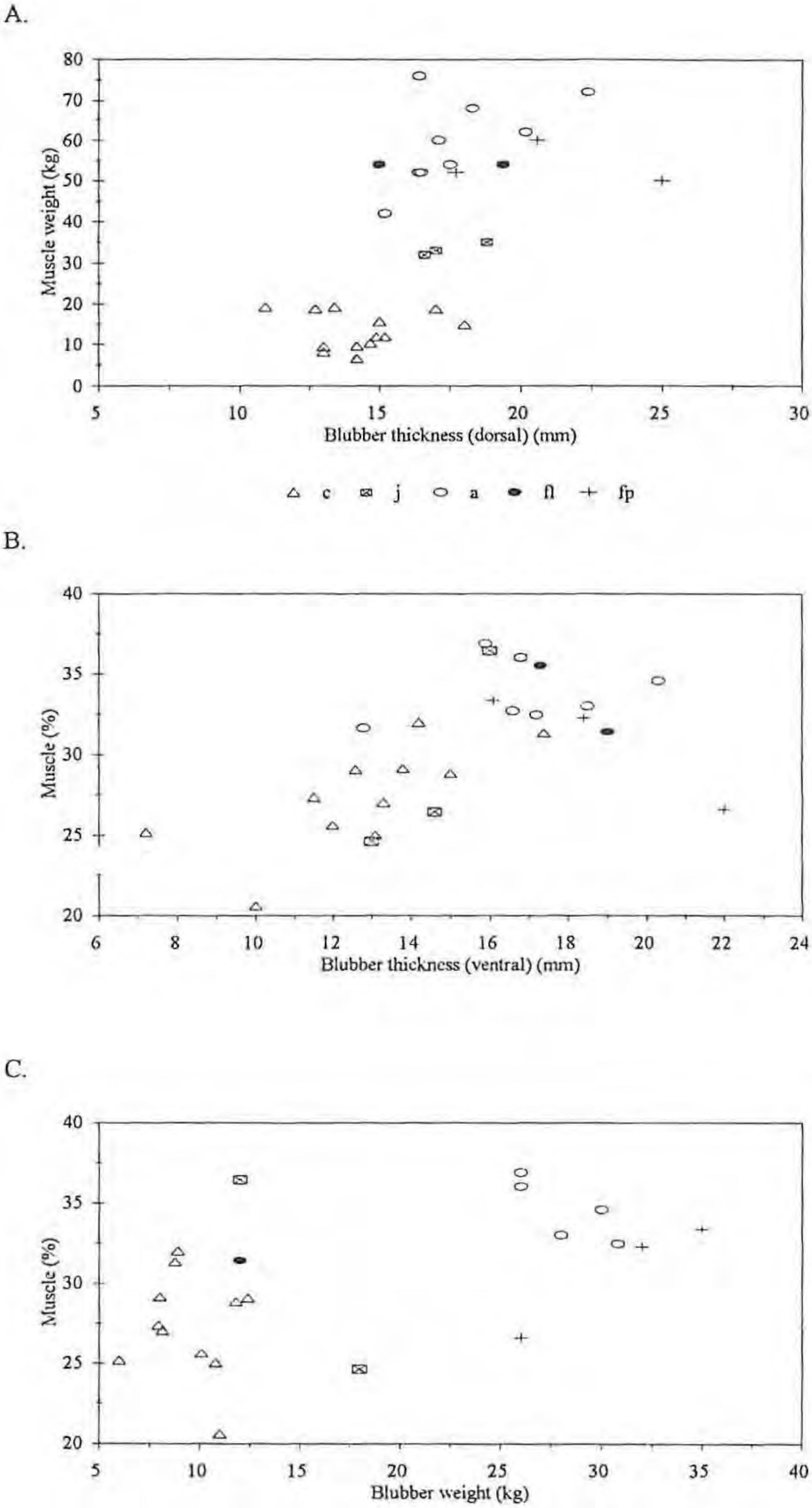
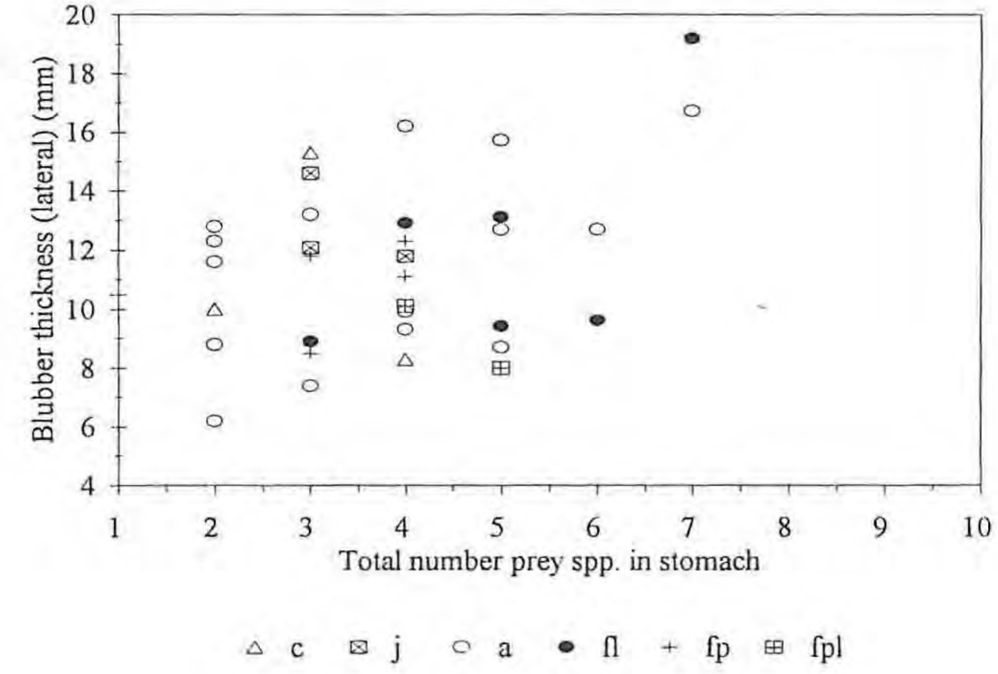


Figure 5.12: Significant correlations between total (A) and proportional (B) muscle weight and blubber thickness and between proportional muscle weight (%M) and total blubber weight (C) for bottlenose dolphins caught in shark nets between 1992 and 1996) (c: calves, j: juveniles, a: adults, fl: lactating females, fp: pregnant females).

A.



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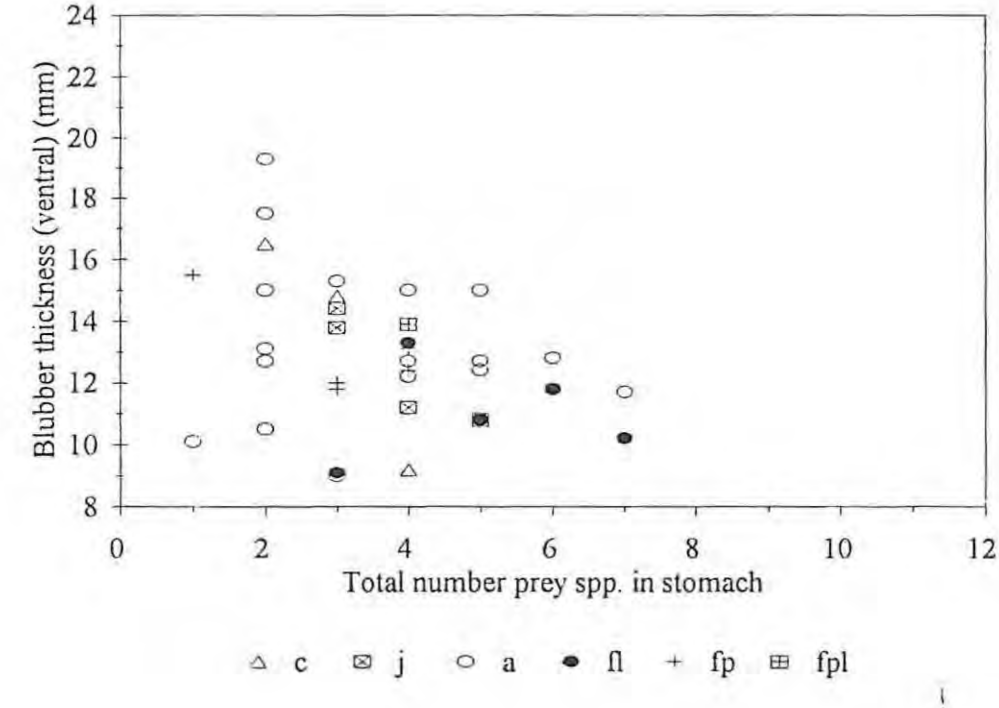


Figure 5.13: Significant correlations between lateral (A) and ventral (B) blubber thickness and the total number of prey species in the stomach for common dolphins (D. delphis) caught in shark nets between 1992 and 1996 (c: calves, j: juveniles, a: adults, fl: lactating females, fp: pregnant females, fpl: simultaneously pregnant and lactating females).

Dorsal blubber thickness was negatively correlated with mean reconstituted prey length (r=-0.30; p=0.03; n=54) and weight (r=-0.28; p=0.04; n=54) as well as the mean energy content of prey (r=-0.28; p=0.05; n=49). However, this trend showed age-related changes, which occur in both dorsal blubber thickness and the consumption of different sized prey.

b. Common dolphins

Lactating common dolphin females consumed approximately twice as many prey species as resting adult females (t=2.28 ; p=0.04; df=14). A higher prey diversity in the diet was positively related with lateral blubber thickness in this species (r=0.35; p=0.007; n=59) (Fig. 5.13A). In contrast, ventral blubber thickness was negatively correlated with the total number of prey species in the stomach (r=-0.34; p=0.05; n=35) (Fig. 5.13B). Data were variable and specific trends within age groups were difficult to distinguish.

Total muscle weight and %M were positively related to several dietary parameters (the total number of prey items in the stomach (r=0.38; p=0.02; n=37), mean prey length (r=0.43; p=0.001; n=55), mean prey weight (r=0.42; p=0.001; n=55) and mean energy content of prey (r=0.36; p=0.009; n=51). However, data were highly variable and these relationships were difficult to confirm, both between and within age groups.

c. Bottlenose dolphins

The only relationship between diet and morphology specific to bottlenose dolphins was between ventral blubber thickness and the number of prey species in the stomach (r=0.52; p=0.02; n=19) (Fig. 5.14). Generally, within the different age groups, ventral blubber thickness was higher when a larger number of prey species were consumed. This was the inverse of the same relationship described above for common dolphins.

3. WATER TEMPERATURE

a. Common dolphins

Dorsal blubber thickness of common dolphins was positively correlated with the mean local water temperature of the month of capture (r=0.22; p=0.05; n=84). Although data were variable, there was a tendency for thicker blubber at warmer water temperatures, even within age groups (Fig. 5.15A). Almost all lactating females had thinner blubber than other animals at all temperatures (Fig. 5.15A). Similarly, total blubber weights were positively correlated with the mean local (r=0.34; p=0.02; n=49) and regional (r=0.34; p=0.02; n=49) water temperature of the month of capture. Blubber weights in all age and reproductive classes appeared to be greater at higher water temperatures (Fig. 5.15B).

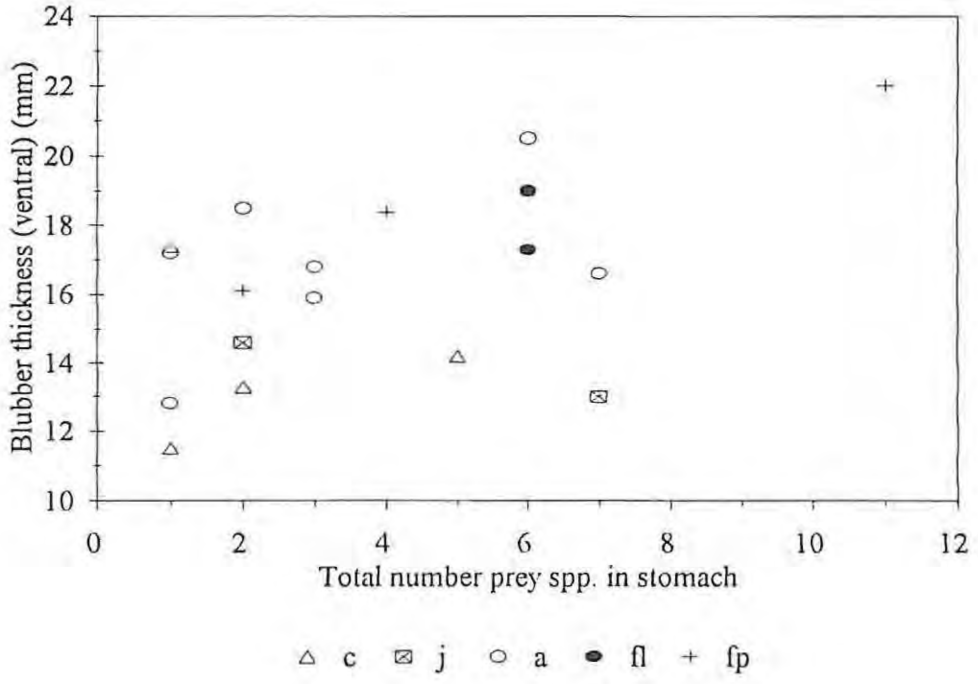


Figure 5.14: Significant correlation between ventral blubber thickness and the total number of prey species in the stomach for bottlenose dolphins (T. truncatus) caught in shark nets between 1992 and 1996 (c: calves, j: juveniles, a: adults, fl: lactating females, fp: pregnant females).

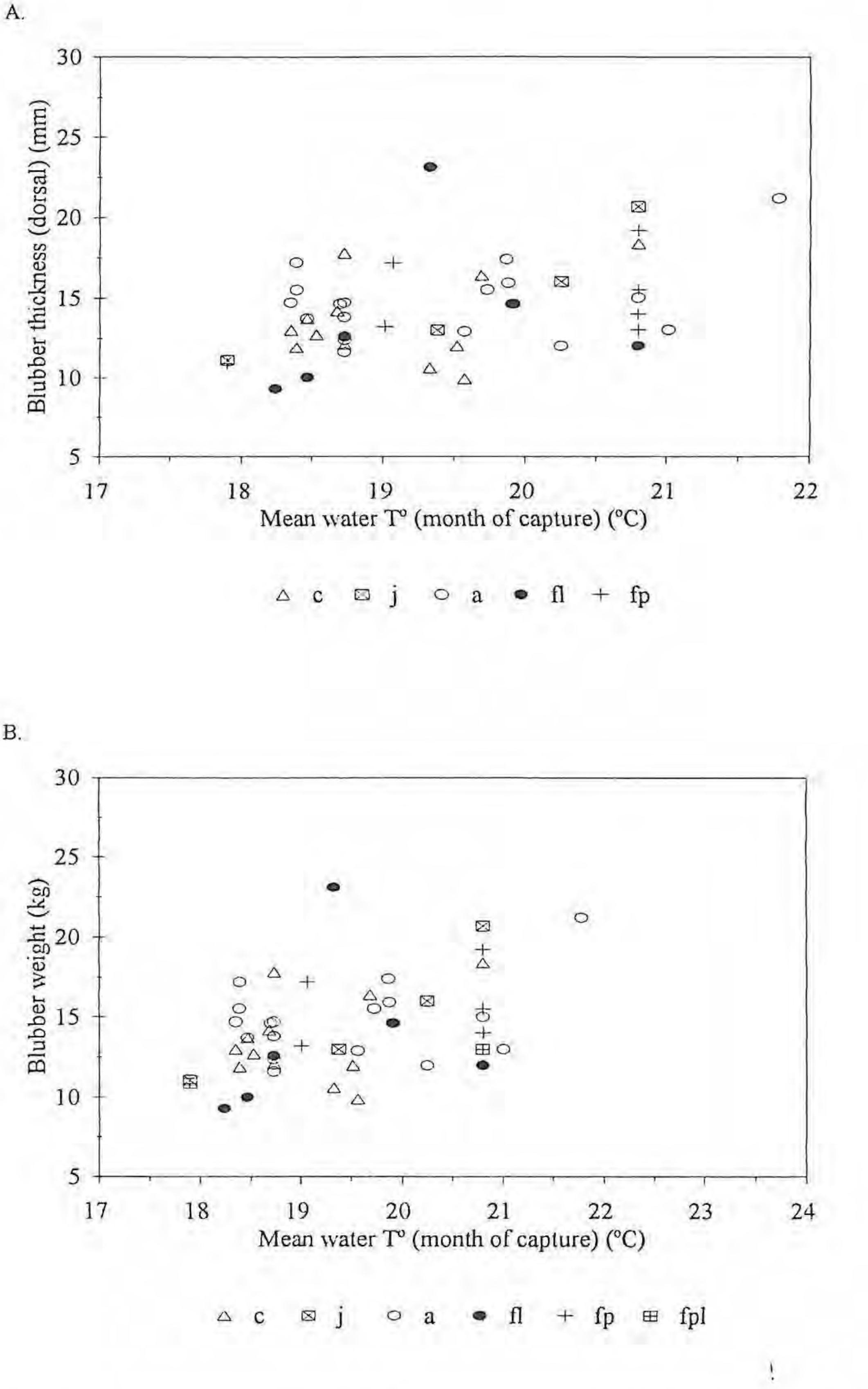


Figure 5.15: Significant correlations ($p<0.05$) between dorsal blubber thickness (A) and total blubber weight (B) and the mean local water temperature of the month of capture for common dolphins caught in shark nets between 1992 and 1996 (c: calves, j: juveniles, a: adults, fl: lactating females, fp: pregnant females, fpl: simultaneously pregnant and lactating females).

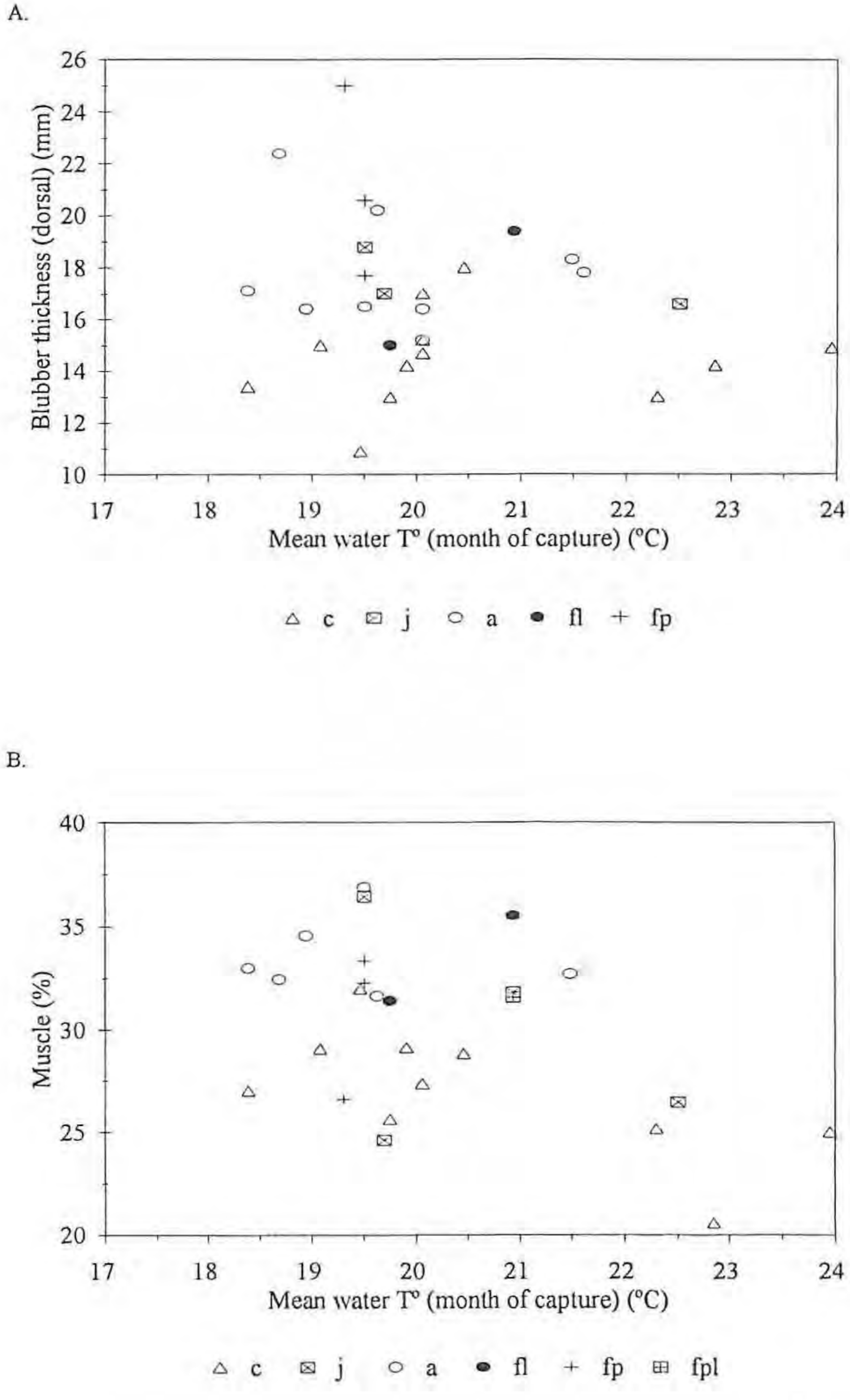


Figure 5.16: Relationships between dorsal blubber thickness (A) and proportional muscle weight (B) and the mean local water temperature of the month of capture for bottlenose dolphins caught in shark nets between 1992 and 1996 (c. calves, j. juveniles, a. adults, fl. lactating females, fp. pregnant females, fpl. simultaneously pregnant and lactating females).

b. Bottlenose dolphins

Blubber thickness data from bottlenose dolphins were not significantly related to water temperature (Fig. 5.16A). The proportional weight of muscle was negatively correlated with both the mean local ($r=-0.35$; $p=0.001$; $n=87$) and regional ($r=-0.27$; $p=0.01$; $n=87$) water temperature of the month of capture. %M appeared to decrease with increasing water temperature in most age groups, but particularly in calves and juveniles (Fig. 5.16B).

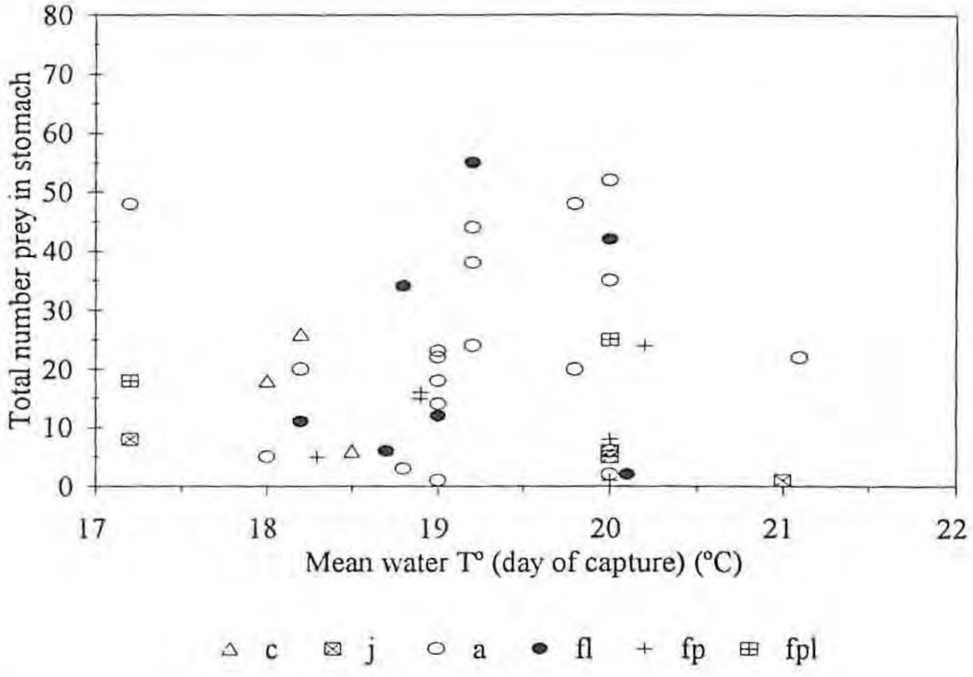
4. DIET AND WATER TEMPERATURE

Stomach contents were assumed to be indicative of the most recent feeding bout, therefore relationships between diet and water temperature were assessed in terms of the water temperature on the day of capture. The number of prey items in the stomachs of common dolphins was highly variable (Fig. 5.17A). There was a significant increase in the number of prey items in the stomachs of bottlenose dolphins when water temperatures were warmer ($r=0.38$, $p=0.001$, $n=71$) (Fig. 5.17B). In conjunction, the mean reconstituted weight ($r=-0.29$, $p=0.02$, $n=65$) and mean energy content (-0.38, $p=0.003$, $n=60$) of prey were negatively correlated with the water temperature on the day of capture. However, this was due primarily to more adult dolphins being captured at lower water temperatures. The relationships could not be verified for specific age groups.

DISCUSSION

The strong relationships between length, weight, girth and blubber for animals used in this study enforced their use as valuable tools in assessing overall condition. Girth, together with length, was identified as an important predictor of weight in this study. Originally, only total body weight was used to assess the condition of marine mammals, however, the more recent use of girth and blubber thickness measurements in the assessment of marine mammal condition has proved extremely valuable. When toothed whales lose weight, it is usually first visible in depressions in the area lateral of the dorsal fin and if emaciation continues, a pronounced "neck" region develops posterior to the head (Ridgway and Fenner 1982, Kastelein and van Battum 1990). This change in blubber and muscle during a decline in condition has a direct effect on the girth and for this reason, girth has become a vital parameter in the more accurate assessment of overall condition (Lockyer *et al.* 1985, Lockyer 1986, 1993a, Wells 1991). Early studies relied only on length to estimate weight (Bryden 1986), but the incorporation of girth measurements into estimations has emphasised the limitations of using only total body length in the estimation of overall weight and condition (Kastelein and Vaughan 1989, Castellini and Kooyman 1990, Kastelein and van Battum 1990, Wells 1991, Lockyer 1993a, Read *et al.* 1993).

A.



B.

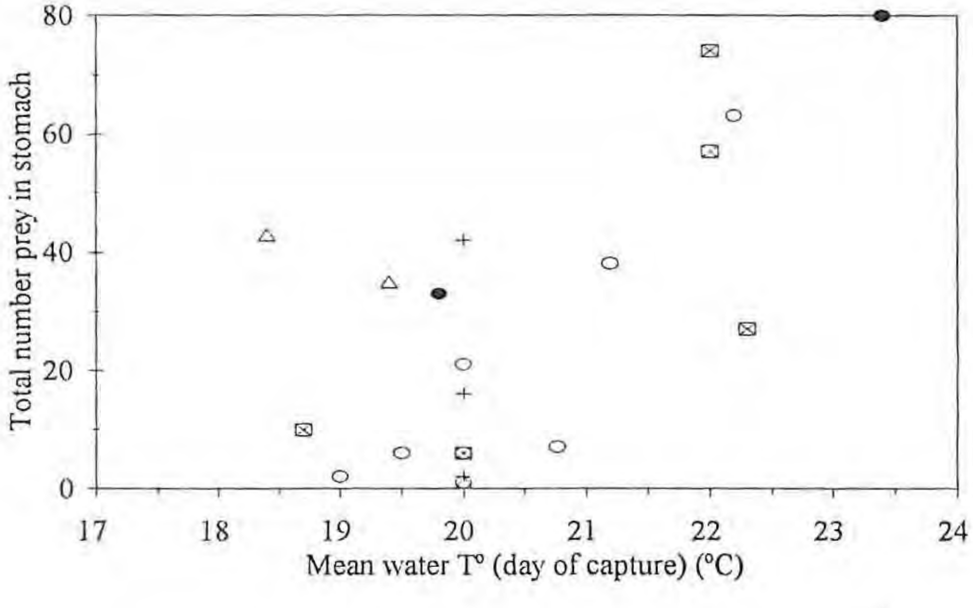


Figure 5.17: Significant correlations between water temperature on the day of capture and the number of prey items in the stomach for common (A) and bottlenose (B) dolphins caught in shark nets between 1992 and 1996 (c: calves, j: juveniles, a: adults, fl: lactating females, fp: pregnant females, fpl: simultaneously pregnant and lactating females).

The use of girth and blubber measurements, in conjunction with total body weight and/or length, have proved to be invaluable for assessing overall condition in many species of marine mammal, including fin whales (*Balaenoptera physalus*) (Lockyer *et al.* 1984, 1985, Lockyer 1986), pilot whales (*Globicephala melas*) (Lockyer 1993a), harbour porpoise (*Phocoena phocoena*) (Kastelein and van Battum 1990), Antarctic fur seals (*Arctocephalus gazella*) (Arnould 1995) and other pinnipeds (Boyd 1984, Fedak and Anderson 1987, Gales and Burton 1987, Boyd and McCann 1989, Castellini *et al.* 1993, Lunn and Boyd 1993, Baker *et al.* 1994a, 1994b, Gales *et al.* 1994). Several authors have related a deterioration in morphological condition, particularly weight, with the energetic costs of reproduction, reproductive success and/or survival potential of offspring (Arnbom *et al.* 1993, Walker and Bowen 1993, Baker *et al.* 1994a, 1994b, Derocher and Stirling 1995). Blubber thickness has been used previously as an indicator of potential nutritional and/or thermoregulatory problems (Castellini *et al.* 1993).

The species differences in ventral blubber thickness, as well as blubber and muscle weights, reflect the size and robustness of the two species, with the slender common dolphins having thinner blubber and lower total and proportional blubber weight. This trend appeared to contradict the habitat types and water temperatures generally inhabited by the two species, but these differences reflected rather the different behaviour, swimming speed and movements of bottlenose and common dolphins (Ross 1984). Although bottlenose dolphins move over large distances, they are coastal animals that typically swim slower and are more residential than common dolphins, which are pelagic, faster swimmers and migrate over larger distances (Ross 1984). The significantly greater muscle proportion in common dolphins supports this interpretation. The faster swimming speed of common dolphin necessitates a larger muscle proportion and would compensate for a lower blubber proportion. Increased swimming speed and higher levels of activity in these animals produces more metabolic heat, which reduces the need for a thick layer of blubber in a relatively cooler, pelagic environment. Similar comparisons have been made between belugas (*Delphinapterus leucas*) and killer whales (*Orcinus orca*) (Kastelein *et al.* 1994). In this respect, a thinner layer of blubber is beneficial in terms of efficient thermoregulation. Thinner blubber, in this case, was seen as an adaptation to behavioural and environmental differences, as opposed to reduced morphological condition in relation to bottlenose dolphins. Several studies have documented the variation (in thickness and quality) in blubber layers from species to species (Ross 1984, Lockyer *et al.* 1984, 1985, Lockyer 1986, 1993a, Ognetov 1990, Wells 1993, Pohlod *et al.* 1997).

Results from this study emphasise the importance of considering factors, such as species differences in morphology, when investigating the morphological condition of dolphins. Other factors that must also be considered include age, sex, physiological condition and season (Tomilin

1937, Kleinberg *et al.* 1964, Lockyer *et al.* 1984, 1985, Lockyer 1986, Ognetov 1990). Blubber has been successfully used in the assessment of marine mammal condition, through measurements of thickness, weight and more recently, its lipid composition (Lockyer *et al.*, 1984, 1985, Lockyer 1986, 1993, Ognetov 1990, Gales *et al.* 1994, Arnould 1995). In this respect, it is also possible that the blubber of common and bottlenose dolphins have different insulating qualities, similar to those described by Worthy (1991) and Kastelein *et al.* (1994). However, this was impossible to verify within the confines of the present study.

Only the ventral blubber thickness differed significantly between species. Age-related changes in blubber thickness were evident at the dorsal and ventral site. Dorsal blubber thickness was particularly reduced in calves, increasing markedly in juveniles and adults. Reductions in blubber thickness during lactation were evident only at the dorsal site. If blubber thickness is assumed to reflect blubber quality (in terms of lipid content), as concluded by Feltmann *et al.* (1948 in Lockyer *et al.* 1985), these differences suggested that different areas of blubber serve different functions. An increasing dorsal blubber thickness as an animal ages illustrated that this site may be important in terms of storage, blubber increasing in thickness at this site as metabolic stores are increased with time. The use of dorsal blubber as a storage site was also shown in lactating animals, which are under a certain degree of physiological and nutritional stress as a result of the demands of lactation (Lydersen *et al.* 1995, Lydersen and Kovacs 1996). The ventral blubber may play a larger role in thermoregulation, which accounts for thinner blubber at this site in common dolphins. Besides aiding in streamlining and providing insulation, the subdermal blubber layer functions as energy store and changes in the thickness and composition of blubber at different positions on the body (within a species) have been ascribed to the fact that different areas of blubber serve different functions (Ackerman *et al.* 1975, Lockyer *et al.* 1984, 1985; Kvadsheim *et al.* 1996, Pohlod *et al.* 1997). Large variations have been found in lipid and water contents of blubber samples from different regions of the body of various species of marine mammal, including fin, sei (*B. borealis*), minke (*B. acutorostrata*) and humpback whales (*Megaptera novaeangliae*) (Ackerman *et al.* 1975, Lockyer *et al.* 1984, 1985, Kvadsheim *et al.* 1996) as well as harbour porpoise and spotted dolphins *(Stenella attenuata*) (Worthy and Edwards 1990). Because of the negative effect lipid content has on blubber conductivity, variations in the lipid content of the dorsal and ventral blubber could create a 45 % difference in blubber conductivity in the same animal (Kvadsheim *et al.* 1996). Lateral and abdominal blubber sites were identified as important storage areas in minke whales. The dorsal region of fin and sei whales was highest in lipid content and an important storage area (Lockyer *et al.* 1984, 1985). Ventral blubber was found to be thinnest, contain the least lipid and vary least between age classes. Differences in blubber composition between the ventral and dorsal areas have also been related to buoyancy (Lockyer *et al.* 1984).

Although blubber thickness is an important tool in assessing body condition, it may be important to use this measurement in conjunction with estimates of blubber composition and/or quality, keeping in mind the different metabolic and thermoregulatory roles blubber has in maintaining physiological homeostasis. Blubber thickness measurements carried out during this study were significantly related to both the total and proportional blubber and muscle weights. If an increased proportional blubber weight is indicative of an improvement in blubber quality, as is increased blubber thickness (Feltmann *et al.* 1948, Lockyer *et al.* 1984), it may be possible to use blubber thickness measurements to assess the overall condition of blubber. Further examination of the relationship between blubber weight, thickness and composition will provide a valuable tool in marine mammal condition assessment.

The species-specific nature of blubber weights and proportional blubber and muscle weights were indicative of the different habits and habitats of the species. Different species of marine mammal are known to have different blubber proportions, depending on their size, swimming speed and habitat. In the larger baleen whales, the blubber layer appears to function more for storage than for insulation (Lockyer et al. 1984). In the smaller toothed whales, the insulation properties of blubber play a more important role, with blubber thickness inversely related to environmental water temperature (Ross 1984, Ognetov 1990, Kastelein *et al.* 1994). Harbour porpoises, for example, have a very unfavourable body weight to body surface ratio (Anderson 1981) may have blubber proportions of up to 40 % in the Baltic Sea (Slijper 1958, Tomilin 1957), where temperatures may be as low as 8°C (Sergeant 1978). Beluga whales have blubber proportions amounting to 52 % of body weight, blubber being thickest in winter (Kleinberg *et al.* 1964, Ognetov 1990). Tomilin (1957) recorded an increase in fat content and blubber thickness of common dolphins in the Black Sea during winter. The summer decrease for this species was associated with the deterioration of food supplies and an increase in water temperature. Bottlenose dolphins in the Black Sea had blubber proportions averaging 30.9 % (Tomilin 1957).

The change in the proportional contributions of muscle and blubber weight with increasing size and age was certainly related to changes in thermoregulatory demands and locomotion. The proportion of blubber to total body weight is greater than that of muscle at birth, but this trend is reversed at a weight of approximately 22 kg or an age of four to five weeks (Cockcroft and Ross 1990a). Muscle proportions in pilot whales increased from 22.5 % in juveniles to 30.5 % in adults, in conjunction with a decrease in blubber proportion (26 % in juveniles, 24 % in adults) (Lockyer 1993a). The muscle proportion of calves in this study was almost identical to that of adults. However, the larger blubber proportions reflected the larger surface to volume ratio in calves and associated increased thermoregulatory demands. As the ability to swim efficiently develops, and the surface to volume ratio decreases, so the proportional contribution of blubber decreases, in conjunction with an increased contribution by muscle (Cockcroft and Ross, 1990). A reduced proportion of blubber in beluga whales, between the age of two and five years, was related to the increase in energy expenditure during the transference to active feeding and the relatively rapid growth rate (Ognetov 1990). The relatively higher blubber proportions in common dolphin calves, compared to bottlenose dolphins of the same size may be related to the seasonality of calf catches in the nets for the different species. Common dolphin calves were caught predominantly in the winter months, whereas the majority of bottlenose calves were caught in periods of warmer water temperatures. The fact that common dolphins are typically found in waters colder than those along the Natal coast, the species difference may have adaptive significance in an age group, which is most vulnerable to thermoregulatory demands.

Although %B is not an indication of total body lipid, it has been used as a fairly objective and precise index of the nutritional condition of an animal (Lockyer *et al.* 1984, 1985 Lockyer 1986, Ognetov 1990). The results of this study reinforce the fact that many factors have an important influence on blubber thickness, weight and condition. These include species specific differences in morphology and habitat, age, environment and reproductive condition. Assessments of condition using blubber parameters must therefore take all of these into account. However, blubber remains an important indicator of nutritional and overall body condition, within specific age groups.

Lactation had a marked effect on morphological condition, including severely reduced weight, thinner blubber and significantly reduced blubber weight. Pond (1984 in Lockyer *et al.* 1985) considered that fat reserves in mammals were a direct adaptation to the demands of lactation. Although it is unclear which fat deposits are used in times of nutritional and physiological stress, it is known that energy stores in the blubber of marine mammals are used in the production of milk. In this respect, a reduction in blubber stores in lactating marine mammals is well documented (Boyd 1984, Lockyer *et al.* 1984, Lockyer 1993a). Although lactating animals in this study were of similar age and length to resting adult females, they weighed significantly less. In conjunction with smaller girths, thinner blubber and lower blubber weights, these differences reflected a reduction in overall body condition during lactation. Similar lactation-associated reductions in weight, blubber thickness and blubber volume have been described by Ross (1984) and Peddemors *et al.* (1992). In contrast, condition appears to be greatly improved during pregnancy, with pregnant bottlenose dolphins having a mean blubber weight of almost 10 kg more than other adult females. These results comply with data from fin and sei whales (Lockyer *et al.* 1984, 1985, Lockyer 1986) but contrast with those of pregnant beluga whales, which had the thinnest blubber, thicknesses being greatest in resting females (Ognetov 1990).

It has been suggested that lactating common dolphins make use of the annual sardine run along the south-east coast of South Africa to replenish energy stores (Cockcroft and Peddemors 1990, Young and Cockcroft 1994). However, it appears that lactation remains a huge metabolic drain on the condition of these females and the sardine run may be used to sustain and maintain, rather than replenish. Pregnant females, on the other hand, appear to be able to build up the necessary stores, in preparation for the spring/summer calving period. The build up of ventral blubber stores in particular may illustrate a need to protect the foetus (Kvadsheim 1996).

The energetic demands of lactation in marine mammals are well documented (for example, Lockyer 1981c, Lydersen *et al.* 1995, Lydersen and Kovacs 1996). These energetic demands are estimated to elevate food intake (above those required for maintenance) by 29-41 % in Icelandic fin whales (Lockyer 1978) and by 32-63 % in sperm whales (*Physeter catodon*) (Lockyer 1981a). Energy requirements of captive dolphins increased by approximately 50 % during lactation (Reddy *et al.* 1994). Free-ranging bottlenose dolphins in Sarasota Bay, Florida double their food intake during lactation (Wells 1991). Grey seals (*Halichoerus grypus*) are believed to cover all costs of lactation from maternal energy stores without any additional feeding (Lydersen *et al.* 1995). Cockcroft and Ross (1990) record an additional 3.1 % (of total body mass) food intake per day by a bottlenose dolphin during lactation. This corresponded to an extra energy intake of approximately 37 000 kJ/day, 96 % of which was used in the production of milk (Cockcroft and Ross 1990a). These data emphasise the immense energetic demands of lactation and the fact that unless energy reserves are sufficient to last the first five months of intense suckling, lactating females must be capable of either reducing their own energy requirements or increasing food consumption. The calculated energy reserve of pilot whales, built up during the winter feeding period was slightly less than the annual estimated cost of lactation (Lockyer 1993a). This again emphasises the extremely high energetic demands of lactation, which must be met initially through feeding in autumn and early winter and through energy stores in spring (Lockyer 1993a).

The sex composition of common dolphins caught in shark nets shows that a large proportion of the catch comprises pregnant females and lactating females with small calves (Cockcroft 1990, Cockcroft and Ross 1991). Results from the current study indicated that pregnant common dolphins are able to make use of the annual fish migration to build up energy stores, reflected by larger blubber stores. However, lactating common dolphins appear to use the resource for the production of milk and homeostasis, with the elevated demands of lactation preventing energy storage. Lactating females of this species are thought to use "nursery groups" during the sardine run (Cockcroft and Ross 1990b, Young and Cockcroft 1994) as a possible behavioural/social means of facilitating energy conservation and acquiring of additional food supplies. Lactating females were less selective in their dietary habits than other animals. In order to maintain condition, lactating females may be forced to become more opportunistic, consuming a greater number of prey species, with a lower mean energy content. Pregnancy did not have a marked effect on dietary diversity or food consumption. These reproductive-related changes in diet have been documented in other species of cetacean (Bernard and Hohn 1989, Young and Cockcroft 1994). Reproductively active sperm whales were found to feed more frequently and more actively than males (Lockyer 1981a).

The importance of condition during reproductively active periods, and the importance of diet in maintaining condition during this time, is accentuated by the fact that calving periods for different species of marine mammal correspond with seasonal availability of local food resources (Urian *et al.* 1996, Boyd 1996). The milk quality of Antarctic fur seals was associated with feeding activity and efficiency (Arnould *et al.* 1996). Additionally, maternal size and condition of Grey seals had a significant effect on the amount of energy transferred to the suckling pup (Iverson *et al.* 1993), in turn affecting growth efficacy of the pup (Hindell *et al.* 1994, Lydersen *et al.* 1995).

The difference in dietary intake between the two species of dolphin examined here was linked directly to the habitats and habits of the different species (Cockcroft 1990, Young and Cockcroft 1994). The mean dietary intake of common dolphins had more than double the energy value of those species consumed by bottlenose dolphins. These differences in diet reflect the energetic demands generated by colder water temperatures, faster swimming speed and larger muscle proportions of common dolphins. In this respect, certain aspects of morphological condition are inherently linked to dietary and environmental features that are characteristic for each species. Overall, diet-associated changes in morphological condition were difficult to interpret during this study, as stomach contents are an indication of short-term food intake only, whereas morphological parameters are an indication of food intake and diet composition over a longer period. However, a higher dietary diversity, accompanied by a reduction in the mean energy content of prey was related to reduced blubber thickness in certain age groups of common dolphins. In bottlenose dolphins, increased prey diversity was generally associated with a higher mean energy content. This may explain greater blubber thickness measurements in adult bottlenose dolphins. The positive relationship between food supply and blubber, which has been recorded for other marine mammals (Lockyer 1986, Ognetov 1990) indicates that these morphological measures may have potential as indicators of habitat quality.

Dolphins, during this study, generally consumed prey of higher energy value when water temperatures were colder, which corresponded to an increase in blubber thickness. The consumption of fish with a higher energy value in colder water temperatures may result in a build up of energy stores, above that needed for metabolic maintenance, and consequently allow for an increase in blubber thickness. Thicker blubber is usually associated with an increased lipid content in the blubber and greatest blubber thicknesses are generally displayed in mid-winter (Lockyer *et al.* 1984, 1985, Ognetov 1990, Lockyer 1993a, Wells, 1993). Bottlenose dolphins in Sarasota Bay, Florida consumed about 10 % to 20 % more calories in autumn (to build up the insulative layer of blubber) than in spring (Costa *et al.* 1993, Wells 1993). Blubber stores in these animals, together with food consumption, was reduced as water temperatures increased, assumedly due to the utilisation of energy stores in the blubber. Similar temperature-related elevations in food intake have been recorded for bottlenose dolphins (Cates and Schroeder 1986) and beluga whales (Kastelein *et al.* 1994).

The increased blubber thickness and total blubber weight in warmer water temperatures in common dolphins is difficult to explain, although it concurred with the relationship between dietary diversity, energy intake and temperature discussed above. Common dolphins caught on the lower south coast (mean water temperature July-September: 18.7°C) had a mean dorsal blubber thickness of 12.25 mm, reflecting the morphological adaptation to warmer waters in the Eastern Cape during summer. Catches on the upper south coast (mean water temperature July-September: 18.9°C) had an increased mean blubber thickness of 14.31 mm. Mean blubber thickness of common dolphins caught on the north coast (mean water temperature June-November: 19.70°C) was 15.54 mm. This would suggest that common dolphins that had moved further up the coast, and were caught further north, had more blubber stores than animals caught further south. These increased blubber stores resulted from the intense bouts of feeding, which occur as the dolphins follow the fish migration northwards. Although blubber thickness at the dorsal site was specific to different age groups, there was no significant regional difference in the capture of calves, juveniles and adults that would explain this phenomenon.

Common dolphins are typically found in colder offshore waters, and are purely transitory in the warmer inshore water of the Natal coast (Ross 1984, Cockcroft and Peddemors 1991). Therefore, this species makes use of the abundant food supplies of the sardine run to build up energy stores and thicken the blubber layer before returning to the colder waters off the continental shelf, and to a lower latitude. The fact that common dolphins were not caught further north than Salt Rock may indicate that the relatively large blubber stores, built up during the feeding migration, prevents animals from losing heat effectively in water temperatures warmer than those found north of Salt Rock. Common dolphins may actually be deleteriously affected by the warmer inshore waters and in fact, have to actively lose heat. Studies of the larger whale species have shown that following the feeding season, some of the animals are effectively over-insulated, with blubber used predominantly as a food store, rather than for insulation (Lockyer 1978, 1981a, 1981b, 1986). Smaller cetaceans are also known to suffer from overheating (Costa *et al.* 1993, Wells 1993). Exposure of common dolphins to extraordinarily warm water temperatures may cause an increase in metabolic rate in order to move blood to the extremities in an effort to lose heat. This energetically demanding thermoregulation may have a negative effect on the maintenance of common dolphin condition during this time. This effect may be magnified in lactating females of this species, as their energetic demands are additionally elevated through milk production.

Summary

Results obtained during this study reflected an important and valuable relationship between morphological condition and diet, temperature and reproductive status. The complexity of these relationships and their influence on condition must be accounted for when using morphological parameters to assess marine mammal condition. Similar to other studies, measurements of weight, girth and blubber thickness were valuable indicators of condition in net-caught dolphins, and in conjunction, these may be used to predict the condition of other aspects of morphology. Blubber thickness and proportional blubber weight appeared to be the best indicators of nutritional quality and therefore habitat status. However, these parameters were not only affected by diet, but also by age, pregnancy, lactation and water temperature. The relationship between blubber quality, composition, thickness and weight should be further investigated to improve the use of these parameters as condition indices in free-ranging dolphins.

CHAPTER 6:

PHYSIOLOGICAL INDICATORS OF CONDITION IN NET-CAUGHT BOTTLENOSE (*Tursiops truncatus*) AND COMMON (*Delphinus delphis*) DOLPHINS:

I. HAEMATOLOGY.

INTRODUCTION

Terrestrial studies have proved there to be valuable relationships within and between haematology, physiology, morphology and the environment, which reflect the nutritional condition of animals, as well as overall habitat quality. The majority of these relationships have been established through strictly controlled studies on collared peccaries (*Tayassu tajacu*) (Lochmiller *et al.* 1985b, c, 1988) and white-tailed deer (*Odocoileus virgianus*) (Seal *et al.* 1978a, 1978b, Warren *et al.* 1981, 1982, Dinkines *et al.* 1991). A number of studies on other terrestrial mammal species have reinforced the valuable use of haematological parameters not only as animal condition indices, but also as indicators of nutritional status and habitat quality (Bailey 1968, Smith 1970, Franzmann 1972, Hyvarinen *et al.* 1975, Seal *et al.* 1975, Hanks *et al.* 1976, Brooks 1978, Robinson 1979, Melton and Melton 1982, DelGuidice *et al.* 1987b, Henke and Demarais 1990, Grant *et al.* 1992, Altmann *et al.* 1993, Boren *et al.* 1993, Choquenot 1993, Crete *et al.* 1993, Hellgren *et al.* 1993, Knick *et al.* 1993, Van Rooyen 1993, Rietkerk *et al.* 1994, Wolkers *et al.* 1994a, Gallivan *et al.* 1995, Huot *et al.* 1995, Clode *et al.* 1995). Studies have also identified interconnected relationships between body condition, nutrition, fertility and reproductive success (Robinette *et al.* 1973, Allaye Chan-McLeod *et al.* 1994, 1995, White *et al.* 1997). Recently, particular physiological and morphological condition indices have been shown to be density-dependent (Blumstein and Foggin 1997, Sams *et al.* 1996, 1998).

The haematology of marine mammals has been extensively reviewed by Bossart and Dierauf (1990). The majority of studies have shown that haematological parameters are invaluable in the assessment of captive animals health (Ridgway 1965, Ridgway *et al.* 1970, Medway and Geraci 1978, Engelhardt 1979, Van Foreest 1980, McConnell and Vaughan 1983, Cornell 1983, Clarke 1990, Asper *et al.* 1990, Thurman and van der Elst 1995, Neilsen 1995). In addition, haematological parameters have provided comprehensive information on the physiological characteristics and adaptations of different marine mammal species to their respective environments (Medway and Geraci 1964, Medway and Moldovan 1966, De Monte and Pilleri 1968a, 1968b, 1972, 1977, 1983, Harkness and Grayson 1969, Lane *et al.* 1972, Korzhuev and

Glazova 1973, Bogdanova and Lebedev 1973, White *et al.* 1976). The relationship between marine mammal haematology and diving capabilities has been particularly well investigated (Horvath *et al.* 1968, Bryden and Lim 1969, Geraci 1971, Ridgway *et al.* 1984, Ridgway 1986, De Monte and Pilleri 1988). However, more recently, marine mammal haematology has been increasingly used in the assessment of not only physiological health, but also overall body and nutritional condition (Medway and Cornell 1989, Asper *et al.* 1990). Although the potential use of haematology as an index of environmental quality was mentioned in the early 1980's (Medway 1983), few studies since have examined the possibility of using haematological parameters in marine mammals to reflect habitat quality.

The relationship between certain morphological and/or physiological measures often allows for generalisations about relative changes in morphology and/or physiological processes over a wide range of body sizes (Ridgway and Fenner 1982, Ridgway and Kohin 1995). The usefulness of these relationships is apparent when applied to free-ranging populations, where extensive physiological measurements are not readily available. These relationships can additionally be interpreted with respect to age and habitat type (Ridgway and Kohin 1995). The influence of age, reproduction and other factors, such as diet and water temperature, on morphological, particularly blubber, condition of marine mammals is well documented (e. g. Ridgway and Fenner 1982, Ross 1984, Lockyer *et al.* 1984, 1985, Lockyer 1986, 1993a, Ognetov 1990, Wells 1993, see Chapter 5). However, it remains unclear how physiological, morphological and environmental variables interact.

Long-term monitoring of free-ranging marine mammal condition is almost impossible. An exceptional, isolated study in Sarasota, Florida has successfully monitored the morphology and physiology of a local population of bottlenose dolphins (*Tursiops truncatus*) for over two decades (for example Rhinehart *et al* 1991, 1992, Wells 1991, 1993). There are no similar studies ongoing, particularly in the Southern Hemisphere. This study comprises the first haematological investigation of coastal dolphins along the south-east coast of Southern Africa. The south-east coast of South Africa supports 37 species of marine mammal; however, knowledge of the condition of any of these species is lacking. In light of increasing coastal development and the associated increase of factors such as marine pollution and depleted food resources (Brown 1986, Lundin and Lindén 1993, Ngoile and Horrill 1993, Agardy 1994, Lubke and Britz, 1994, Simmonds 1994), it is vital to assess the condition of marine mammals in the region. Although the environmental conditions and geography of the area prevent regular capture and sampling of free-ranging animals, annual incidental catches of dolphins in anti-shark nets along the Kwazulu-Natal coast provide a representative sample of the local dolphin population (Cockcroft and Ross, 1989, 1991, Cockcroft 1990, Young and Cockcroft 1994). Physiological studies, particularly from incidentally-caught animals are not ideal. However, these animals provide a valuable opportunity to investigate the potential use of physiological and morphological measures of condition for the monitoring of wild animal populations.

In this context, by using net-caught dolphins, this study addressed the following objectives:

- To obtain haematological data from blood samples taken from dolphins incidentally-caught in shark nets.
- To identify and assess the effect of species, age, sex and reproduction on the haematology of net-caught dolphins.
- To examine relationships between haematology and morphological parameters, such as weight, girth, blubber thickness and blubber and muscle weight (see Chapter 5).
- To identify and assess associations between haematology and environmental variables, such as diet and water temperature.
- To investigate the potential use of haematological parameters in assessing the overall condition of net-caught dolphins
- To investigate the potential use of interactions between net-caught dolphin morphology and physiology to assess and monitor the condition of the local dolphin population.

MATERIALS AND METHODS

All haematological analyses described in this chapter are from those animals for which morphological measurements, dietary and environmental variables are described in Chapter 5.

1. BLOOD SAMPLING, STORAGE AND ANALYSIS

Blood was sampled from net-caught animals as soon as possible after capture. Blood was drawn directly from a caudal vessel with an 18-guage needle and syringe; or directly into a vac-u-tube from the aorta. Each vac-u-tube was filled with 10 ml of blood. Equipment used for blood sampling and storage, including all vac-u-test ® tubes, were identical to those described in Chapter 3. All samples were refrigerated at 4°C before transportation (on ice) by courier to the South African Institute for Medical Research (S.A.I.M.R.) laboratory in Port Elizabeth. The majority of analyses were completed within 48 hours of sampling, although some were delayed due to logistical problems, including obtaining blood samples from animals caught at beaches not near the Natal

Sharks Board headquarters, timing of courier services and batching of samples obtained during weekends.

Tests used for haematological analysis were identical to those described in Chapter 3. Although an attempt was made to include all analyses used in Chapter 3, certain logistical problems prevented this. Leukocytic and haematological analyses carried out on blood from net-caught animals included counts of white blood cells (WBC), lymphocytes, red blood cells (RBC) and platelets as well as measurements of haemoglobin (HB), packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC).

Materials and methods used to investigate the morphology, age, growth, diet and environment of the animals used for haematological analyses are described in Chapter 5. Statistical analyses were carried out using the same statistical computer packages used in the previous chapters. For all data sets, outliers were determined using box-whisker plots. Data were analysed including and excluding these outliers to determine their influence, if any, on the data. Relationships between haematological parameters and morphology, diet and water temperature were examined using multiple correlation analysis.

RESULTS

Blood was sampled from a total of 96 animals (58 common dolphins (*Delphinus delphis*) and 38 bottlenose dolphins) caught in shark nets between 1993 and 1996. Blood was sampled from 39 animals in 1993, 59 in 1994 and four in 1995. Blood was sampled from a single bottlenose dolphin in January of 1996. These data exclude all samples found to be badly haemolysed on arrival from Natal Sharks Board. In addition to common and bottlenose dolphins, blood was also sampled from seven humpback dolphins (*Sousa chinensis*). The limited sample size of this species prevented the description of any haematological trends, but as these results comprise the first haematological results for this species, mean values are included in Table 6.1.

The seasonal and regional distribution of the dolphin captures is described in Chapter 5. In addition, the composition of the sampled common and bottlenose dolphin catch is discussed in the previous chapter. Animals were divided in age classes, using the same characteristics, reported by Cockcroft and Ross (1990b) and Mendolia (1990), as used in Chapter 5. Parameters (dependent and independent) which were significantly different between species were considered separately. Data from the two dolphin species that were not significantly different were pooled. Within a species, morphological, dietary and environmental (water temperature) variables did not differ significantly between males and females of different ages (Two sample t-test, $p<0.05$) (see Chapter 5). Where physiological parameters were significantly different between males and females of the same age group, these were treated separately.

LEUKOCYTIC PARAMETERS

A. White blood cells (WBC)

a. Common and Bottlenose dolphins (pooled data)

There were no significant species differences in mean WBC counts (t=0.05, p=0.95, df=80), therefore WBC data from the two species were pooled. The mean WBC count of the juvenile age group (21.29 ± 14.36 x10^9 cells/l) was significantly higher than that of calves' (13.16 ± 6.47 x10^9 cells/l) and adults (11.35 ± 5.84 x10^9 cells/l) (Table 6.1). However, two WBC counts above 45.00 x 10^9 cells/l (both from juveniles) were identified as outliers (box-whisker plot) and these biased this groups mean WBC count. The removal of these outliers resulted in the mean WBC count of different age groups becoming similar (F=1.44, p=0.24, df=71) (Table 6.2). However, the mean WBC count of adult females was significantly higher (23.10 ± 5.27 x10^9 cells/l) than adult males (11.54 ± 6.11 x10^9 cells/l) (t=3.13, p=0.004, df=26).

The WBC count was not significantly related with any aspect of the animals' morphology and/or diet. Although pooled WBC data from common and bottlenose dolphins were positively correlated with the mean local water temperature of the month prior to capture (r=0.25; p=0.03; n=75), counts for the two species were highly variable (~5-30 x10^9 cells/l) and no distinct trend was apparent.

b. Common dolphins

Although mean WBC counts from the two species were similar, only common dolphin data differed significantly between groups of different reproductive status. The total number of animals in each group was limited, but the mean WBC count of resting adult female common dolphins (23.10 ± 5.27 x10^9 cells/l) was significantly higher than that of pregnant (9.28 ± 3.70 x10^9 cells/l) (t=-2.95, p=0.01, df=11), lactating (10.99 ± 5.07 x10^9 cells/l) (t=-3.50, p=0.007, df=9) and simultaneously pregnant and lactating females (9.09 ± 5.32 x10^9 cells/l) (t=-3.24, p=0.03, df=4).

B. Lymphocytes

a. Common and bottlenose dolphins (pooled data)

The mean percentage of lymphocytes was significantly different between species, with common dolphins having a mean lymphocyte proportion almost 20 % higher than that of bottlenose dolphins (t=4.47, p<0.001, df=15) (Table 6.1). Two lymphocyte proportions, below 49 %, both of which were recorded from male bottlenose dolphins, were identified as outliers (Box-whisker plot). The

Table 6.1: Mean values of leukocytic and haematological parameters from dolphins caught in shark nets between 1992 and 1996. (Data are presented as means and standard deviations (in parentheses). (Parameters are measured in units identical to those listed in Table 3.2). (Data from humpback dolphins have not been included in the calculation of overall means and/or species differences. ND: no data).

Blood parameter		**Overall mean**	**N**	***D. delphis*** **Common dolphin**	**n**	***T. truncatus*** **Bottlenose dolphin**	**n**	***S. chinensis*** **Humpback dolphin**	**n**
White blood cells (WBC)		13.46 (8.92)	87	12.93 (9.11)	51	12.83 (6.02)	31	20.17 (13.52)	7
	Adults	11.35 (5.84)	53						
	Juveniles	21.29 (14.36)	15						
	Calves	13.16 (6.47)	19						
Lymphocytes*		65.83 (10.67)	17	70.18 (4.80)	13	51.73 (12.98)	4	59.14 (5.69)	5
Red blood cells (RBC)*		5.65 (1.22)	75	6.10 (0.97)	51	4.70 (1.16)	24	4.64 (1.31)	6
Haemaoglobin (HB)*		21.24 (3.92)	82	22.20 (3.00)	51	19.67 (4.70)	31	18.16 (2.25)	7
Packed cell volume (PCV)*		0.63 (0.17)	75	0.66 (0.16)	51	0.57 (0.18)	24	0.48 (0.20)	6
	Adults			0.70 (0.11)	36				
	Juveniles			0.60 (0.17)	5				
	Calves			0.55 (0.22)	10				
Mean cell volume (MCV)*		112.98 (17.90)	59	108.00 (15.52)	38	121.98 (17.96)	21	100.23 (18.80)	6
	Adults			112.09 (9.93)	28				
	Juveniles			107.63 (3.29)	3				
	Calves			91.80 (24.09)	7				
Mean cell haemoglobin (MCH)		38.44 (8.26)	59	37.66 (9.37)	38	39.87 (5.69)	21	42.25 (15.05)	6
Mean cell haemoglobin conc. (MCHC)		33.64 (9.20)	58	34.56 (10.41)	38	31.90 (5.52)	20	44.57 (20.28)	6
Platelets		411.59 (518.31)	17	411.23 (542.28)	13	412.75 (344.80)	4	762.00 (543.41)	5

Table 6.2: Mean values of leukocytic and haematological parameters (excluding statistical outliers) from common and bottlenose dolphins caught in shark nets between 1992 and 1996. (Data are presented as means and standard deviations (in parentheses). (Parameters are measured in units identical to those listed in Table 3.2)

Blood parameter	**Overall mean**	**N**	***D. delphis* Common dolphin**	**n**	***T. truncatus* Bottlenose dolphin**	**n**
White blood cells (WBC)	12.29 (6.34)	79	12.02 (6.47)	50	12.83 (6.02)	31
Lymphocytes	68.88 (6.69)	15	70.18 (4.8)	13	60.45 (13.65)	2
Red blood cells (RBC)*	5.70 (1.15)	74	6.10 (0.97)	51	4.81 (1.04)	23
Haemaoglobin (HB)*	21.72 (2.89)	75	22.29 (2.53)	49	20.81 (3.19)	28
Packed cell volume (PCV)*	0.64 (0.16)	74	0.67 (0.14)	50	0.57 (0.18)	24
Mean cell volume (MCV)*	116.95 (12.18)	54	112.31 (8.75)	34	124.83 (13.31)	20
Mean cell haemoglobin (MCH)*	38.12 (4.80)	56	36.56 (4.85)	36	40.91 (3.24)	20
Mean cell haemoglobin conc. (MCHC)	32.06 (3.85)	53	31.67 (3.59)	34	32.75 (4.30)	19
Platelets	297.19 (221.90)	16	258.67 (132.06)	12	412.75 (398.14)	4

removal of these outliers served to increase the mean lymphocyte percentage of bottlenose dolphins, increasing its similarity with that of common dolphins (Table 6.2). There were no sex-, age- or reproductive-related differences in lymphocyte percentages within species, although these analyses were limited by the lack of lymphocyte data from common dolphin calves, juveniles and resting adult females.

Lymphocytes were not related to morphological data from either dolphin species. Lymphocytes were, however, negatively correlated with the total number of prey species in the stomach (r=-0.53; p=0.05; n=16) and the total number of prey items in the stomach (r=-0.58; p=0.02; n=16). However, these relationships appeared to be indicative of species differences in this parameter, as opposed to dietary-associated changes in the number of lymphocytes and it was difficult to establish whether this relationship was directly related to diet. A similar trend was evident between lymphocytes and the local water temperature on the day of capture (r=-0.49; p=0.02; n=22) and the mean regional water temperature of the month of capture (r=-0.45; p=0.03; n=22). Common dolphins were generally caught in colder water (18-20°C) and had significantly higher lymphocyte counts; as opposed to bottlenose dolphins, which were caught in warmer waters (21-23.5°C) and had lower lymphocyte counts.

HAEMATOLOGICAL PARAMETERS

A. Red blood cells (RBC)

a. Common and bottlenose dolphins (pooled data)

The mean RBC count from common dolphins was significantly higher than that of bottlenose dolphins (t=5.45, p<0.001, df=73) (Table 6.1). RBC counts below 2.40 x10^{12} cells/l were identified as outliers (Box-whisker plot). The removal of outliers did not have a marked effect on the mean RBC counts of the two dolphin species (Table 6.2). There were no significant reproductive-related changes in RBC counts.

Morphological parameters that were significantly correlated with RBC counts from both species included ventral blubber thickness (r=-0.36; p=0.003; n=69), proportional weight of blubber (%B) (r=-0.28; p=0.02; n=70) and proportional weight of muscle (%M) (r=0.26; p=0.03; n=72) (Fig. 6.1). Data were generally highly variable. This was particularly true for the relationship between RBC and ventral blubber thickness (Fig. 6.1A). Investigation of this relationship within age groups of the two dolphin species did not reveal any significant trends (Fig. 6.2), although lactating common dolphins showed a decline in RBC counts as ventral blubber thickness increased (Fig. 6.2A). Data were similarly variable for the latter two correlations, but RBC counts were predominantly lower and higher at increased %B and %M, respectively (Fig 6.1B & C).

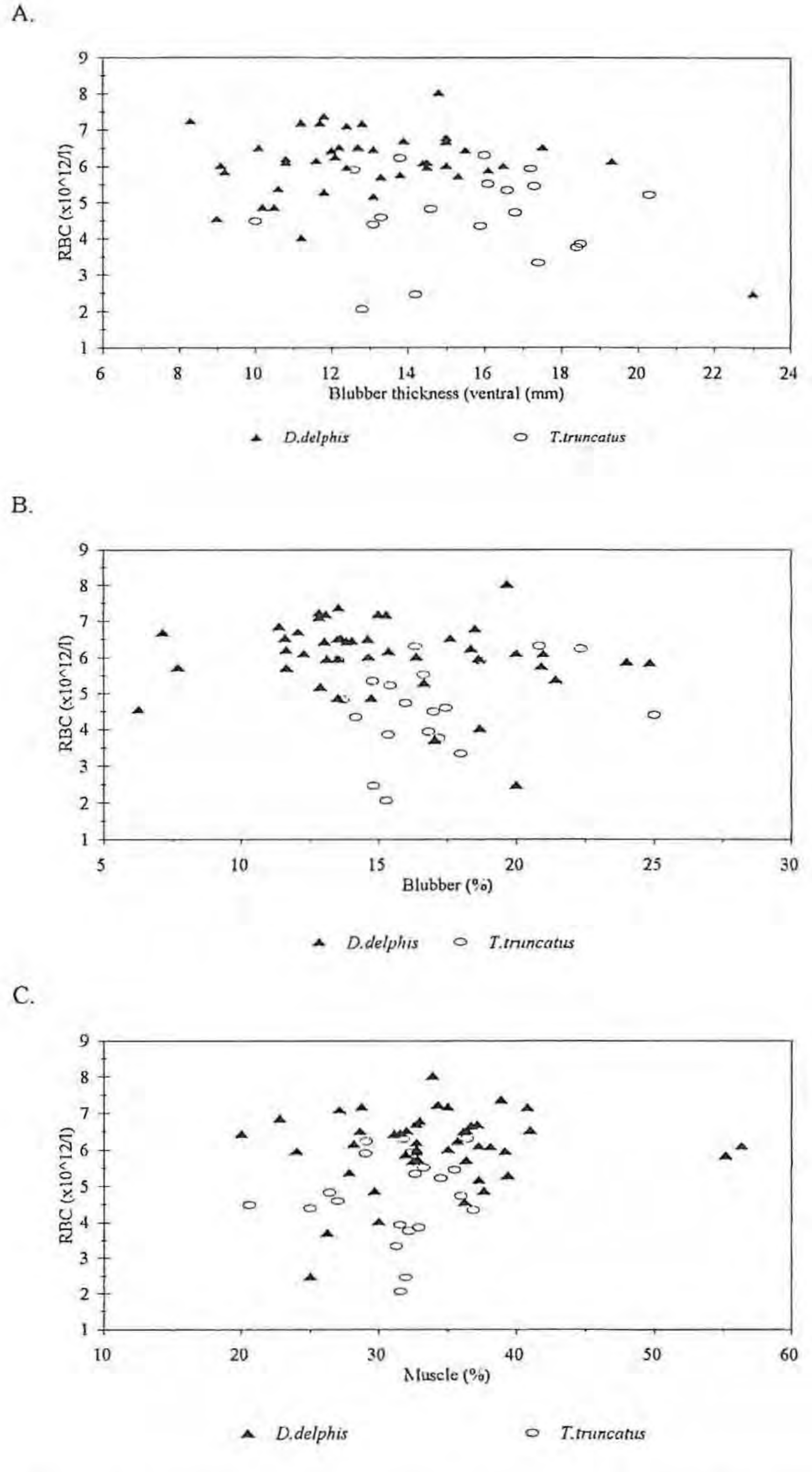


Figure 6.1: Significant correlations between red blood cell (RBC) count and ventral blubber thickness (A), proportional blubber weight (B) and proportional muscle weight (C) for common (D. delphis)and bottlenose (T. truncatus) dolphins caught in shark nets between 1992 and 1996.

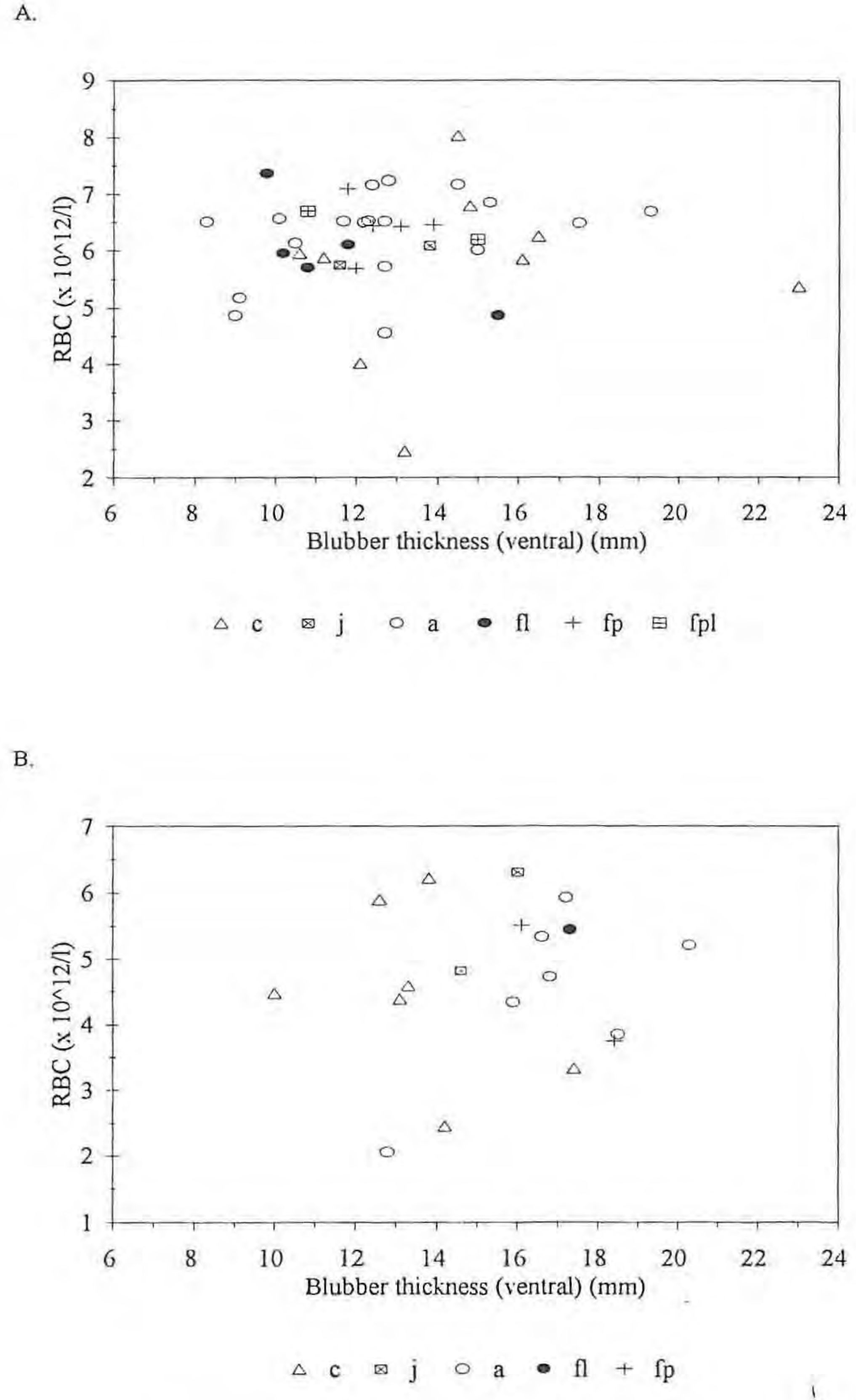


Figure 6.2: Significant correlations ($p<0.05$) between RBC count and ventral blubber thickness for common (A) and bottlenose (B) dolphins caught in shark nets between 1992 and 1996 (c: calves, j: juveniles, a: adults, fl: lactating females, fp: pregnant females).

b. Common dolphins

Adult male common dolphins had a significantly higher mean RBC count ($6.39 \pm 0.71 \times 10^{12}$ cells/l) than calves ($5.12 \pm 1.38 \times 10^{12}$ cells/l) and juveniles ($5.43 \pm 1.16 \times 10^{12}$ cells/l) of the same sex (Two sample t-test, $p<0.05$). In this context, common dolphin RBC counts were positively correlated with total body length ($r=0.30$; $p=0.03$; $n=51$), total body weight ($r=0.28$; $p=0.04$; $n=51$) and age ($r=0.35$; $p=0.05$; $n=32$) (Fig. 6.3). This increasing trend was difficult to verify, but data variability was markedly increased in smaller, younger animals (Fig. 6.3). A similar trend was evident between common dolphin RBC count and anal girth ($r=0.30$, $p=0.05$, $n=44$).

Although pooled RBC data was significantly correlated with the local water temperature of the day of capture, this trend remained significant only for common dolphins when data were considered separately ($r=-0.37$; $p=0.001$; $n=58$). The different nature of these relationships reflected species-specific RBC counts and differences in environmental variables at the capture locations of the two species. Bottlenose dolphins were caught in waters of temperatures between 18.3°C and 22.5°C and typically had lower RBC counts than common dolphins. In contrast, common dolphins, with their typically higher RBC counts, were caught in water temperatures of between approximately 17°C and 20°C (Fig. 6.4). Variability of common dolphin RBC counts was highest in warmer waters (~20°C), in conjunction with a higher frequency of reduced RBC counts. Calves, in particular, had reduced RBC counts at higher water temperatures (Fig. 6.4A). Bottlenose dolphin RBC counts from all age groups were variable over the temperature range (Fig. 6.4B).

B. Haemoglobin (HB)

a. Common and bottlenose dolphins (pooled data)

Mean HB concentrations were significantly higher in common dolphins ($t=2.96$, $p=0.003$, $df=80$) (Table 6.1). HB concentrations below 14.00 g/dl were identified as statistically outlying (Box-whisker plot). The HB levels of 28.40 g/dl (adult male common dolphin) and 31.00g/dl (adult (resting) female common dolphin) were also identified as outliers. However, these outliers did not have a marked effect on the mean HB levels of the two species (Table 6.2).

b. Common dolphins

Common dolphin HB levels were positively correlated with both total body length ($r=0.28$; $p=0.05$; $n=51$) and age ($r=0.55$; $p=0.001$; $n=32$). HB levels were generally stable (~22 g/dl) at lengths between 200 cm and 260 cm, but appeared to be slightly lower and more variable at lengths between 120 cm and 180 cm. In conjunction, HB levels appeared to be more variable, and possibly slightly lower, at ages younger than five years. Other morphological measurements with which common dolphin HB was significantly correlated included axillary girth ($r=0.32$; $p=0.03$; $n=44$) and ventral blubber thickness ($r=-0.32$; $p=0.006$; $n=75$). The positive relationship with girth

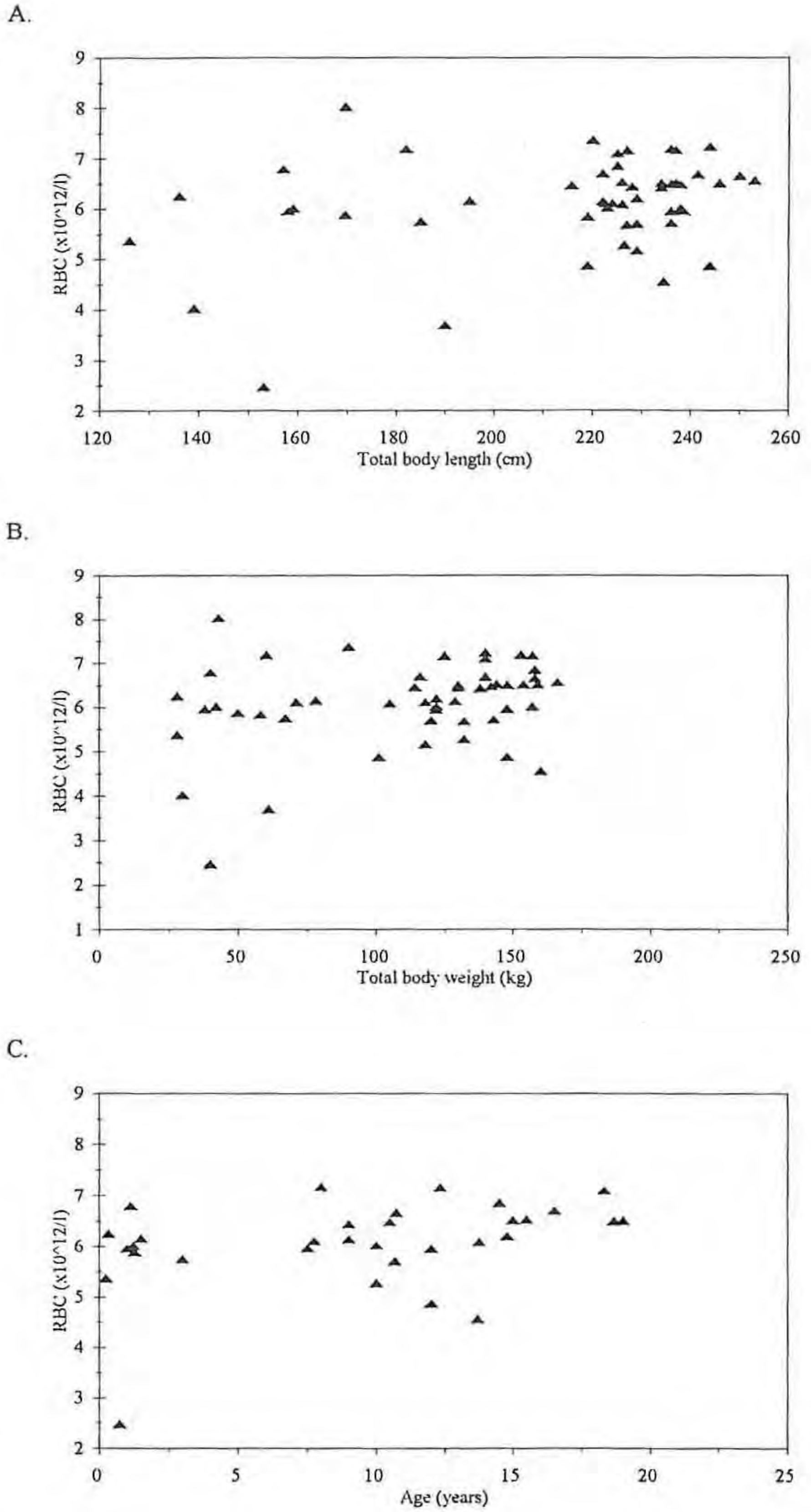
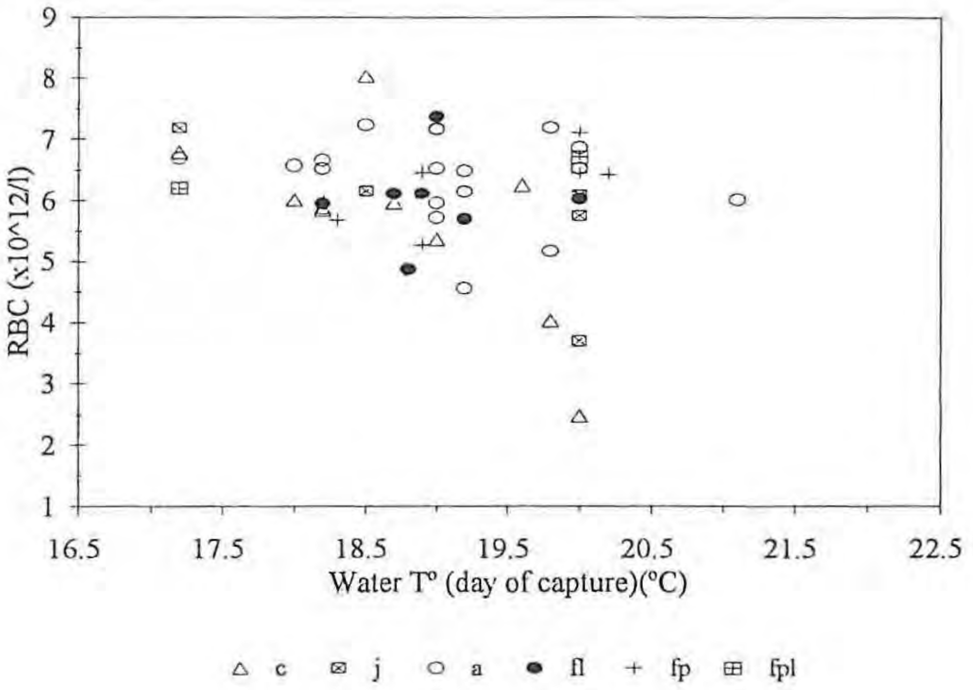


Figure 6.3: Significant correlations between red blood cell (RBC) count and total body length (A), weight (B) and age (C) for common dolphins caught in shark nets between 1992 and 1996.

A.



B.

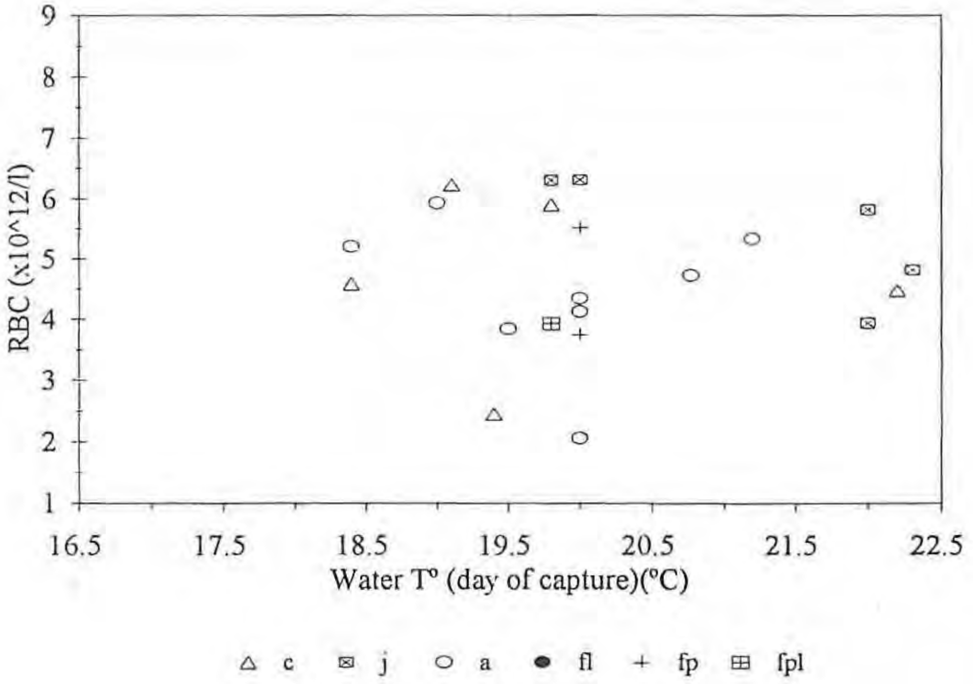


Figure 6.4: Relationship between red blood cell (RBC) count and the water temperature on the day of capture for different age and reproductive classes of common dolphin (A) and bottlenose dolphin (B) caught in shark nets between 1992 and 1996.

reflected the differences in ventral blubber thickness between age and reproductive classes (Fig. 6.5A). However, animals of the same age group (particularly calves and adults) appeared to have lower HB concentrations when ventral blubber was thicker (Fig. 6.5B). Common dolphin HB levels were also related to %M (r=0.26; p=0.02; n=78) and %B (r=-0.36; p=0.001; n=76). The former relationship was complicated by highly variable data, showing no distinct trend. Levels of HB remained relatively stable over a wide range of %B measurements. Levels of HB in calves were slightly increased with reduced %B.

Haemoglobin concentrations from the two dolphin species were not significantly affected by diet. Similarly, HB levels were variable over a wide range of water temperatures, with no distinct trends identified for either common or bottlenose dolphins.

C. Packed cell volume (PCV)

a. Common and bottlenose dolphins (pooled data)

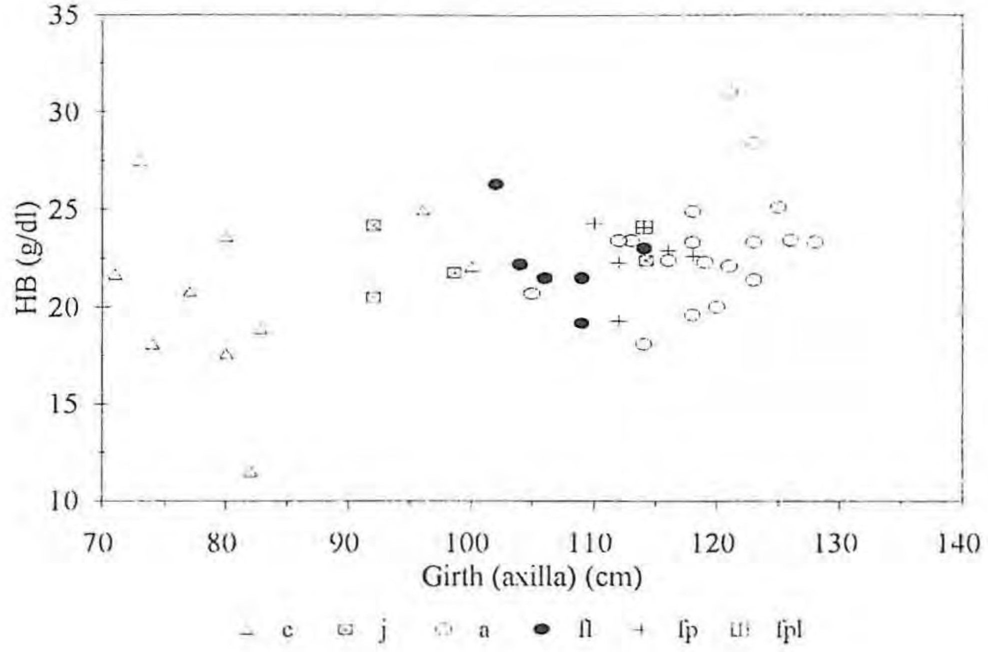
Mean PCV values were significantly higher in common dolphins (t=2.18, p=0.03, df=73), similar to the elevated RBC and HB described previously (Table 6.1).

b. Common dolphins

Mean PCV values were significantly different between common dolphin calves, juveniles and adults. A single PCV values (0.13 l/l), from a male common dolphin calf, was identified as an outlier. The removal of this value resulted in the age differences in PCV becoming non-significant (Table 6.2). However, there was a significant positive correlation between common dolphin PCV and total body length (r=0.22; p=0.05; n=81) and age (r=0.50; p=0.003; n=32) (Fig. 6.6). This relationship reflected increased variability and a greater frequency of reduced PCV values in smaller, younger common dolphins.

Common dolphin PCV data were also positively correlated with all measures of girth (axilla (r=0.40; p=0.008; n=44), maximum (r=0.35; p=0.02; n=44) and anus (r=0.38; p=0.01; n=44)). However, data were highly variable and no clear trend was evident. As described above, a high variability in PCV corresponded to smaller girths and body size. PCV values from common dolphins were also significantly correlated with total blubber (r=0.32; p=0.04; n=44) and muscle (r=0.33; p=0.03; n=45) weights, as well as %B (r=-0.25; p=0.03; n=70) and %M (r=0.33; p=0.005; n=72) (Fig. 6.7). These relationships paralleled those described above for age and total body length. The PCV values from calves were highly variable within a narrow range of blubber and/or muscle weights, contrasting to adult animals, which showed relatively stable PCV values over a wide range of blubber weights (Fig. 6.7A). Extensive variability made any trend between %M and PCV difficult to confirm. However, PCV values of calves and juveniles appeared slightly higher

A.



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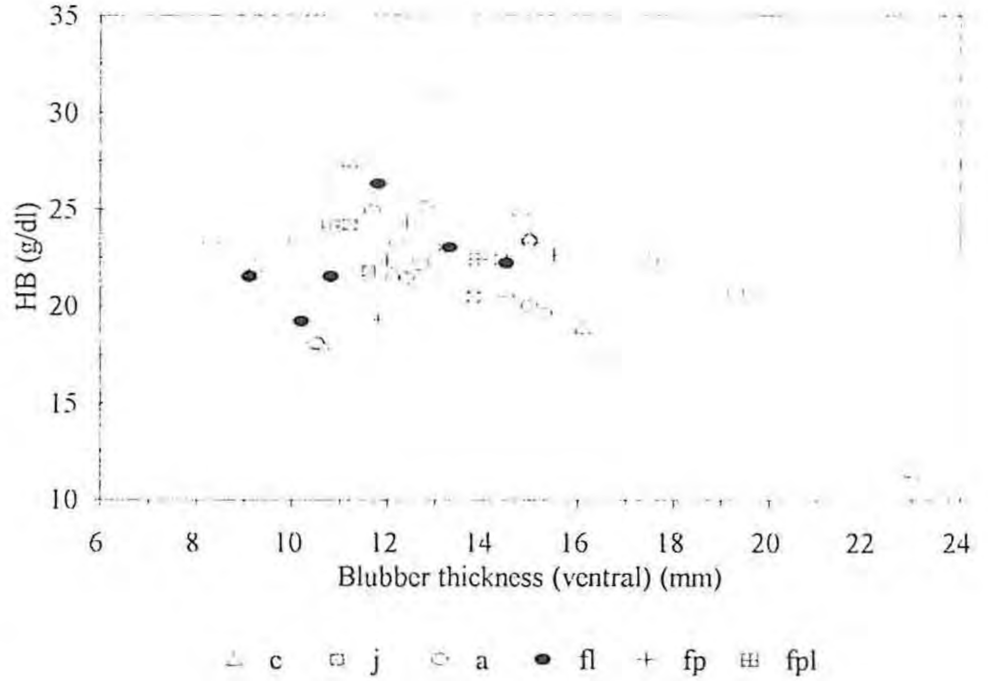


Figure 6.5: Significant correlations ($p<0.05$) between haemoglobin (HB) concentration and axillary girth (A) and ventral blubber thickness (B) for common dolphins caught in shark nets between 1992 and 1996 (c: calves, j: juveniles, a: adults, fl: lactating females, fp: pregnant females, fpl: simultaneously pregnant and lactating females).

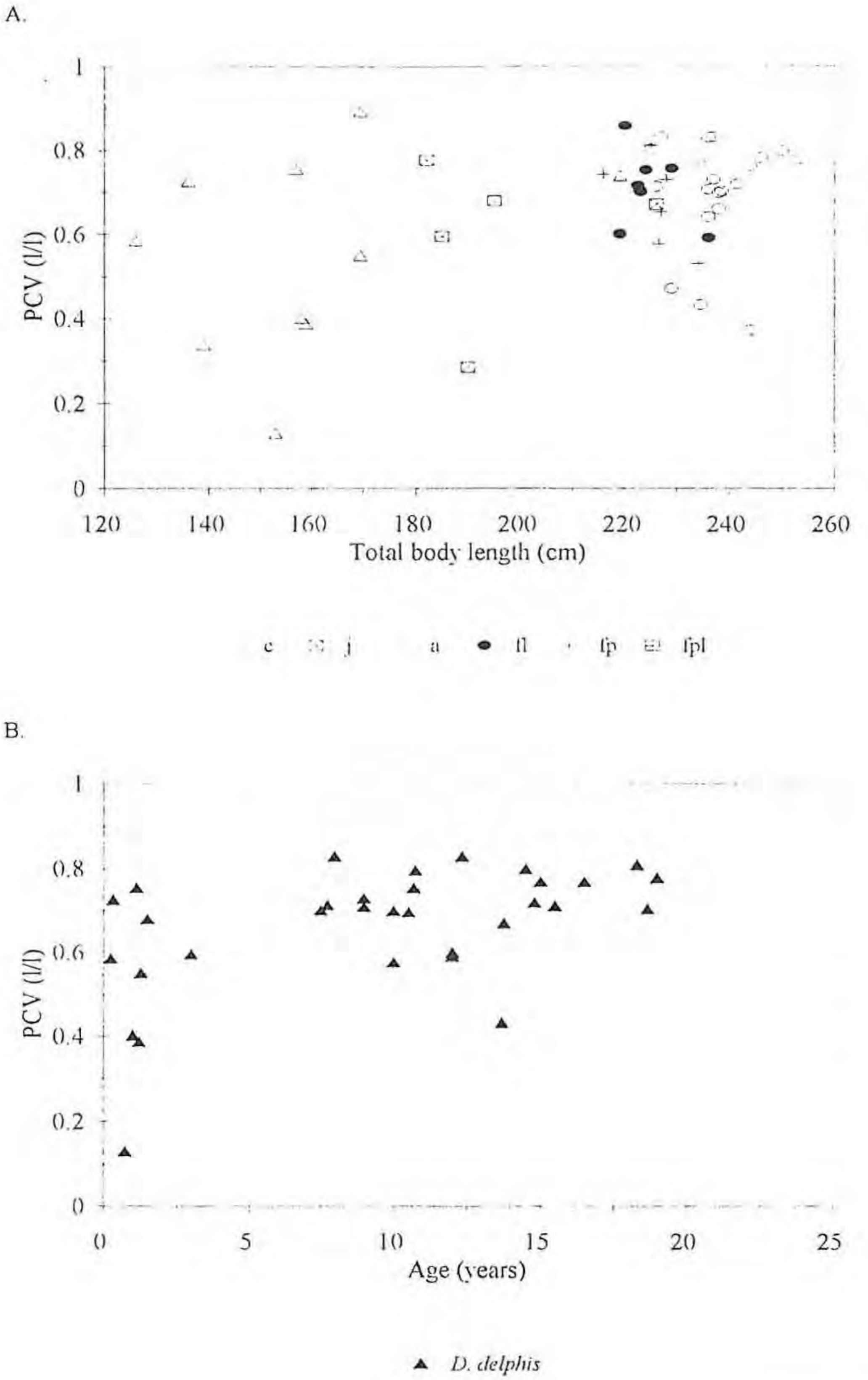


Figure 6.6: Significant correlations ($p<0.05$) between packed cell volume (PCV) and total body length (A) and age (B) for common dolphins (D. delphis) caught in shark nets between 1992 and 1996 (c. calves, j. juveniles, a. adults, fl. lactating females, fp. pregnant females, fpl. simultaneously pregnant and lactating females).

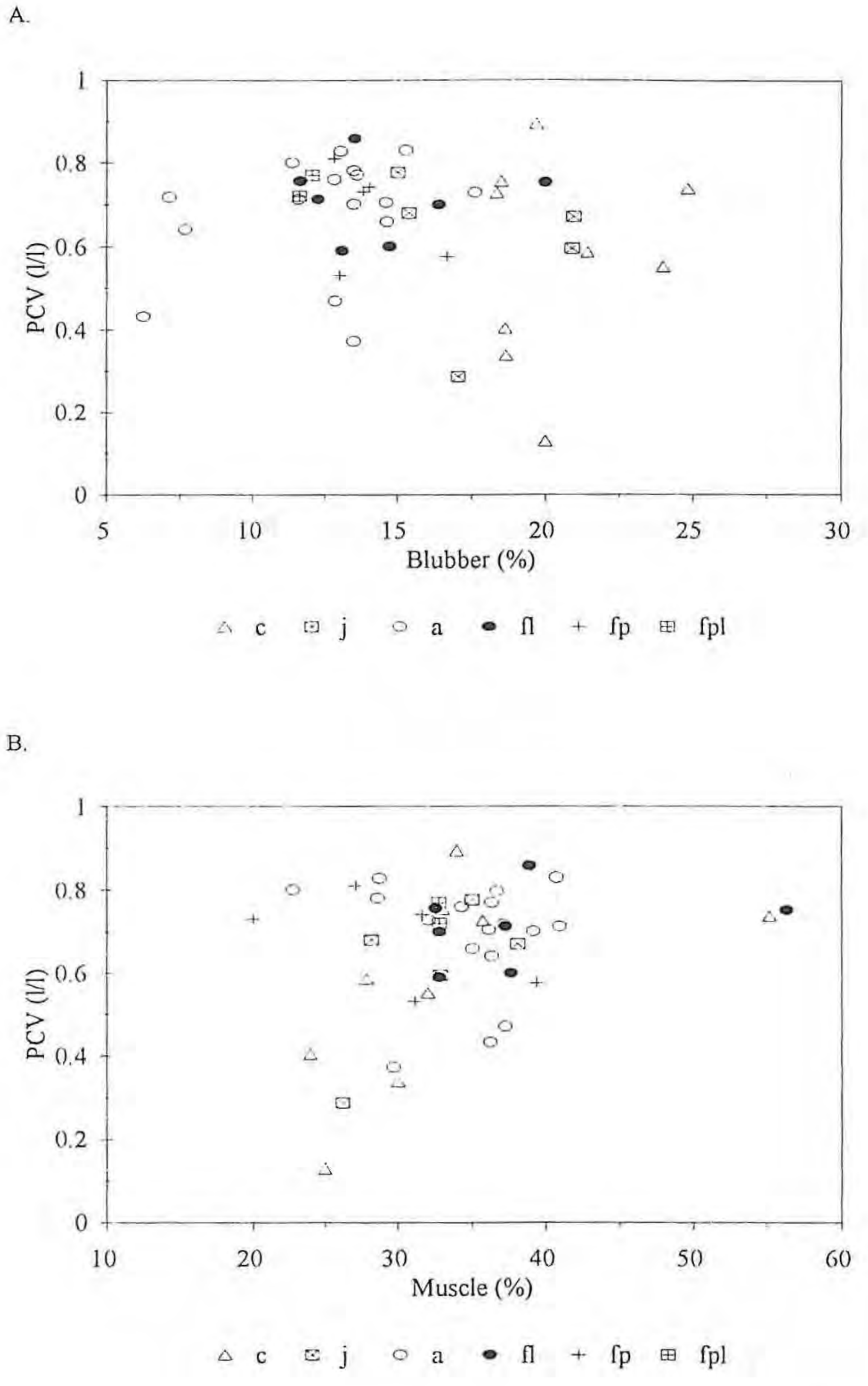


Figure 6.7: Significant correlations ($p<0.05$) between packed cell volume (PCV) and proportional blubber (A) and muscle weight (B) for common dolphins caught in shark nets between 1992 and 1996 (c. calves, j. juveniles, a. adults, fl. lactating females, fp. pregnant females, fpl. simultaneously pregnant and lactating females).

when %M was increased (Fig. 6.7B).

Although combined PCV data from both dolphin species were positively correlated with the mean energy content of prey (r=0.28; p=0.04; n=52), data from each species and age group did not reflect any significant changes in PCV with this dietary category (Fig. 6.8). Common dolphin PCV was relatively stable over a wide range of energy intake levels (Fig. 6.8B & C). The range of energy intake for bottlenose dolphins was more restricted than that of common dolphins, but no significant trends could be identified for this species (Fig. 6.8A). In addition, PCV data from both species were negatively correlated with water temperature on the day of capture (r=-0.25; p=0.02; n=79). However, this relationship reflected the species specificity of PCV data and the distinct temperature ranges in which the different species were caught, as described for RBC. This relationship was almost identical to that described for RBC (see Fig. 6.6).

D. Mean cell volume (MCV)

a. Common and bottlenose dolphins (pooled data)

Measures of MCV differed significantly between species, with bottlenose dolphins having a significantly higher mean MCV value (121.98 ± 17.96 fl) than common dolphins (108.00 ± 15.52 fl) (t=-3.07, p=0.003, df=57) (Table 6.1). Five MCV values below 88.00 fl were considered extreme (box-whisker plot). These originated from four common dolphins (three calves, one pregnant female) and one adult male bottlenose dolphin. The removal of these outliers served to increase the mean MCV values of both species, but they remained significantly different (Table 6.2).

Although the different age groups of common dolphins initially had significantly different mean MCV values (Table 6.1), these differences were eliminated after the exclusion of outliers (Table 6.2). However, MCV data for both species were positively correlated with total body length (r=0.26; p=0.04; n=65). Besides male (111.65 ± 6.15 fl) and female (135.50 ± 9.33 fl) bottlenose dolphin calves having significantly different MCV's (t=-3.19, p=0.03, df=4), this parameter was not significantly related to the sex or reproductive condition of animals.

b. Common dolphins

Common dolphin MCV was positively correlated with age (r=0.54, p=0.005, n=25). As described for other haematological parameters, this relationship was due primarily to increased variability in MCV values from calves. Common dolphin MCV was similarly positively related with girth and total blubber and muscle weights ($p<0.05$); and negatively correlated with ventral blubber thickness (r=-0.39, p=0.03, n=32). This relationship was due primarily to the declining MCV values associated with increased blubber thickness in calves (Fig. 6.9). However, within the calf age

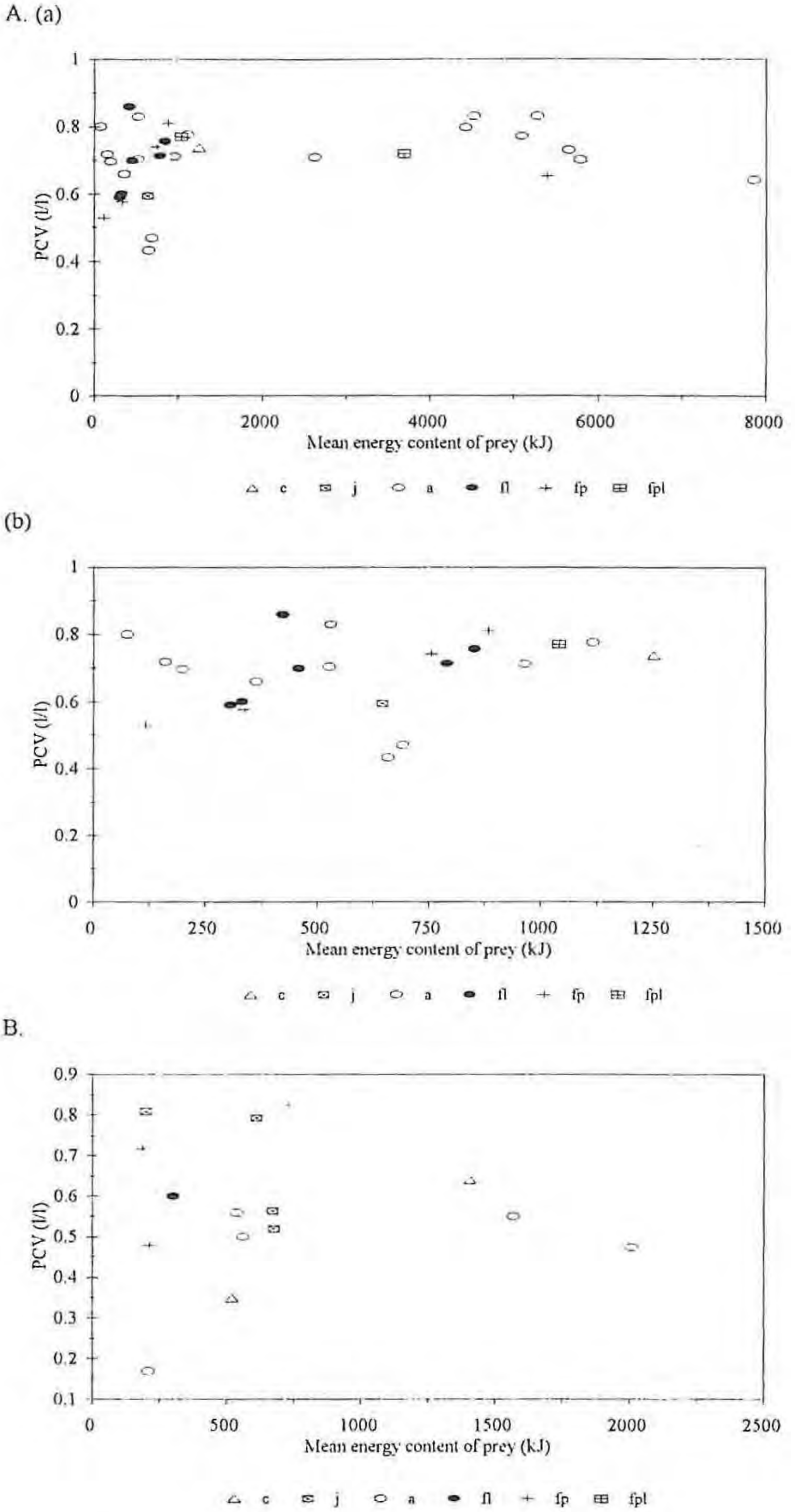


Figure 6.8: Significant correlations ($p<0.05$) between packed cell volume (PCV) and the mean energy content of prey in the stomachs of common (A.a&b) and bottlenose (B) dolphins caught in shark nets between 1992 and 1996 (c. calves, j. juveniles, a. adults, fl. lactating females, fp. pregnant) females, fpl. simultaneously pregnant and lactating females).

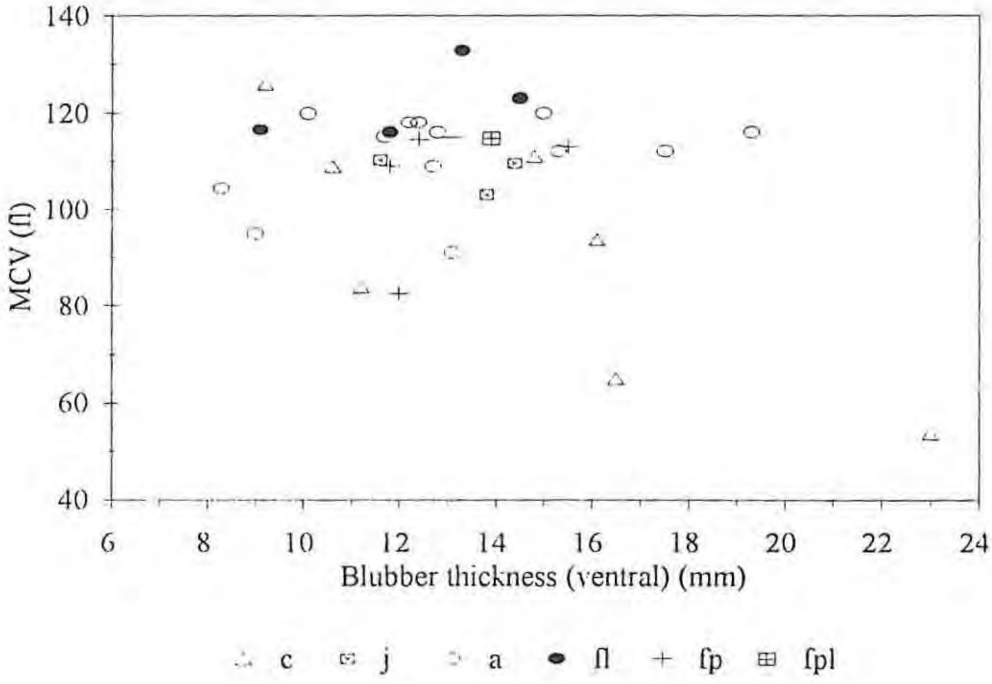


Figure 6.9: Significant correlation ($p<0.05$) between ventral blubber thickness and mean cell volume (MCV) for common dolphins caught in shark nets between 1992 and 1996 (c: calves, j: juveniles, a: adults, fl: lactating females, fp: pregnant females, fpl: simultaneously pregnant and lactating females).

group, MCV values appeared to decline with increased blubber thickness. All other age and reproductive classes maintained relatively stable MCV's at blubber thicknesses between 8 mm and 24 mm (Fig. 6.9).

MCV was not related to dietary parameters for either dolphin species. However, common dolphin MCV was negatively correlated with the mean local water temperature of the month prior to capture (r=-0.32, p=0.05, n=38) (Fig. 6.10A). Although total numbers of animals in each age class were limited, the relationship reflected a decline in MCV with increased temperature, particularly for calves, adults and lactating females (Fig. 6.10B). Interestingly, reduced MCV values from common dolphins were measured only in waters warmer than 19.5°C, whereas low MCV values from bottlenose dolphins were only recorded from animals when water temperature during the previous month averaged below 20°C (Fig. 6.10A).

E. Mean cell haemoglobin (MCH)

a. Common and bottlenose dolphins (pooled data)

Initial analyses showed that mean MCH values were not significantly different between species (t=-0.98, p=0.33, df=57) (Table 6.1). However, levels below 29.00 pg and above 50.00 pg were considered extreme (box-whisker plot) and the removal of these outliers resulted in bottlenose dolphins having a significantly higher mean MCH levels than common dolphins (Table 6.2).

Mean cell haemoglobin was not significantly influenced by different levels of food intake or dietary composition. Combined MCH data from both dolphin species were positively correlated with the water temperature on the day of capture (r=0.25, p=0.05, n=64), however, this correlation was biased by outliers (Fig. 6.11A). Species-specific relationships between MCH and water temperature were not significant for common or bottlenose dolphins. Generally though, common dolphin MCH increased slightly with increased water temperature, especially for adults and calves (Fig. 6.11B). The restricted temperature range made this trend difficult to elucidate. MCH values of bottlenose dolphins were more stable over a wider range of water temperatures (Fig. 6.11C).

b. Common dolphins

Lactating common dolphins had MCH levels that were higher (47.04 ± 21.68 pg), and more variable, than levels from other females of the same species (33.40 - 35.60 pg). There was only one resting adult female common dolphin, which had a MCH level of 50.00 pg, but this was considered extraordinary (box-whisker plot). In this respect, reproductive-related changes were difficult to assess.

c. Bottlenose dolphins

MCH values for bottlenose dolphins were positively correlated with total body length (r=0.47,

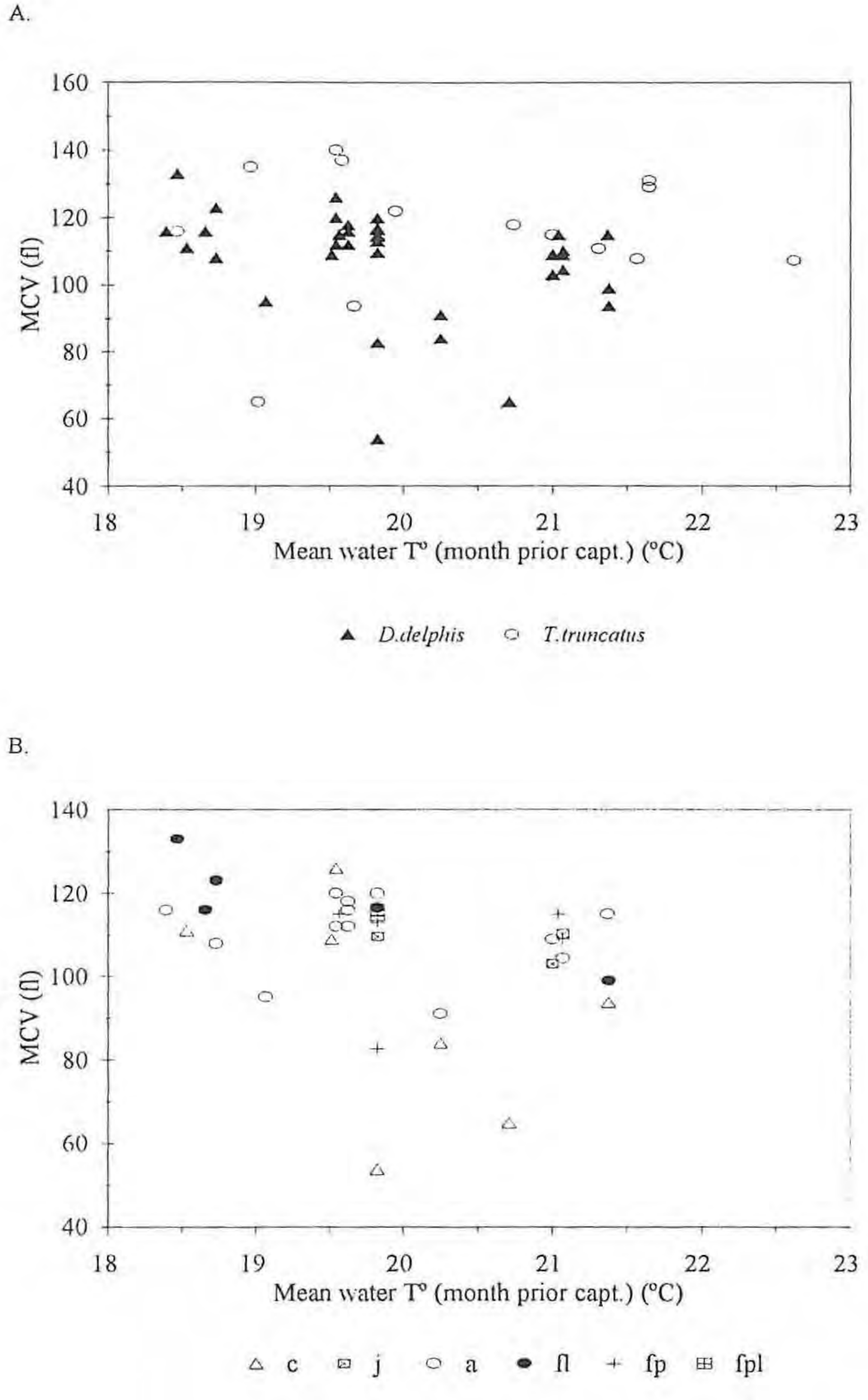


Figure 6.10: Significant correlation ($p<0.05$) between mean cell volume (MCV) and the mean water temperature of the month prior to capture for all common dolphins (A) and the different age and reproductive classes of common dolphins (B) (c: calves, j: juveniles, a: adults, fl: lactating females, fp: pregnant females, fpl: simultaneously pregnant and lactating females).

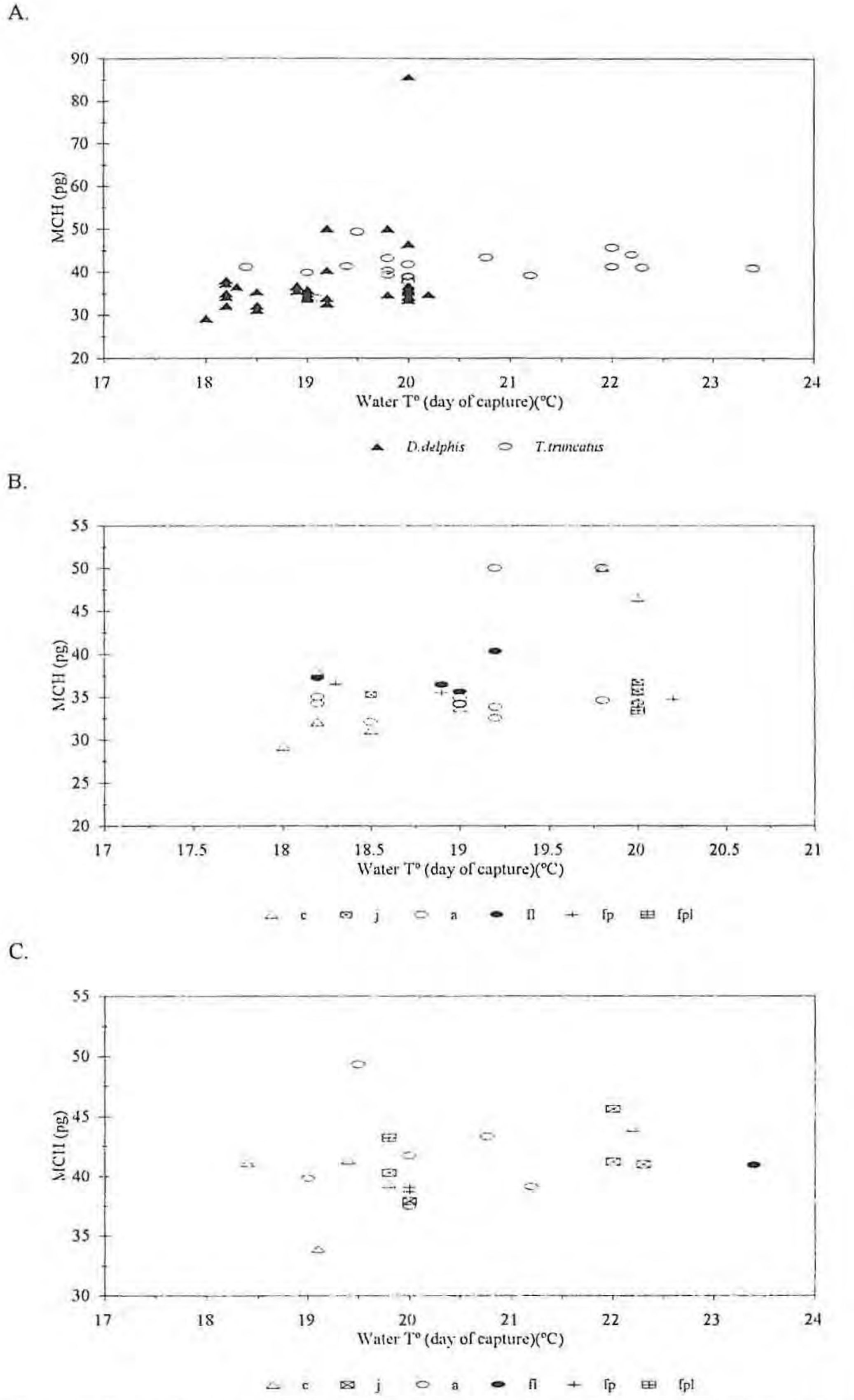


Figure 6.11: Significant correlation ($p<0.05$) between mean cell haemoglobin (MCH) and the water temperature on the day of capture for common (D. delphis) and bottlenose (T.truncatus) dolphins (A); and the relationship for the different age and reproductive classes of common (B) and bottlenose (C) dolphins caught in shark nets between 1992 and 1996 (c: calves, j: juveniles, a: adults, fl: lactating females fp. pregnant females, fpl. simultaneously pregnant and lactating females).

p=0.03, n=21) and axillary girth (r=0.50, p=0.04, n=17). However, all animals generally maintained a stable MCH (~30-45 pg) between lengths of 120 cm and 260 cm and associated girths, and a positive trend was difficult to confirm.

F. Mean cell haemoglobin concentration (MCHC)

a. Common and bottlenose dolphins (pooled data)

Mean cell haemoglobin concentrations were not significantly different between species (t=1.05, p=0.30, df=56) (Table 6.1). Measurements of MCHC below 27.00 g/dl, and above 42.00 g/dl, were classified as outliers (Box-whisker plot). The removal of outlying MCHC values resulted in a change in the mean MCHC values of both common and bottlenose dolphins but they remained similar (Table 6.2). In addition, there were no significant sex-, age- or reproductive-related changes in this parameter.

Combined MCHC data, from both species, were negatively related to total body length (r=-0.37, p=0.002, n=64) and weight (r=-0.30, p=0.02, n=64) (Fig. 6.12). These relationships were similar to those described for other haematological parameters, with smaller, younger animals having more variable MCHC values. In this respect, the majority of outliers (described above) were associated with younger smaller animals. For older, larger animals the range of MCHC values was very restricted (29.65-35.40 g/dl) (Fig. 6.12). Similar significant correlations (p<0.05) between MCHC and girth paralleled the relationships described for total body length and weight.

Changes in blubber thickness and/or blubber and muscle weight did not appear to have a significant effect on MCHC, although these parameters were significantly correlated (p<0.05). Generally, MCHC was remarkably stable over a wide range of blubber thicknesses and blubber and muscle weights.

b. Bottlenose dolphins

Bottlenose dolphin MCHC values were positively correlated with the total number of prey species in the stomach (r=0.57, p=0.03, n=15), but this was a reflection of increased variability in calves, for which dietary diversity was low. MCHC values from this species were also positively correlated with the water temperature on the day of capture (r=0.64, p=0.003, n=19), the mean local water temperature for the month of capture (r=0.79, p=0.001, n=18) and the mean regional water temperature of the month prior to capture (r=0.69, p=0.002, n=18). Although differences were slight, MCHC values appeared to be slightly elevated at water temperatures above 20°C (Fig. 6.13).

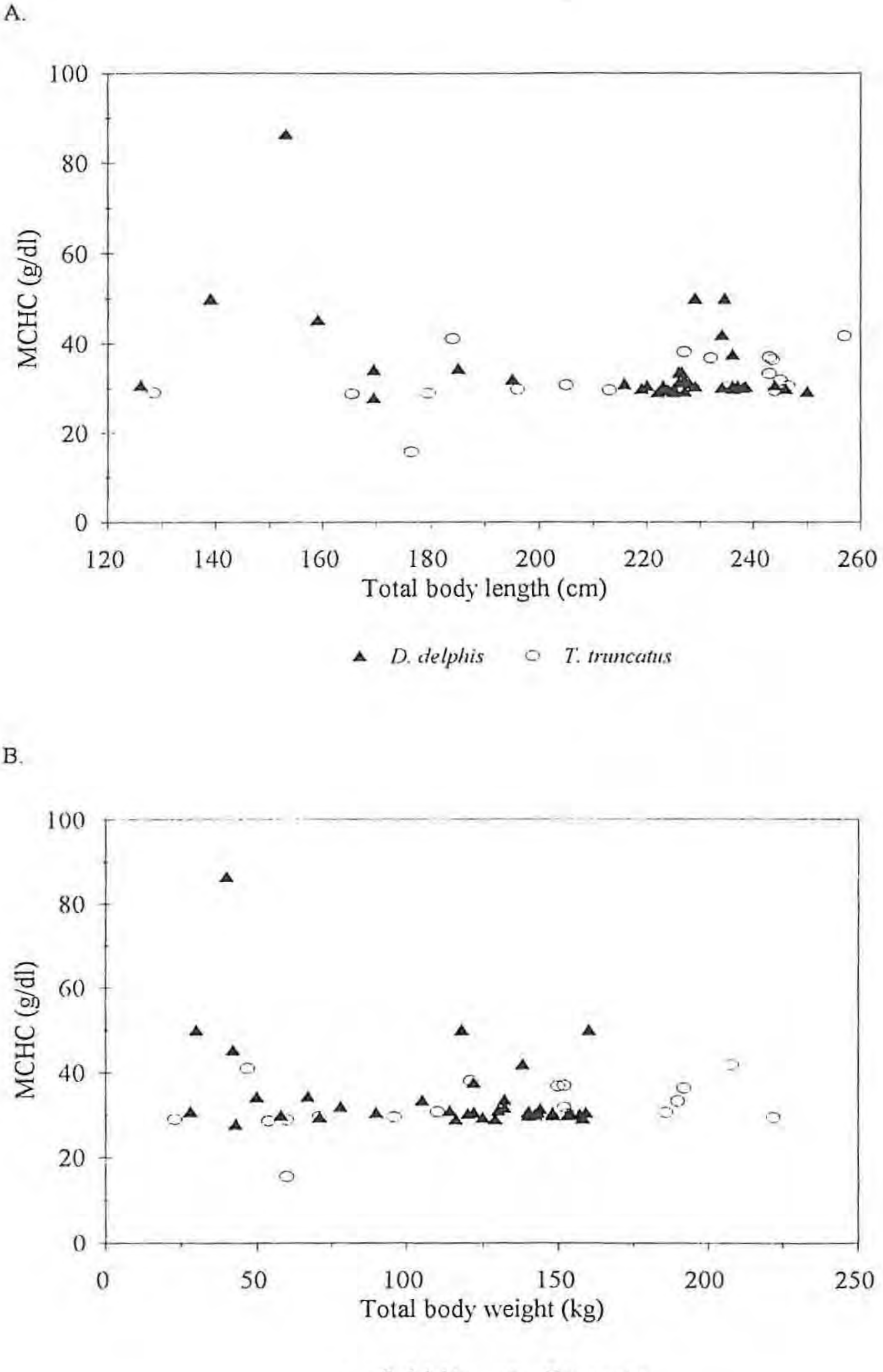
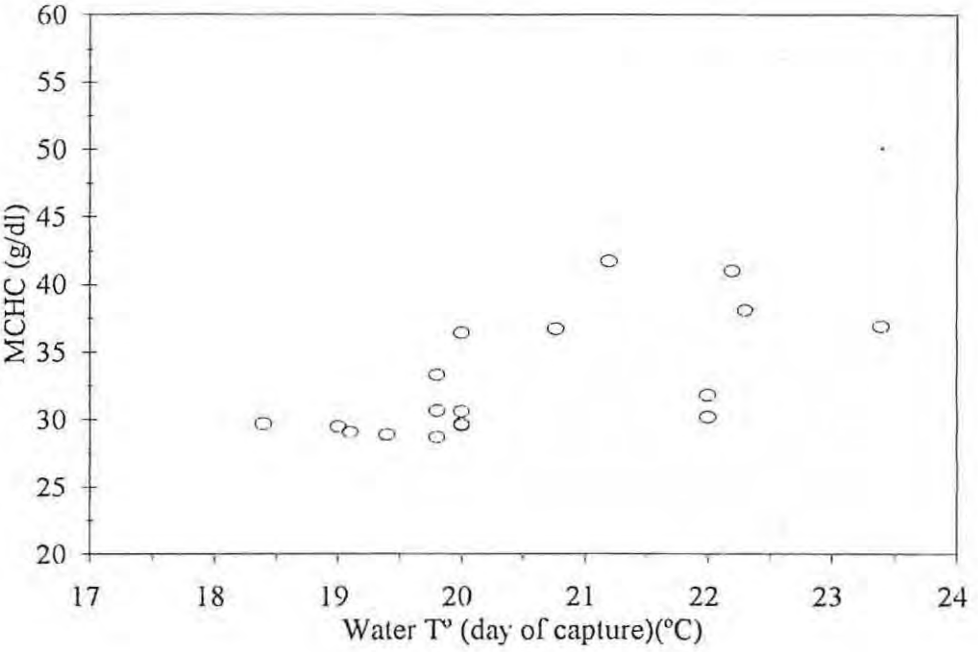


Figure 6.12: Significant correlations ($p<0.05$) between mean cell haemoglobin concentration (MCHC) and total body length (A) and total body weight (B) for common (D. delphis) and bottlenose (T. truncatus) dolphins caught in shark nets between 1992 and 1996.

A.



B.

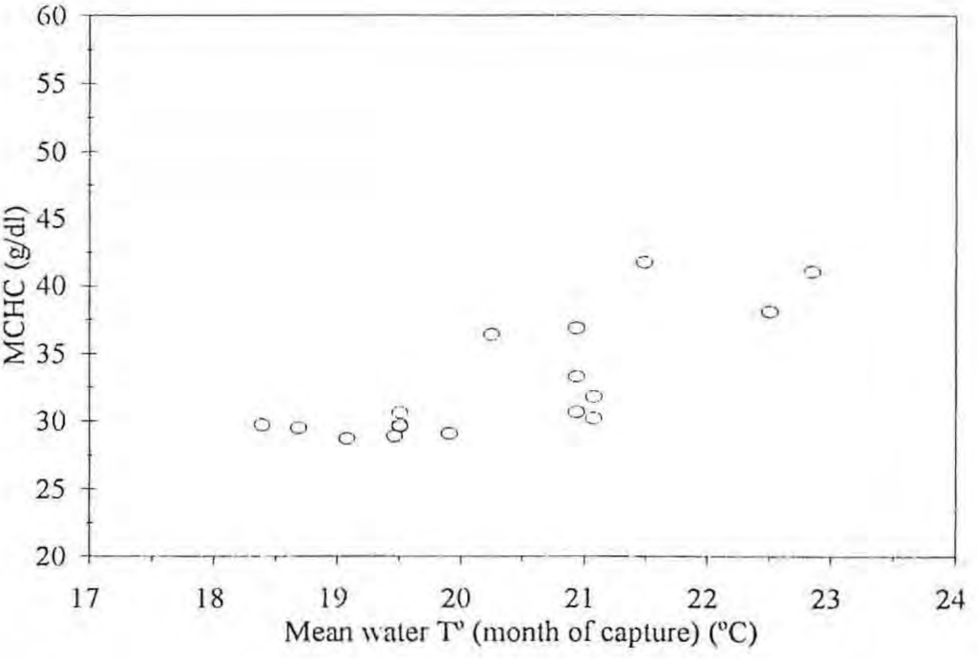


Figure 6.13: Significant correlations ($p<0.05$) between mean cell haemoglobin concentration (MCHC) and the water temperature on the day of capture (A) and the mean water temperature for the month of capture (B) for bottlenose dolphins caught in shark nets between 1992 and 1996.

G. Platelets

a. Common and bottlenose dolphins (pooled data)

Mean platelet counts for common and bottlenose dolphins were similar (t=-0.005, p=0.99, df=15) (Table 6.1). However, due to logistical problems, platelets were not counted for all animals, which resulted in a small sample size for this parameter. Consequently, the lack of significant species-, age-, sex- and/or reproductive-related differences should be treated with caution. One common dolphin calf had a significantly high platelet count (> 1 000 $\times 10^9$/l) (Box-whisker plot). Platelet counts in other age groups were relatively stable (see below). The removal of this outlier had a dramatic effect on the mean platelet count for common dolphins (Table 6.2).

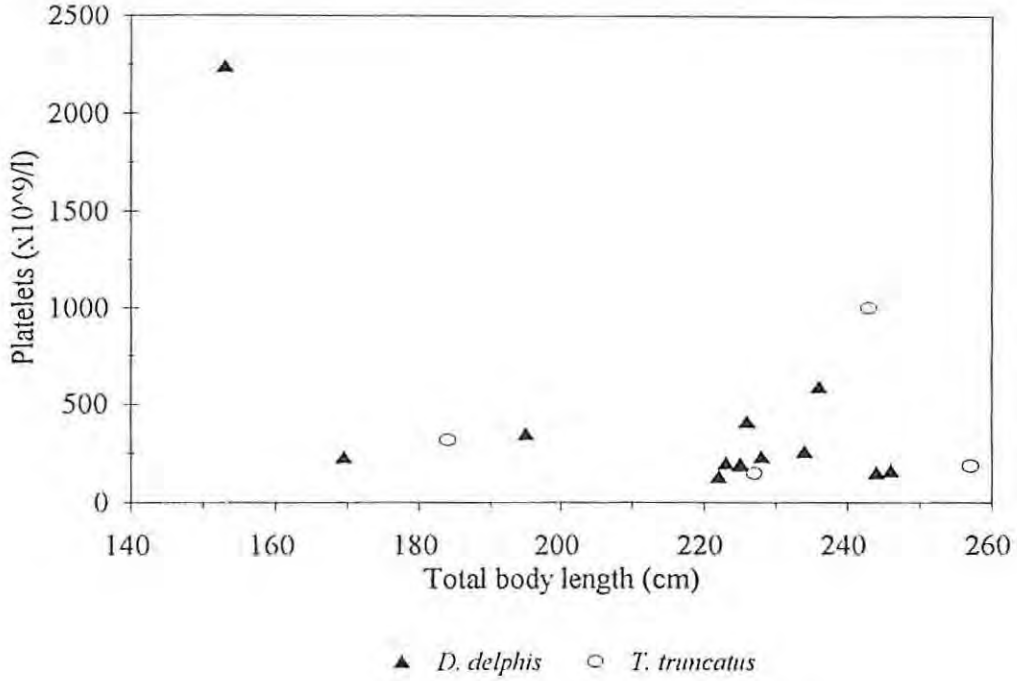
Negative correlations between platelet count and total body length (r=-0.58, p=0.005, n=22), weight (r=-0.52, p=0.01, n=22) and axillary girth (r=-0.61, p=0.05, n=11) suggested that platelet count declined with growth in both dolphin species (Fig. 6.14). However, without additional data, this was difficult to clarify. Limited data made other relationships between platelet counts and independent variables similarly difficult to confirm. Additional blood samples, from animals in all age groups, are needed to investigate any potential relationships between platelet count, diet and temperature.

DISCUSSION

Assessments of physiological status in net-caught animals were complicated by possible *post-mortem* changes which take place in the blood (Young, unpublished data), as well as stress-associated changes that take place during capture (Young *et al.* 1998). In all cases, an attempt has been made to take these effects into account during interpretation of results obtained during the current study. However, further investigations are needed to fully understand these effects. The causes of dolphin captures in the Natal shark nets have been extensively examined (Cockcroft and Ross 1991, Cockcroft 1992, 1994). However, the exact time at which dolphins are caught in the shark nets and therefore the length of time they have been dead before retrieval from the nets is unclear. It has been reported, by Natal Sharks Board staff, that the majority of bottlenose dolphins are entangled during early morning feeding, which implies that incidentally caught specimens of this species died between two and four hours before retrieval and blood sampling. It is unknown at what time common dolphins are caught.

Mean WBC counts of bottlenose and common dolphins during this study were higher than previous reports (Appendix 4), indicating that dolphins along the Natal coast may be exposed to a greater immunological challenge. Marine pollution levels in this region are reportedly high (Cockcroft *et al.* 1989) and this may be the cause of their elevated WBC counts. In this respect, the markedly

A.



B.

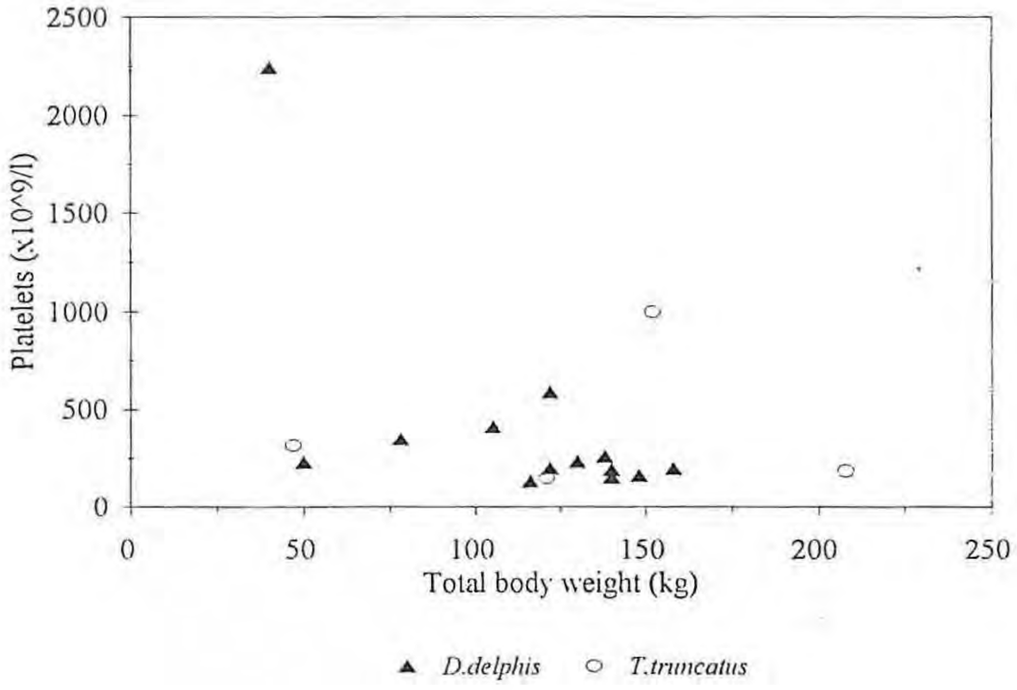


Figure 6.14: Significant correlations ($p<0.05$) between platelet count and total body length (A) and total body weight (B) for common (D. delphis) and bottlenose (T. truncatus) dolphins caught in shark nets between 1992 and 1996.

higher WBC counts of humpback dolphins, relative to the other two species, may be attributed to their primary habitat on the Kwazulu-Natal coast - the industrial harbour of Richards Bay, with which notable marine pollution is associated (Cockcroft *et al.* 1989).

Preliminary investigations show that WBC counts may be elevated in the first three hours *post-mortem* (Young, unpublished data) but show an overall decrease over 24 hours. Leukocytosis has been linked with different types of stress (Lee *et al.* 1977, Medway and Geraci 1978, Medway and Cornell 1989, Bossart and Dierauf 1990, Young *et al.* 1998) and it is likely that WBC's were increased by the stress associated with net capture and drowning. In this respect, a proportion of the increase in WBC counts may be more indicative of stress-associated physiological changes, rather than increased exposure to disease, infection and/or pollution. This reduces the effectiveness of WBC count as an indicator of condition, although WBC counts remain a valuable indicator of immunological status. Increased WBC count (in humpback dolphins) associated with a polluted habitat suggests that this parameter may be useful as a biomonitor of environmental condition.

Reports of the exact dynamics of WBC changes with age in different marine mammal species are conflicting (Engelhardt 1979, McConnell and Vaughan 1983, Cornell 1983, Asper *et al.* 1990, Thurman and van der Elst 1995, Neilsen 1995). Harbour seals (*Phoca vitulina*) are one species for which WBC counts have been found to peak in juvenile animals (nine months old) and then decline with age. The reasons for an elevated WBC count in juvenile net-caught dolphins are unclear. Juvenile animals may be more susceptible to WBC-increasing factors, which include pollution, parasitism, infection, diseases, etc. The fact that both extremely high WBC counts were associated with juvenile animals would appear to support this hypothesis. *Post-mortems* of the animals in question revealed no clear reason for these elevated counts. It is possible that these animals were suffering from infections or illness that were not identified through dissection alone. Both animals were on the lower limit of the weight range for the juvenile age group and the reduced weight may be a morphological manifestation of illness and/or infection. The relationship between fluctuating WBC count, weight and diet has been recorded for Baikal (*Pusa sibrica*) (Ronald and Kay 1982) and harbour (Kuiken 1985) seals and terrestrial mammals (Chandra *et al.* 1982 in Lochmiller *et al.* 1988). Reasons for the reduced WBC counts in pregnant and lactating animals are unknown. They contrast with the results of Thurman and van der Elst (1995) which report an increased WBC count during pregnancy. More data are needed to examine this relationship further.

The increased proportion of lymphocytes in common dolphins may be attributed to the pelagic nature of the species and their higher degree of visible parasite infestation. Bottlenose and humpback dolphins, both inhabiting similar coastal regions, and presumably exposed to a similar level of parasitism, have similar mean lymphocyte counts. Lymphocytosis has previously been associated with infection and disease (Penington *et al.* 1978, Thurman and van der Elst 1995), but a direct link between lymphocytes and parasitism has not been documented. Lymphocyte counts could not definitely be related to morphological condition during this study. Additionally, there was no apparent relationship between lymphocytes and diet. Elevated lymphocyte counts associated with chronic infection, may indicate a potential "health" problem and possibly lead to a decline in blubber quality, weight and other aspects of morphological condition but as discussed for WBC counts, lymphocytes better reflect immunological status and are not good indicators of physiological, morphological and/or nutritional condition.

Haematological parameters are subject to changes based on the emotional and physical state of the animal (Lane *et al.* 1972, De Monte and Pilleri 1988, Thurman and van der Elst 1995). Problems such as these must be kept in mind when assessing the haematological parameters of net-caught animals. Although *post-mortem* changes in haematological parameters do occur (Young, unpublished data), they did not appear to have a significant effect on the results obtained during this study. The mean values for RBC and HB obtained for bottlenose dolphins during this study were slightly higher than the majority of mean values reported in the literature, but within the overall range (Appendix 4). Mean PCV and MCV values for this species were generally higher than those reported previously (Appendix 4). Common dolphin RBC and PCV were higher than those in the literature were, but HB levels were similar (Appendix 4). Although a number of haematological values recorded in the literature originate from captive animals, results showed that mean haematological values of net-caught common and bottlenose dolphins were also higher than those reported for free-ranging populations (Appendix 4). This may be attributed to the stressful nature of net capture (Young *et al.* 1998). Stress-related haematological changes have been documented in moulting seals and acclimitising captive animals (Ronald and Kay 1982) and have been attributed to an increased demand for oxygen.

The significant species differences in haematological parameters were all attributable to the different habitats, swimming speeds and diving capabilities of the different dolphin species. Common dolphins had significantly higher RBC counts, HB concentrations and PCV values, reflecting the deeper water in which they typically feed, the faster speeds at which they swim and the colder water temperatures they are usually exposed to. The small size of the common dolphin RBC, in conjunction with a higher PCV allows for more efficient oxygen transport in these animals (Geraci 1971, De Monte and Pilleri 1988). The relationship between haematological parameters and diving capabilities and/or habitat of marine mammals is well documented (Andersen 1966, Horvath *et al.* 1968, Lenfant 1969, Bryden and Lim 1969, Ridgway *et al.* 1970, 1984, Lane *et al.* 1972, Korzhuev and Gazlova 1973, Medway and Geraci 1978, De Monte and Pilleri 1983, 1988, Ridgway 1986, Bossart and Dierauf 1990).

Significantly higher RBC counts and HB levels in adult male common dolphins and potentially elevated PCV and MCV values in adults of both sexes corresponded with the change in haematological parameters with age that has been reported for terrestrial (Lochmiller *et al.* 1985c, Rietkerk *et al.* 1994) and marine mammals (Bryden and Lim 1969, Geraci 1971, Lane *et al.* 1972, Geraci and Smith 1975, Engelhardt 1977, McConnell and Vaughan 1983, Roletto and Dougherty 1983 in Bossart and Dierauf 1990, Kuiken 1985, Bossart and Dierauf 1990). This age-associated adjustment has been attributed to the gain in weight and increased ability to dive with growth. The fact that this difference was so apparent in male common dolphins may reflect that male dolphins of this species were relatively more active and dived more often and to greater depths than their female counterparts. The highly variable RBC counts, PCV's and MCV's measured in younger, smaller animals reflected the initial exposure to diving and the physiological adjustments needed for this activity (Bryden and Lim 1969, Geraci 1971, Bossart and Dierauf 1990). Low values for these parameters were measured only in calves. This physiological anemia, described previously in grey seals (*Halichoerus grypus*) is apparently normal at a young age (Medway and Cornell 1989).

A number of morphological parameters were related to haematological indicators of physiological status. Increased ventral blubber thickness in common dolphin calves was associated with a reduction in HB levels and MCV. One of the calves, from which a significantly low HB level and MCV was measured, had unusually thick blubber for an animal of this age group. The overall changes in RBC, HB and PCV in relation to the proportional weight of blubber and muscle were almost certainly related to age and the different adjustments to locomotion, diving and thermoregulation. However, the variation of certain haematological parameters, within an age group, with blubber thickness is interesting. Relative reductions in HB and MCV with increased blubber thickness in common dolphin calves may be related to changing thermoregulatory demands and oxygen transport. Within age groups, RBC counts were generally slightly higher when proportional blubber weights were reduced. This may indicate an increase in activity (Ridgway *et al.* 1984, Wells 1993) to meet thermoregulatory demands, when blubber proportion is reduced. Similarly, increased PCV with increased muscle proportion in calves and juveniles may be attributed to increased activity and locomotory capabilities. In this context, the relationship between haematological parameters and morphological condition is potentially useful when attempting to use aspects of morphology to gain an insight into physiological status and visa versa. The value of these types of relationships in condition assessment is emphasised by the established links between increased fat stores, high RBC counts and good condition and/or health in terrestrial mammals (Anderson *et al.* 1970, Cameron and Luick 1972 in Hellgren *et al.* 1993, Franzmann 1972, Hellgren *et al.* 1993, Gallivan *et al.* 1995).

Alterations in stored body fat result in changes in total body water, which, in turn, has an effect on plasma volume (Hellgren *et al.* 1993). These changes in total body water may also account for changes in PCV with fluctuating blubber proportions. In this context, it is possible that the relationships between haematological parameters, blubber thickness and proportion in marine mammals was based on seasonal changes in diet, activity levels and water temperatures, but without further samples, this relationship is difficult to explain.

Generally, haematological variables did not appear to be dramatically affected by water temperature, in terms of the temperature categories used in this study. However, the greater frequency of low RBC counts, PCV's and MCV's measured for common dolphins in water temperatures of approximately 20°C, combined with the fact that this species was hardly ever caught in waters warmer than this, is suggestive of a thermo-barrier above which common dolphins may be physiologically compromised. The thermoneutral zone of bottlenose dolphins is reportedly 20°C (Ross and Cockcroft 1990, Cockcroft 1991) and the energy requirements for maintenance of body temperature by this species is thought to increase and decrease in water temperatures below and above 22°C, respectively (Cockcroft 1991, see Chapter 5). Bottlenose dolphin HB levels were more variable in temperatures below 20°C, indicating a change in haematological status at water temperatures below the thermoneutral zone. Although the thermoneutral zone of common dolphin is unknown, it is likely to be lower than that of bottlenose dolphins, due to their pelagic habitat. Based on the results of this study, common dolphins may be physiologically stressed in water temperatures of approximately 20°C, reflected in reduced haematological parameters. This would support the idea that these animals may be susceptible to overheating along certain regions of the coast, as discussed previously in Chapter 5. Variable data from younger, smaller animals implied that adult animals are able to respond physiologically to thermoregulatory changes more effectively than younger, smaller animals. These trends were not evident for bottlenose dolphins, which suggested that the physiology of the pelagic common dolphins (calves in particular) experiences more significant changes during exposure to coastal water temperatures. MCH values also reflected that common dolphin haematology was more unstable in warmer waters, whereas that of bottlenose dolphins was more stable in the range of water temperatures typically associated with the Kwazulu-Natal coast.

Food intake and dietary composition did not appear to have a marked affect on haematological values in net caught animals, although further investigations are warranted. The effect of diet on marine mammal haematology is presently not known although some attempt has been made to investigate the relationship between vitamin deficiencies and diet quality in captive dolphins (McConnell and Vaughan 1983). Dietary restriction has led to a reduction in several haematological values in terrestrial mammals (Seal *et al.* 1975, 1978a, 1978b, Lochmiller *et al.* 1985b, 1985c, Henke and Demarais 1990, Wolkers *et al.* 1994a). Changes in the quality (i. e. protein and/or energy content) of terrestrial mammal diets have yielded contrasting results, reflecting the complexity of the relationship between diet and haematology (Harlow and Seal 1981 in Knick *et al.* 1993, Melton and Melton 1982, Warren *et al.* 1982, DelGuidice *et al.* 1987b, Henke and Demarais 1990, Hellgren *et al.* 1993, Knick *et al.* 1993, Wolkers *et al.* 1994a). However, many of these studies were extended investigations into long-term dietary changes on haematology. It is unlikely that similar changes would be reflected in net-caught dolphins, as diet was assessed using stomach contents, indicative only of food intake for the 24 hours prior to capture (Ross 1984). Investigations over a longer period of time are needed to validate the relationship between haematology and diet in marine mammals.

However, blood results from animals that were emaciated, therefore showing signs of longer-term dietary changes, depicted changes in haematological values, similar to those described in terrestrial animals. Deviant haematological values were associated with calves (with only milk in their stomachs); adults with stomach ulcers and a severely emaciated and parasitised humpback dolphin with evidence of pneumonia in the lungs. Most animals with deviant haematological values showed no signs of recent feeding. A similar relationship between haematology and poor nutrition, gastric ulcers and intestinal malabsorption has been reported for other marine mammals (Bossart and Dierauf 1990). Additionally, extensive parasitism in three calves may have been the cause of significantly low haematological parameters. Anemia, as a result of parasitism, has been described in terrestrial mammals (Gallivan *et al.* 1995) and fish (Rand and Cone 1990). These results highlighted the potential use of haematological values in the assessment of nutritional condition. However, the time periods for which these nutritional condition indices are most effective need to be validated.

The parameters of MCH and MCHC remained relatively stable in a wide range of animals of different ages and sizes. This, together with the fact that there were limited relationships between MCH and MCHC and morphology, diet and/or temperature, reduced their usefulness as condition indices. However, it must be noted that the majority of outlying values identified for these particular parameters were associated with young animals and they may be reflective of age-related changes, rather than abnormal values associated with illness, infection, etc. Further investigation of this possibility is needed before confirmation of trends resulting from this study.

Summary

Body and nutritional condition of net-caught dolphins were difficult to assess in terms of leukocytic and haematological parameters. Stress- and *post-mortem* associated changes reduced the usefulness of some haematological parameters as indicators of condition. In addition, several of the parameters reflected highly variable data, which obscured any potential trends. However, the relationships between certain morphological parameters, leukocytic and haematological values and dietary and environmental variables presented some interesting relationships that have potential as indicators of condition and habitat quality.

In light of the fact that WBC counts were apparently not affected by age and/or morphology, this parameter directly reflects the immunological status of an animal and, although not necessarily linked to overall condition, this parameter served to highlight certain changes in health which may lead to a deterioration in condition. Counts of WBC's also show potential as an indicator of habitat quality, in terms of parasite load and/or pollution.

Haematological values largely reflected the activity levels of animals; and this, in conjunction with *post-mortem* changes, limits the use of these parameters as condition indices in net-caught dolphins. However, the results of this study show that important interactions between RBC, HB, PCV, MCV and dietary, morphological and environmental factors may prove valuable in the monitoring of dolphin condition in the marine habitat. Several haematological parameters were significantly correlated with various aspects of morphological condition. Although data were generally highly variable, the possibility of using morphological parameters such as weight, girth and blubber thickness to predict haematological status should be further investigated in terms of developing non-invasive indices of condition. The relationship between diet, morphology and temperature reinforced the potential usefulness of such an index. Relationships between haematology and water temperature suggested that both common and bottlenose dolphins have physiological and environmental "zones of comfort", in which morphological and physiological condition is optimised. The maintenance of this condition depends on interacting dietary and environmental variables, within a particular temperature range.

In conclusion, although haematology can be used to indicate condition in free-ranging dolphins, it is imperative to assess results in terms of diet, morphology, and temperature and activity levels, all of which have an important influence on haematological status. The establishment of additional relationships, and the defining of existing trends, may result in haematology being used to effectively identify changes taking place in the marine habitat. However, because haematology is an ideal index of an animal's immediate physiological activity and nutritional condition, it may be more difficult to use haematology to monitor long term changes in population and/or habitat status.

CHAPTER 7:

PHYSIOLOGICAL INDICATORS OF CONDITION IN NET-CAUGHT BOTTLENOSE (*Tursiops truncatus*) AND COMMON (*Delphinus delphis*) DOLPHINS:

II. BLOOD CHEMISTRY.

INTRODUCTION

The condition of animals can provide valuable information about the potential survival, reproductive success and "well-being" of a population (Kirkpatrick 1980). Body condition can reflect the nutritional state of animals, as well as the factors influencing animal-habitat interactions (Virgl and Messier 1993). Body, and nutritional, condition has traditionally been assessed and monitored using morphological factors such as weight, changes in body fat and growth dynamics (Eberhardt and Sniff 1977, Fowler and Sniff 1992, Krebs and Singleton 1993, Hanks 1981). Other analyses that have been used successfully to monitor animal condition include physiological parameters such as blood and urine. Analyses of haematological and blood chemistry components of blood have been reliably used in the assessment of physiological condition; and successfully implemented as indices of condition in terrestrial animals. Blood chemistry components, such as blood urea nitrogen (BUN), have been extensively used in the assessment of terrestrial animal nutritional condition and food resource quality (Franzmann 1972, Torrell *et al.* 1974, deCalesta *et al.* 1975, Kirkpatrick *et al.* 1975, Seal *et al.* 1975, 1978a, Melton and Melton 1982, Warren *et al.* 1982, Lochmiller *et al.* 1985d, 1986, DelGuidice *et al.* 1987b, Hellgren *et al.* 1993, Knick *et al.* 1993, Zaugg *et al.* 1993, Wolkers *et al.* 1994a). Serum chemistry enzymes have proved useful in investigations into both animal and habitat condition (Seal *et al.* 1978b).

Although morphological condition, and its relationship to population and habitat status, has been investigated using whaling specimens (Lockyer 1986, 1987) and stranded or incidentally-caught animals, marine mammal blood chemistry has not typically been used as a condition index. The earlier studies of marine mammal blood chemistry were carried out using captive animals, comparing marine mammal physiology to that of terrestrial mammals (Medway and Geraci 1965). As the number of dolphins in captivity increased, so did the need for base-line blood chemistry values, in order to monitor health and identify illness (Ridgway 1965, Andersen 1968, White *et al.* 1976, Medway and Geraci 1978, Engelhardt 1979, Irvine *et al.* 1980, Ronald and Kay 1982, Cornell

1983, Kuiken 1985, Asper *et al.* 1990, Clarke 1990, Fothergill *et al.* 1991, Thurman and van der Elst 1995).

Stranded and rehabilitated animals, in conjunction with specimens killed during whaling and other similar operations, have provided valuable information about marine mammal blood chemistry, and enabled a comparison between the physiology of captive and wild animals (Boice and Roberts 1966, Nelson 1970, De Monte and Pilleri 1972, 1977, Bogdanova and Lebedev 1973, Allen *et al.* 1976, Geraci *et al.* 1979, Irvine *et al.* 1980, Neilsen and Andersen 1982, McConnell and Vaughan 1983, Medway 1983, Medway and Cornell 1989, Kastelein *et al.* 1990, Montoya-Ospina 1994, Heidel *et al.* 1996). Only recently have physiological investigations of free-ranging marine mammals been successfully carried out (Medway *et al.* 1982, Asper *et al.* 1990, Rhinehart *et al.* 1991, 1992, Schumacher *et al.* 1992, Nordøy *et al.* 1993, Koopman *et al.* 1995). Although many of these studies recognised the important influence the environment has on marine mammal physiology, studies of potential relationships between blood chemistry and body condition, in relation to habitat status, are still in their infancy (Castellini *et al.* 1993). Studies such as that of Castellini *et al.* (1993) have proposed that factors such as relocation, disease, environmental perturbations (indirectly affecting quantity and/or quality of prey) and the synergistic effects of fisheries may all influence body condition of marine mammals. As early as the early 1980's, Medway (1983) suggested that blood values might be useful in assessing the effects of oil spills or noise harassment during oil exploration and drilling. Changes in physiological parameters (Medway 1983), and more recently body composition, have been used to reflect environmental quality (Croxall *et al.* 1988, Costa *et al.* 1989, Gales *et al.* 1994).

Although the south-east coast of South Africa supports a large diversity of marine mammals, little is known of their physiology and how this is related to their health, population status and environment. Using the same net-caught animals described in Chapter 5, this particular facet of the study aimed to investigate the blood chemistry of net-caught dolphins from the south-east coast of southern Africa. It is recognised that physiological studies from incidentally-caught animals are not ideal, but these animals provide a valuable opportunity to investigate the potential use of physiological and morphological measures of condition for the monitoring of wild animal populations.

The specific aims of the study were therefore:

- To obtain blood chemistry data from blood samples taken from bottlenose (*Tursiops truncatus*) and common (*Delphinus delphis*) dolphins incidentally-caught in shark nets.

- To identify and assess the effects of species, age, sex and reproduction on the blood chemistry of net-caught dolphins.

- To examine relationships between serum chemistry of net-caught dolphins and morphological parameters, such as weight and blubber thickness.

- To identify and assess associations between blood chemistry and environmental variables such as diet and water temperature.

- To investigate the potential use of blood chemistry parameters in assessing the overall condition of net-caught dolphins.

- To investigate the potential use of net-caught dolphin morphology and physiology to assess and monitor the condition of the local dolphin population along the south-east coast of southern Africa.

MATERIALS AND METHODS

Chemistry analyses of blood from net-caught dolphins were done in conjunction with the leukocytic and haematological analyses described in Chapter 6. Blood drawn for chemistry analyses was collected at the same time and location, using the same methodology, as that used for haematological analyses (Chapter 6). Storage and transport procedures were identical to those described in Chapter 6. Blood chemistry tests used to analyse blood samples from net-caught animals were identical to those used to analyse samples from captive dolphins in Chapter 4. Due to financial and logistic constraints, two tests were omitted from the blood chemistry analyses for net-caught dolphins: alanine aminotransferase (ALT), aspartate aminotransferase (AST)

Materials and methods used to assess morphology, age, dietary and reproductive status and environmental conditions for net-caught animals are described in Chapter 5. The classification of animals into age and sex classes according to GLG counts, total body weight and reproductive condition is also described in Chapter 5.

RESULTS

Blood was sampled from a total of 101 animals between 1992 and 1995 and a single bottlenose dolphin caught in January 1996. Of the total, 58 were common dolphins, 38 were bottlenose dolphins and seven were humpback dolphins (*Sousa chinensis*). The small sample size for humpback dolphins prevented the examination of any potential relationships between blood chemistry of this species and dietary and environmental variables. However, for the sake of information, mean values for blood chemistry results obtained from humpback dolphins are included in Table 7.1. The seasonality, location and composition of the bottlenose and common dolphin catches are discussed in Chapter 5.

There were no significant differences in morphological, dietary and/or environmental parameters between calves, juveniles and adults of different sexes, of either dolphin species (Two sample t-test, $p>0.05$). Therefore males and females of the same age group were pooled. Some physiological parameters were significantly different between males and females of the same age group. These are discussed separately. Results obtained from morphological, dietary and environmental assessments of net-caught dolphins are presented in Chapter 5.

LIVER-, MUSCLE- AND KIDNEY-ASSOCIATED PARAMETERS

A. Creatinine

a. Common and bottlenose dolphins (pooled data)

Serum creatinine levels were significantly higher in common dolphins (607.14 ± 668.31 μmol/l) than in bottlenose dolphins (183.15 ± 48.23 μmol/l) ($t=2.22$, $p=0.03$, $df=32$) (Table 7.1). As is evident from the large standard deviations, levels of creatinine were highly variable. Box-whisker plots showed only two creatinine levels to be statistically outlying. These originated from an adult male common dolphin (1 606 μmol/l) and a simultaneously pregnant and lactating common dolphin (2 921 μmol/l). The variability of creatinine levels was reduced once these outliers had been removed, although mean levels remained species specific (Table 7.2). There were no sex- or age-related differences in this parameter. The number of lactating and/or pregnant females was limited, which made the assessment of reproductive-induced changes in blood chemistry difficult. However, creatinine levels did not appear to be markedly affected by pregnancy and/or lactation.

Serum creatinine from net-caught common and/or bottlenose dolphins was not significantly correlated with water temperature or any of the dietary categories.

b. Common dolphins

Common dolphin creatinine concentrations were positively correlated with girth at the anus ($r=0.50$, $p=0.04$, $n=17$) (Fig. 7.1A), although creatinine concentrations of between 100 μmol/l and 250

Table 7.1: Mean values of blood chemistry parameters from dolphins caught in shark nets between 1992 and 1996. (Data are presented as means and standard deviations (in parentheses). (Parameters are measured in units identical to those listed in Table 3.2). (Data from humpback dolphins have not been included in the calculation of overall means and/or species differences. ND: no data).

Blood parameter		**Overall mean**	**N**	***D. delphis*** **Common dolphin**	**n**	***T. truncatus*** **Bottlenose dolphin**	**n**	***S. chinensis*** **Humpback dolphin**	**n**
Creatinine*		445.03 (573.42)	34	607.14 (668.31)	21	183.15 (48.23)	13	1258.50 (1016.07)	6
Urea		23.28 (7.31)	35	24.16 (7.44)	21	21.97 (7.18)	14	24.28 (4.60)	6
Urea:creatinine		0.10 (0.06)	33	0.09 (0.07)	20	0.12 (0.04)	13	0.04 (0.04)	6
Blood urea nitrogen (BUN)		10.88 (3.42)	35	11.28 (3.40)	21	10.27 (3.23)	14	11.33 (2.15)	6
BUN:creatinine		0.05 (0.03)	33	0.04 (0.03)	20	0.06 (0.02)	13	0.02 (0.02)	6
Total bilirubin		88.86 (188.19)	14	136.89 (224.23)	9	2.40 (0.80)	5	3.00 (1.00)	2
Alkaline phosphotase (ALP) ^		1126.48 (1045.46)	23	763.00 (717.49)	10	1406.08 (1192.86)	13	477.00	1
	Adults	451.83 (192.03)	12						
	Juveniles	2351.67 (699.09)	3						
	Calves	1392.67 (1253.86)	6						
ALP:creatinine ^		3.28 (2.97)	17	2.50 (2.94)	9	4.15 (2.92)	8	3.00	1
	Adults	2.24 (1.09)	12						
	Juveniles	9.94	1						
	Calves	2.69 (2.41)	3						
Lactate dehydrogenase (LDH)		5065.21 (5660.54)	14	4964.22 (41112.94)	9	5247.00 (8380.87)	5	2168.75 (2755.91)	4
LDH:creatinine		17.00 (15.96)	12	20.31 (17.53)	8	10.37 (11.36)	4	11.58 (18.46)	4
Creatine phosphokinase (CPK)*		5720.65 (4724.22)	20	7984.44 (3982.30)	9	3868.46 (4618.69)	11	102.50 (86.5)	2
CPK:creatinine		28.670 (28.724)	14	23.70 (23.81)	9	26.68 (32.49)	4	0.26 (0.16)	2
Calcium		2.57 (1.05)	20	2.31 (1.23)	9	2.77 (0.84)	11	0.19 (0.09)	2
Potassium		ND		10.00	2	ND		ND	
Sodium		132.22 (26.95)	32	130.91 (29.50)	21	134.73 (22.37)	11	133.33 (14.27)	6
Sodium:creatinine*		0.59 (0.33)	31	0.47 (0.33)	20	0.81 (0.24)	11	0.25 (0.29)	6

Table 7.1: contd.

Blood parameter		**Overall mean**	**N**	***D. delphis*** **Common dolphin**	**n**	***T. truncatus*** **Bottlenose dolphin**	**n**	***S. chinensis*** **Humpback dolphin**	**n**
Glucose		8.39 (7.67)	60	7.60 (6.12)	41	10.10 (10.16)	19	5.33 (3.37)	3
Total protein		101.88 (18.42)	16	96.50 (3.30)	6	105.10 (22.55)	10	ND	
Albumin		42.82 (11.21)	16	45.50 (1.50)	6	41.20 (13.88)	10	ND	
Globulin		59.06 (19.32)	16	51.00 (4.43)	6	63.90 (22.87)	10	ND	
Albumin:globulin		0.84 (0.48)	16	0.90 (0.11)	6	0.80 (0.60)	10	ND	
Cholesterol		4.45 (0.88)	12	4.72 (0.89)	6	4.18 (0.77)	6	ND	
Triglycerides (TGL)		3.24 (0.89)	12	3.69 (0.76)	6	2.79 (0.78)	6	ND	
HDL-cholesterol ^		2.71 (0.64)	8	2.05 (0.91)	2	2.93 (0.28)	6	ND	
	Adults	2.88 (0.25)	5						
	Juveniles	3.05 (0.24)	2						
	Calves	1.14	1						
LDL-cholesterol		0.18 (0.33)	8	0.05 (0.05)	2	0.22 (0.37)	6	ND	
Cholesterol:HDL-cholesterol ^		1.64 (0.52)	8	2.25 (0.65)	2	1.43 (0.21)	6	ND	
	Adults	1.36 (0.16)	5						
	Juveniles	ND							
	Calves	1.85 (2.17)	4						

* Mean values significantly different ($p<0.05$) between species (two sample t-test)

^ Mean values significantly different ($p<0.05$) between age groups (ANOVA)

Table 7.2: Mean values of blood chemistry parameters (excluding statistical outliers) from common and bottlenose dolphins caught in shark nets between 1992 and 1996. (Data are presented as means and standard deviations (in parentheses). (Parameters are measured in units identical to those listed in Table 3.2) (ND: no data).

Blood parameter		**Overall mean**	**N**	***D. delphis*** **Common dolphin**	**n**	***T. truncatus*** **Bottlenose dolphin**	**n**
Creatinine*		303.55 (270.23)	31	390.50 (328.85)	18	183.15 (48.23)	13
Urea		23.91 (6.40)	34	25.27 (5.57)	20	21.97 (7.18)	14
Urea:creatinine		0.10 (0.06)	33	0.09 (0.07)	20	0.12 (0.04)	13
Blood urea nitrogen (BUN)		11.47 (2.82)	32	11.80 (2.61)	20	10.92 (3.18)	12
BUN:creatinine		0.05 (0.03)	33	0.04 (0.03)	20	0.06 (0.02)	13
Total bilirubin		2.50 (1.27)	10	2.60 (1.67)	5	2.40 (0.89)	5
Alkaline phosphotase (ALP) ^		1126.48 (1045.46)	23	763.00 (680.67)	10	1406.08 (1146.07)	13
	Adults	451.83 (192.03)	12				
	Juveniles	2351.67 (699.09)	3				
	Calves	1392.67 (1253.86)	6				
ALP:creatinine*		2.33 (1.34)	15	1.57 (0.99)	8	3.20 (1.19)	7
Lactate dehydrogenase (LDH)		3916.39 (3833.38)	13	4964.22 (3877.72)	9	1558.75 (1721.61)	4
LDH:creatinine		17.00 (15.96)	12	20.31 (16.40)	8	10.37 (9.84)	4
Creatine phosphokinase (CPK)*		5720.65 (4724.22)	20	7984.44 (3982.30)	9	3868.46 (4403.75)	11
CPK:creatinine		24.62 (25.38)	13	23.70 (23.81)	9	26.68 (32.49)	4
Calcium ^		2.96 (0.42)	17	2.90 (0.43)	7	3.01 (0.44)	10
	Adults	2.67 (0.23)	9				
	Juveniles	2.94 (0.40)	2				
	Calves	3.43 (0.25)	4				
Potassium		ND		10.00	2	ND	
Sodium		138.34 (19.41)	29	140.56 (17.68)	18	134.73 (21.33)	11
Sodium:creatinine*		0.59 (0.33)	31	0.469 (0.315)	20	0.806 (0.227)	11

Table 7.2: contd.

Blood parameter		**Overall mean**	**N**	***D. delphis*** **Common dolphin**	**n**	***T. truncatus*** **Bottlenose dolphin**	**n**
Glucose		6.38 (4.42)	54	6.37 (4.42)	38	6.39 (4.54)	16
Total protein		98.00 (7.72)	13	96.50 (3.30)	6	99.29 (10.21)	7
Albumin ^		44.69 (2.06)	13	45.50 (1.50)	6	44.00 (2.24)	7
	Adults	44.14 (1.07)	7				
	Juveniles	47.50 (0.71)	2				
	Calves	43.50 (2.12)	2				
Globulin		53.29 (12.45)	14	51.00 (4.43)	6	55.00 (16.22)	8
Albumin:globulin*		0.77 (0.22)	14	0.90 (0.11)	6	0.68 (0.23)	8
Cholesterol		4.45 (0.88)	12	4.72 (0.89)	6	4.18 (0.77)	6
Triglycerides (TGL)		3.24 (0.89)	12	3.69 (0.76)	6	2.79 (0.78)	6
HDL-cholesterol		2.93 (0.28)	7	2.95	1	2.93 (0.28)	6
LDL-cholesterol		0.06 (0.07)	7	0.05 (0.06)	2	0.06 (0.08)	5
Cholesterol:HDL-cholesterol		1.46 (0.22)	7	1.60	1	1.43 (0.21)	6

* Mean values significantly different ($p<0.05$) between species (two sample t-test)

^ Mean values significantly different between age groups (ANOVA)

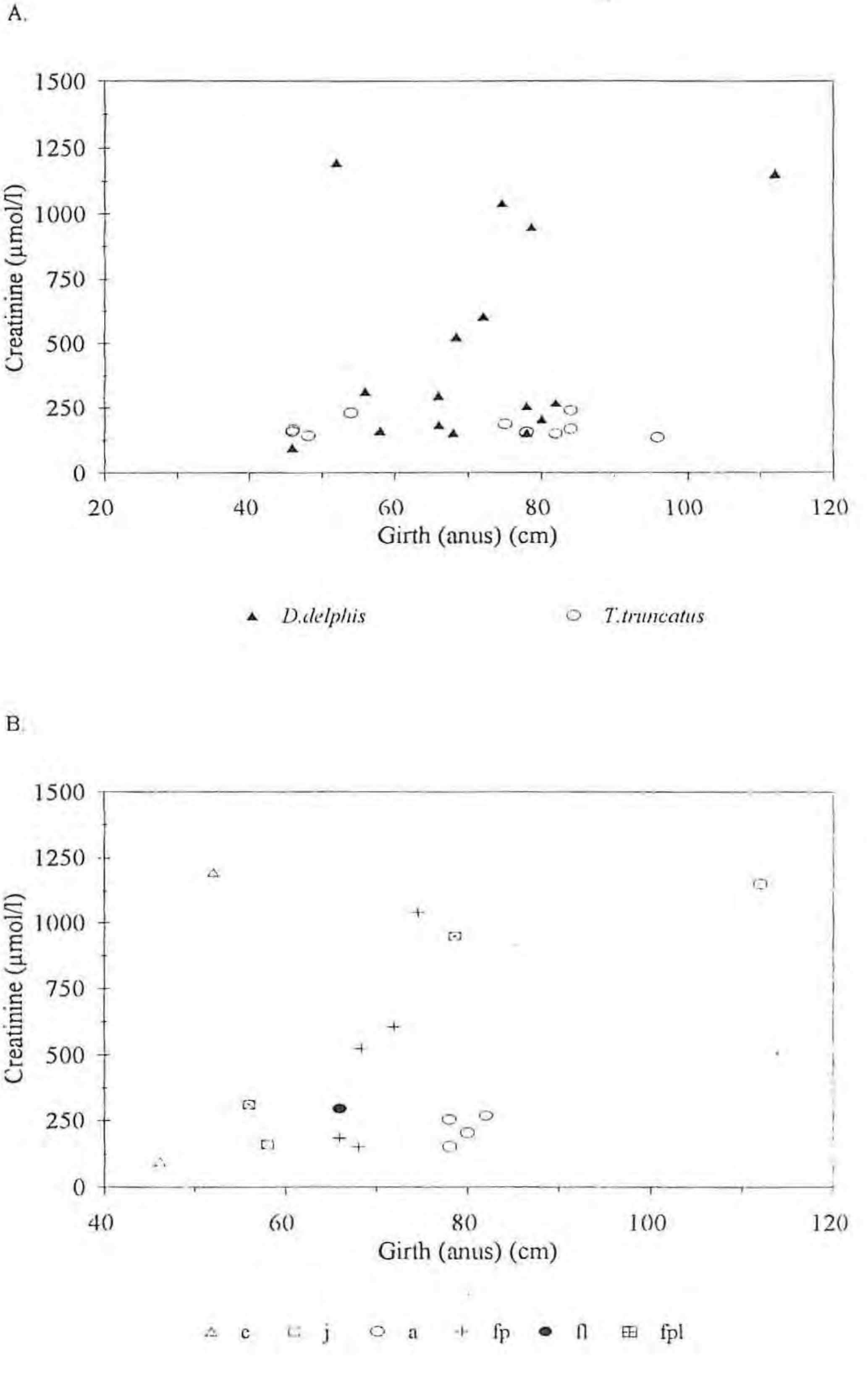


Figure 7.1: Significant correlation ($p<0.05$) between levels of serum creatinine and girth (anus for all common dolphins (A) (and respective age and reproductive classes (B)) caught in shark nets between 1992 and 1996 (c: calves, j: juveniles, a: adults, fp: pregnant females, fl: lactating females, fpl: simultaneously pregnant and lactating females).

μmol/l were measured in animals of various sizes. Within age groups, creatinine concentrations were higher in animals with larger anal girths (Fig. 7.1B).

c. Bottlenose dolphins

Creatinine concentrations from bottlenose dolphins were positively correlated with both dorsal blubber thickness (r=0.63, p=0.04, n=11) and the proportional contribution of blubber to the total body weight (%B) (r=0.62, p=0.04, n=11). Although the number of animals in each age group was low, creatinine concentrations appeared to be increased with greater blubber thickness and greater %B in most age groups, but particularly calves (Fig. 7.2).

B. Blood urea nitrogen (BUN) / BUN:creatinine

a. Common and bottlenose dolphins (pooled data)

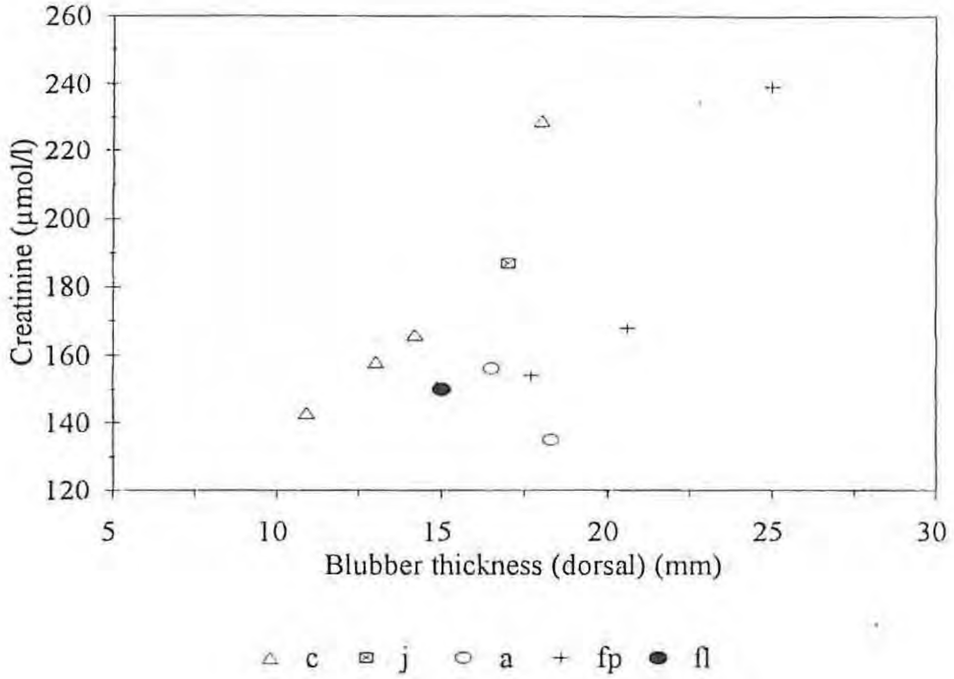
There were no significant differences between the mean BUN concentrations of common dolphins (11.28 ± 3.40 mmol/l) and bottlenose dolphins (10.27 ± 3.23 mmol/l) (t=0.85, p=0.40, df=33). Similarly, the mean BUN:creatinine value was similar for the two species (Table 7.1). The range of BUN levels from both species was similar, but concentrations being most variable in common dolphins. BUN concentrations below 6.50 mmol/l were identified as statistically outlying. An extremely low BUN level (0.90 mmol/l) was measured in a juvenile male common dolphin which, together with parasites in the blubber and testes, showed no signs of recent feeding. Blood results from this particular animal had statistically outlying values for several parameters. The removal of outliers did not have a marked effect on mean BUN levels (Table 7.2). No outlying BUN:creatinine ratios were identified. BUN and BUN:creatinine from net-caught dolphins did not change significantly with age, sex or reproductive status.

The only relationship between BUN and any of the independent variables, common to both species, was between BUN:creatinine and the number of prey items in the stomach (common dolphins: r=0.59, p=0.02, n=15; bottlenose dolphins: r=0.79, p=0.01, n=9) (Fig. 7.3). The numbers of animals in each age and reproductive group was limited but overall, BUN:creatinine appeared to increase with a greater number of prey items in the stomach – particularly for adults.

b. Common dolphins

The only morphological parameter with which BUN was significantly related was %B (r=-0.50, p=0.02, n=21), and this was only for common dolphins. Although data were variable, BUN levels appeared to decline slightly as the proportional weight of blubber increased (Fig. 7.4). This relationship, however, was biased by outliers and the decline was not evident for any age groups (Fig. 7.4B).

A.



B.

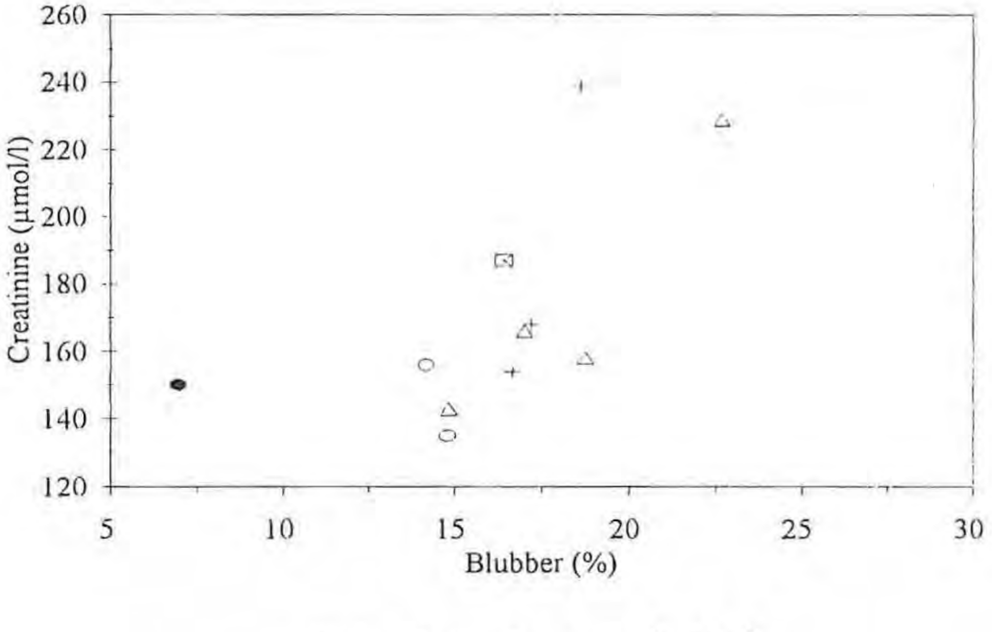
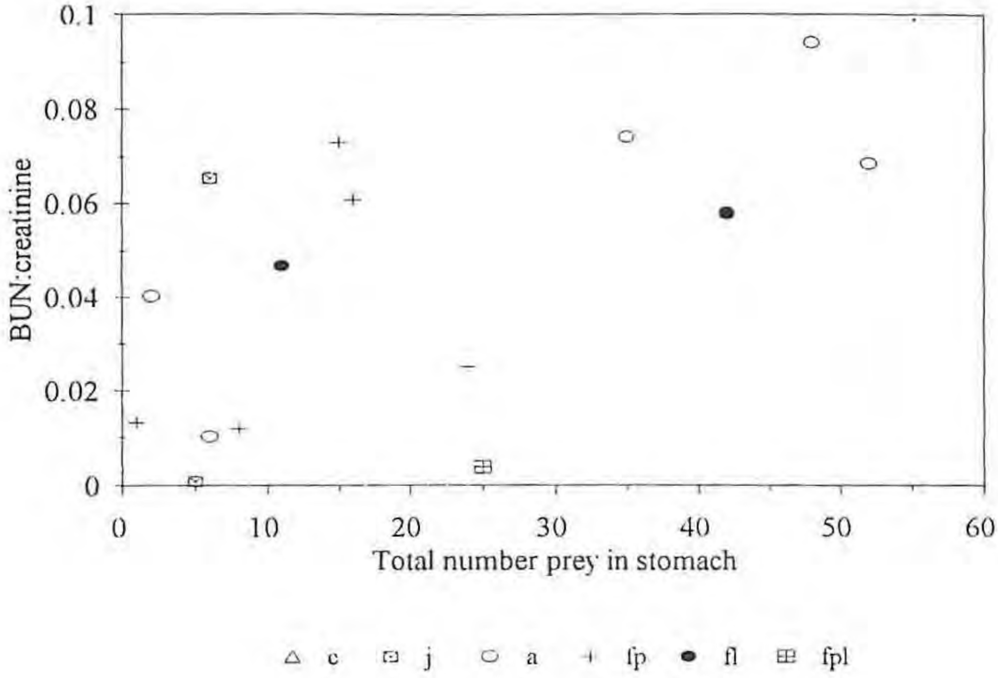


Figure 7.2: Significant correlations ($p<0.05$) between serum creatinine and dorsal blubber thickness (A) and proportional blubber weight (B) for bottlenose dolphins caught in shark nets between 1992 and 1996 (c: calves; j: juveniles; a: adults; fp: pregnant females; fl: lactating females).

A.



B.

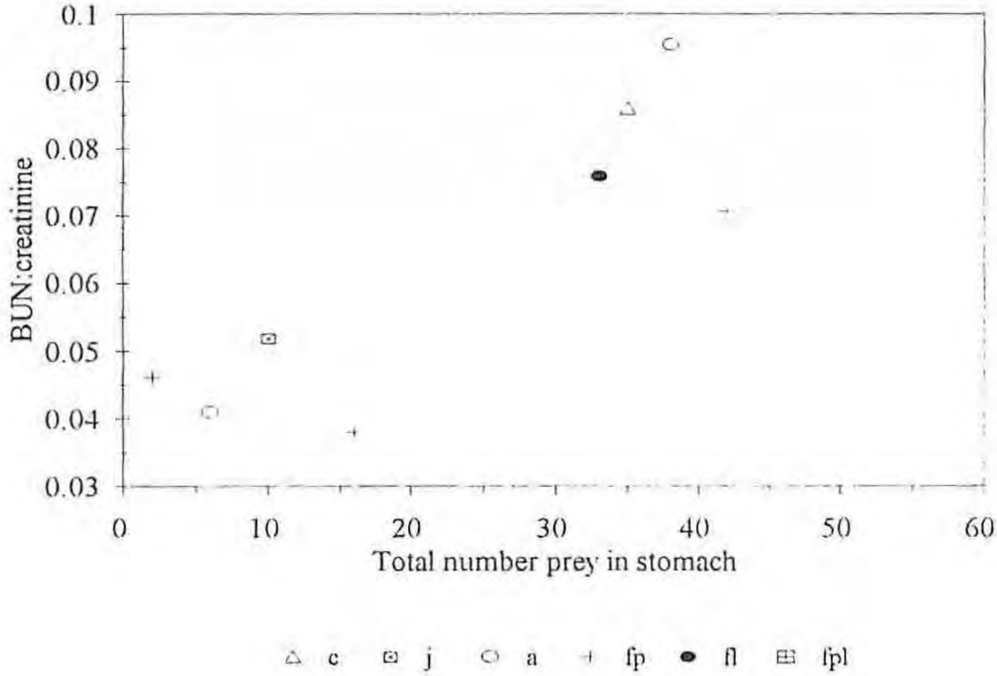


Figure 7.3: Significant correlations ($p<0.05$) between BUN:creatinine and the total number of prey items in the stomachs of different age groups of common (A) and bottlenose dolphins (B) caught in shark nets between 1992 and 1996 (c: calves; j: juveniles; a: adults; fp: pregnant females; fl: lactating females; fpl: simultaneously pregnant and lactating females).

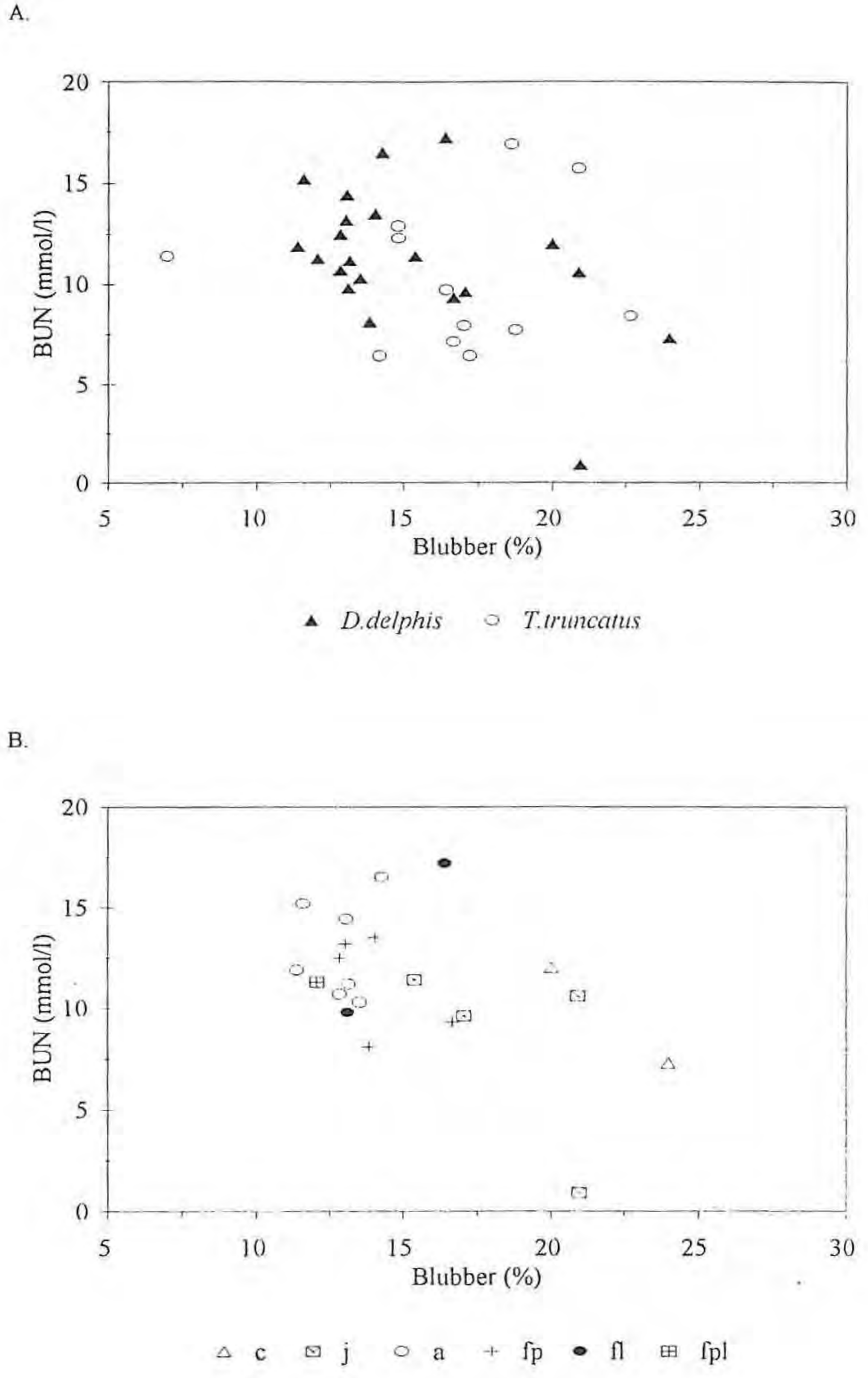


Figure 7.4: Significant correlation ($p<0.05$) between blood urea nitrogen (BUN) and proportional blubber weight for common dolphins (D. delphis) caught in shark nets between 1992 and 1996 (A); and for the respective age and reproductive classes (B) (c: calves; j: juveniles; a: adults; fp: pregnant females; fl: lactating females; fpl: simultaneously pregnant and lactating females).

Levels of BUN, from common dolphins only were positively related with the number of prey items in the stomach (r=0.49, p=0.006, n=15). Although not apparent for all age groups, BUN was slightly elevated when the number of prey items was greater (Fig. 7.5).

C. Total bilirubin

Although mean values of total bilirubin were not species-specific, the variability of values was significantly higher in samples from common dolphins (136.89 ± 224.23 μmol/l) (Table 7.1). The exclusion of statistical outliers (> 75.00 μmol/l) (box-whisker plot) reduced this variability markedly and resulted in similar mean total bilirubin levels from both species (Table 7.2). Total bilirubin was not related to age, sex or reproductive condition. Although a number of bilirubin levels were significantly correlated with morphological and dietary parameters, outliers biased all trends. Without additional data, it was impossible to validate these trends. Morphology, diet and water temperature had no marked effect on data, exclusive of outliers.

D. Alkaline phosphotase (ALP) / ALP:creatinine

a. Common and bottlenose dolphins (pooled data)

The mean ALP level of bottlenose dolphins (1406.08 ± 1192.86 U/l) was markedly, but not significantly, higher than that of common dolphins (763.00 ± 717.49 U/l) (t=-1.5, p=0.15, df=21) (Table 7.1). However, the majority of values (50 %) from both species (common: 204-2 444 U/l; bottlenose: 319-3 000 U/l) were within a similar range (box-whisker plot). There were no significant outliers for this enzyme. A significant age-related difference in ALP concentration (F=9.75, p=0.001, df=18) showed that ALP levels were highest in juvenile animals (2351 ± 699.09 U/l) and lowest in adults (451.83 ± 192.03 U/l). In addition, the mean ALP:creatinine ratio was significantly similar between species (t=-1.16, p=0.26, df=15), although the mean ratio of bottlenose dolphins was markedly higher than that of common dolphins (Table 7.1). As described previously for ALP, the ratio was age related (F=14.40, p=0.0005, df=13) (Table 7.1), being lowest in calves and highest in juveniles. Ratios higher than 6.00 were statistically outlying (box-whisker plot). The animals from which these ratios originated included a juvenile male common dolphin with parasites in the intestine, stomach and muscle. Additionally, this animal had no recently consumed food in the stomach. The exclusion of outliers resulted in the age differences in ALP:creatinine becoming non-significant (F=0.26, p=0.62, df=9).

Levels of ALP from both species of dolphin were negatively correlated with both total body length (r=-0.49, p=0.02, n=22) and total body weight (r=-0.47, p=0.03, n=22). Although the decline in

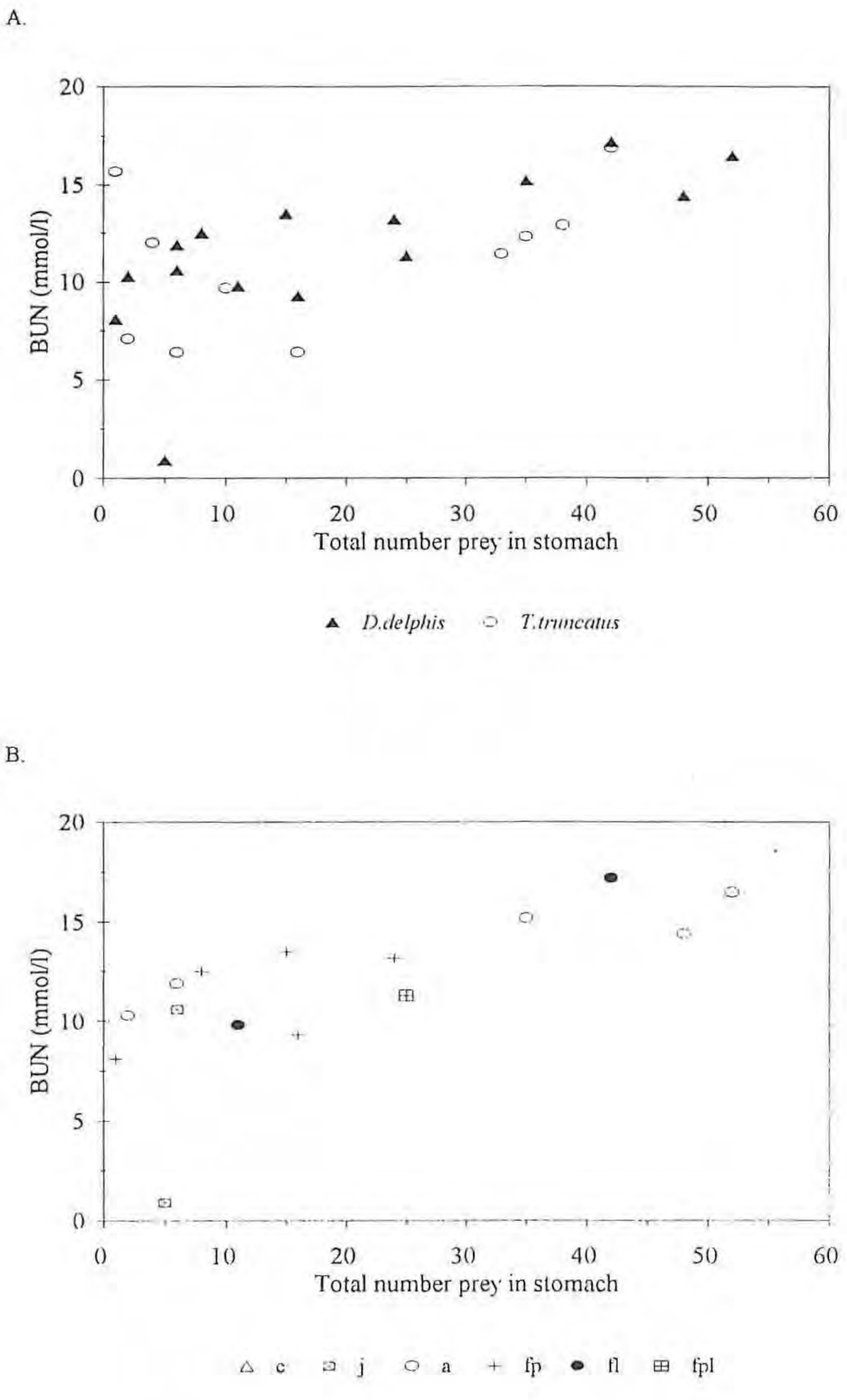


Figure 7.5: Significant correlations ($p<0.05$) between blood urea nitrogen (BUN) and the total number of prey items in the stomachs of common dolphins (D. delphis) (A) (and the respective age groups (B)) caught in shark bets between 1992 and 1996 (c: calves; j: juveniles; a: adults; fp: pregnant females, fl: lactating females, fpl: simultaneously pregnant and lactating females).

ALP with increased size was not distinct, ALP concentrations were more variable at lengths and weights below 200 cm and 100 kg, respectively (Fig. 7.6).

b. Common dolphins

Common dolphin ALP levels declined significantly with increased total blubber ($r=-0.75$, $p=0.01$, $n=10$) and muscle weight ($r=-0.54$, $p=0.009$, $n=10$) (Fig. 7.7). Both these relationships were almost identical to those described for total body length and weight.

Levels of ALP, from either dolphin species, were not related to food consumption and/or the composition of the diet. Although concentrations of this enzyme were correlated with water temperature, trends were more indicative of the high variability associated with young animals, rather than a direct effect of water temperature on ALP levels. Bottlenose dolphin ALP:creatinine was also significantly correlated with water temperature. However, ratios were also related rather to age, than to water temperature. Without additional data, it was impossible to examine any potential temperature-related changes in ALP within specific age groups.

E. Lactate dehydrogenase (LDH) / LDH:creatinine

a. Common and bottlenose dolphins (pooled data)

Mean levels of LDH were not significantly different between species ($t=-0.08$, $p=0.93$, $df=12$), although levels from both species were extremely variable (Table 7.1). Similarly, LDH:creatinine values varied markedly within the two species (Table 7.1). Because of this variability, only a male bottlenose dolphin calf had an outlying high LDH concentration (> 20 000 U/l). No parasites were found in this animal and both milk and squid remains were found in the stomach. The removal of this outlier had a marked effect on the mean LDH level and LDH:creatinine ratio of bottlenose dolphins (Table 7.2).

Mean LDH levels did not differ significantly between different age, sex, or reproductive groups (Two sample t-test, $p>0.05$). LDH and LDH:creatinine were not significantly related to food consumption and/or diet composition, nor did they show any clear trends when related to water temperature.

b. Common dolphins

Common dolphin LDH was positively related to total body length ($r=0.86$, $p=0.003$, $n=9$) and total muscle weight ($r=0.79$, $p=0.01$, $n=9$). Although trends within age groups were difficult to verify, animals with greater muscle weights had higher levels of LDH (Fig. 7.8).

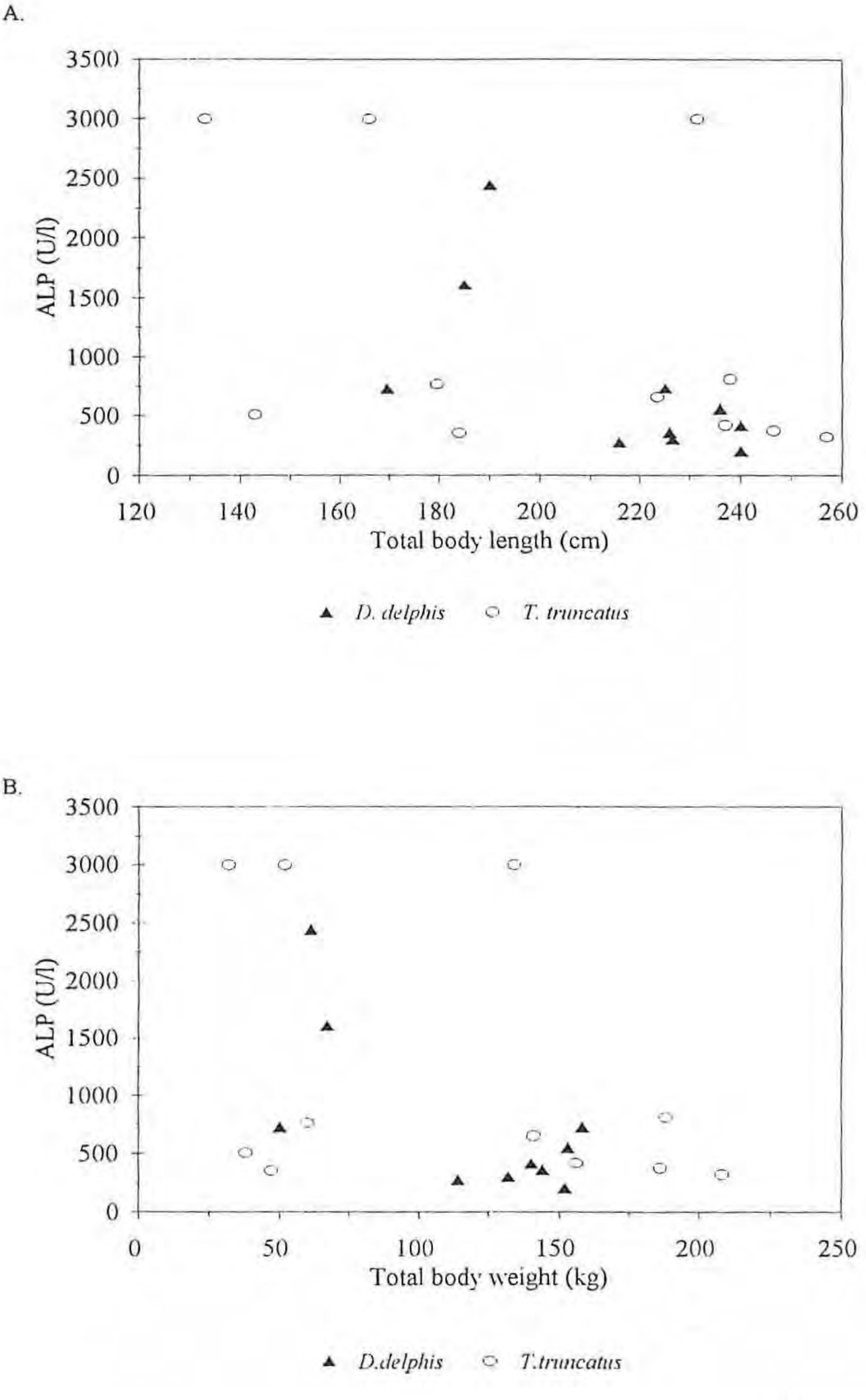
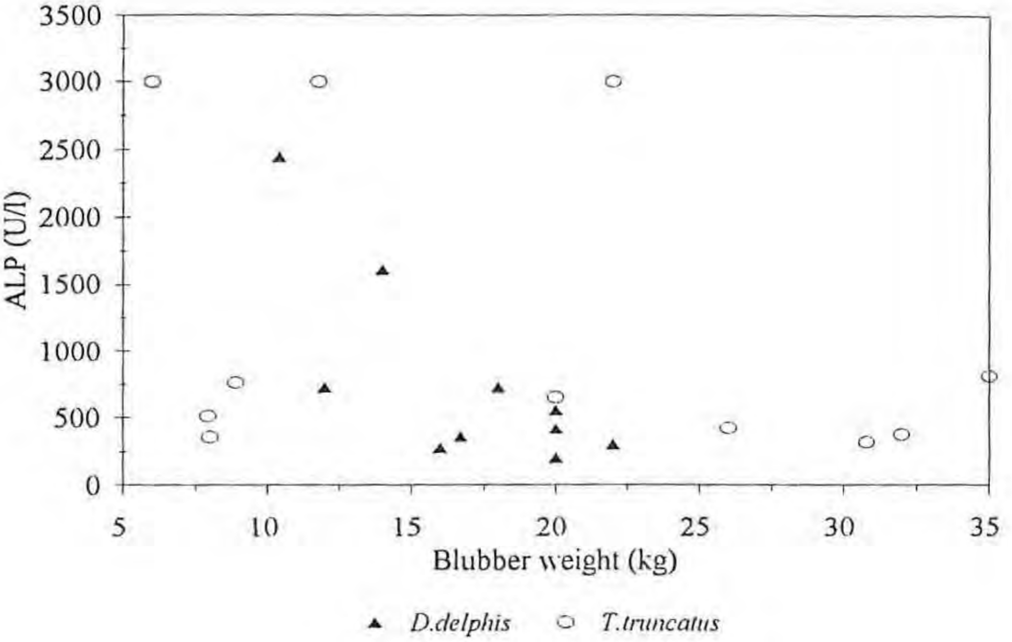


Figure 7.6: Significant correlations ($p<0.05$) between alkaline phosphotase (ALP) and total body length (A); and total body weight (B) for common (D. delphis) and bottlenose (T. truncatus) dolphins caught in shark nets between 1992 and 1996.

A.



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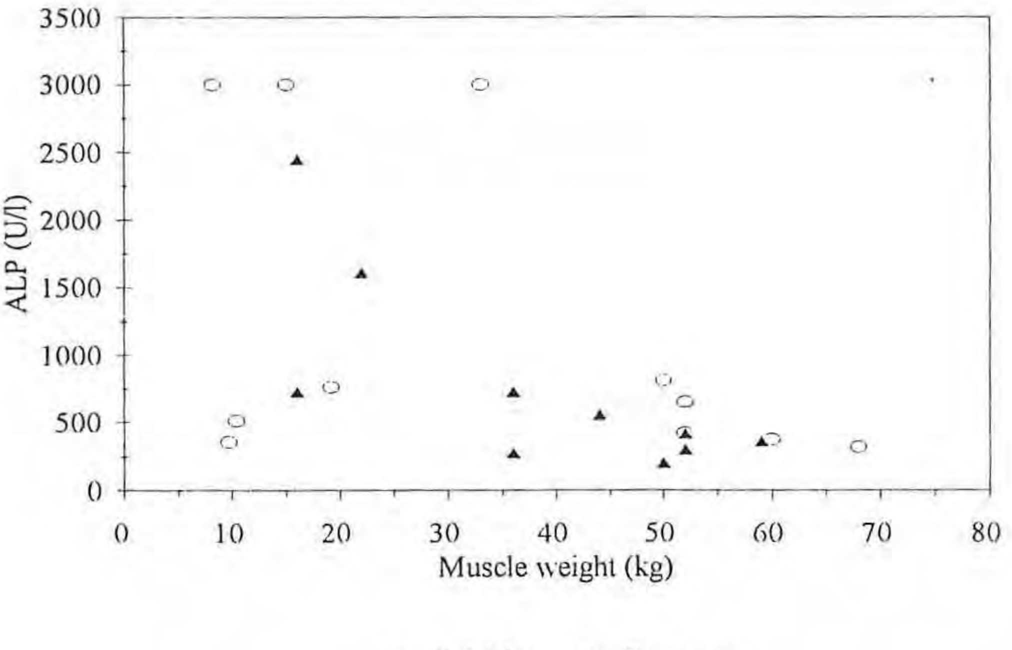


Figure 7.7: Significant correlations ($p<0.05$) between alkaline phosphotase (ALP) and total blubber (A) and muscle (B) weight for common dolphins (D. delphis) caught in shark nets between 1992 and 1996.

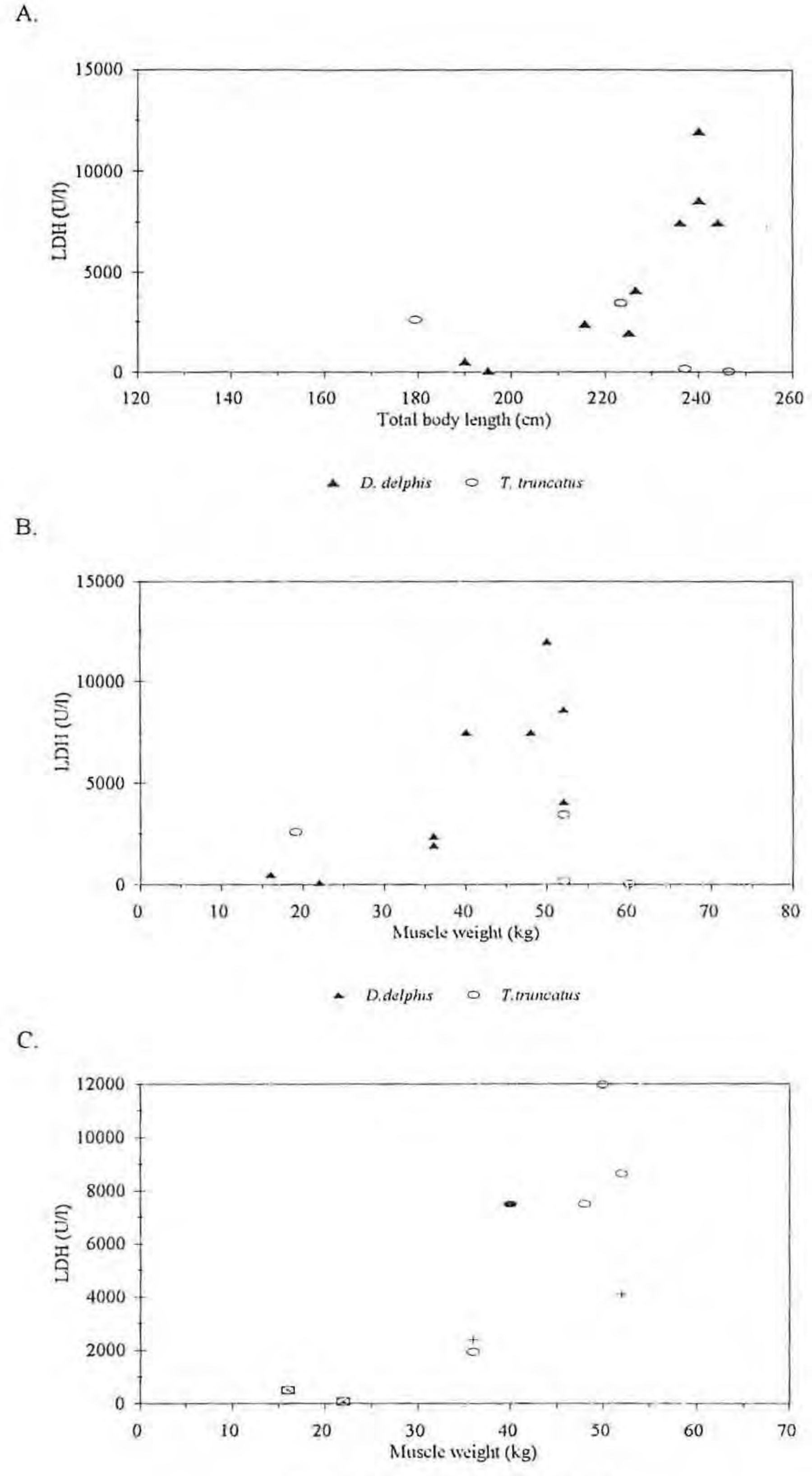


Figure 7.8: Significant correlations ($p<0.05$) between lactate dehydrogenase (LDH and total body length (A) and total muscle weight (B) for common dolphins (D. delphis) (and the respective age and reproductive groups (C)) caught in shark nets between 1992 and 1996.

F. Creatine phosphokinase (CPK) / CPK:creatinine

a. Bottlenose and common dolphins (pooled data)

Mean levels of CPK were significantly different between common (7 984 ± 3 982.30 U/l) and bottlenose dolphins (3 868.46 ± 4 618.69 U/l) (t=2.11, p=0.05, df=18) (Table 7.1). However, mean values for the CPK:creatinine ratio were not species specific (t=-0.86, p=0.41, df=12) (Table 7.1). Due to the large variability, no CPK levels and/or CPK:creatinine ratios were classified as significantly outlying. Neither CPK nor CPK:creatinine were significantly different between different age and/or sex groups. However, CPK levels measured in the two pregnant bottlenose dolphins were significantly lower (1 198.33 U/l) than those recorded from a resting female (12 699.00 U/l).

There were few significant relationships between these blood chemistry parameters and independent variables. The CPK:creatinine ratio from common dolphins was positively related to total muscle weights (r=0.64, p=0.05, n=10) and %M (r=0.56, p=0.02, n=17). Although data from common dolphins also reflected that both CPK and CPK:creatinine were positively correlated ($p<0.05$) with water temperature, limited and highly variable data made all these relationships impossible to verify. CPK and CPK:creatinine were not significantly related to any of the dietary parameters.

ELECTROLYTES

A. Sodium / Sodium:creatinine

a. Bottlenose and common dolphins (pooled data)

Levels of sodium were similar for common (130.91 ± 29.50 mmol/l) and bottlenose (134.73 ± 22.37 mmol/l) dolphins (Table 7.1). Three sodium concentrations, below 83.00 mmol/l, were classified as significant outliers (box-whisker plot). Although the exclusion of these outliers resulted in a higher mean sodium level for common dolphins, it did not make mean sodium concentrations species-specific (Table 7.2). However, sodium:creatinine was significantly different between species, being twice as high for bottlenose dolphins (0.81 ± 0.24) than in common dolphins (0.47 ± 0.33) (Table 7.1). No sodium:creatinine ratios were outlying (box-whisker plot). Both sodium and sodium:creatinine showed no significant changes with age, sex and/or reproductive condition.

b. Common dolphins

Common dolphin sodium levels were negatively related to ventral blubber thickness (r=-0.49, p=0.04, n=18), with serum sodium being slightly reduced when blubber thickness was increased. Although this appeared to be true within age groups, additional data are needed to verify this trend, particularly in light of the bias effect of outliers. In addition, sodium levels from this species were negatively correlated with the mean reconstituted weight of prey in the stomach (r=-0.74, p=0.004, n=13), as well as the mean energy content of prey items in the stomach (r=-0.39, p=0.04, n=12) (Fig. 7.9). To some extent, this decline was associated with significantly reduced sodium

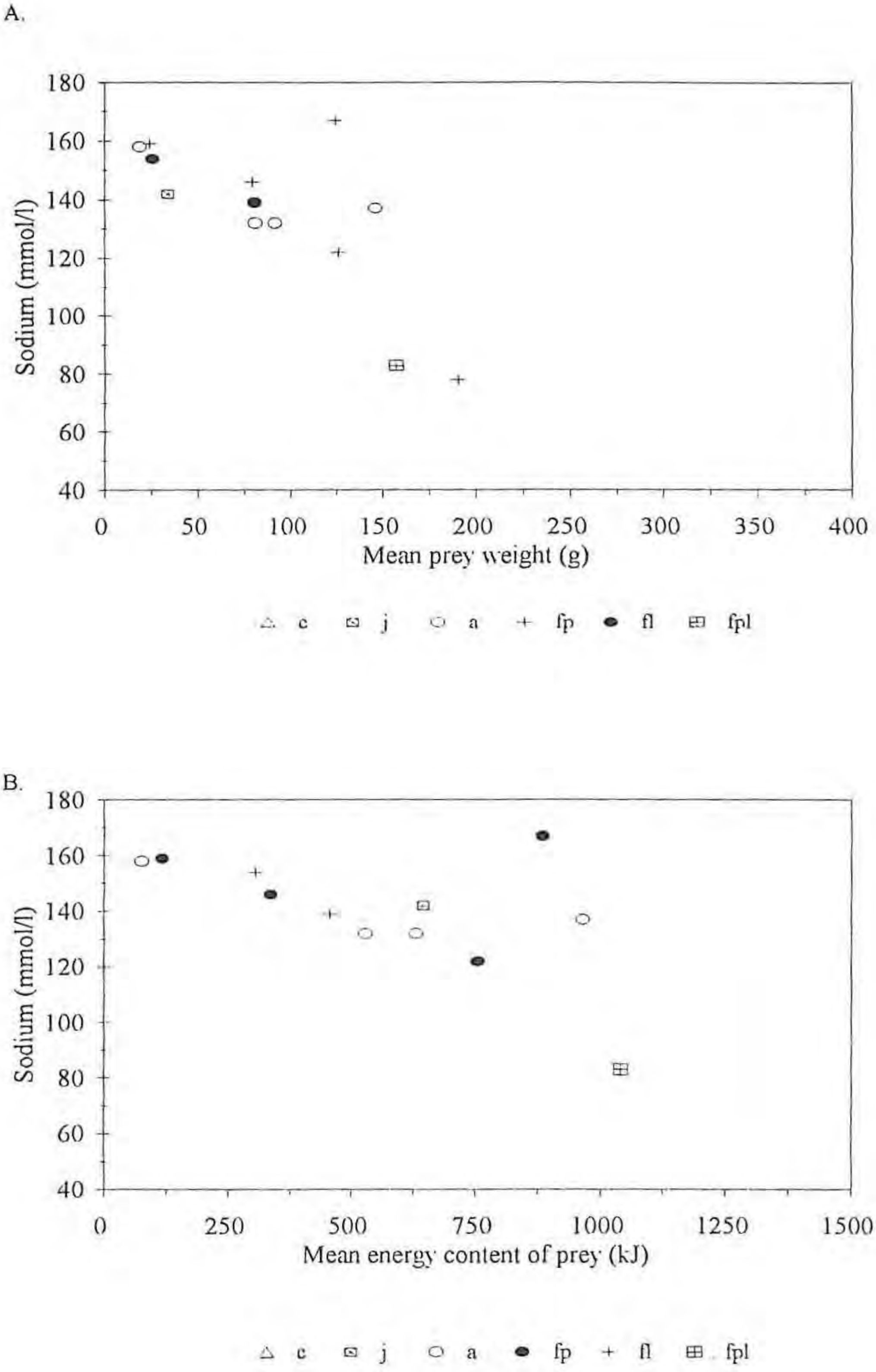


Figure 7.9: Significant correlations ($p<0.05$) between serum sodium and the mean weight (A) and the mean energy content (B) of prey in the stomachs of common dolphins caught in shark nets between 1992 and 1996 (c: calves; j: juveniles; a: adults; fp: pregnant females; fl: lactating females; fpl: simultaneously pregnant and lactating females).

concentrations associated with greater prey size and energy content. However, there was a slight decline in sodium concentrations as the mean prey weight (Fig. 7.9A) and mean energy content (Fig. 7.9B) of prey increased.

B. Potassium

The analysis of serum potassium in blood samples taken from net-caught dolphins proved extremely difficult and results were obtained from only two adult male common dolphins. In both cases, levels of potassium in the serum were greater than 10.00 mmol/l (Table 7.1). Due to this lack of data, no relationships between serum potassium and age, sex, reproduction, morphology, diet and/or environmental parameters could be assessed.

C. Calcium

a. Common and bottlenose dolphins (pooled data)

Mean levels of calcium for the two dolphin species were similar (t=-0.98, p=0.34, df=18) (Table 7.1). Several calcium concentrations below 2.35 mmol/l were classified as outliers (box-whisker plot). The animals from which these results came included a pregnant common dolphin, a juvenile male common dolphin and a lactating bottlenose dolphin, which had an ulcerated stomach. The removal of these outliers changed the mean value of both species (Table 7.2). In addition, data, exclusive of outliers, illustrated that calcium concentrations differed significantly between age groups (F=12.59, p=0.001, df=12). Calves had significantly higher calcium levels (3.43 ± 0.25 mmol/l) than both juveniles (2.94 ± 0.40 mmol/l) and adults (2.67 ± 0.23 mmol/l).

b. Bottlenose dolphins

The significantly different mean calcium levels from different age groups was paralleled by a negative correlation between bottlenose dolphin calcium levels and total body length (r=-0.69, p=0.04, n=9) (Fig. 7.10 A). A similar decline was evident for common dolphins, although the trend was not significant (p>0.05). Serum calcium from bottlenose dolphins was positively related to %B (r=0.79, p=0.01, n=9) (Fig. 7.10B). Limited data made this relationship impossible to confirm. A single lactating female had an extremely low %B associated with a severely reduced calcium level (Fig. 7.10B).

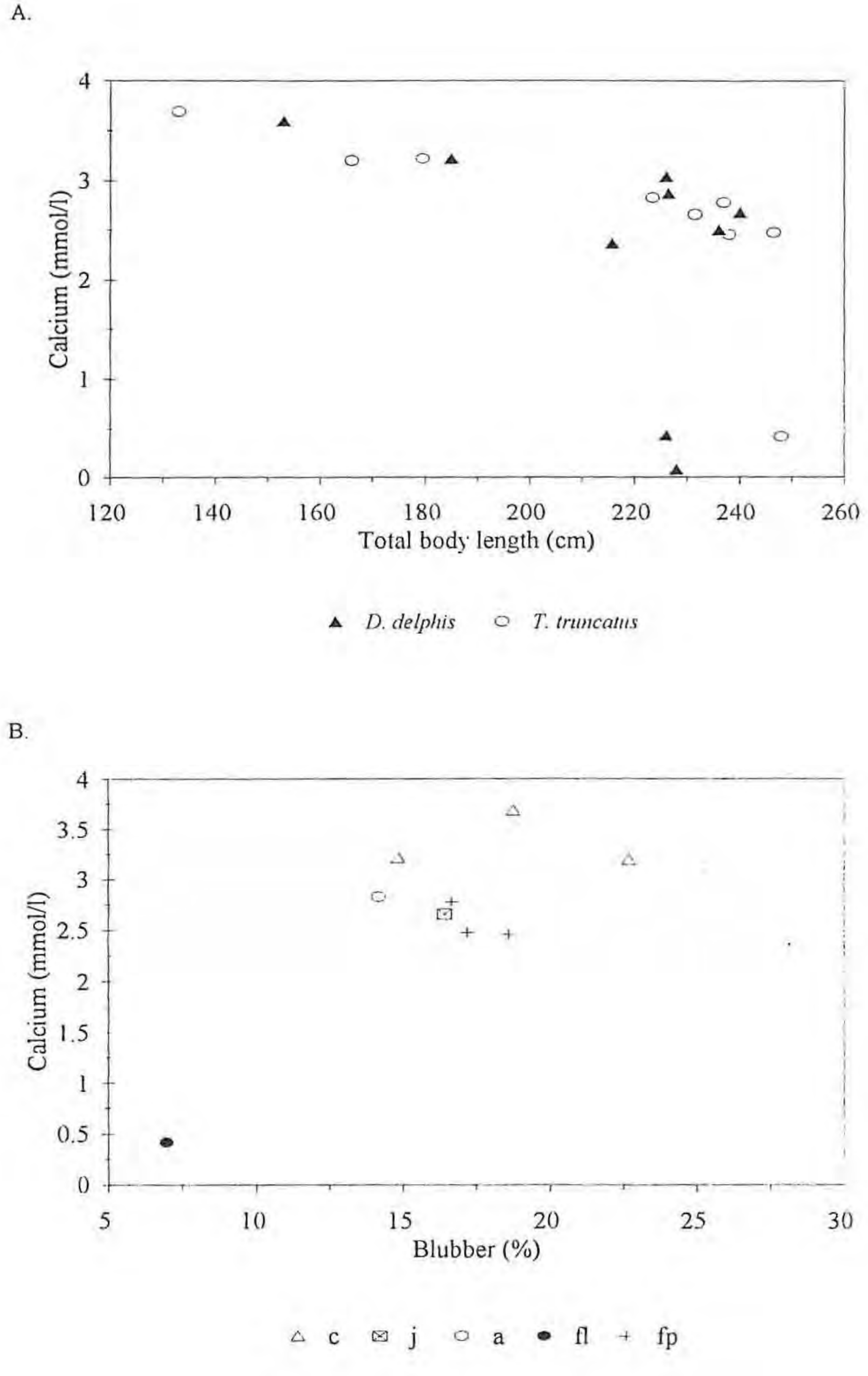


Figure 7.10: Significant correlations ($p<0.05$) between serum calcium and total body length for bottlenose dolphins (<u>T. truncatus</u>) (A); and between calcium and proportional blubber weight (B) for the different age and reproductive groups of bottlenose dolphins caught in shark nets between 1992 and 1996 (c: calves; j: juveniles; a: adults; fp: pregnant females; fl: lactating females).

PROTEINS

A. Total protein

a. Common and bottlenose dolphins (pooled data)

Mean levels of total protein in the two dolphin species were not significantly different (Table 7.1). Box-whisker plots showed total protein levels above 120.00 g/l and below 80.00 g/l to be significant outliers. A protein concentration of 154.00 g/l was measured in blood from a pregnant bottlenose dolphin with a slightly ulcerated stomach, although fish remains were found in the stomach. An outlying low total protein level (71.00 g/l) was associated with a bottlenose dolphin calf, which had both fish and squid in the stomach but also a particularly heavy parasite load. The exclusion of these outliers magnified the similarity of mean total protein levels for common and bottlenose dolphins (Table 7.2). Total protein did not differ significantly between animals of different sex, age and reproductive status.

b. Bottlenose dolphins

Although data were limited, total protein concentrations from bottlenose dolphins were positively correlated with a number of morphological indices, including total body weight (r=0.56, p=0.04, n=8), axillary girth (r=0.62, p=0.02, n=8), anal girth (r=0.55, p=0.05, n=8), dorsal blubber thickness (r=0.74, p=0.004, n=8) and total blubber weight (r=0.68, p=0.008, n=8). Overall, total protein remained relatively stable (~90-100 g/l) (in both species), over a wide range of dolphin sizes (25-200 kg) (Fig. 7.11 & 7.12). Low outliers were associated with smaller animals, whereas significantly elevated levels of total protein were recorded only from larger animals (Fig. 7.11 & 7.12).

Although combined total protein data were positively related with the total number of prey species in the stomach (r=0.71, p=0.01, n=12), further analyses are needed to confirm this relationship. An outlying high level of total protein was associated with a particularly large number of prey species in the stomach of a bottlenose dolphin (Fig. 7.13A). In addition, bottlenose dolphin data implied that that levels of total protein were negatively related to the mean regional water temperature of the month prior to capture (r=-0.58, p=0.04, n=7). However, this parameter was highly variable (70-152 g/l) within a severely restricted temperature range (19.5-21°C), precluding the confirmation of any real trends (Fig. 7.13B).

B. Albumin

a. Common and bottlenose dolphins (pooled data)

Mean albumin levels in common (45.50 ± 1.50 g/l) and bottlenose (41.20 ± 13.88 g/l) dolphins were similar (t=0.71, p=0.49, df=14) (Table 7.1), but results from the latter species were more variable.

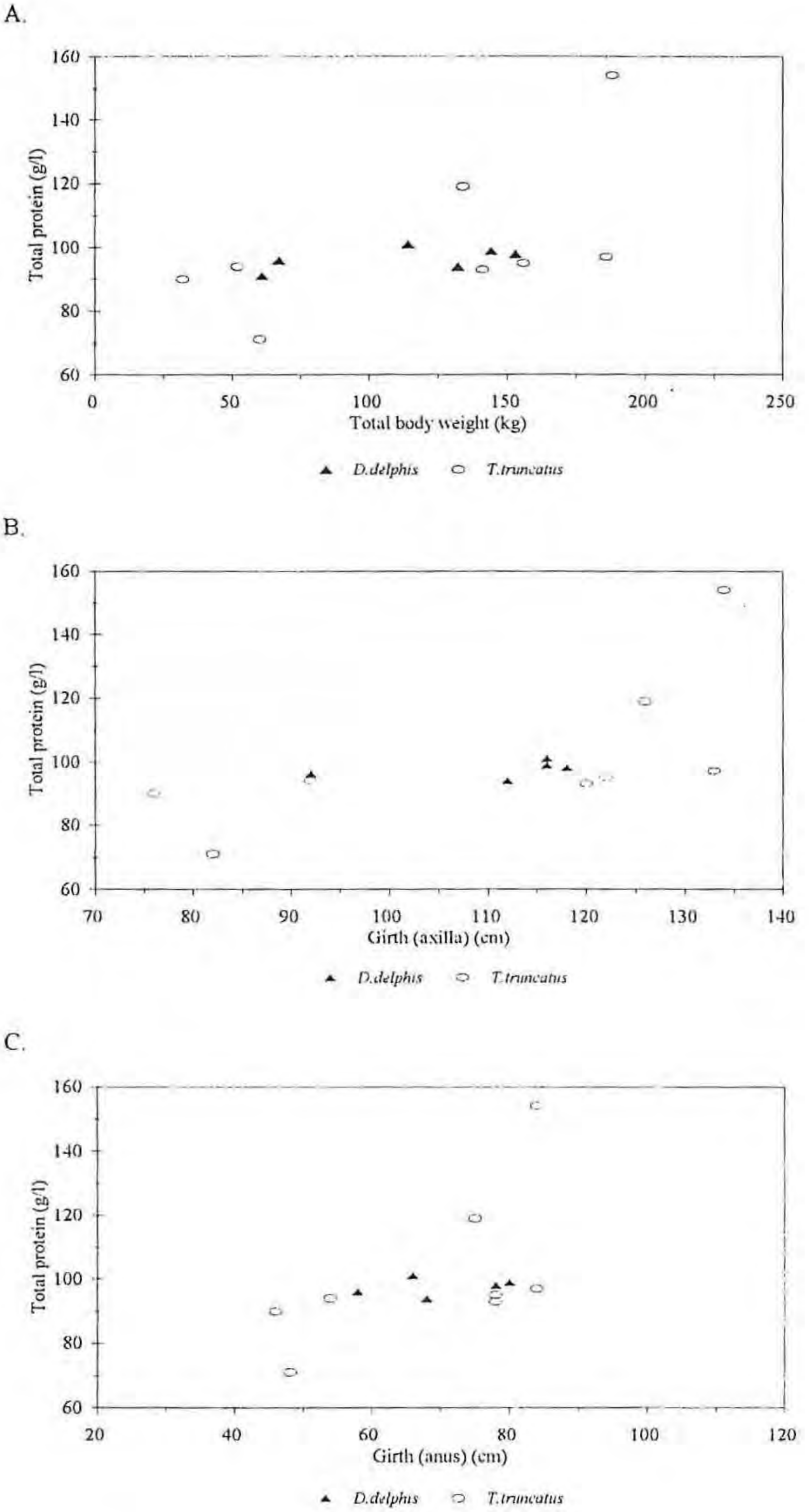
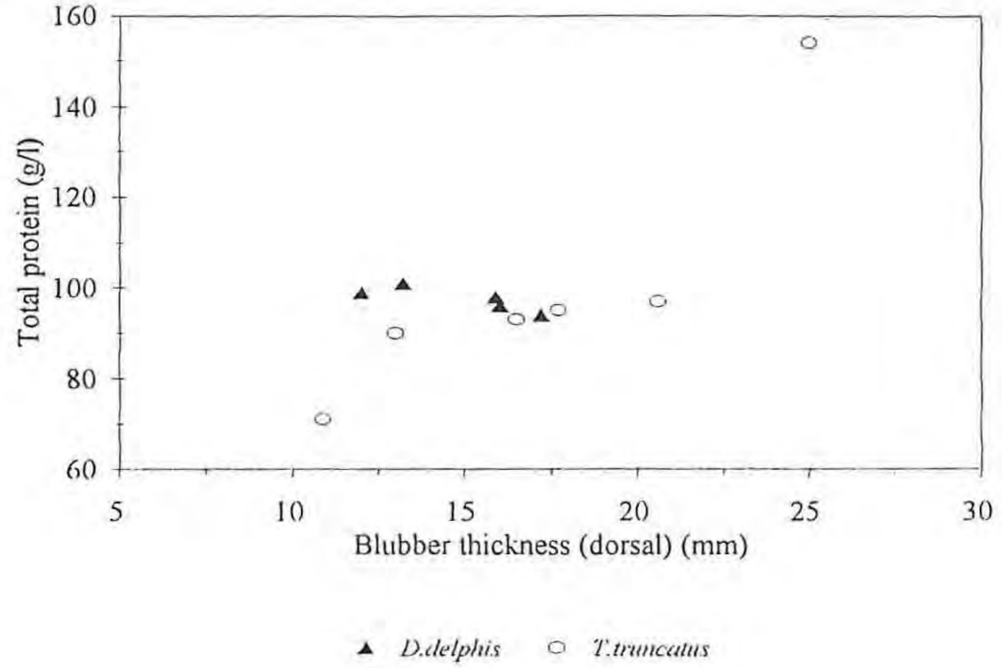


Figure 7.11: Significant correlations ($p<0.05$) between total protein and total body weight (A), girth (axilla) (B) and girth (anus) (C) for common (D. delphis) and bottlenose (T. truncatus) dolphins caught in shark nets between 1992 and 1996.

A.



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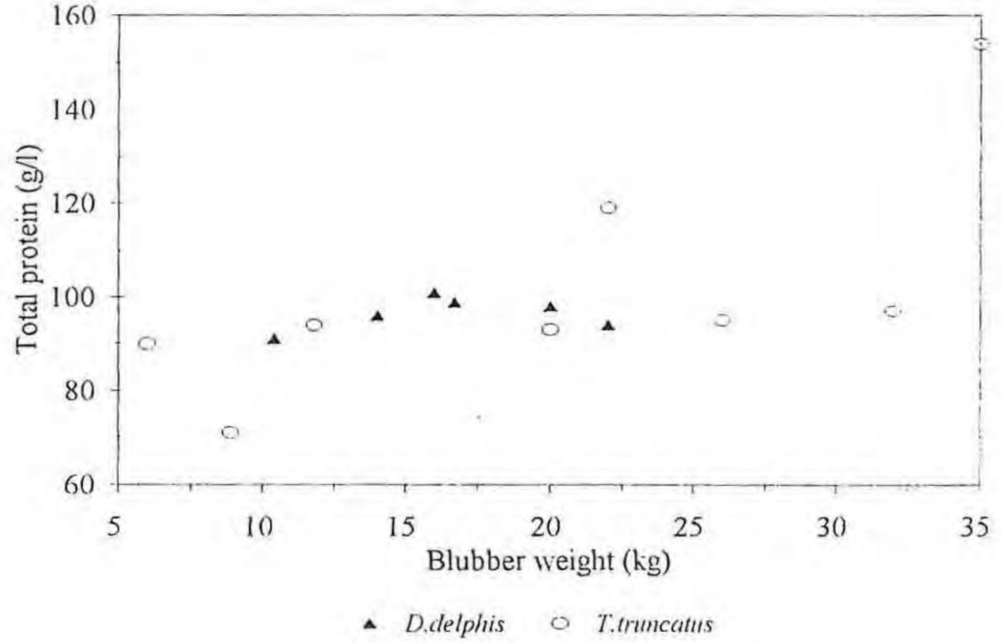
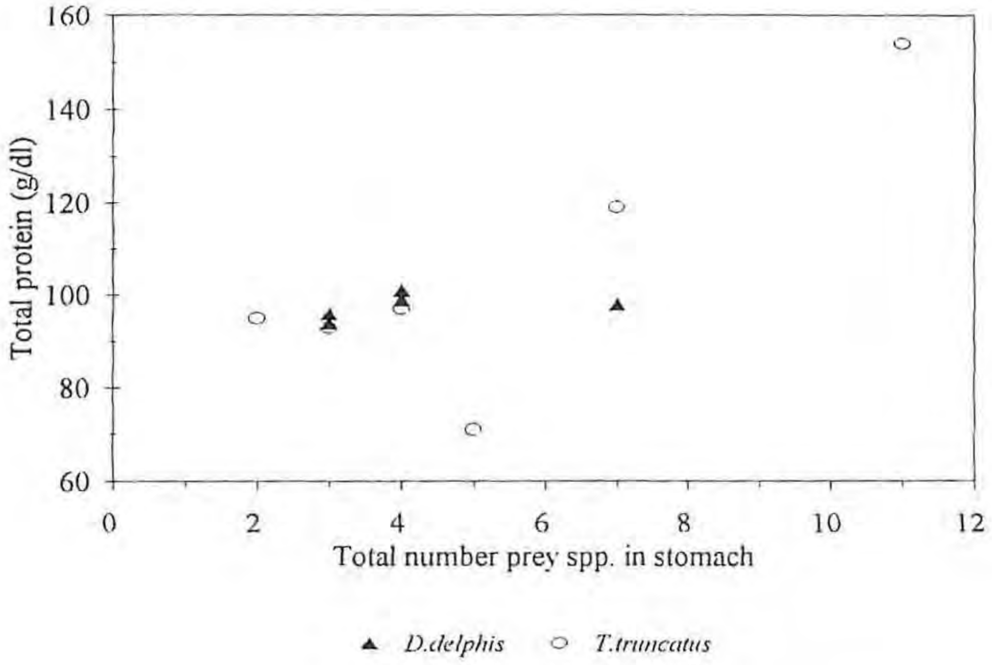


Figure 7.12: Significant correlations ($p<0.05$) between total protein and dorsal blubber thickness (A) and blubber weight (B) for common (D. delphis) and bottlenose (T. truncatus) dolphins caught in shark nets between 1992 and 1996.

A.



B.

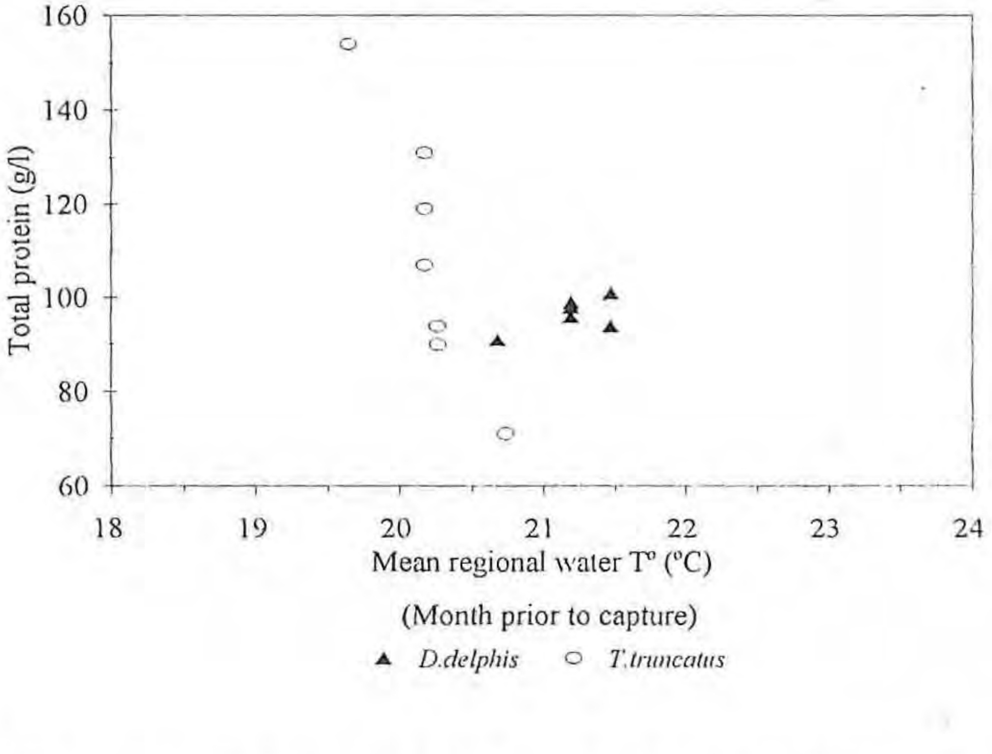


Figure 7.13: Significant correlations ($p<0.05$) between total protein and the total number of prey species in the stomach (A) and the mean regional water temperature of the month prior to capture for common (D. delphis) and bottlenose (T. truncatus) dolphins caught in shark nets between 1992 and 1996.

Albumin levels below 42.00 g/l and above 48.00 g/l were classified as outliers (box-whisker plot). An extremely low albumin level (8.00g/l) was measured in the same animal from which a total protein level of 71.00 g/l was recorded (see above). An adult female bottlenose dolphin, found to have parasites in the blubber and mammary gland, had an albumin level of 66.00 g/l. Fish and squid were found in the stomach of this animal. A juvenile male bottlenose dolphin, which had an empty stomach and swollen lymph nodes, as well as tumours in the pleural cavity, had an albumin level of 30.00 g/l. The exclusion of these values increased the similarity of mean albumin levels for common and bottlenose dolphins (Table 7.2). There was a potentially important age difference in albumin levels (F=6.98, p=0.02, df=8) (Table 7.2), although sample size for the different age groups was too small to confirm this.

Limited data prevented the identification of any meaningful relationships between albumin and morphology, diet and temperature. Extremely low albumin levels were however associated with reduced mean prey weight and low water temperature. Significantly elevated, outlying, albumin levels were, in contrast, associated with extremely heavy mean prey weight and warmer water temperatures.

C. Globulin

a. Bottlenose and common dolphins (pooled data)

Globulin levels measured in serum samples from bottlenose dolphins were markedly higher and more variable (63.90 ± 22.87 g/l) than those recorded from common dolphin samples (51.00 ± 4.43 g/l). However, this difference was not significant (t=-1.28, p=0.22, df=14) (Table 7.1). Two globulin concentrations, both from bottlenose dolphins, were classified as significant outliers. One animal had a globulin level of 110.00 g/l (associated with a total protein level of 154.00 g/l – see above); the other, with a globulin level of 89.00 g/l was the same animal for which an albumin level of 30.00 g/l was recorded. The exclusion of outliers, as was the case for albumin, increased the similarity of mean globulin levels for the two species (Table 7.2). Globulin was not significantly related to age, sex and/or reproductive condition. In conjunction, globulin was not significantly correlated with any morphological parameters. This was due primarily to a limited number of samples.

b. Bottlenose dolphins

Although numbers of analyses were low and therefore made definition of trends difficult, globulin levels of bottlenose dolphins were positively related to the total number of prey species in the stomach (r=0.80, p=0.002, n=7) (Fig. 7.14A). This increase was associated with a significant negative relationship between globulin and the mean reconstituted length of prey (r=-0.63, p=0.03, n=12) (Fig. 7.14C). This trend was difficult to confirm for different age groups, although globulin

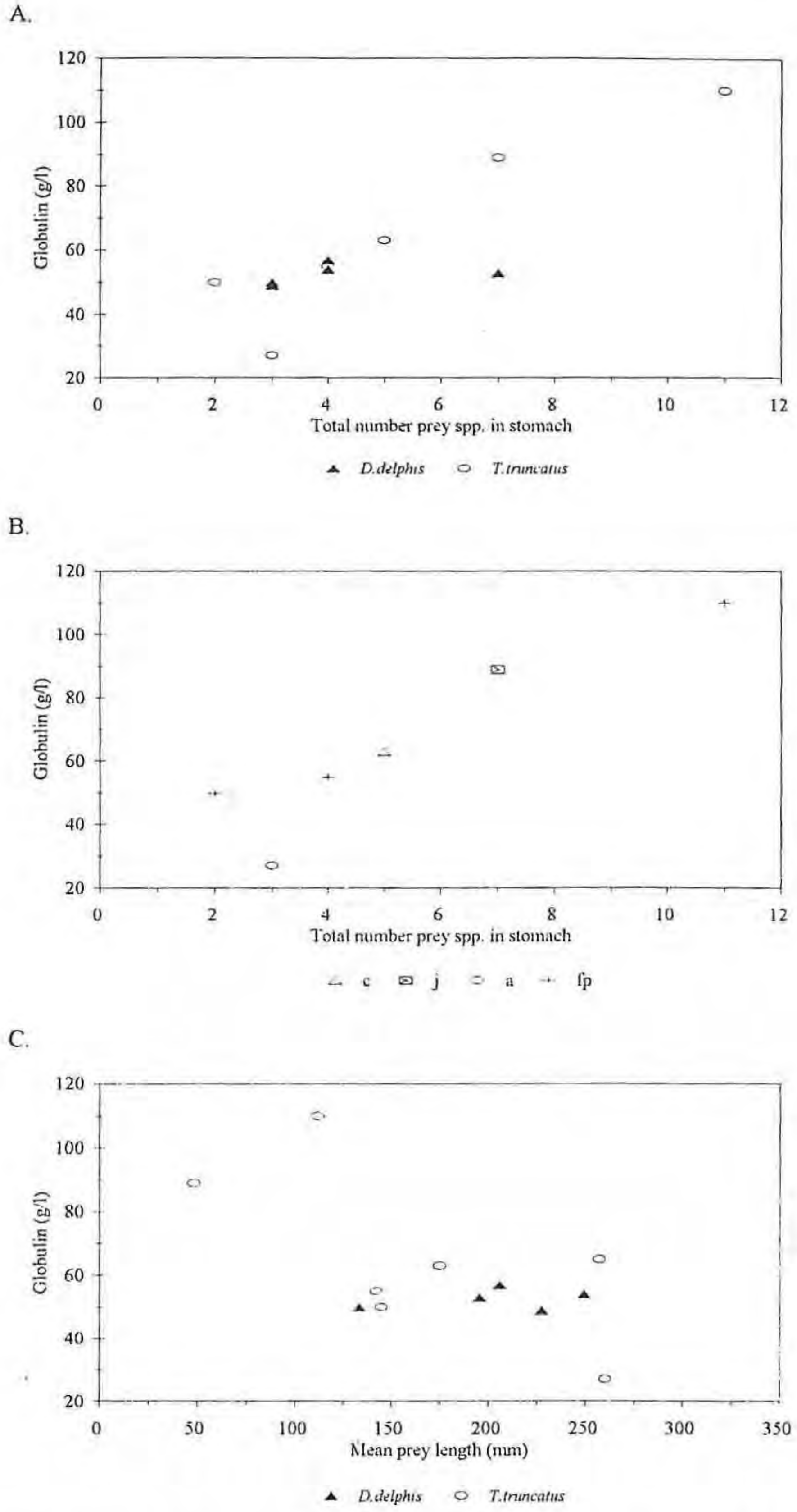


Figure 7.14: Significant correlations ($p<0.05$) between serum globulin and the total number of prey species in the stomachs of common (D. delphis) and bottlenose (T. truncatus) dolphins (A) and for the different age and reproductive classes of bottlenose dolphins (B); and between globulin and the mean reconstituted length of prey in the stomach for common and bottlenose dolphins (C) (c. calves; j. juveniles; a. adults; fp. pregnant females; fl. lactating females).

levels in pregnant females increased dramatically with a higher dietary diversity; and associated smaller mean prey size (Fig. 7.14B). Additional data, from a wider range of age and reproductive classes, are needed to further examine these relationships.

The relationship between globulin and water temperature was interpreted with caution, due to limited analyses and a restricted temperature range. However, levels of globulin in bottlenose dolphins were significantly lower at temperatures above 20.5°C, compared to those associated with water temperatures of approximately 19.5°C ($r=-0.57$, $p=0.02$, $n=10$) (Fig. 7.15).

D. Albumin:globulin

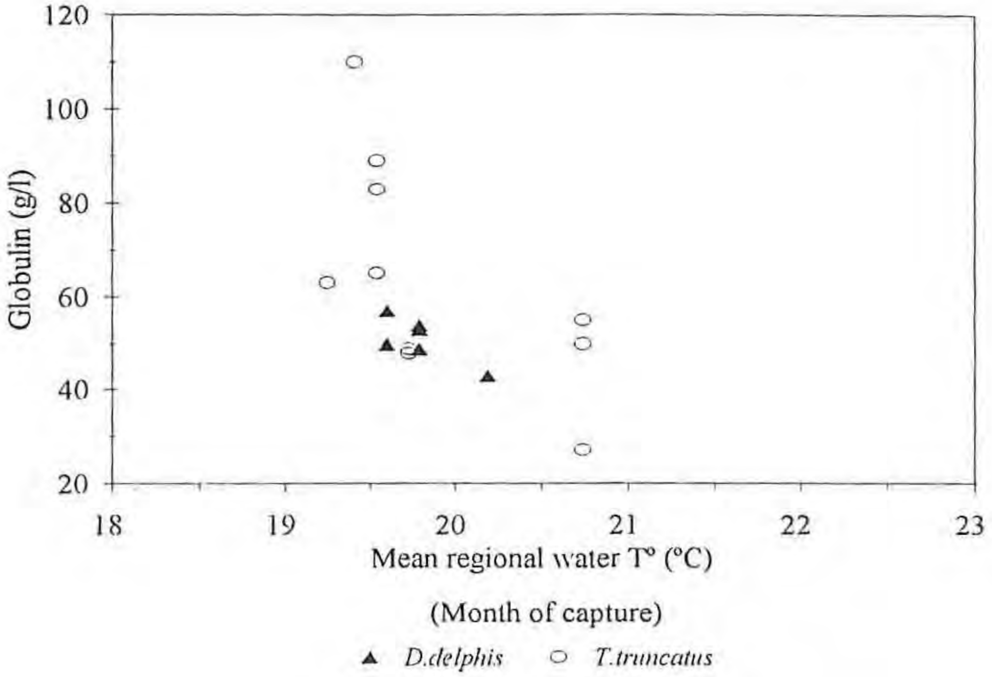
a. Common and bottlenose dolphins (pooled data)

The mean albumin: globulin ratio did not differ significantly between species (Table 7.1). However, when data classified as outliers were excluded (box-whisker plot), the mean albumin:globulin ratio of common dolphins (0.90 ± 0.12) was significantly higher than that of bottlenose dolphins (0.68 ± 0.23) ($t=2.19$, $p=0.05$, $df=12$) (Table 7.2). Outlying high (2.40) and low (0.13) ratios were removed from the analyses. These ratios were associated with a high albumin concentration (66.00 g/l) and low total protein (71.00 g/l) and albumin (8.00 g/l), respectively. This ratio was not significantly different between age and sex classes. Samples were too limited to verify the significant difference between mean albumin:globulin of pregnant (0.74 ± 0.20) and resting (1.14 ± 1.18) females ($t=-7.71$, $p=0.002$, $df=4$).

The ratio between albumin and globulin was not related to morphology and the only significant relationship between bottlenose dolphin albumin:globulin and diet (mean reconstituted prey weight) ($r=0.83$, $p=0.001$, $n=7$) was biased by a single extremely high ratio (2.40) associated with a mean prey weight of over 350g. All other ratios ranged between zero and one, when associated with prey sizes less than 200 g (Fig. 7.16A).

Albumin:globulin ratios, from both common and bottlenose dolphins, were positively correlated with the mean regional water temperature of the month of capture ($r=0.67$, $p=0.005$, $n=16$). Although the temperature range was limited, ratios showed a definite increase as water temperature increased between 19°C and 21°C (Fig. 7.16B). This trend was apparent within some age and/or reproductive groups, calves and pregnant females having higher albumin:globulin ratios at warmer water temperatures. More samples are needed to examine this trend further.

A.



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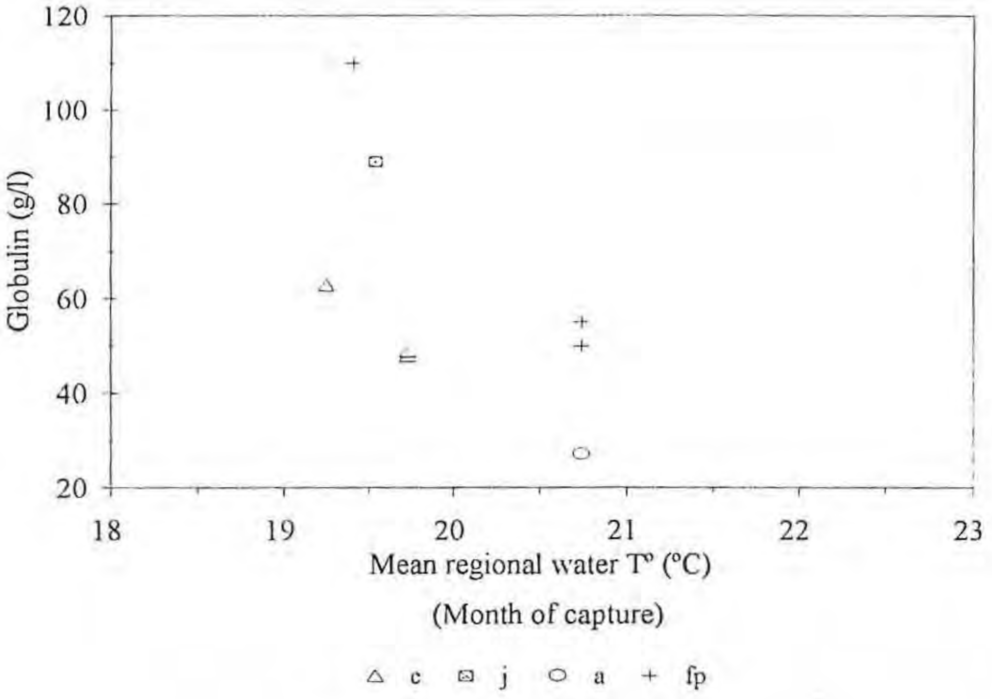
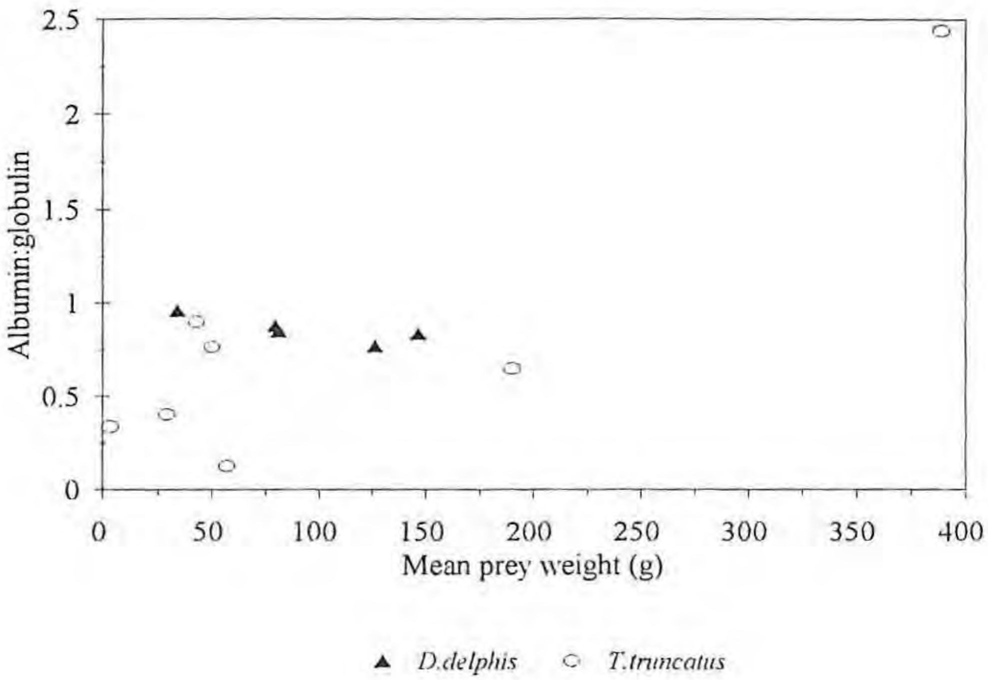


Figure 7.15: Significant correlation ($p<0.05$) between serum globulin and mean regional water temperature of the month of capture for common (D. delphis) and bottlenose (T. truncatus) dolphins (A) and for the different age and reproductive classes of bottlenose dolphins (B) (c: calves, j: juveniles, a: adults, fp: pregnant females).

A.



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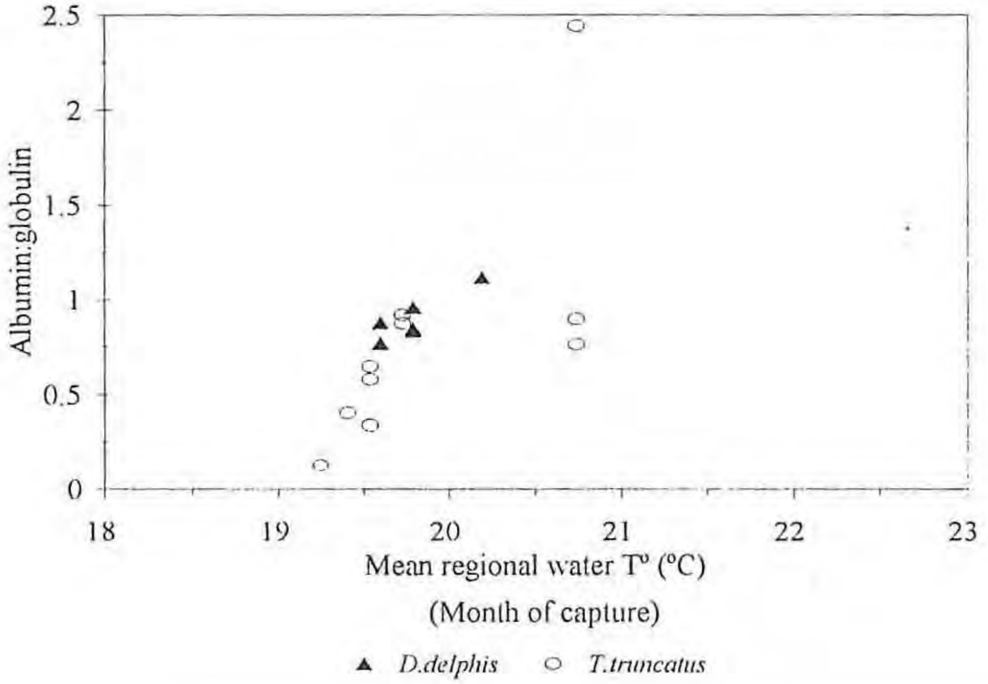


Figure 7.16: Significant correlations ($p<0.05$) between the albumin:globulin ratio and the mean weight of prey in stomachs (A); and the mean regional water temperature of the month of capture (B) for common (D. delphis) and bottlenose (T. truncatus) dolphins caught in shark nets between 1992 and 1996.

BLOOD SUGARS

A. Glucose

a. Common and bottlenose dolphins (pooled data)

Concentrations of plasma glucose were not significantly different between species before (Table 7.1) or after (Table 7.2) the exclusion of outliers. Six plasma glucose results, above 18.00 mmol/l, were classified as outliers (box-whisker plot). Four of the six animals with outlying high glucose levels in the blood showed no signs of recent feeding. There were no age-, sex- or reproductive-associated changes in this parameter.

Although plasma glucose was analysed for in numerous samples, very few significant relationships between this parameter and morphology, diet and water temperature were identified. When data from both species were combined, reduced glucose levels were associated with a greater number of prey species in the stomach (r=-0.34, p=0.02, n=46). The majority of outlying high glucose levels were associated with a low number of prey species in the diet but overall, glucose levels were highly variable (Fig. 7.17A).

b. Common dolphins

Glucose levels from common dolphins were negatively correlated with the mean local water temperature of the month prior to capture (r=-0.37, p=0.02, n=38). Data were highly variable but there was an overall decline in glucose levels as water temperatures increased (Fig. 7.17B).

LIPIDS

Due to logistical problems, lipids were measured irregularly and resulted in limited data and few relationships with independent variables. The need for additional data is acknowledged before defining any of the relationships described below.

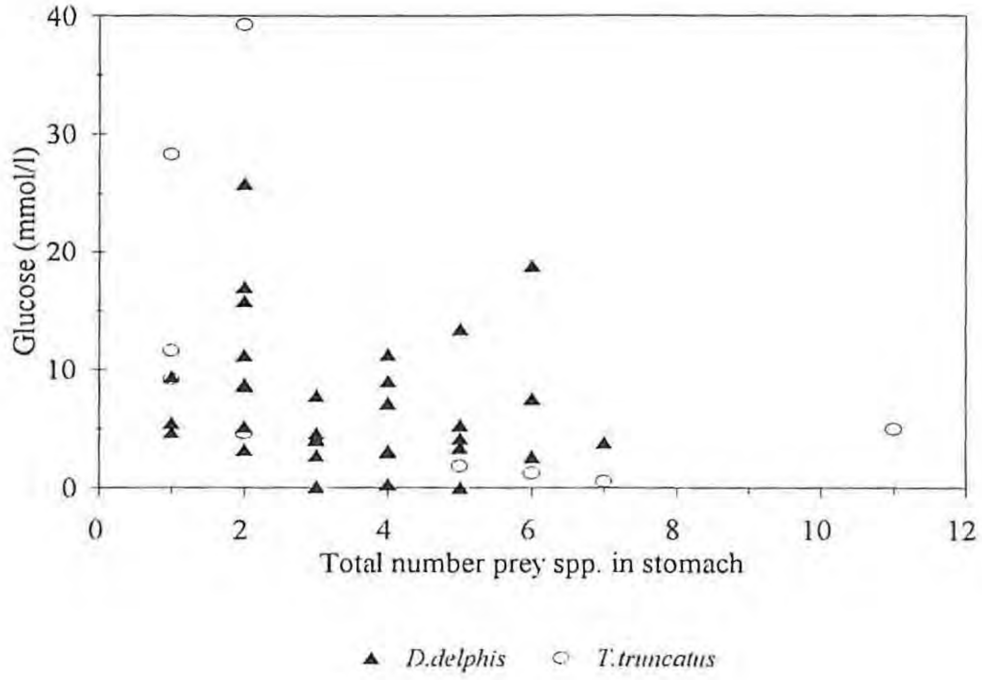
A. Cholesterol

a. Bottlenose and common dolphins (pooled data)

Mean cholesterol levels in common (4.72 ± 0.89 mmol/l) and bottlenose (4.18 ± 0.77 mmol/l) dolphins were similar (t=0.93, p=0.36, df=11) (Table 7.1). No outlying values were identified for this parameter. A low number of samples made any investigation of possible sex-, age- or reproductive-associated differences ineffective.

However, levels of cholesterol were significantly related with a number of morphological and dietary parameters. When data from both species was combined, elevated cholesterol concentrations were associated with thinner blubber at both the dorsal (r=-0.67, p=0.02, n=11) and ventral (r=-0.65,

A.



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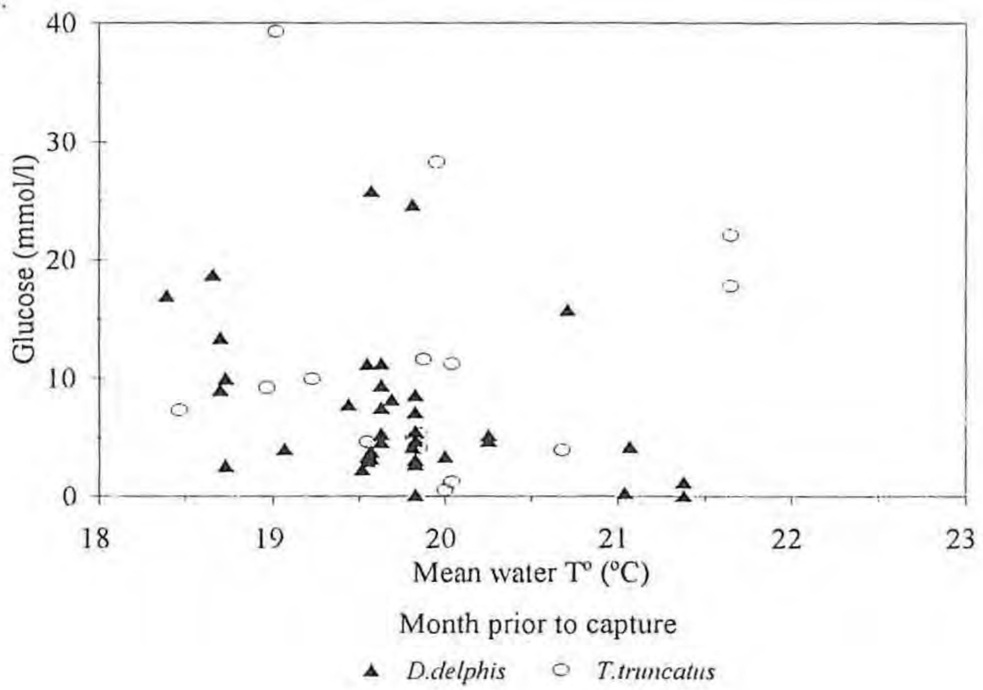


Figure 7.17: Significant correlations ($p<0.05$) between plasma glucose and the total number of prey species in the stomach (A) and the mean local water temperature of the month of capture (B) for common (D. delphis) and bottlenose (T. truncatus) dolphins caught in shark nets between 1992 and 1996.

p=0.03, n=11) sites (Fig. 7.18A). This trend was most distinct for bottlenose dolphins, with common dolphin cholesterol being variable over a limited range of blubber thickness measurements. Limited data made trends within age groups difficult to confirm, although cholesterol was reduced in a calf with thicker blubber (Fig. 7.18B). All animals with cholesterol concentrations below 4.5 mmol/l were adults.

b. Bottlenose dolphins

Bottlenose dolphin cholesterol was also negatively correlated with %M (r=-0.88, p=0.02, n=6). In contrast, although not significant (p>0.05), common dolphin cholesterol was higher when %M was greater (Fig. 7.18C).

c. Common dolphins

Common dolphin cholesterol concentrations increased significantly with increased mean reconstituted prey length (r=0.96, p=0.01, n=5). A similar trend was evident between cholesterol and the mean energy (r=0.91, p=0.03, n=5) content of prey in the stomach of this species (Fig. 7.19). Although more data are needed to further define these trends, adult males and pregnant females had higher cholesterol concentration when prey size, and dietary quality, was increased.

B. Triglycerides (TGL)

a. Bottlenose and common dolphins (pooled data)

Mean TGL levels were similar for both common (3.69 ± 0.76 mmol/l) and bottlenose (2.79 ± 0.78 mmol/l) dolphins (t=3.04, p=0.11, df=11) (Table 7.1). No TGL data were classified as outliers. There were no other significant age- or reproductive-related differences in levels of TGL.

b. Common dolphins

Common dolphin TGL concentrations were negatively correlated with total blubber weight (r=-0.96, p=0.003, n=6). Although data were limited, reduced TGL was associated with heavier blubber in juveniles, adults and pregnant females (Fig. 7.20). Common dolphin TGL concentrations were also significantly related to the mean, reconstituted prey length (r=0.95, p=0.01, n=5) and energy content of prey (r=0.90, p=0.04, n=5) (Fig. 7.21 A&B). Data were limited but this trend was evident for adult male and pregnant females.

c. Bottlenose dolphins

A positive correlation between TGL levels and water temperature for bottlenose dolphins (r=0.86, p=0.03, n=6) was biased by an outlying value, associated with a water temperature of 22.2°C

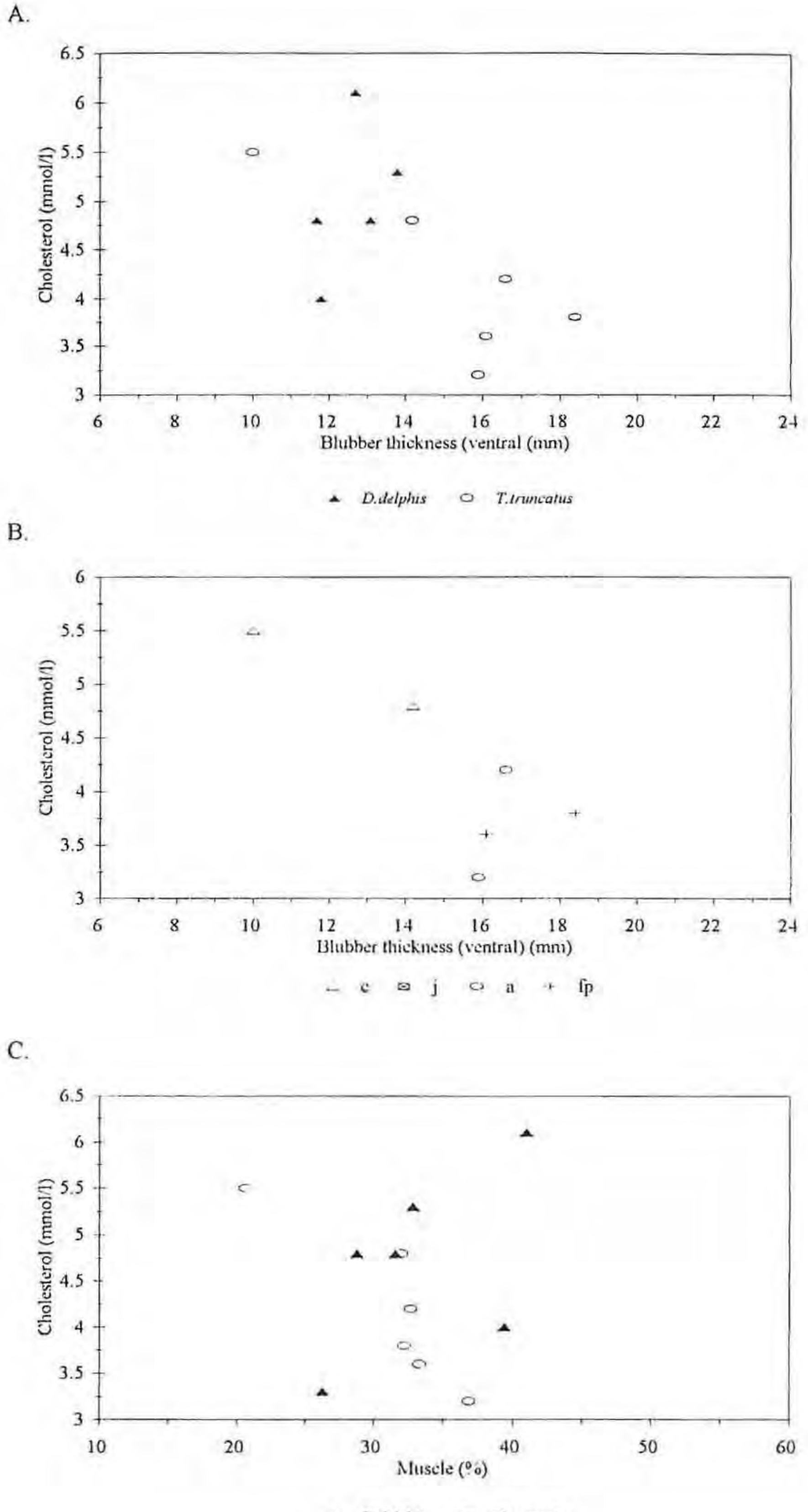


Figure 7.18: Significant correlations ($p<0.05$) serum cholesterol and ventral blubber thickness for common (D. delphis) and bottlenose (T. truncatus) dolphins (A), for the different age and reproductive classes of bottlenose dolphins (B); and between cholesterol and proportional muscle weight for common and bottlenose dolphins (C) caught in shark nets between 1992 and 1996 (c: calves; j: juveniles; a: adults; fp: pregnant females).

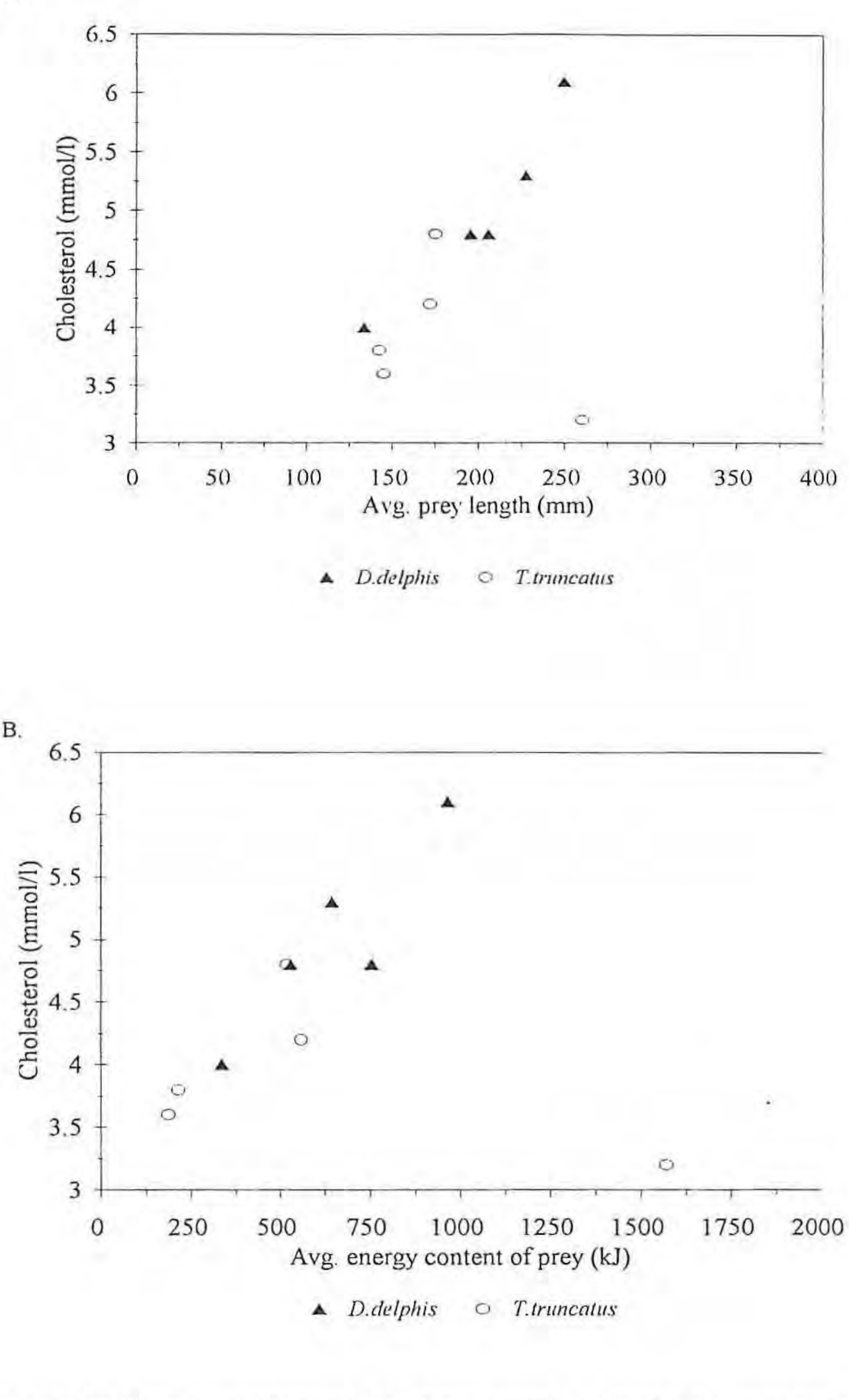
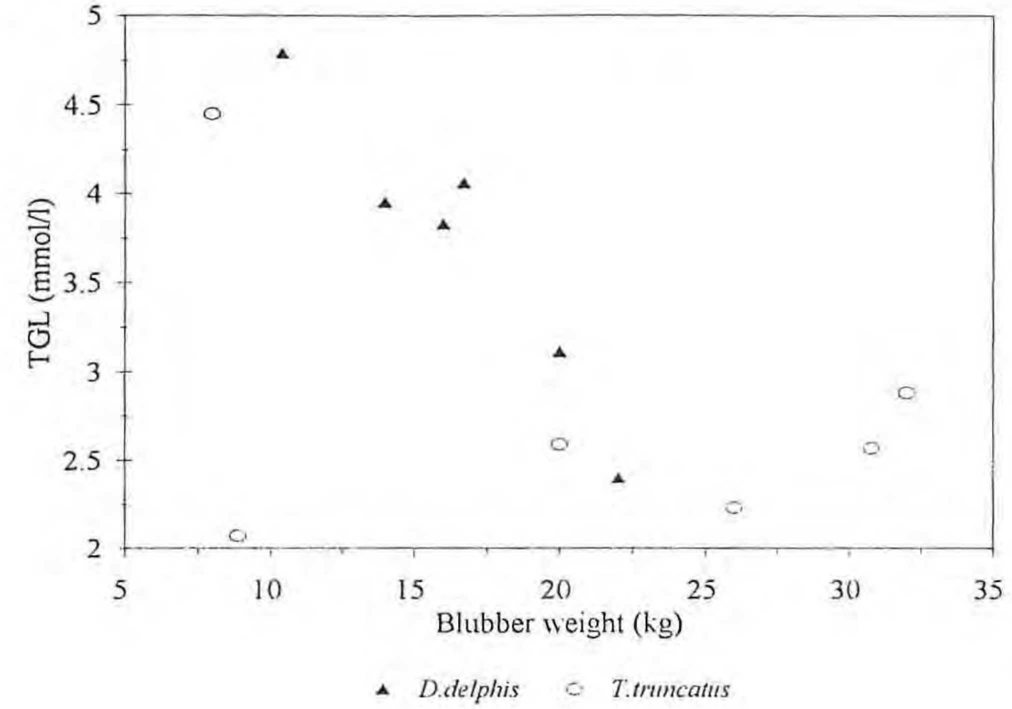


Figure 7.19: Significant correlations ($p<0.05$) between serum cholesterol and the mean length (A) and energy content (B) of prey in the stomachs of common (D. delphis) and bottlenose (T. truncatus) dolphins caught in shark nets between 1992 and 1996.

A.



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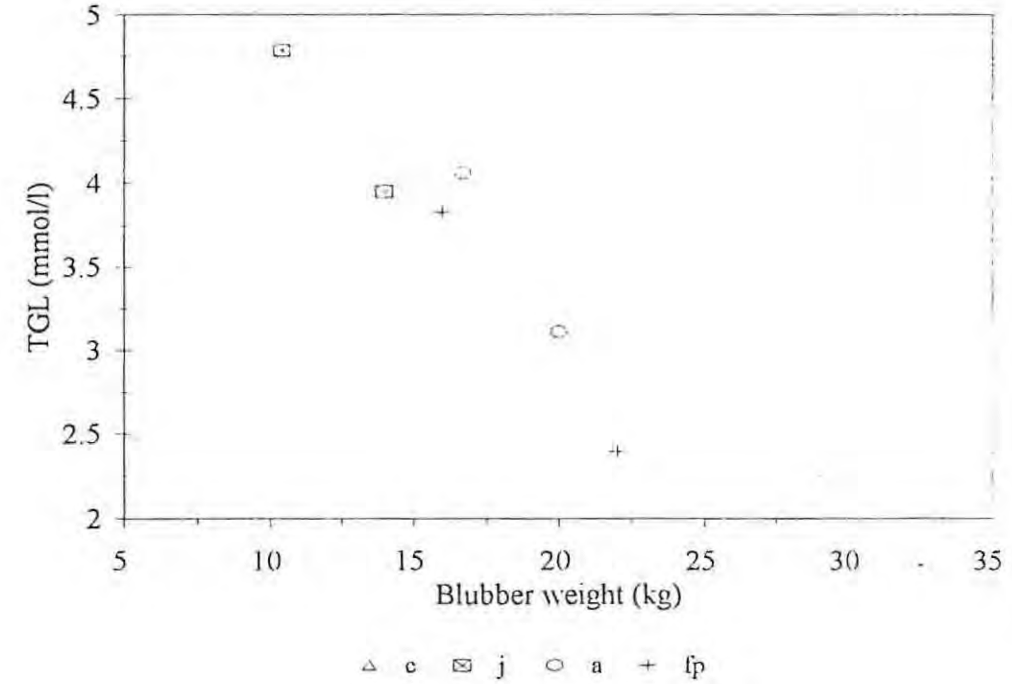

Figure 7.20: Significant correlations ($p<0.05$) between serum triglycerides (TGL) and total blubber weight for common (D. delphis) and bottlenose (T. truncatus) dolphins (A) and for the different age and reproductive classes of common dolphins (B) caught in shark nets between 1992 and 1996 (c: calves, j: juveniles; a: adults; fp: pregnant females).

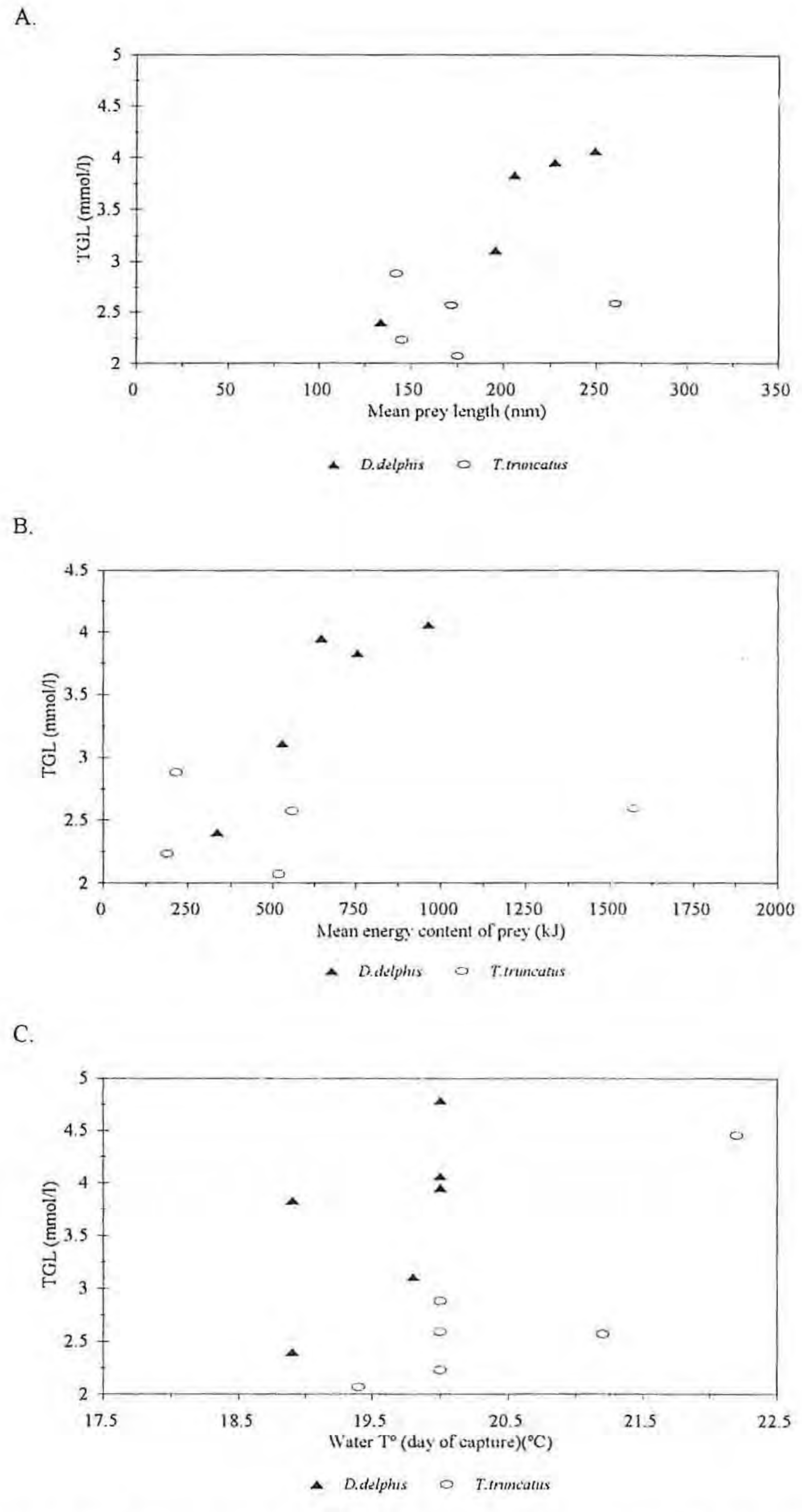

Figure 7.21: Significant correlations ($p<0.05$) between serum triglycerides (TGL) and the mean length (A) and energy content (B) of prey in the stomach; and between TGL and the local water temperature on the day of capture for common (D. delphis) and bottlenose (T. truncatus) dolphins caught in shark nets between 1992 and 1996.

(Fig. 7.21C). Overall, highly variable TGL levels at a water temperature of 20°C on the day of capture illustrated that TGL was not significantly affected by water temperature.

Additional lipid components, including HDL-cholesterol, LDL-cholesterol and cholesterol:HDL-cholesterol were measured in a small number of serum samples. However, the limited number of samples made any potential relationships with morphology, diet and temperature impossible to distinguish. Although some results were outlying (box-whisker plot), analyses were too few to gauge the significance of this. As a result, mean levels of these lipids are given purely for information (Table 7.1 and 7.2). Significant age-related differences in HDL-cholesterol and the cholesterol:HDL-cholesterol ratio were identified but without further data, these differences remain unconfirmed.

DISCUSSION

Assessments of blood chemistry and its relationship with physiological status in net-caught animals are complicated by *post-mortem* changes. In addition, the effects of stress on marine mammal blood chemistry are well documented (Geraci and Medway 1973, Geraci *et al.* 1979, Koopman *et al.* 1995, Heidel *et al.* 1996, Young *et al.* 1998). The potential influence of *post-mortem* and stress-related changes on the results presented in this study is acknowledged, but every attempt has been made to account for these changes wherever possible. Nevertheless, further investigations are needed to fully understand these effects, particularly if blood chemistry is to be successfully used in the assessment of condition in free-ranging marine mammals. As discussed in Chapter 6, the exact time at which dolphins are caught in the Kwazulu-Natal shark nets; and therefore the length of time which they have been dead prior to blood sampling, is unclear. The majority of bottlenose dolphins appear to be caught during early morning feeding, implying that net-caught specimens of this species have been dead between two and four hours before retrieval and blood sampling. There is no indication of the time of capture for common dolphins.

The creatinine values obtained for bottlenose dolphins during this study were similar, but generally slightly higher than values recorded in the literature for this species (Appendix 5). The elevation in values obtained during this study may be due either to stress, as discussed above and/or *post-mortem* changes, although the latter appear to be slight, in the short-term (Young, unpublished data). Unfortunately, there are no published data available for creatinine levels in common dolphins.

The significantly elevated creatinine levels in common dolphins were a direct reflection of the higher proportional muscle weights in this species (see Chapter 5). Increased creatinine has similarly indicated a greater muscle mass in humans (Kaplan and Pesce 1989) as well as other marine mammals (Bossart and Dierauf 1990, Wolkers *et al.* 1994a). This association is also reflected in positive relationships, within a species, between creatinine and other aspects of morphology, such as girth and blubber thickness. The relationships between creatinine concentrations and size have been recorded in larger whale species (Cornell 1983, Heidel *et al.* 1996). Increased concentrations of creatinine with increased anal girth in common dolphins and increased blubber thickness in bottlenose dolphins complies with the size-related increase in creatinine, associated with a greater muscle mass. Creatinine, however, was not directly related to total muscle weight. It must be noted that bottlenose dolphins are typically larger than common dolphins (Ross 1984), and yet they have significantly lower creatinine levels. Therefore, creatinine appears to be an indicator of muscle proportion within a species, rather than total muscle weight. Higher creatinine levels in common dolphins may also be related to their relatively higher levels of activity. Common dolphins are more slender and streamlined, swim faster and dive deeper than bottlenose dolphins (Ross 1984). With increased muscular activity more creatinine is produced and as a result, levels of activity are reflected in serum creatinine concentrations (McConnell and Vaughan 1983). The higher degree of variability in data from common dolphins may therefore reflect different individual levels of activity.

However, this variability may also illustrate that common dolphins are more vulnerable to stress and their increased creatinine levels may be indicative of muscle damage, which occurs as a result of the increased muscular activity during entanglement. A similar increase in creatinine has been recorded from harpooned or restrained marine mammals (Medway 1983, De Monte and Pilleri 1983, Koopman *et al.* 1995). In light of the marked *post-mortem* increase in creatinine levels (in the long-term) (Young, unpublished data), elevated, and highly variable, creatinine results may also illustrate that net-caught common dolphins have been dead longer than bottlenose dolphins, before retrieval from the nets – changes in creatinine levels reflecting a longer *post-mortem* period.

Creatinine is reportedly unaffected by diet (Kaplan and Pesce 1989, Bossart and Dierauf 1990) and results obtained from this study show no significant changes in creatinine with different levels of food consumption and different components of dietary energy. Elevated creatinine levels have been associated with kidney disease (Bossart and Dierauf 1990) and dehydration (Medway *et al.* 1982) in marine mammals. In terrestrial species, elevated creatinine during fasting has been attributed to reduced renal blood flow resulting from reduced protein intake (DelGuidice *et al.* 1987b, Wolkers *et al.* 1994a). Factors, associated with extremely high creatinine levels during this study, included lactation, which may have resulted in the animals being dehydrated.

Levels of BUN measured during this study were within the ranges previously reported for bottlenose dolphins, but higher than most other species of small cetacean (Appendix 5). Mean BUN levels in this study were almost identical to those recorded from a free-ranging population in Florida, U. S. A. (Asper *et al.* 1990). BUN levels measured from common dolphins during this study were also similar to that reported by De Monte and Pilleri (1977) (Appendix 5). A positive relationship between BUN and the number of prey items (and the total energy content of the diet) in the stomach implies that BUN in the blood of free-ranging dolphins along the Natal coast is related to diet. The relationship between BUN and diet is well documented in marine (Anderson 1968, Ridgway *et al.* 1970, De Monte and Pilleri 1972, 1977, Lee *et al.* 1977, Medway and Geraci 1978, Geraci *et al.* 1979, Ronald and Kay 1982, McConnell and Vaughan 1983, Cornell 1983, Medway and Cornell 1989, Asper *et al.* 1990, Bossart and Dierauf 1990, Castellini *et al.* 1993) and terrestrial mammals (Franzmann 1972, Torell *et al.* 1974, deCalesta *et al.* 1975, Kirkpatrick *et al.* 1975, Seal *et al.* 1975, 1978b, Melton and Melton 1982, Warren *et al.* 1982, Lochmiller *et al.* 1985d, 1986, DelGuidice *et al.* 1987b, Hellgren *et al.* 1993, Knick *et al.* 1993, Zaugg *et al.* 1993, Wolkers *et al.* 1994a). The fact that animals of the same age group have higher BUN levels when feeding levels increased; and diet quality (energy content) was improved reflects the usefulness of this parameter as an indication of dolphin nutritional condition. The similarity in BUN levels between the two dolphin species implied that the two dolphin species had similar dietary compositions. However, this needs further investigation, as the mean energy value of prey consumed by common dolphin was significantly higher than that of bottlenose dolphins (Chapter 5). In this respect, it may be the protein, and not the energy, content of the diet, which determines BUN. In some terrestrial mammals, BUN increases appear to be proportional to protein increase in the diet (Franzmann 1972, Kirkpatrick *et al.* 1975).

All outlying BUN values measured during this study were reduced, rather than increased, reflecting that these outlying values relate to a reduction in dietary quality and/or food intake. Several of these outliers were recorded from animals with no signs of recent feeding in their stomachs. This reinforces the use of BUN as an indicator of nutritional condition. The monitoring of BUN may be useful in the interpretation of physiological adjustments to seasonal and regional changes in dietary diversity and/or abundance; and associated changes in nutritional and morphological condition. Depression of BUN levels has been related to low protein diets and starvation (Lochmiller *et al.* 1985d, 1986, Bossart and Dierauf 1990, Castellini *et al.* 1993), due mainly to lipid depletion and subsequent catabolism of muscle tissue. In this context, the analysis of BUN in free-ranging marine mammals may prove invaluable in assessing and monitoring the status of the marine environment, particularly with respect to food resources. BUN has been used successfully as an indicator of nutritional status and habitat quality in the terrestrial environment (Franzmann 1972, Skeen 1974 in Kirkpatrick *et al.* 1975, Seal *et al.* 1978a, 1978b, Hellgren *et al.* 1993). Although the ratio between BUN and creatinine has proved to be a useful index of condition in other studies (Berkow 1982, Kaplan and Pesce 1989, Medway and Geraci 1978), the stress- and *post-mortem*-related changes, which may occur in creatinine reduces the effectiveness of this particular ratio in net-caught animals (De Monte and Pilleri 1977, Young *et al.* 1998, Young, unpublished data).

Levels of ALP, measured for bottlenose dolphins, during this study were dramatically higher than those recorded in the literature for this species (Appendix 5). However, the present results complied with the conclusion of Thurman and van der Elst (1995), who report that values above 700 U/l are indicative of a healthy (captive) bottlenose dolphin. Elevations of ALP in marine mammals are assumed to indicate liver damage (Medway and Geraci 1978, Geraci and St. Aubin 1979, Thurman and van der Elst 1995), intrinsic bone pathology or intestinal inflammation (Thurman and van der Elst 1995). However, there were no visible signs of problems such as these that would explain the markedly higher ALP values recorded during this study. ALP levels are reported to increase during the final stages of pregnancy (Thurman and van der Elst 1995), although no significant reproductive-associated changes were identified.

ALP concentrations in dolphins from this study were highest in juveniles and calves, associated with rapid bone growth and increased metabolism (Seal *et al.* 1978a, Lochmiller *et al.* 1986, 1988, Kaplan and Pesce 1989). The relationship between age and ALP is well documented for marine (Anderson 1968, Lee *et al.* 1977, Geraci *et al.* 1979, MacDonald 1981 in Bossart and Dierauf 1990, McConnell and Vaughan 1983, Asper *et al.* 1990, Bossart and Dierauf 1990, Fothergill *et al.* 1991, Rhinehart *et al.* 1992, Heidel *et al.* 1996) and terrestrial mammals (Seal *et al.* 1975, 1978b, Melton and Melton 1982, Lochmiller and Grant 1984). However, the mean ALP level measured for adults during this study was similar to that of juvenile animals from other regions (Rhinehart *et al.* 1992, Asper *et al.* 1990). The increased activity of ALP in dolphins during this study, may be caused primarily by *post-mortem* changes in the blood, with preliminary investigations showing that levels of this enzyme increase dramatically after death (Young, unpublished data). It is also possible that this enzyme was elevated as a result of net-capture stress (Young *et al.* 1998). Although further analyses are needed, possible *post-mortem* changes, together with the lack of any defined relationships with morphology, diet and/or water temperature reduces the usefulness of this enzyme in the condition assessment of net-caught dolphins.

Levels of LDH and CPK measured for bottlenose dolphins during this study were markedly higher than those previously measured (Appendix 5). Although the different use of units and the unknown status of some of the study animals used in the literature prevented any conclusive comparisons, the increased LDH and CPK levels are more than likely a reflection of *post-mortem*- and capture stress-associated changes. The significantly higher CPK levels in common dolphins implies a longer time period between death and retrieval and/or a greater stress response. LDH and CPK are rapidly elevated by stress (Geraci and Medway 1973, Lee *et al.* 1977, Seal *et al.* 1975, Ronald and Kay

1983, Williams and Pulley 1983, Lochmiller and Grant 1984, DelGuidice *et al.* 1987b, Bossart and Dierauf 1990, Thurman and van der Elst 1995, Young *et al.* 1998). Increased LDH and/or CPK in marine mammals is generally associated with cell damage and necrosis, excess muscular activity, and extensive damage to skeletal or cardiac muscle (Ridgway *et al.* 1970, Geraci and St. Aubin 1979, Medway *et al.* 1982, De Monte and Pilleri 1983, Bossart and Dierauf 1990, Heidel *et al.* 1996). Both enzymes, and their ratios with creatinine, showed some interesting relationships with morphology, particularly those related to muscle. However, the obvious variability and stress- and *post-mortem*-associated changes diminish the usefulness of these particular enzymes as condition indices in captured or restrained marine mammals. LDH and CPK have proved to be useful in condition (and habitat) assessment of terrestrial species (Seal *et al.* 1978a) but a similar use in net-caught dolphins (and marine mammals generally) is not recommended.

Sodium levels measured for net-caught bottlenose dolphins were within the range recorded by Ridgway (1965), but were generally lower than those previously documented (Appendix 7). No comparative data were available for common dolphins. The reduced sodium concentrations in net-caught dolphins were thought to be a result of *post-mortem* changes and stress. Sodium concentrations decreased with time after death (Young, unpublished data) and many stress-related responses in marine mammals are characterised by a reduction in sodium (Geraci 1972, Geraci and Smith 1975, Engelhardt and Geraci 1978, Geraci *et al.* 1979, Engelhardt 1979, Ronald and Kay 1982, Medway 1983, Heidel *et al.* 1996).

Sodium levels were not related to any aspect of morphology, although higher sodium levels have been associated with improved condition in ringed seal pups (*Phoca hispida*) (Geraci *et al.* 1979). Sodium levels were slightly elevated when prey weight and dietary energy value was reduced, implying a relationship between dietary quality and serum sodium. Additional analyses are, however, needed to confirm this trend. Dietary composition and quality has previously been inversely related with sodium levels (Geraci and Smith 1975, Medway and Cornell 1989). Prolonged fasting can lead to haemoconcentration and increases in serum sodium (Medway and Geraci 1965, 1978, Clarke 1990, Thurman and van der Elst 1995). In the same context, it is possible that changes in sodium concentrations (in relation to diet) only become evident following a longer-term change in diet quality. Stomach contents from net-caught dolphins are typically indicative of feeding in the 24 hours prior to capture (Ross 1984).

Potassium is an important cation in the intracellular fluid. Serum concentrations have been found to approximate lean body mass in humans and therefore play an important role in assessing overall human condition (Kaplan and Pesce 1989). Serum potassium has been used as an indicator of muscle integrity in bottlenose dolphins (Thurman and van der Elst 1995). Dietary-related changes in potassium have been documented in marine mammals (Bossart and Dierauf 1990, Thurman and van der Elst 1995). However, although serum potassium is potentially useful as an index of marine mammal condition, the fact that only two results were obtained for serum potassium during this study highlights the limited use of this parameter as an index of condition in net-caught dolphins. This parameter undergoes rapid and dramatic *post-mortem* changes (Young, unpublished data) and is also significantly affected by stress (Medway and Geraci 1978, Medway 1983, Heidel *et al.* 1996, Young *et al.* 1998). Although potassium is an important physiological electrolyte, its use in physiological investigations of net-caught animals is severely limited by the influence of stress and *post-mortem* changes. But, if ways can be found to monitor this parameter in free-ranging animals, it should be considered.

Calcium values obtained for bottlenose and common dolphins during this study were similar to those recorded in the literature (Appendix 7). A decline in calcium levels with age has been previously documented, with higher calcium levels in calves associated with bone growth (Heidel *et al.* 1996). Extremely low calcium levels measured during this study originated from animals with no signs of recent feeding or ulcerated stomachs, reflecting the importance of dietary calcium intake on serum calcium levels. Calcium levels in humans, are partially dependent on dietary calcium intake, gastro-intestinal absorption and renal excretion (Kaplan and Pesce 1989). A reduced calcium level in marine mammals has been associated with renal failure and generalised malabsorption (Thurman and van der Elst 1995). Lactation also appeared to have a significant effect on calcium levels. It is possible that calcium stores in lactating females were depleted in an effort to maximise and maintain the calcium content of milk produced for the suckling calf.

Mean levels of total protein measured for common and bottlenose dolphins were higher than the overall mean for marine mammals (Appendix 7). Early studies calculated the mean total protein level in marine mammals to be 79.60 g/l, with a physiological range of 60-90 g/l (Geraci *et al.* 1968, Engelhardt 1979, De Monte and Pilleri 1982). Although outlying total protein data obtained from this study was associated with extremely high or low morphological and dietary values, overall, total protein did not appear to be significantly affected by morphology, diet and/or water temperature. Measurement of total protein has been found to be of little diagnostic value in terrestrial and marine mammals, as it can be affected by plasma volume – with no change in the albumin:globulin ratio (DelGuidice *et al.* 1987b, Thurman and van der Elst 1995). The different functions of serum proteins have made it necessary and valuable to assess protein status in terms of the different protein fractions, particularly albumin and globulin (Geraci *et al.* 1968, Medway and Geraci 1978, Engelhardt 1979, De Monte and Pilleri 1982). The ability to assess nutritional status, and hence animal condition, by measuring serum levels of different protein fractions has long been recognised in terrestrial mammals (Lochmiller *et al.* 1985b, 1988).

Albumin concentrations recorded for bottlenose dolphins during this study were predominantly higher than previous records from free-ranging animals of the same species, although results were similar to those of Rhinehart *et al.* (1991) (Appendix 7). Incidental capture of dolphins in shark nets along the Kwazulu-Natal coast reportedly occurs when animals are feeding around the nets, which act as fish aggregating devices (FAD's) (Cockcroft 1994). The increased levels of albumin, in animals of this study, above those in the literature could reflect the recent intake of food and associated digestive processes, prior to capture. Albumin has been found to be a valuable guide to nutrition in terrestrial (Hyvarinen *et al.* 1975, Seal *et al.* 1978b, Warren *et al.* 1981, 1982, Lochmiller *et al.* 1984, 1985b, 1985d, 1986, Wolkers *et al.* 1994) and marine mammals (Kuiken 1985, Medway and Cornell 1989, Thurman and van der Elst 1995). The multifunctional role of albumin (nutrition, maintenance of oncotic pressure, transport of calcium, unconjugated bilirubin and free fatty acids) makes this protein an important analyte of liver function (Kaplan and Pesce 1989, De Monte and Pilleri 1982).

Albumin levels imply that juvenile animals have a higher nutritional status than other age groups. This would seem reasonable in terms of the energetic demands of juvenile growth and puberty. Lower total protein values were recorded in younger captive killer whales (*Orcinus orca*) (Cornell 1983) and common seals (*Phoca vitulina*) (McConnell and Vaughan 1983). However, similar differences in albumin have not been recorded previously in marine mammals. Older chacma baboons (*Papio ursinus*) were found to have lower albumin concentrations (Melton and Melton 1982). Outlying albumin values in this study were associated with lack of recent feeding and the presence of parasites, emphasising the relationship between diet, digestion and serum levels of this parameter. Albumin is reduced during lack of adequate diet and malnutrition and as a result of gastrointestinal parasites (Bossart and Dierauf 1990). Increased albumin has been related to dehydration (Bossart and Dierauf 1990, Thurman and van der Elst 1995). The association between an outlying albumin value and enlarged lymph nodes in one animal indicated that there may be a relationship between albumin and stress and/or shock (Bossart and Dierauf 1990, Thurman and van der Elst 1995).

Globulin concentrations were higher than those previously recorded for free-ranging bottlenose dolphins (Rhinehardt *et al.* 1991, 1992) (Appendix 7). Globulin does not undergo any marked *post-mortem* changes in the short term (Young, unpublished data) and, in conjunction with albumin data (which is not dissimilar to published data), increased globulins implied a greater level of immunological stimulation. Globulins increase with liver disease and the stimulation of the immune system (Asper *et al.* 1990, Bossart and Dierauf 1990). Globulin distribution is reportedly species specific (Geraci *et al.* 1968, Ridgway 1972, Engelhardt 1979) but it is unknown whether the elevated globulin levels in dolphins along the south-east coast of South Africa is a species difference or, rather reflects a habitat difference, in terms of immunological response. Pollutant loads in dolphins along the Kwazulu-Natal coast are reportedly high enough to interfere with reproduction (Cockcroft *et al.* 1989) and, in this respect, the elevated globulin levels may be linked to marine pollution in the area. If this can be confirmed, globulin levels may prove to be useful in assessing and monitoring not only dolphin health but also that of the surrounding marine habitat.

Outlying high globulin values were associated with a high dietary diversity, coupled with a smaller mean prey size, implying a higher degree of opportunistic feeding. These high globulin levels were also associated with reduced albumin levels, emphasising the interaction between nutritional (as measured by albumin) and immunological (as measured by globulin) condition. Albumin:globulin ratios calculated for common and bottlenose dolphins during this study were lower than all but two previous records (Appendix 7). These differences suggest that the nutritional status of the dolphins used during this study was below that of animals used in prior studies, as increased albumin:globulin ratios have been associated with good nutritional condition (Medway *et al.* 1982, Asper *et al.* 1990) and the relationship between albumin and globulin has long been used to investigate protein status in mammals (Geraci *et al.* 1968, Ridgway 1972, Medway and Geraci 1978, Engelhardt 1979). However, the reduced ratios calculated during the study were due primarily to the markedly increased globulin levels, as discussed above. Although higher albumin levels reflected that net-caught animals had generally been feeding regularly and on high quality food, it appears that the increased immunological challenge, and related elevated globulin concentrations, resulted in a reduced albumin:globulin ratio, interpreted as reduced nutritional condition and protein status (Geraci *et al.* 1968, Ridgway 1972, Medway and Geraci 1978, Engelhardt 1979). These interpretations are based on limited data and although these parameters show potential as condition indices, further investigations are needed to verify the relationships.

Glucose concentrations measured during this study fall within the range reported for other marine mammals. Normal values for blood sugar in dolphins range between 100-150 mg% (5.60-8.40 mmol/l) (Ridgway *et al.* 1970). The analysis of plasma glucose in cetaceans provides an approximate assessment of basic glycaemia, however glucose is also dramatically affected by stress (Ridgway *et al.* 1970, De Monte and Pilleri 1977, Thurman and van der Elst 1995). However, glucose levels of several species of cetacean have been found to be similar to that of man (De Monte and Pilleri 1977).

All significantly low glucose levels measured during this study were associated with animals that had empty stomachs, implying that glucose was reduced in animals that had not recently fed. Increased glucose in net-caught dolphins was associated with a low dietary diversity (indirectly related to a more selective diet and an improved dietary composition (Chapter 5)). Similar relationships between glucose and food intake have been recorded in other marine mammals (Kuiken 1985, Nordoy *et al.* 1993, Castellini *et al.* 1993, Thurman and van der Elst 1995). High levels of glucose in marine mammals have been attributed to a fish diet (high in protein and fat) (Bossart and Dierauf 1990). Levels of glucose in fasting marine mammals are normally higher than those seen in terrestrial mammals, reportedly an adaptation to deep and prolonged diving, to assure adequate nutrient supplies to the brain (Ridgway *et al.* 1970, Bossart and Dierauf 1990).

Highly variable data prevented the establishment of any other meaningful relationships between glucose and independent variables. The association between low glucose levels and warmer water temperatures may reflect a change in activity levels in waters of different temperatures, although this was not confirmed. Glucose values in marine mammals are typically highly variable within species, and even within individuals, depending on the state of activity and fasting interval before sampling (Medway and Geraci 1978). Variable glucose levels in Steller sea lion pups (*Eumetopias jubatus*) were thought to reflect the time since the pups had last nursed (Castellini *et al.* 1993). These changes reduce the potential usefulness of this parameter as a condition index. However, in light of the similarities of present results with other studies, and potentially important relationships between glucose and nutrition, further examination of the use of plasma glucose in the assessment of condition is needed.

Although analyses of serum lipids during this study were limited, the fact that lipids do not appear to change dramatically *post-mortem* or with stress, suggests that the analysis of serum lipids, in conjunction with diet and blubber composition analyses, may provide an important insight into some of the most important aspects of dolphin condition. The use of serum lipids as a tool in the assessment of marine mammal condition has proved potentially valuable, with earlier studies showing relationships between the diets of different marine mammal species and their respective serum lipid profiles (Nelson 1970, De Monte and Pilleri, 1982).

The mean concentrations of cholesterol measured in the serum of net-caught common and bottlenose dolphins were only slightly lower than those reported for bottlenose dolphins by Davis *et al.* (1991). Cholesterol content in marine mammals varies with species, possibly related to metabolic rate and body size, ranging from 4.94 ± 0.05 mmol/l in bottlenose dolphins to 8.40 ± 0.68 mmol/l in the Californian sea lion (*Zalophus californianus*) (Davis *et al.* 1991). Although there were no age and/or sex differences in cholesterol measured in dolphins during this study, cholesterol in human males is higher than premenopausal females. After menopause, cholesterol is higher in females (Kaplan and Pesce 1989). Comparative age differences in cholesterol for marine mammals are not available. TGL levels measured during this study were markedly higher than those recorded previously (De Monte and Pilleri 1983, Davis *et al.* 1991, Schumacher *et al.* 1992). TGL levels in marine mammals range between 0.49 ± 0.08 mmol/l in harbour seals and 1.33 ± 0.23 mmol/l in killer whales (Davis *et al.* 1991, Schumacher *et al.* 1992). Exact reasons for this difference are unclear but it may relate to the fact that many of the dolphins in this study were actively feeding a short time prior to capture, whereas records in the literature may originate from fasted animals; or from animals for which the fasting period is not known. Increased levels of TGL's in the human diet tend to promote cholesterol absorption. Increases are relatively non-specific, but, like cholesterol, levels do vary with age (Kaplan and Pesce 1989).

In adult humans, the liver and intestinal wall supplies over 90 % of endogenous cholesterol and the amount of cholesterol absorbed from the diet is self-regulating (Kaplan and Pesce 1989). Cholesterol, and TGL, levels, in both species of dolphin, during this study were similar, although levels in common dolphins were slightly higher, reflecting the significantly different energy, and fat, content of the common dolphin diet (see Chapter 5). It has been assumed that marine mammal cholesterol, like that of humans, is only partially affected by diet; and endogenous biosynthesis of cholesterol is the major factor in determining the amount of blood cholesterol in marine mammals (Ridgway et al. 1970). However, despite this, cholesterol and TGL levels, during this study, appeared to increase with a greater intake of dietary energy. Levels of TGL vary widely with diet in marine mammals (Kuiken 1985, Bossart and Dierauf 1990). Both cholesterol and TGL in marine mammals have previously been found to be diet related (Asper *et al.* 1990). Fish vary considerably in fat and protein composition (Geraci 1977, Reiter and Crissey 1991, Walsh and Dover 1997) and changes in serum lipids have been attributed to differences in diet type and levels of consumption (Nelson 1970, Asper *et al.* 1990). Feeding resulted in increased TGL's in the blood of seals. Neonatal harbour seals (*Phoca vitulina*) and California sea lions had very low TGL levels following prolonged fasting (Kuiken 1985, Bossart and Dierauf 1990). No reproductive-related changes were observed for TGL during this study, although the number of samples analysed for serum lipids prevented any in-depth investigation of reproductive-related trends.

This work has shown a potentially important relationship between serum lipids and blubber condition. This needs to be examined further, especially as it has been shown that pregnancy causes an increase in TGL in bowhead whales (*Balaena mysticetus*) (Heidel *et al.* 1996) and harbour seals (Kuiken 1985). The need for further investigation is emphasised by the increased blubber stores measured in pregnant females (Chapter 5). Both cholesterol and TGL were significantly related to morphological parameters, especially those measures of blubber condition. The relationship between serum lipids, blubber condition and composition, and dietary content is potentially vital in the assessment and monitoring of condition in marine mammals. Many species of marine mammal undertake extensive migrations, during which the female does not feed. In this respect, the important role of fat deposits in these animals has long been realised (Lockyer 1986). Therefore, large-scale mobilisation and consumption of deposited fat is responsible for the provision of calories and metabolic water (De Monte and Pilleri 1982). For this reason, the role that lipoproteins and lipids play in lipid metabolism and transport in marine mammals is extremely important. Protein and lipid metabolism in cetaceans is more active than in land mammals, mainly because of the energy required for the huge muscles which are constantly in motion, but also to meet thermoregulatory demands (Slijper 1962 in De Monte and Pilleri 1977).

Summary

Many blood chemistry components of net-caught dolphins are severely influenced by the stress of entanglement and drowning; and *post-mortem* changes in the blood. The former is particularly true for serum enzymes, which are elevated rapidly during capture. However, several parameters were not significantly influenced by stress and/or *post-mortem* changes (although the latter does need further investigation). These include BUN, albumin, globulin and albumin:globulin and lipids, such as total cholesterol and TGL. The results of this study show that all these parameters are potentially valuable indicators of condition in net-caught and/or free-ranging dolphins, particularly with respect to nutritional status. Further detailed studies of the relationships between serum lipids, blubber composition and condition, diet quality and nutritional status will provide an invaluable index of condition which may be further developed to produce a non-invasive index of condition for use in the field. In this context, these parameters may reflect habitat and food resource quality within a particular region, based on the physiological and morphological condition of the top marine predators.

CHAPTER 8:

COMPARATIVE ASPECTS OF CONDITION IN CAPTIVE AND NET-CAUGHT DOLPHINS: GENERAL DISCUSSION AND CONCLUSIONS.

Morphology

Morphological data obtained from captive and net-caught animals during this study reflected an important and valuable relationship between morphological condition and diet, temperature and reproductive status. The complexity of these relationships and their influences on morphological condition must be accounted for when using morphological parameters to assess marine mammal condition. The traditional measure of condition, total body weight, was a good overall indicator of condition in both captive and net-caught dolphins. In captive animals, illness was generally associated with a reduction in weight. Similarly, net-caught animals, which were obviously out of condition, with a high degree of parasitism and/or evidence of disease and/or infection, weighed considerably less than other animals of the same size and age. Pregnancy and lactation (in net-caught animals) had a marked effect on weight. Increased weight in captive and net-caught dolphins during pregnancy reflected improved overall body condition, emphasised by an improvement in other morphological parameters, such as blubber thickness. Although the effects of lactation could not be investigated in captive animals, net-caught lactating animals weighed significantly less and had thinner blubber, emphasising the deleterious effects that the energetic demands of lactation have on body condition. These data accentuated the importance and value of total body weight as an overall indicator of condition. However, it was apparent that body weight, blubber thickness and other morphological measures were long-term indices, changes occurring after long-term dietary changes and associated physiological fluctuations.

The implementation of other morphological measures into the regime of body condition assessment for marine mammals has proved highly successful (Ridgway and Fenner 1982, Kastelein and van Battum 1990, Wells *et al.* 1992, Castellini and Calkins 1993, Wells 1993). Girth measurements are particularly important in the overall assessment of condition (Lockyer *et al.* 1984, 1985, Lockyer 1986, Castellini and Kooyman 1990, Kastelein *et al.* 1990, Rosen and Renouf 1997) and are important predictors of total body weight. The monitoring of blubber thickness in captive animals during the current study was potentially an important indicator of body and blubber condition, although measurements were unfortunately too irregular to adequately define their value. Relationships between blubber thickness, age, diet and water temperature in net-caught animals highlighted the fact that measures of blubber thickness allow for an insight, not only into overall body condition, but also nutritional condition with respect to environmental variables such as food resources and water temperature. Blubber thickness also provided information on blubber weight, proportional blubber weight and overall blubber condition, similar to calculations of blubber volume described by other authors (Slip 1992, Worthy *et al.* 1992, Renouf *et al.* 1993, Rosen and Renouf 1997). However, it must be noted that blubber thickness is not necessarily a reliable indicator of blubber composition and/or quality. A *post-mortem* investigation of one of the captive animals illustrated that although blubber thickness was optimal, lipid content was reduced. Further investigations are needed into the relationship between blubber thickness and composition, particularly in small cetaceans. If a relationship is confirmed, blubber thickness, a morphological parameter that can be used non-destructively in the field, can be used to predict other aspects of blubber condition.

Haematology

Overall, WBC counts were not an ideal indicator of condition in captive and/or net caught animals. They have been used extensively in marine mammal medicine (Ridgway *et al.* 1970, Medway and Geraci 1978, Cornell 1983, Clarke 1990, Bossart and Dierauf 1990), and this study underscored their more effective use in assessing clinical health, rather than overall condition. It must be acknowledged that condition and clinical health are interactive. For example, a change in nutritional condition, due to a change in the availability and/or quality of food resources, may result in an animal becoming susceptible to parasites, infectious processes and/or disease. In the same context, infection, parasites and/or disease will, if left untreated, lead to deterioration in condition. The mean WBC count in net-caught dolphins ($12.29 \pm 6.34 \times 10^9$ cells/l) was higher than that of captive animals ($9.13 \pm 2.49 \times 10^9$ cells/l), and was within the "precautionary" range of the captive dolphin haematological condition index, established during the current study (Table 3.14). This reflected the higher level of antigenic stimulation in the wild, and implied that the captive dolphins, in their specially controlled environment, were immunologically more healthy, exposed to fewer parasites and lower levels of pollution, than their free-ranging counterparts. This parameter, therefore, directly reflected on the immunological status of an animal and, although not necessarily linked to overall condition, it highlighted certain changes in health, which may lead to deterioration in condition. WBC's in net-caught dolphins show potential as an indicator of habitat quality, in terms of parasite load and/or pollution. Immunological status has been previously linked to pollution in the marine environment (Anon. 1991, de Swart *et al.* 1994, 1995, Motluk 1995).

The use of the differential count of WBC's as a condition index was difficult to assess because of the different cell types measured for captive and net-caught animals. The proportion of lymphocytes was the only parameter measured in the WBC differential of net-caught dolphins. Four different cell types were measured in the differential of captive dolphins. Lymphocytes were excluded from analyses of captive animal blood, due to lack of significant correlations with independent variables. However, a comparison of mean lymphocyte values of captive (19.00-31.51

%) and net-caught dolphins (60.45-70.18 %) showed that lymphocyte proportions were almost three times higher in the latter group. Stress has been reported to cause lymphopenia (a reduction in lymphocytes) (Bossart and Dierauf 1990), which would seem to contradict the results obtained in the current study. Increased lymphocytes have been attributed to viral infections, hypoadrenocorticism and leukemia (Penington *et al.* 1978, Bossart and Dierauf 1990, Thurman and van der Elst 1995). The mean lymphocyte percentage recorded for net-caught dolphins is markedly higher than most previous records (Bossart and Dierauf 1990) and only just falls within the range (3-70 %) reported for harbour porpoise (*Phocoena phocoena*) by Neilsen and Andersen (1982). Mean lymphocyte values from the captive animals used in this study were more similar to previous records (Appendix 4). Engelhardt (1979) reported that lymphocytes generally comprise a third of the marine mammal differential. Therefore it would appear that the lymphocyte proportion in the blood of net-caught animals has undergone a significant increase. As stress reportedly causes a decrease in this cell type (Bossart and Dierauf 1990), and it is unlikely that almost all individuals of the two dolphin species would be exposed to viral infections and/or leukemia, this elevation may be due to *post-mortem* changes in the blood. The nature of these changes are unknown and must be examined further if this parameter, and other WBC types, are to be used as an index of condition for incidentally-caught marine mammals.

Mean RBC counts of captive bottlenose dolphins (*Tursiops truncatus*) during this study were markedly lower ($4.32 \pm 0.28 \times 10^{12}$ cells/l) than that of net-caught common dolphins (*Delphinus delphis*) ($6.10 \pm 0.97 \times 10^{12}$ cells/l) but only slightly reduced relative to their net-caught counterparts ($4.81 \pm 1.04 \times 10^{12}$ cells/l). PCV and MCV data from net-caught dolphins were also only slightly higher than that of captive dolphins. These differences were a direct reflection of the habitats and associated swimming speeds, diving depths and activity levels of the different groups of dolphins (Ross 1984) and illustrated the increased fitness of free-ranging dolphins. The diving depths and activity levels of captive animals are limited in comparison to the long distances travelled by wild dolphins; hence the haematology of the captive animals reflects physiological adaptations to different environments. Slightly reduced RBC counts and PCV and MCV values reflected that captive animals, relative to their restrained environment, were slightly "out of condition" or less fit than their free-ranging counterparts. HB concentrations of captive dolphins were markedly reduced below that of free-ranging dolphins of the same species, implying that oxygen transport efficiency was reduced in captive dolphins, not by a significant decline in numbers of RBC and cell volumes but by a decrease in HB concentration. Although these haematological changes have been associated with a lack of condition in some marine mammal species (Bossart and Dierauf 1990, Thurman and van der Elst 1995), they emphasise the distinctive haematological nature of species, behaviour and habitat, which must be taken into account when using these parameters as condition indices. However, it must be noted that HB may be more noticeably elevated by stress and *post-mortem* changes than RBC, PCV and MCV and thus may be the reason for the marked HB differences between captive and net-caught dolphins.

The relationships between diet and haematological parameters in captive dolphins indicated that RBC, HB and PCV were useful as short-term indicators of nutritional condition. A change in erythropoetic function resulting from changes in nutritional plane has rapid effects on haematological values (Hellgren *et al.* 1993). The importance of blubber thickness measurements as an index of condition was re-iterated by the interesting relationships between blubber thickness and haematological parameters in both captive and net-caught dolphins. These relationships reflected a potentially valuable interaction between morphological and physiological assessments of condition in that morphological condition, which can be assessed non-destructively, may be used to gain an insight into haematological condition. Although data were generally highly variable, the possibility of using morphological parameters such as weight, girth and blubber thickness to predict haematological status should be further investigated in terms of developing non-invasive indices of condition. The interactions between morphology, diet and temperature only emphasise the potential usefulness of such an index. Haematology has long been recognised as a vital tool in monitoring not only the state of hydration in an animal (Medway and Geraci 1978, Clarke 1990, Thurman and van der Elst 1995), but also the status of the animal's oxygen transport system (Lenfant 1969, Ridgway *et al.* 1970, Medway and Geraci 1978, Bossart and Dierauf 1990), two vital components of physiological condition in free-ranging and captive dolphins. Blubber thickness and condition is related to these components of physiological condition through the storage and mobilisation of energy stores and thermoregulatory homeostasis (Ridgway and Fenner 1982, Lockyer *et al.* 1984, 1985, Lockyer 1986, 1993a, Ognetov 1990, Costa *et al.* 1993, Wells 1993). The interactions between haematology and blubber condition may provide an important insight into an animal's condition relative to seasonal changes in food resources and other environmental variables.

Haematological parameters exhibit strong and important relationships with all aspects of the animal's life and provide a valuable indication of condition with respect to the environment and responses to it. However, the highly complex interactions between haematology, diet, temperature and weight demand that all possible influential factors be taken into account when considering haematological and overall physical condition. Water temperature has an important effect on both physiological and morphological condition. The maintenance of homeostasis, both physiologically and morphologically, is highly dependent on fluctuating energetic demands within and outside of the respective thermoneutral zones (Ross and Cockcroft 1990, Cockcroft 1991). Within the thermoneutral zone, metabolic and thermoregulatory demands are met through food and energy intake, in conjunction with optimal activity levels, thus maintaining ideal condition. Outside of this zone, metabolic and thermoregulatory demands vary, resulting in a change in the requirements of homeostasis. Changes in thermoregulatory and dietary requirements can deleteriously affect the ability to maintain both morphological and physiological condition. In this respect, the effects of thermoneutral zones should also be taken into account when assessing condition of dolphins, based on their important influence on those factors which are so vital in the maintenance of peak condition.

In light of the many similarities between haematological data of captive and net-caught animals in this and other studies (Appendix 4), it would appear that stress and/or *post-mortem* changes had little effect on the mean haematological values of net-caught dolphins (except possibly HB). This provides for future opportunities in which comparisons between captive and net-caught haematological analyses can be used in the assessment of condition. However, it must be noted that overall, haematology is typically affected by both stress- and *post-mortem*-associated haemolysis. This must be taken into account when making use of these parameters, as these changes detract from their use as ideal indices of condition in free-ranging marine mammals.

Blood chemistry

The mean creatinine level from net-caught bottlenose dolphins was similar to mean values from previous studies (Appendix 5), although there appeared to be slight changes in creatinine associated with stress, *post-mortem* and/or storage-associated changes in the blood. As a physiological indicator of muscle mass and activity, the relative differences in the mean creatinine levels of the two groups of study animals reflected not only species differences in muscle mass and activity but also implied a reduced muscle mass (and possibly muscle condition) and activity in captive animals. The mean creatinine level of common dolphins was approximately 300 U/l higher than that of the captive dolphin group. The mean creatinine level of net-caught bottlenose dolphins was similar to that of the captive dolphins, although it did correspond to the level at which mature captive dolphins were deemed to be in less than ideal condition (Table 4.10). As the majority of net-caught animals were healthy, it is feasible that this increase was due to stress, increased muscular activity during entanglement and *post-mortem* changes. Without further analyses, it is difficult to confirm whether the elevated creatinine levels in net-caught animals were indicators of stress or physiological indicators of improved muscle condition, mass and activity in free-ranging dolphins. Creatinine values from both captive and net-caught animals were variable, limiting the conclusive identification of any relationships between this parameter and diet and/or temperature. However, the influence of muscle mass on creatinine provides an opportunity for further investigations into the influence of muscle condition on circulating levels of creatinine in the serum.

Blood urea nitrogen in net-caught dolphins was higher (~2 mmol/l) than those measured from most of the captive dolphins, but corresponded with the BUN levels that reflected optimal condition in captive dolphins (Table 4.10). The slight difference in BUN levels was representative of the different diets of the two groups, higher BUN in the net-caught animals indicating a more diverse diet of slightly higher quality. The importance of BUN as an indicator of nutritional status was reinforced by the data from both captive and net-caught dolphins, both groups displaying several important interactions between BUN and food intake and/or dietary composition. The BUN:creatinine ratio has proved effective in other condition studies (Berkow 1982, Kaplan and Pesce 1989, Medway and Geraci 1978) and similarly, BUN:creatinine was positively related to several dietary parameters in the captive animals. The BUN:creatinine ratio from net-caught animals corresponded with the range of ratio values attributed to optimum nutritional condition in captive dolphins (Table 4.10). In addition, although only evident for Domino, the relationship between this ratio and blubber thickness reflected a potentially vital interaction between nutritional and blubber condition. A tentative relationship between BUN:creatinine and diet in net-caught dolphins consolidated the usefulness of this ratio in assessing nutritional condition. Seasonal changes in BUN evident for captive animals may prove useful in monitoring seasonal changes in food resources, and hence nutritional condition, in free-ranging dolphins. The nutrient and calorific contents of fish are known to vary seasonally (Reiter and Crissey 1991, Walsh and Dover 1997), and, in the wild, the abundance and diversity of locally available food resources vary on a seasonal and regional basis. In this context, BUN (and its relationship with blubber thickness) may provide an invaluable index of animal nutritional status, with respect to seasonal changes in food abundance and diet quality. Further investigations into the affects of activity, stress and *post-mortem* changes on creatinine, and therefore BUN:creatinine, are needed to confirm the application of this ratio in the assessment of condition in net-caught and/or free-ranging marine mammals.

Enzymes, generally, did not appear to be ideal indices of condition. Results from enzyme analyses during this study confirmed that they are typically highly variable, are dramatically changed during stress and appear to increase markedly after death. This was particularly true for the muscle- and heart-associated enzymes, LDH and CPK. However, ALP appeared to be an effective indicator of growth processes and maturity status. Age differences in the levels of this enzyme were apparent for both captive and net-caught dolphins. The mean ALP level for juvenile net-caught dolphins (2 351.67 ± 699.09 U/l) was more than double the "normal" mean ALP level of the captive juvenile animal, Domino (958.40 ± 280.20 U/l). Mean levels from adult net-caught animals were similar to those of Dolly, Thandi and Simo. Reasons for the low ALP levels of Domino, relative to free-ranging animals of the same age, are unclear. Domino, although classified as a juvenile, may have been relatively older than the majority of net-caught juvenile dolphins. Overall, ALP levels in net-caught dolphins corresponded with the range of ALP levels, which identified a captive dolphin as being in optimal condition (Table 4.10). Relationships between diet and ALP in captive animals reflected that this enzyme might be a useful index of nutritional stress, which may indirectly affect growth, especially in young animals. These trends were not confirmed in net-caught animals. The important relationship between age, growth and nutrition highlights the need for further investigations into this enzyme and it's potential use as a condition index.

Although relationships between diet, LDH and CPK in captive animals indicated that heart and muscle activity may change according to nutritional status, these enzymes were typically variable, fluctuated according to activity levels and rose rapidly with stress. Mean LDH and CPK values were approximately one and a half, and 25 times higher, respectively, in net-caught bottlenose dolphins. Although fluctuations in LDH and CPK were associated with periods of physiological stress, whether nutrition- or water temperature-related, they were most seriously affected by artificial stresses, such as net capture and *post-mortem* changes, which detracts from their use in the assessment of condition.

Sodium levels in net-caught dolphins were approximately 20 mmol/l less than those of captive dolphins, as well as the majority of previous results (Appendix 7). Serum sodium has been used successfully in previous studies of condition (Geraci *et al.* 1979) and nutritional status (Medway and Geraci 1965, 1978, Medway and Cornell, 1989, Clarke 1990, Thurman and van der Elst 1995) and although increased sodium levels were indicative of nutritional stress and dehydration, the obvious artificial changes, which occurred in net-caught animals, limits the use of sodium as a condition index for free-ranging marine mammals. Elevations in serum potassium, above 10.0 mmol/l were evident in net-caught dolphins, reflecting *post-mortem*- (Young, unpublished data), stress- and storage-associated changes (Geraci and Medway 1973, 1974, Geraci *et al.* 1979, Engelhardt 1979, Ronald and Kay 1982, Medway 1983, Heidel *et al.* 1996). Calcium levels from captive dolphins were only 0.5-0.7 mmol/l less than those measured from net-caught dolphins. Although no base-line relationships were established for this parameter, data from net-caught animals reflected an age-associated decrease in this electrolyte, implying that levels of serum calcium positively reflected growth activity. The usefulness of this parameter as a condition index, however, remained unconfirmed, as there were no distinct relationships between calcium and the independent variables of morphology, diet and water temperature.

Total serum protein was higher, by approximately 30 g/l, in net-caught dolphins than in captive dolphins. This increase was attributed an elevation of globulins, of between 30 % and 194 %, relative to the captive animals. Albumin levels were similar in net-caught and captive dolphins. In association with these differences, the albumin:globulin ratio was reduced in net-caught animals, by between 28 % and 73 %. The similar albumin levels reflected similar nutritional status in net-caught and captive dolphins. Albumin has been identified as an important indicator of nutritional status by several authors (Hyvarinen *et al.* 1975, Seal *et al.* 1978a, 1978b, Warren *et al.* 1981, 1982, Lochmiller and Grant 1984, Lochmiller *et al.* 1985b, 1985d, 1986, Kuiken 1985, Medway and Cornell 1989, Wolkers *et al.* 1994a, Thurman and van der Elst 1995). This potential was tentatively confirmed by the results of this study, although further analyses are needed. The increased globulin levels paralleled the higher WBC counts in net-caught dolphins and, according to the captive dolphin index of condition, reflected that net-caught animals were in "less than ideal" condition (Table 4.10). Globulin levels measured in net-caught animals were also higher than the majority of mean globulin values reported in the literature (Appendix 7). Serum proteins appeared relatively unaffected by stress and *post-mortem* changes and reasons for this marked elevation in globulin levels are unclear. In terms of the increased immunological activity they represent (Asper *et al.* 1990, Bossart and Dierauf 1990), they should be investigated with respect to a potential problem in the marine habitat from which the animals originate. Along the South African coastline, approximately 60 pipelines discharge over 700 000 m^3 of effluent per day. Of these, 33 discharge below the high-water mark, into coastal waters. The Kwazulu-Natal coast is for responsible for 73 % of the total effluent discharge; the south and east Cape coasts contributing only 24 %. Agricultural insecticides, used on inland sugar cane farms, as well as river-borne industrial effluent from numerous coastal-based factories, form a large part of marine pollution in the region (Cockcroft *et al.* 1989, 1990). The most recent assessments of pollutant loads in dolphins from the area were undertaken prior to 1990 (Cockcroft *et al.* 1989, 1990), which reported that pollutant loads in coastal bottlenose dolphins along the Kwazulu-Natal coast were high enough to interfere with reproductive processes (Cockcroft *et al.* 1989). Pollutant loads in both the dolphins and coastal waters of Kwazulu-Natal should be urgently re-assessed, with respect to a potentially debilitating affect on immunological activity. Serum proteins in net-caught dolphins were not obviously related to morphology and/or temperature, however, there were several relationships between albumin, globulin and albumin:globulin and dietary parameters. Although additional data are needed to confirm many of these relationships, they serve to highlight the positive relationship between good nutrition and albumin; as well as increased susceptibility to immunological challenges when nutrition was compromised. The fact that stress and/or post-mortem changes least affected these parameters makes them ideal indicators of nutritional condition in free-ranging marine mammals.

Plasma glucose was lower, by between 0.90 mmol/l and 2.50 mmol/l, in captive dolphins than net-caught dolphins, illustrating the sensitivity of this parameter to stress and *post-mortem* changes, as well as the fasting period to which captive animals are usually exposed to prior to blood sampling. Although glucose appears to be a reliable indicator of feeding in some cases, variable activity levels have a marked effect on plasma glucose. Glucose values in marine mammals are typically highly variable, depending on the state of activity and fasting interval before sampling (Medway and Geraci 1978, Castellini *et al.* 1993). These fluctuations reduce the potential usefulness of this parameter as a condition index. However, in light of the similarities of the present results with other studies, and potentially important relationships between glucose and nutrition, further examination of the use of plasma glucose in the assessment of condition is needed.

Mean cholesterol concentrations were variable in captive dolphins, ranging from 2.87 mmol/l in Simo to 6.12 mmol/l in Dimple but cholesterol concentrations in net-caught dolphins were within the same range. Levels of TGL's were similar only between net-caught dolphins and Dimple. All other captive animals had significantly reduced TGL levels, relative to net-caught specimens. In addition, TGL levels from net-caught dolphins during the current study (3.24 ± 0.89 mmol/l) were higher than the range reported for other marine mammals: 0.49 ± 0.08 mmol/l in harbour seals (*Phoca vitulina*) and 1.33 ± 0.23 mmol/l in killer whales (*Orcinus orca*) (De Monte and Pilleri 1983, Davis *et al.* 1991, Schumacher *et al.* 1992). Therefore, the captive animals, excluding Dimple, had TGL levels within the normal range reported for marine mammals. The elevated TGL in net-caught dolphins was attributed to the fact that many of the dolphins in this study were actively feeding a short time prior to capture, whereas captive animal samples were taken after at least eight hours fasting. In this respect, Dimple's TGL results reflected that the majority of samples, used for lipid analyses, from this animal did not correspond with the same period of fasting as the other four captive animals. As a result, the mean TGL level for this animal was biased by the predominance of increased TGL levels in blood samples taken after a shorter fasting period.

Both cholesterol and TGL levels from net-caught dolphins were positively related with dietary energy intake, confirming that these lipids were meaningful indicators of diet quality. Levels of TGL vary widely with diet in marine mammals (Kuiken 1985, Bossart and Dierauf 1990). Fish vary considerably in fat and protein composition (Geraci 1977, Reiter and Crissey 1991, Walsh and Dover 1997) and changes in serum lipids have been attributed to differences in diet type and levels of consumption (Nelson 1970, Kuiken 1985, Asper *et al.* 1990, Bossart and Dierauf 1990). The relationship between lipids, particularly TGL concentrations, and food intake, diet quality and fasting makes this serum component an ideal indicator of animal nutritional condition. Serum cholesterol and TGL were also related to morphological parameters, especially those pertaining to blubber. In this respect, the interaction between serum lipids, blubber and diet demands further investigation. Further analyses of serum lipids, in conjunction with measures of blubber condition (thickness, lipid content), will provide vital information concerning seasonal changes in animal nutritional status and morphological condition, with respect to seasonal and regional changes in food resources.

The potential value of serum lipids as indicators of condition is accentuated by the fact that they appear to remain remarkably stable after death (Young, unpublished data) and did not appear to change dramatically with stress. In addition, Geraci and Medway (1974) reported that cholesterol was stable during storage, for at least eight days. Further detailed studies of the relationships between serum lipids, blubber composition and condition, diet quality and nutritional status will contribute vital information to the understanding of interactions between diet, physiology and morphology.

The results from the captive animals used in this study have shown that certain blood chemistry parameters are valuable indicators of morphological and nutritional condition and provide an insight into seasonal physiological changes with respect to diet, temperature and overall condition. Blood parameters in terrestrial mammals offer information on short-term and recent metabolic function in response to dietary changes (Lochmiller *et al.* 1986, Dinkines *et al.* 1991). Results from this study indicated that several blood chemistry components offer the same perspective for marine mammals. Parameters found to be most useful in the monitoring of terrestrial mammal metabolism and morphological and nutritional condition include BUN, BUN:creatinine, ALP (Franzmann 1972, Seal *et al.* 1978a, Lochmiller *et al.* 1986, Dinkines *et al.*, 1991, Wolkers *et al.* 1994a) and albumin:globulin (Lochmiller *et al.* 1985b). Creatinine, BUN:creatinine and ALP are reportedly more effective in the short-term, although BUN and BUN:creatinine are valuable indicators of long-term undernutrition (Wolkers *et al.* 1994a). Based on the results from this study creatinine, BUN, BUN:creatinine, albumin and serum lipids provide the best indication of nutritional condition and diet quality for captive dolphins (Table 8.2). Levels of serum sodium are an important indication of diet quality and hydration status, but their use is limited by stress- and *post-mortem*- related changes. In addition, creatinine, BUN:creatinine, cholesterol and TGL can be used to reflect changes in morphological condition (Table 8.2). Many of these parameters effectively illustrate the influence that water temperature has on physiology and that physiological condition can be compromised outside of the thermoneutral zone of the species. Although, many blood chemistry components, but particularly LDH, CPK and potassium, of net-caught dolphins were influenced by capture stress and *post-mortem* changes, several parameters were not affected by these factors (although the latter does need further investigation). These included BUN, albumin, globulin and albumin:globulin and lipids such as total cholesterol and TGL. Relationships between these parameters and morphology, diet and water temperature support their use as condition indices in net-caught and/or free-ranging dolphins, and highlight their potential as indicators of habitat quality and environmental health.

Conclusions

The current study reinforced the usefulness of captive dolphins in physiological studies. Data obtained from captive specimens can not only be used to monitor captive animal condition, but also form a vital base-line database for comparison with free-ranging dolphins. Relationships between different aspects of condition, established for captive animals, in this and other studies, provide an important base-line with which to compare the condition of free-ranging, incidentally-caught and stranded marine mammals. Free-ranging animals are not easily accessible, but the current study shows that net-caught specimens provide a more accessible sub-sample of the population, allowing for an insight into the condition of free-ranging marine mammals in the region. Where incidental captures occur on a regular basis, animal condition may be used to monitor population status and environmental quality. Results and relationships obtained from captive and net-caught dolphins during the current study are summarised in Table 8.1. This summary provides information on changes in haematological and blood chemistry parameters observed during the study, and how these fluctuations related to changes in morphological, nutritional and environmental status. In addition, Table 8.1 also highlights those parameters, which showed the most potential as indicators of animal condition and environmental health. Physiological and morphological condition indices of dolphins show potential as indicators of short- and long-term changes in food resource abundance and quality, as well as fluctuations in environmental stresses, such as marine pollution. In this regard, the monitoring of the condition of top marine predators has vital conservation implications for the management and protection of coastal habitats.

Certain relationships established during the current study indicate that non-invasive condition assessment of free-ranging marine mammals is certainly feasible. This is particularly true for those trends that reflect an interaction between morphological and physiological condition. Blubber biopsies are already a relatively non-invasive means of obtaining samples from free-ranging marine mammals. Worthy and Abend (1997) have explored the option of using fatty acids in blubber biopsy samples to identify and monitor prey species eaten by killer whales. Although differential blubber composition prevented any conclusive results from being obtained, these types of samples, with further research, will provide indispensable information pertaining to morphological, physiological, nutritional and, possibly even, reproductive condition. With this in mind, several future research prospects are discussed below, which may be useful in expanding the knowledge of condition assessment in marine mammals, as well as the development of non-invasive condition evaluation and monitoring techniques.

Future research prospects

Particular parameters were removed from analyses during the current study in an attempt to streamline data and associated investigations. The potential of these parameters as condition indices, particularly AST, ALT, calcium and glucose, is recognised and further analyses need to be completed in order to fully understand and interpret the relationship between these blood components and dolphin condition.

The trends described in this study using "curves of best fit" constitute a basic analysis of how physiological parameters relate to morphological, dietary and environmental variables in dolphins. In so doing, it was hoped that certain of these trends could be used in a predicative capacity, such that aspects of physiology could be used to estimate morphological and nutritional condition and visa versa. The value of this being that oceanarium staff and marine mammal researchers could use

Table 8.1: A review of interactions between leukocytic, haematological and blood chemistry parameters and morphological, nutritional and environmental factors in captive and net-caught dolphins. Parameters which were found to be the most effective indices of animal condition and nutritional status during the study, and are potential habitat indices, are highlighted (? denotes the need for further investigation, T°: water temperature) (Groups for which the trends were evident are denoted by C (captive) or N (net-caught); the nature of the change is indicated by increasing or decreasing arrows).

Parameter	**Age/sex changes**	**Nature of change**	**Group**		**Cause/Implication**	**Biomonitor potential**
WBC		↑	C/N	↑	Immunological stimulation	**Habitat quality - pollution**
			C/N	↓	Nutritional condition (long-term)	
			C	↑	Environmental stress (T°) - elderly animals	
Neutrophils	Age	↓	C	↑	Nutritional stress	
			C	↑	Environmental stress (T°)	
Eosinophils	Age	↑	C	↑	Nutritional stress	
			C	↑	Environmental stress (T°)	
Lymphocytes		↑	N	↑	Immunological stimulation	
			N	↑	Parasitism?	
RBC, HB, PCV, MCV	Age	↑	C/N	↑	Nutritional status (short-term)	
			C/N	↑	Fitness and overall condition	
			C/N	↑	Activity levels and diving capabilities	
	Seasonal	Variable	N		Stress/post mortem changes	
			N		Environmental stress (T°) - young animals	
			N		Nutritional stress (ulcers/malabsorption)	
Creatinine		↑	C/N	↑	Muscle condition and/or activity	
			C	↑	Nutritional condition?	
BUN		↑	C/N	↑	Nutritional condition	**Food resource quality**
		↓	C/N		Nutritional stress/Malnutrition	
BUN:creatinine		↑	C	↑	Blubber condition - related to improved nutritional condition	
	Seasonal	↑	C		Seasonal changes in nutritional condition	
		Variable	N		Stress/post mortem changes	

Table 8.1: contd.

Parameter	**Age/sex changes**	**Nature of change**	**Group**		**Cause/Implication**	**Biomonitor potential**
ALP	Age Seasonal?	↑	C/N	↑	Osteoblastic activity and growth	
		↑	C		Nutritional stress?	
		↑	N		Stress/post mortem changes	
Total bilirubin		↑	C	↓	Nutritional condition (long-term)	
			C	↓	Hepatic function	
LDH		↑	C	↑	Activity levels	
			C		Stress/cellular damage	
			N		Stress/post mortem changes	
CPK		↑	C	↑	Activity levels - possibly optimised by improved nutritional condition?	
			C		Stress/cellular damage	
			N		Stress/post mortem changes	
Sodium		↑	C/N	↓	Nutritional condition	
					Dehydration	
		↓	C		Environmental stress (T°)	
			N		Stress/post mortem changes	
Potassium		↑	N		Stress/post mortem changes	
Calcium		↓	N		Nutritional stress	
Albumin	Seasonal	↑	C/N	↑	Nutritional condition	**Food resource quality**
		↓	N	↓	Dietary quality	
				↑	Parasitism?	
Globulin		↑	C/N	↑	Immunological stimulation	**Habitat quality - pollution**

Table 8.1: contd.

Parameter	**Age/sex changes**	**Nature of change**	**Group**	**Cause/Implication**	**Biomonitor potential**
Albumin:globulin	↑	N	↑	Nutritional condition	**Food resource quality**
	↓		↑	Nutritional condition/improved immunological status	**Habitat quality**
Glucose	↑	N	↑	Nutritional condition Stress?	
Lipids - Cholesterol	↑	N	↑	Nutritional condition	**Food resource quality**
- TGL			↑	Blubber condition	**Morphological condition**

a selection of accessible, easily available parameters to estimate those parameters which are less obtainable. Although an attempt was made to form "condition models" which could be used to predict condition in marine mammals, the data showed that there was large-scale variation, even within and between individual animals. Inconsistencies made it impossible, within the realms of this study, to build models, which could be used for this purpose. However, certain relationships indicate that the formation of these types of models is feasible and with larger numbers of animals and more comprehensive analyses, these types of models could prove effective in predicting and monitoring condition in both captive and free-ranging marine mammals.

There were several aspects of this study that deserve further attention and may provide future research opportunities. Net-caught dolphins comprise a valuable sub-sample of the free-ranging population and allow for an efficacious insight into a population that is generally otherwise inaccessible. Unlike stranded dolphins, net-caught dolphins are generally healthy and blood samples therefore provide important physiological information, which could not be obtained easily from free-ranging animals. In this respect, *post-mortem* changes in blood need to be further examined to further detail those parameters that are least affected after death, and consequently can provide realistic estimates of physiological condition prior to capture.

The results of this study emphasise the stressful nature of net capture and entanglement and the physiological changes which occur during these processes. The stress-related physiological imbalances evident in the current study are typically associated with overexertion, particularly of muscle and the cardiac and respiratory systems. They are disturbingly similar to the symptoms of the often fatal stress syndrome 'capture myopathy', described for terrestrial wildlife (Basson and Hofmeyer 1973, Hofmeyer *et al.* 1973, Seal and Hoskinson 1978, Chalmers and Barrett 1982, Kock *et al.* 1987a, 1987b, Joubert and Stander 1990, Chapple *et al.* 1991, Dabbert and Powell 1993, Laurensen and Caro 1994, De Villiers *et al.* 1995, Whittington and Grant 1995). It has long been recognised that marine mammals are highly susceptible to capture and handling stress (Colgrove 1978, Stuntz and Shay 1979, Marsh and Anderson 1983). This raises concern for the survival of marine mammals captured and released elsewhere, as well as those exposed to other forms of artificial stress, such as research procedures involving the capture and release of dolphins. Worldwide, various types of fisheries catch dolphins incidentally. Large numbers of these animals are reportedly released alive (Lennert and Hall 1995). However, no cognisance is given to the effects of chase and capture stress on the recovery and survival of these animals. Mortalities associated with these interactions may be dramatically higher because of the stress experienced by these animals. It is possible that a large number of these fishery-related mortalities may be delayed, similar to cases of terrestrial capture myopathy, and therefore unobserved. Despite release, some dolphins may need some time to recover from the stress experienced. Nothing is known of the recovery period needed after release, or their chances of survival.

Harassment in any form may also elicit a stress response. Field studies that use physical restraint, tissue sampling and the attachment of tags or radio transmitters are also responsible for stress (Weinrich *et al.* 1992, Laurensen and Caro 1994, Whittington and Grant 1995, Serfass *et al.* 1996). In this context, the expanding cetacean watching industry could involve a form of harassment, particularly if undirected and uncontrolled. The short- and long-term physiological effects of close human and/or boat contact, harassment, increased noise and water pollution and behavioural and social disruption, especially in areas of intense tourist activity, are unknown. Terrestrial animals show both short-term physiological changes and long-term changes in behaviour and social structure as a result of this non-trivial handling, or harassment and there is every indication that captured and restrained marine mammals may exhibit similar short and long-term responses. However, at present, there are few measures of short-term stress. Although, several behavioural studies have shown that marine mammals react adversely to human activity in the marine environment (Acevedo 1991, Anon. 1996, Wilson *et al.* 1997), the long-term effects of human activity on marine mammals remains unclear (Watkins 1986, Acevedo, 1991, Corkeron 1995, Richardson *et al.* 1995). There is almost no evidence of long-term changes in distribution, population structure and/or reproductive success due to these human-related activities. These problems are magnified when attempting to identify immediate, short-term changes in behaviour and physiology, which could result in stress, potentially reducing the long-term health and condition of the animal or population (Anon. 1996). Long-term changes in reproductive success of spotted dolphins (*Stenella attenuata*) in the Eastern Tropical Pacific have been attributed to tuna fishery-related stress (Chivers and Myrick 1993). In addition, acute physiological changes have been recorded from dolphins incidentally caught and released from fishery operations (Myrick *et al.* 1987, Myrick and Perkins 1994). In view of the expansion of cetacean watching there is an urgent need to measure what levels of disturbance elicit a high enough physiological response to result in long-term effects. Direct interpolation suggests that after any significant stress, a lengthy recovery period may be needed to regain metabolic homeostasis. It is in this context, that the consequences of potentially stressful situations, such as incidental capture and release, tagging procedures, and possibly even whale-watching, on the short- and long-term biology of the animal/s in question needs investigation. The effective interpretation of stress-related responses can only improve the management and protection of marine mammals.

Studies of human condition have emphasised the usefulness of urine components in the assessment of condition (Vestergaard and Leverett 1958, Standard *et al.* 1959, Bleiler and Schedl 1962, Van Niekerk *et al.* 1963, Krehl and Hodges 1965, Pollack 1970). Urinalysis has also been shown to be a sensitive means of monitoring nutritional status in certain species of terrestrial wildlife (DelGiudice *et al.* 1987b, 1988, 1991b, 1994). Urinary creatinine and hydroxyproline are two parameters, which have provided valuable information pertaining to both human and animal condition, with relation to growth and nutrition (Jasin *et al.* 1962, Smiley and Ziff 1964, Whitehead 1965, Howells and Whitehead 1967, Howells *et al.* 1967, McCullagh 1969, DelGiudice *et al.* 1988). The ratio between these two parameters has been used successfully in the assessment of food resource and habitat quality (McCullagh 1969, DelGiudice *et al.* 1988). Similar marine mammal studies are lacking, due primarily to the difficulties of obtaining urine samples from marine animals. However, in light of the valuable information obtained from terrestrial mammals, the use of urine in marine mammal condition assessment does provide certain opportunities. The establishment of base-line urinary values, using captive animals, will allow for the provision of valuable comparative data for use in the field. Urine components are apparently less affected by stress and short-term *post-mortem* changes and therefore, offer a more reliable estimate of condition in net-caught and stranded dolphins. Urine samples obtained from incidentally-caught and released dolphins will offer additional opportunities for the monitoring of condition of free-ranging marine mammals. In this respect, urine samples were collected (wherever possible) from net-caught animals used in the current study. The results obtained from these samples will be examined in conjunction with the morphological, dietary and physiological data presented in this study, to further investigate the interactions between blood and urine and morphology, diet and water temperature.

Certain mineralisation anomalies in teeth are suspected to reflect the effect of "stressors" on the deposition of dentine and cementum in teeth during growth (Lockyer 1993b). These stressors include sexual maturation, pregnancy and/or parturition, periods of starvation, pollution as well as changes in health and life style (Klezeval and Myrick 1984, Simons 1984, Lockyer 1993b, 1995). Global environmental changes, such as the El Niño phenomenon, are reflected in the growth layers of Peruvian dusky dolphins (*Lagenorhynchus obscurus*) (Manzanilla 1989). In effect, these anomalies indicate periods at which odontocetes have undergone a marked change in physiological condition, which has manifested itself in a morphological change in tooth structure. The structure and chemical composition of teeth is inherently linked to the animal's physiology and metabolism, particularly with respect to calcium and phosphorous. However, little is known about the mechanisms responsible for the deposition of incremental lines in marine mammal teeth (Langvatn 1995). Myrick (1988) hypothesised that systemic alteration in tooth tissues is connected to stress-induced low serum calcium, which interferes with normal rates of tissue calcification. In this context, further investigation into the relationships between changes in physiological and nutritional condition and the effects these have on tooth deposition is imperative.

The increased WBC count, lymphocyte percentage and globulin fraction in net-caught dolphins from the Kwazulu-Natal coast may highlight a potential environmental problem in the region. Although the results from this study cannot conclude with any certainty that this type of problem exists, pollution levels in the area are reportedly high (Cockcroft *et al.* 1989, 1990) and these physiological changes may be an early indicator of a debilitating marine- and/or terrestrial-based influence, which is deleteriously affecting the immunology of dolphins in the area. To investigate this possibility, future research in the area should include analyses of pollutants, both in the tissues of dolphins, their prey species and the local coastal waters. The pollutant analysis should coincide with more detailed investigations of the immunological status of net-caught dolphins.

Although data were limited, relationships between albumin, globulin, cholesterol and TGL and dietary parameters suggest that these parameters, and other similar serum components, are potentially reliable and insightful indicators of the nutritional condition of marine mammals. Recent studies have shown important relationships between milk-based fatty acids and nutrition in seals (Iverson *et al.* 1997). The fact that serum lipids and proteins appear to change little after death (Young, unpublished data) highlights the capabilities these components have as indices of condition in both stranded and net-caught animals, able to reliably reflect the nutritional status of these more accessible specimens. The relationships between serum lipids and aspects of blubber condition also serve to emphasise their potential use in interpreting morphological changes with respect to changes in diet and nutrition. Biopsy sampling is a well-known and extensively used technique in marine mammal field studies (Worthy and Abend 1997, Marsili *et al.* 1996). Presently, this technique is used predominantly in obtaining skin samples from free-ranging whales and dolphins for genetic studies (Marsili *et al.* 1996, Smith-Goodwin 1997). However, the small blubber sample, obtained through this method, offers opportunities for examination of the relationships between blubber thickness and condition, nutritional condition and serum lipids and proteins. These interactions offer important opportunities for the development of a non-invasive index of condition for use in the field, which may be used to monitor condition in different species and age groups, as well as seasonal changes in diet, reproductive status and environmental variables such as pollution (Worthy and Abend 1997, Marsilli *et al.* 1996). Other non-invasive condition indices, such as total body water or lipid content, have successfully been used to reflect environmental quality (Croxall *et al.* 1988, Costa *et al.* 1989, Gales *et al.* 1994, Arnould 1995), fertility (Smith 1987, Testa 1987) and energy requirements (Fedak and Anderson 1987). However, these techniques involve some form of restraint, whereas biopsy samples may be obtained from free-ranging animals, without actually restraining the animal. In this respect, the interconnected relationships between serum lipids and proteins, blubber thickness and composition, dietary intake and quality and water temperature offer the best opportunity to develop non-invasive condition assessment techniques - not only for marine mammal populations, but also for some marine habitats.

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APPENDICES

Appendix 1.1: Box-whisker plots for morphological data obtained from the five captive dolphins.

Appendix 1.2: Box-whisker plots for dietary data (food consumption and energy intake) obtained from the five captive animals.

Appendix 1.3: Box-whisker plots for the leukocytic parameters of the five captive dolphins.

Appendix 1.4: Box-whisker plots for the haematological parameters of RBC, HB, PCV and MCV for the five captive dolphins.

Appendix 1.5: Box-whisker plots for the haematological parameters of MCH, MCHC, platelet and reticulocyte counts and corrected index for the five captive dolphins.

Appendix 2.1: Equations for curves of best fit.

Appendix 2.2a: Curves of best fit, coefficients (a, b, c, d), standard error (S), levels of significance (r) and sample sizes (n) for Dimple.

Appendix 2.2b: Curves of best fit, coefficients (a, b, c, d), standard error (S), levels of significance (r) and sample sizes (n) for Dolly.

Appendix 2.2c: Curves of best fit, coefficients (a, b, c, d), standard error (S), levels of significance (r) and sample sizes (n) for Thandi.

Appendix 2.2d: Curves of best fit, coefficients (a, b, c, d), standard error (S), levels of significance (r) and sample sizes (n) for Simo.

Appendix 2.2e: Curves of best fit, coefficients (a, b, c, d), standard error (S), levels of significance (r) and sample sizes (n) for Domino.

Appendix 3.1: Box-whisker plots for serum creatinine, blood urea nitrogen (BUN) and the BUN:creatinine ratio measured in captive dolphins.

Appendix 3.2: Box-whisker plots for serum alkaline phosphotase (ALP) and the ALP:creatinine ratio measured in captive dolphins.

Appendix 3.3: Box-whisker plots for liver-associated enzymes and total bilirubin measured in captive dolphins.

Appendix 3.4: Box-whisker plots for serum lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) measured in captive dolphins.

Appendix 3.5: Box-whisker plots for the serum LDH:creatinine and CPK:creatinine ratios measured in captive dolphins.

Appendix 3.6: Box-whisker plots for serum sodium and the sodium:creatinine ratio measured in captive dolphins.

Appendix 3.7: Box-whisker plots for serum total protein, albumin, globulin and the albumin:globulin ratio measured in captive dolphins.

Appendix 3.8: Box-whisker plots for serum potassium and calcium and plasma glucose measured in captive dolphins.

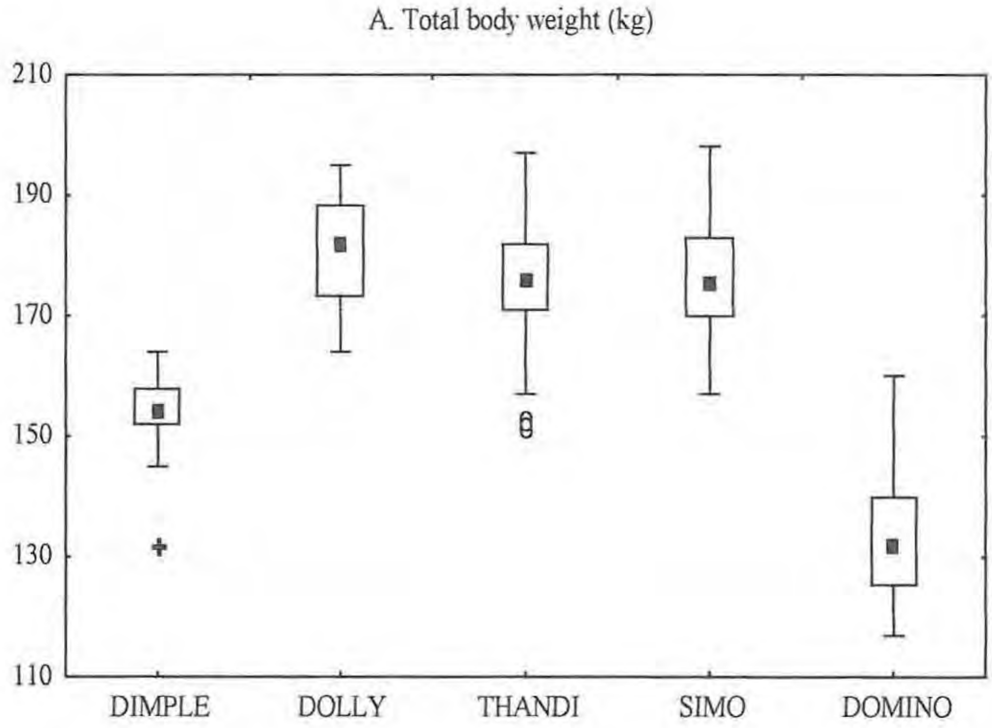
Appendix 3.9: Box-whisker plots for serum lipids measured in captive dolphins.

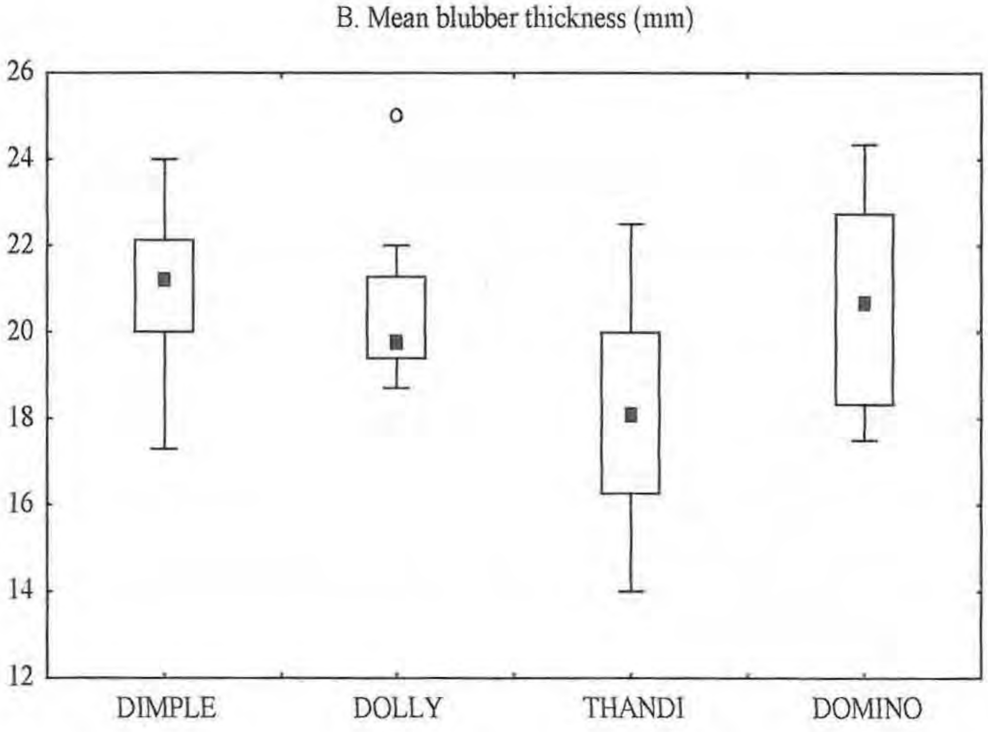
Appendix 4: Haematological data reported in the literature for other marine mammal species.

Appendix 5: Blood chemistry (liver-, muscle- and kidney associated enzymes) data reported in the literature for other marine mammal species.

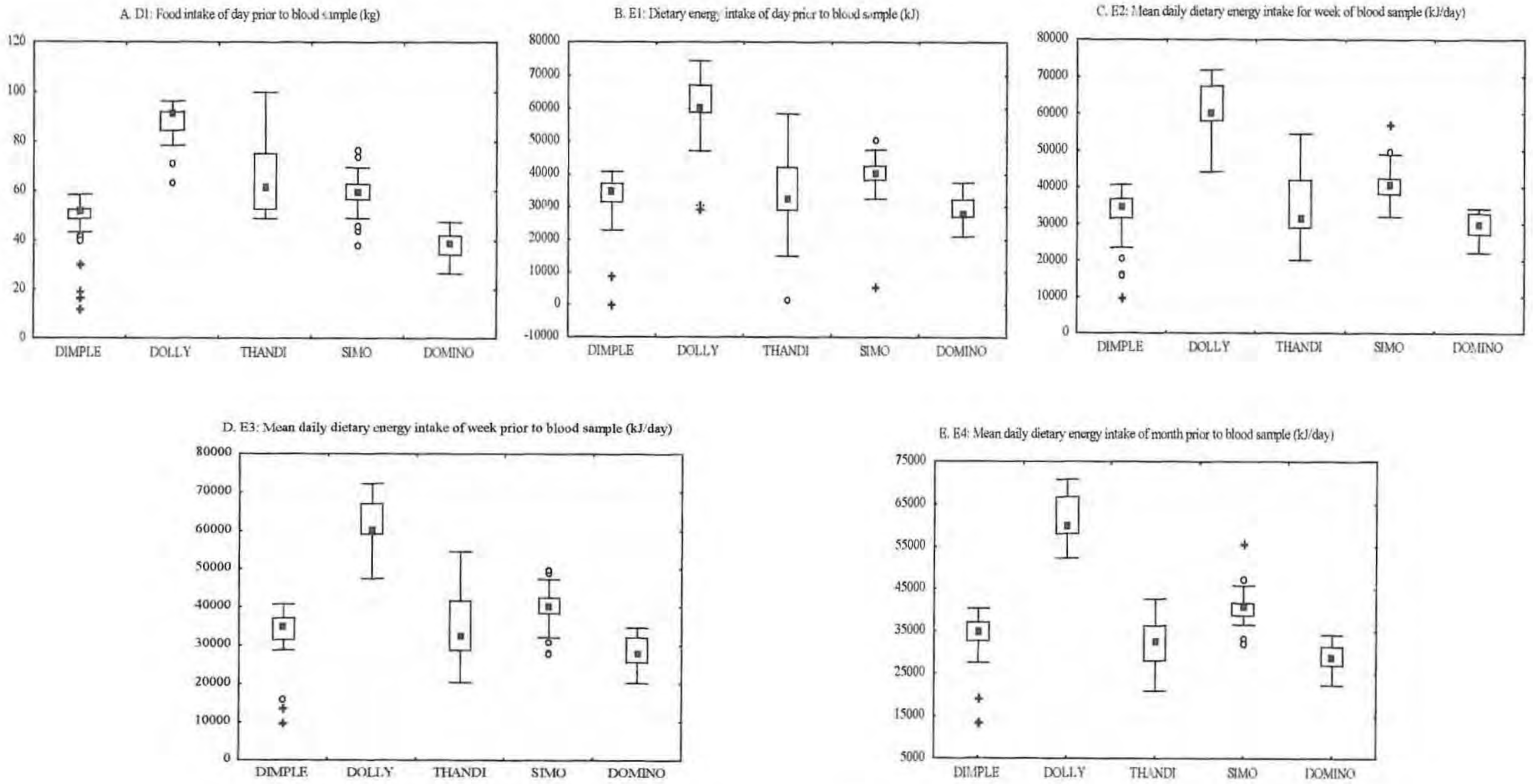
Appendix 6: Blood chemistry (liver-associated enzymes and bilirubin) data reported in the literature for other marine mammal species.

Appendix 7: Blood chemistry (proteins and electrolytes) data reported in the literature for other marine mammal species.

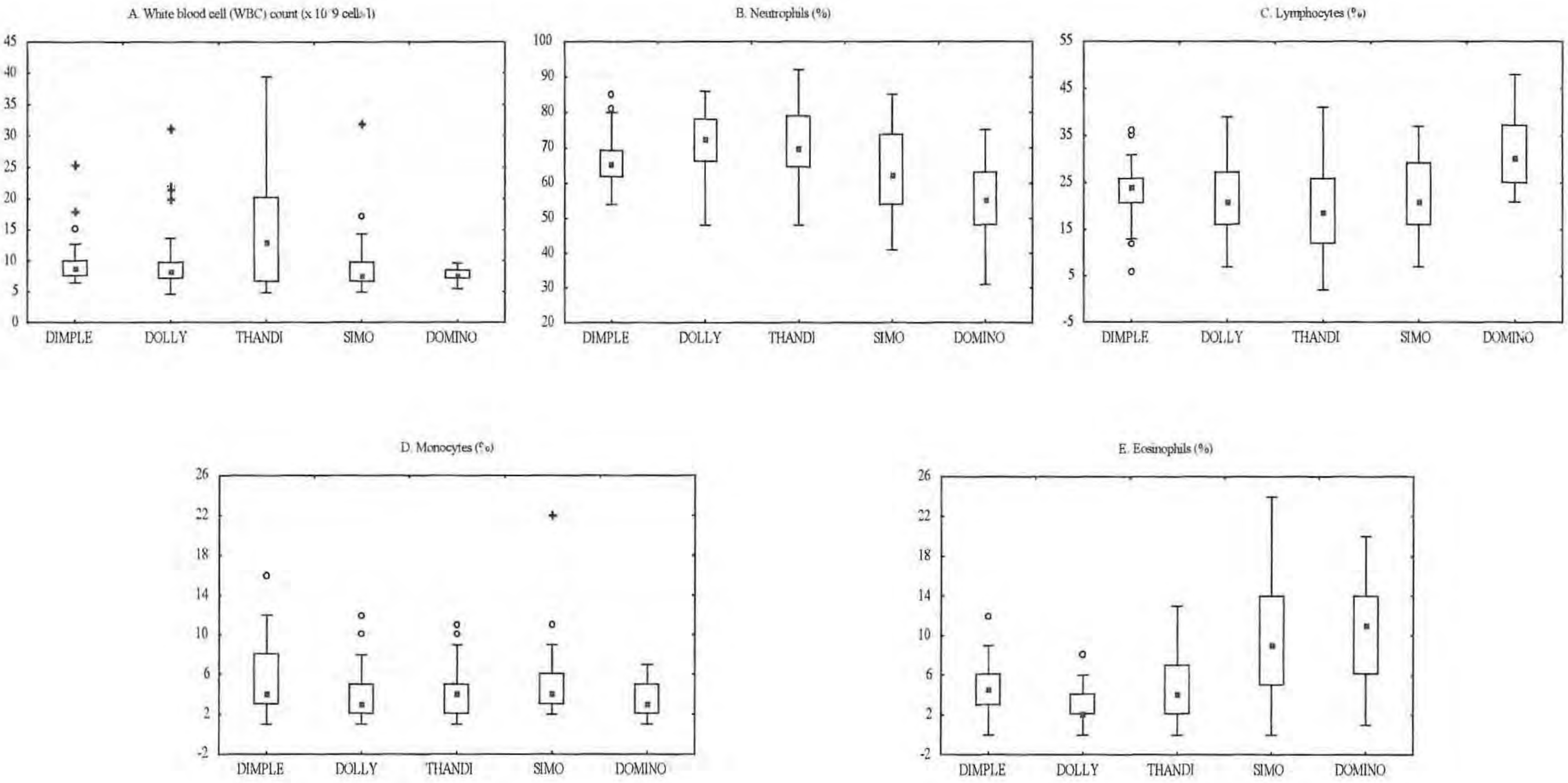




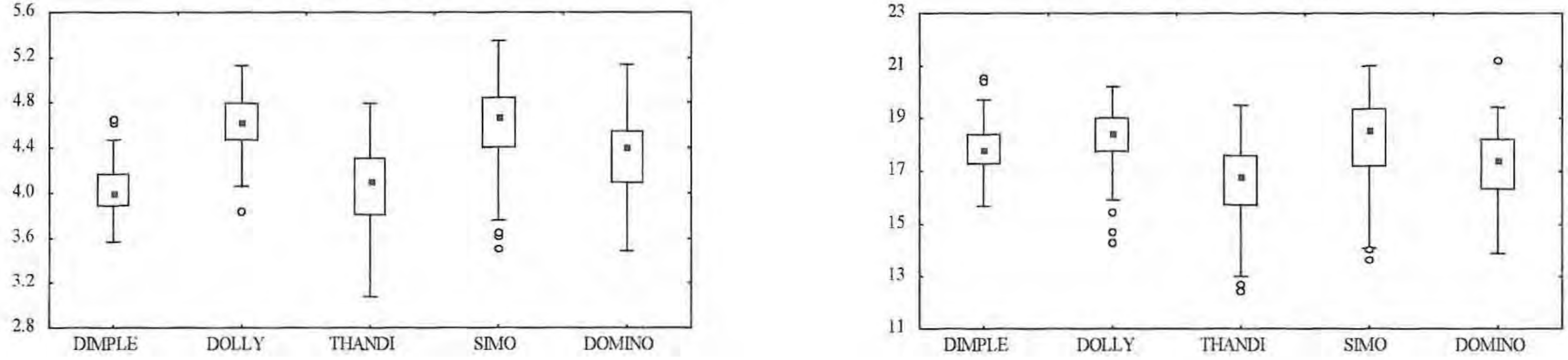
Appendix 1.1: Box-whisker plots for morphological data obtained from the five captive dolphins. Plots include the median, 50 % range, minimum, maximum and outlying (o) and/or extreme (+) values.



Appendix 1.2: Box-whisker plots for dietary data (food consumption and energy intake) obtained from the five captive animals. Plots include the median, 50 % range, minimum, maximum and outlying (o) and/or extreme (*) values.

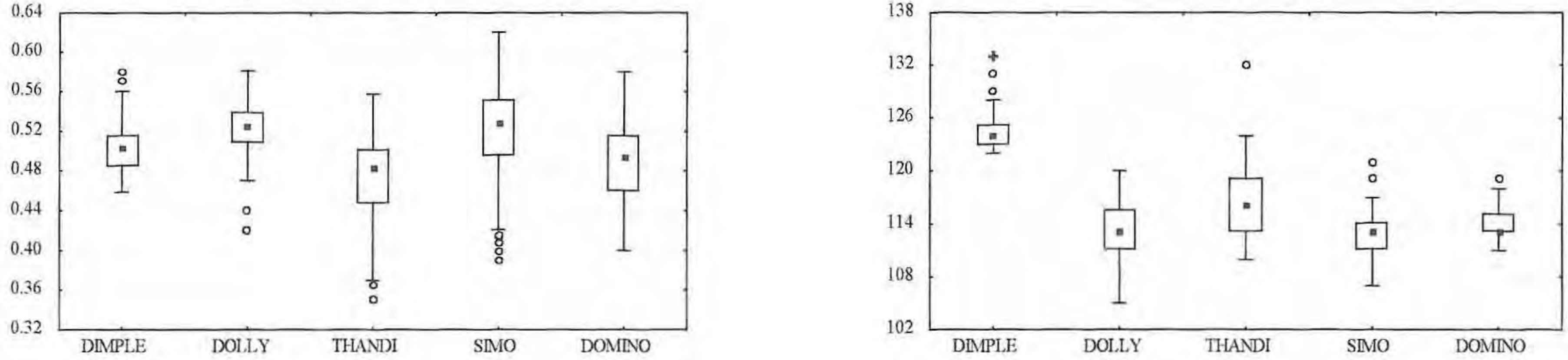


Appendix 1.3: Box-whisker plots for the leukocytic parameters of the five captive dolphins. Plots include the median, 50 % range, minimum, maximum and outlying (o) and/or extreme (+) values.



A. Red blood cell (RBC) count (x 10 12 cells/l)

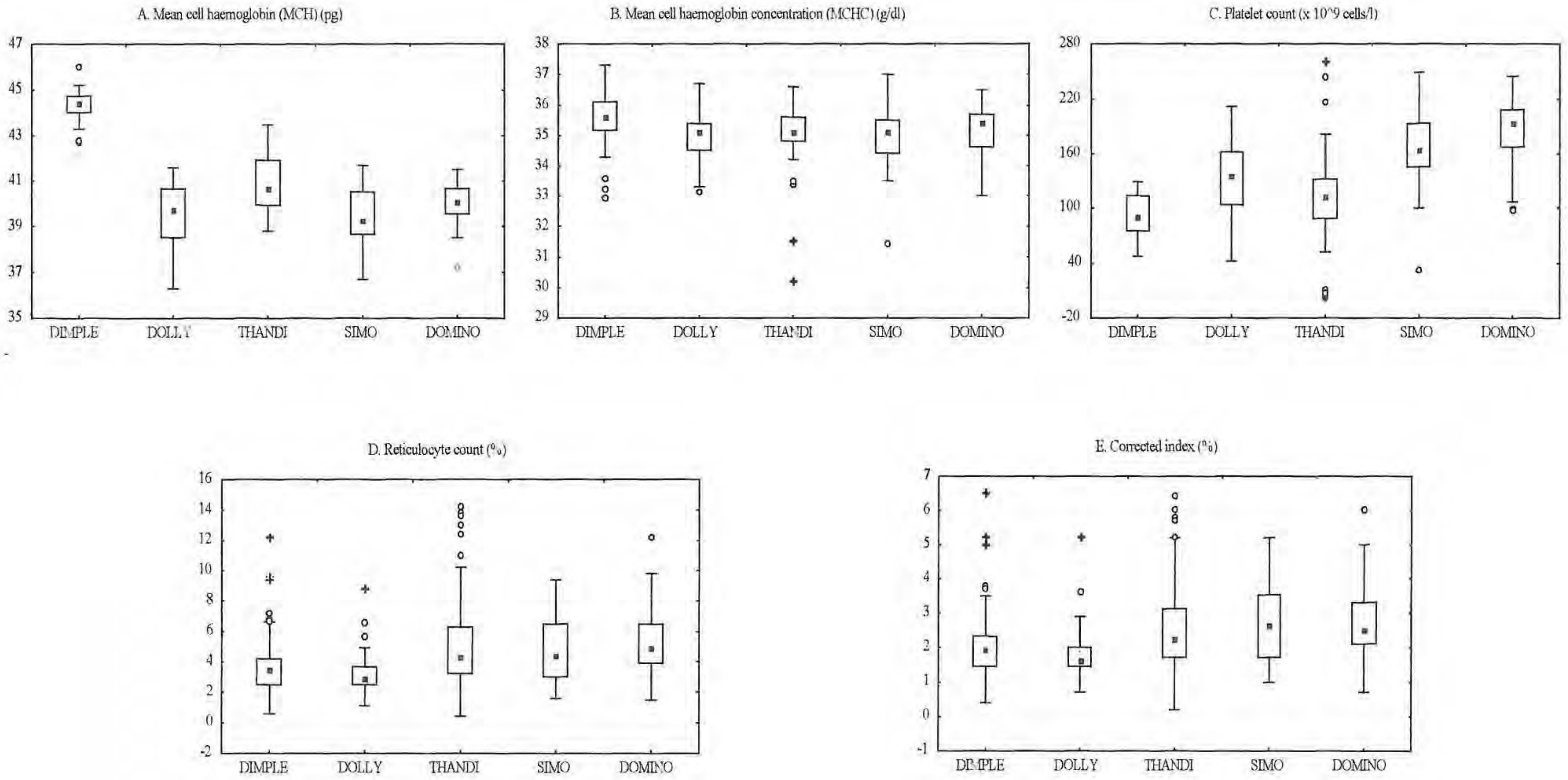
B. Haemoglobin (HB) concentration (g/dl)



C. Packed cell volume (PCV) (l/l)

D. Mean cell volume (MCV) (fl)

Appendix 1.4: Box-whisker plots for the haematological parameters of RBC, HB, PCV and MCV for the five captive dolphins. Plots include the median, 50 % range, minimum, maximum and outlying (o) and/or extreme (+) values.



Appendix 1.5: Box-whisker plots for the haematological parameters of MCH, MCHC, platelet and reticulocyte counts and corrected index for the five captive dolphins. Plots include the median, 50 % range, minimum, maximum and outlying (o) and/or extreme (+) values.

Appendix 2.1: Equations for curves of best fit (in alphabetical order).

Exponential model: $y = ae^{bx}$
Geometric fit: $y = ax^{bx}$
Heat capacity model: $y = a + bx + c/x^2$
Hoerl model: $y = ab^x x^c$
Hyperbolic fit: $y = a + b/x$
Gaussian model: $y = ae\^(-(x - b)^2/2c^2)$
Linear fit: $y = a + bx$
Logarithm fit: $y = a + b\ln x$
Logistic model: $y = a/(1 + be^{-cx})$
Modified exponential: $y = ae^{b/x}$
Modified geometric: $y = ax^{b/x}$
Modified power: $y = ab^x$
Polynomial fit: $y = a + bx + cx^2 + dx^3$
Power fit: $y = ax^b$
Quadratic fit: $y = a + bx + cx^2$
Reciprocal logarithm fit: $y = 1/(a + b\ln x)$
Reciprocal model: $y = 1/(ax + b)$
Reciprocal Quadratic: $y = 1/(a + bx + cx^2)$
Saturation growth rate model: $y = ax/(b + x)$
Sinusoidal fit: $y = a + b\cos(cx + d)$
Vapour pressure model: $y = e^{a + b/x + c\ln x}$

Appendix 2.2a: Curves of best fit, coefficients (a, b, c, d), standard error (S), levels of significance (r) and sample sizes (n) for Dimple (T1. Mean water temperature of th week prior to the blood sample; T2. Mean water temperature of the month in which blood was sampled; T3. Mean water temperature of the month prior to the blood sample; D1. Total food consumed in week prior to blood sample; E1. Energy intake of day prior to blood sample; E2. Mean daily energy intake of week in which blood was sampled; E3. Mean daily energy intake of week and month (E4) prior to blood sample).

	Curve of best fit	a	b	c	d	S	r	n
AGE								
Alkaline phosphotase (ALP)	Vapour pressure model	-2619.0943	22496.672	559.02161		89.49	0.70	47
Lactate dehydrogenase (LDH)	Heat capacity model	-211969.25	3604.1786	1.1010273		219.94	0.90	47
Platelets	Polynomial fit	578583.95	-43260.507	1077.8681	-8.9477416	18.01	0.67	22
Total bilirubin	Vapour pressure model	-8838.0319	75559.006	1883.85		2.64	0.86	43
WEIGHT								
Alkaline phosphotase (ALP)	Polynomial fit	1112864.7	-21692.733	140.80206	-0.30422709	106.75	0.58	44
Total bilirubin	Polynomial fit	52560.328	-1027.1712	6.6844409	-0.014483777	4.50	0.51	43
BLUBBER THICKNESS								
Mean cell volume (MCV)	Saturation growth rate model	114.38164	-1.61434			0.77	0.73	10
MONTH								
Blood urea nitrogen (BUN)	Quadratic fit	8.4879637	-0.32772342	0.020310951		0.70	0.40	45
White blood cells (WBC)	Sinusoidal fit	8.6077698	1.3492997	0.59992844	-4.7913521	1.34	0.59	45
T1								
ALP:creatinine	Modified geometric	0.5737993	12.03501			1.24	0.49	43
Alkaline phosphotase (ALP)	Saturation Growth Rate model	155.58243	-10.539886			106.78	0.51	47
Blood urea nitrogen (BUN)	Reciprocal model	-0.0016605321	0.16991173			0.66	0.45	44
Creatine phosphokinase (CPK)	Power fit	6239.118	-1.2289685			60.48	0.46	38
Creatinine	Gaussian model	107.57151	24.432409	11.201931		10.32	0.66	45
Haemoglobin (HB)	Polynomial fit	-34.525586	7.4632257	-0.34450749	0.0051554266	0.72	0.50	45
Lactate dehydrogenase (LDH)	Polynomial fit	-32863.328	5093.5103	-246.05843	3.8629034	444.47	0.46	47
Sodium:creatinine	Sinusoidal fit	1.5749061	0.14648619	0.41539696	-6.9080333	0.21	0.48	46
White blood cells (WBC)	Sinusoidal fit	8.3864937	1.2903325	0.47812577	-8.4767229	1.30	0.62	45

Appendix 2.2a: contd.

	Curve of best fit	**a**	**b**	**c**	**d**	**S**	**r**	**n**
T2								
ALP:creatinine	Saturation growth rate model	1.3096823	-12.142621			1.18	0.56	43
Alkaline phosphotase (ALP)	Heat capacity model	-1536.6472	52.154596	316465.99		103.78	0.56	47
Blood urea nitrogen (BUN)	Geometric fit	6.1551893	0.00298128			0.69	0.40	45
Creatinine	Hoerl model	1.953964E-05	0.72675197	7.2981238		9.01	0.75	45
Haemoglobin (HB)	Vapour pressure model	7.3530698	-21.261032	-1.1328782		0.74	0.43	45
Sodium:creatinine	Sinusoidal fit	1.5579277	0.17374173	0.51305978	-8.7653402	0.21	0.54	46
Total bilirubin	Modified geometric fit	0.030307354	34.596029			4.34	0.53	43
White blood cells (WBC)	Saturation growth rate model	4.9724123	-8.4364566			1.32	0.58	45
T3								
ALP:creatinine	Geometric fit	7.1041119	-0.011188284			1.32	0.37	43
Alkaline phosphotase (ALP)	Saturation growth rate model	179.31739	-9.4802137			115.87	0.36	47
Blood urea nitrogen (BUN)	Heat capacity model	-4.3336598	0.39262668	1442.8319		0.69	0.43	45
Creatinine	Reciprocal model	-0.0002569485	0.015742324			10.70	0.61	45
Reticulocyte count	Quadratic fit	18.202536	-1.3425385	0.028791672		0.93	0.39	40
Sodium:creatinine	Polynomial fit	-20.637846	3.1120119	-0.14199012	0.0021130174	0.21	0.49	46
White blood cells (WBC)	Vapour pressure model	20.560493	-83.434813	-4.7285729		1.27	0.64	45
D1								
Alkaline phosphotase (ALP)	Modified exponential	47.463318	97.466947			105.31	0.40	37
Eosinophils	Sinusoidal fit	4.190892	1.9024843	0.35117922		2.17	0.45	35
E1								
White blood cells (WBC)	Saturation growth rate model	5.1408682	-13792.101			1.55	0.41	41
E2								
Alkaline phosphotase (ALP)	Quadratic fit	8281.3968	-0.45043769	6.3345767E-06		100.47	0.63	38
Creatine phosphokinase (CPK)	Quadratic fit	3224.3147	-0.17122163	2.3644917E-06		44.78	0.66	32
Creatinine	Sinusoidal fit	96.88696	9.1427672	0.0005242939	-0.31229756	9.78	0.61	36
Sodium:creatinine	Polynomial fit	-48.272334	0.0046548214	-1.427177E-07	1.4387848E-12	0.19	0.56	38
Total bilirubin	Quadratic fit	289.46437	-0.015657681	2.1393297		3.44	0.74	38
White blood cells (WBC)	Linear fit	17.300188	-0.0002525944			1.38	0.48	39

Appendix 2.2a: contd.

	Curve of best fit	a	b	c	d	S	r	n
E3								
Alkaline phosphotase (ALP)	Polynomial fit	-17905.814	1.8969769	-6.333688E-05	6.8536994E-10	94.99	0.70	40
Blood urea nitrogen (BUN)	Hyperbolic fit	10.162701	-93823.92			0.64	0.34	40
Creatine phosphokinase (CPK)	Polynomial fit	18712.365	-1.4776221	3.8873715E-05	-3.379401E-10	44.69	0.73	33
E4								
Neutrophils	Quadratic fit	227.0112	-0.0088548414	1.1973886E-07		5.03	0.39	43
Packed cell volume (PCV)	Polynomial fit	-1.0157807	0.00013523375	-4.084969E-09	4.1604201E-14	0.02	0.38	38

Appendix 2.2b: Curves of best fit, coefficients (a, b, c, d), standard error (S), levels of significance (r) and sample sizes (n) for Dolly (T1. Mean water temperature of the week prior to the blood sample; T2. Mean water temperature of the month in which blood was sampled; T3. Mean water temperature of the month prior to the blood sample; D1. Total food consumed (kg) in week prior to blood sample; E1. Energy intake of day prior to blood sample; E2. Mean daily energy intake of week in which blood was sampled; E3. Mean daily energy intake of week and month (E4) prior to blood sample).

Blood parameter	**Curve of best fit**	**a**	**b**	**c**	**d**	**S**	**r**	**n**
AGE								
Alkaline phosphotase (ALP)	Sinusoidal fit	419.90139	251.24084	1.4593217	11.572102	112.60	0.88	44
Corrected index	Quadratic fit	148.44237	-11.44062	0.22259642		0.43	0.53	40
LDH:creatinine	Hyperbolic fit	22.440769	-331.87749			1.58	0.32	43
Lactate dehydrogenase (LDH)	Sinusoidal fit	995.70567	82.936297	1.6419601	6.7191388	132.32	0.44	43
Reticulocyte count	Sinusoidal fit	2.9350927	-0.50003296	1.5237632	61.12202	0.80	0.40	38
WEIGHT								
Alkaline phosphotase (ALP)	Gaussian model	629.65693	191.76645	12.627241		114.09	0.86	40
Corrected index	Quadratic fit	48.396011	-0.4981663	0.0013214531		0.47	0.40	38
Haemoglobin (HB)	Polynomial fit	-2000.6752	34.304172	-0.19420094	0.00036625942	0.76	0.59	40
Neutrophils	Saturation growth rate model	42.154953	-74.71878			7.75	0.30	43
Red blood cells (RBC)	Modified exponential	7.6670383	-91.458186			0.21	0.47	42
Reticulocyte count	Sinusoidal fit	2.8480802	0.48154637	0.23601971	-22.111663	0.81	0.40	36
Total bilirubin	Harris model	48.608049	-32.82211	0.073470035		1.04	0.63	36
BLUBBER								
Red blood cells (RBC)	Reciprocal model	-0.0054120898	0.31874343			0.19	0.63	10
T1								
Creatine phophokinase (CPK)	Saturation Growth Rate Model	108.91952	-5.1637063			17.47	0.40	42
Lactate dehydrogenase (LDH)	Saturation Growth Rate Model	724.22922	-5.5241989			131.35	0.40	43
Mean cell volume (MCV)	Vapour pressure model	7.3292637	-12.409528	-0.65903951		3.50	0.38	47
Sodium	Sinusoidal fit	158.22827	2.122088	0.71925836	-8.7462769	3.32	0.40	45
T2								
Mean cell volume (MCV)	Geometric fit	119.00698	-0.0008516327			3.50	0.35	47
Sodium	Sinusoidal fit	157.64143	2.48705	0.62977063	0.0011501088	3.11	0.51	45
Total bilirubin	Heat capacity model	22.737533	-0.68631948	-2461.329		1.24	0.43	40

Appendix 2.2b: contd.

Blood parameter	Curve of best fit	a	b	c	d	S	r	n
D1								
BUN:creatinine	Quadratic fit	-0.72626662	0.017248136	-9.105002E-05		0.01	0.47	36
CPK:creatinine	Gompertz relation	1.4781925	11.151782	0.1551163		0.22	0.44	36
Corrected index	Modified geometric fit	0.17295633	44.871967			0.48	0.34	33
Creatinine	Heat capacity model	-1215.0399	9.7313262	3594381.3		11.71	0.49	36
Red blood cells (RBC)	Heat capacity model	-4.0825579	0.071650734	18499.116		0.21	0.38	38
Sodium:creatinine	Gaussian model	1.5169367	91.272503	18.197874		0.18	0.42	37
E1								
BUN:creatinine	Polynomial fit	-1.743177	8.8927332E-05	-1.443218E-09	7.8142206E-15	0.01	0.47	37
Blood urea nitrogen (BUN)	Gaussian model	9.644357	70296.66	34077.726		0.69	0.55	38
Eosinophils	Log fit	-53.257037	5.0840923			1.45	0.36	32
Platelets	Linear fit	408.35975	-0.0043153083			29.40	0.62	22
E2								
BUN:creatinine	Polynomial fit	-2.4090352	0.00013004881	-2.248285E-09	1.2877879E-14	0.01	0.50	35
Eosinophils	Heat capacity model	-17.538092	0.00025874475	1.5848229e+10		1.42	0.43	33
E3								
Blood urea nitrogen (BUN)	Vapour pressure model	25.870102	-147096.97	-1.9272022		0.74	0.50	39
LDH:creatinine	Quadratic fit	35.317639	-0.0009581089	8.5765178E-09		1.49	0.38	38
E4								
CPK:creatinine	Polynomial fit	-144.57104	0.0072375081	-1.193427E-07	6.5406621E-13	0.22	0.42	39
Eosinophils	Polynomial fit	-602.15816	0.030454186	-5.10263E-07	2.8433406E-12	1.45	0.43	33
LDH:creatinine	Reciprocal log fit	1.2673277	-0.10503754			1.47	0.45	40
Packed cell volume (PCV)	Polynomial fit	2.6680993	-8.931539E-05	1.1713147E-09	-4.677146E-15	0.02	0.47	40
Red blood cells (RBC)	Polynomial fit	-12.927237	0.00095530739	-1.732891E-08	1.0450601E-13	0.20	0.42	42

Appendix 2.2c: Curves of best fit, coefficients (a, b, c, d), standard error (S), levels of significance (r) and sample sizes (n) for Thandi (T1. Mean water temperature of th week prior to the blood sample; T2. Mean water temperature of the month in which blood was sampled; T3. Mean water temperature of the month prior to the blood sample); D1. Total food consumed (kg) in week prior to blood sample; E1. Energy intake of day prior to blood sample; E2. Mean energy of week in which blood was sampled; Mean daily energy intake of week (E3) and month (E4) prior to blood sample).

Blood parameter	Curve of best fit	a	b	c	d	S	r	n
AGE								
ALP:creatinine	Sinusoidal fit	3.5479089	2.2051328	1.2315304	-7.3812493	1.18	0.72	82
Albumin:globulin	Reciprocal quadratic fit	1638.4337	-127.19213	2.4697027		0.11	0.82	27
Globulin	Saturation growth rate model	1.4483474	-24.580774			2.93	0.86	28
Lactate dehydrogenase (LDH)	Sinusoidal fit	916.58935	293.33981	1.5500382	5.1293943	238.49	0.64	83
Neutrophils	Sinusoidal fit	68.328919	6.9672378	1.5442364	5.6616833	9.07	0.44	86
White blood cells (WBC)	Sinusoidal fit	10.972568	6.3305438	1.4451264	7.7301235	5.78	0.61	85
WEIGHT								
Albumin	Gaussian model	46.297678	195.20709	25.841071		2.58	0.81	17
Albumin:globulin	Linear fit	-3.7001592	0.025745614			0.12	0.79	16
Globulin	Linear fit	128.59199	-0.47910058			4.84	0.54	17
Haemoglobin (HB)	Sinusoidal fit	16.978898	0.742945	0.16151226	-1.6919795	1.12	0.40	60
Neutrophils	Saturation growth rate model	37.408068	-80.507865			9.57	0.29	63
Red blood cells (RBC)	Sinusoidal fit	4.0987685	0.24871896	0.15221663	0.038198054	0.30	0.47	64
Total bilirubin	Quadratic fit	-61.713921	0.67181211	-0.0017367848		1.31	0.39	54
White blood cells (WBC)	Richards model	13.412518	-42.74967	-0.21881083	0.76698711	7.04	0.29	62
BLUBBER								
CPK:creatinine	Linear fit	3.9492828	-0.13786157			0.49	0.62	12
Creatine phosphokinase (CPK)	Gaussian model	181.84423	16.639516	4.7709699		37.04	0.70	12
MONTH								
Albumin:globulin	Polynomial fit	18.981924	-7.3898728	0.97891047	-0.041787426	0.08	0.90	27
CPK:creatinine	Sinusoidal fit	1.3453985	0.38801647	0.6446336	-4.9632977	0.70	0.38	81
Globulin	Polynomial fit	-119.53933	75.250574	-10.756336	0.47778116	2.83	0.88	28
Lactate dehydrogenase (LDH)	Sinusoidal fit	990.07255	212.62605	0.62310846	0.96775075	273.47	0.48	85
Platelets	Sinusoidal fit	106.75367	16.921199	0.61369757	0.10469274	27.01	0.41	48
T1								
Albumin:globulin	Sinusoidal fit	1.062164	0.12989083	1.3812419	-8.1724728	0.15	0.59	27
BUN:creatinine	Polynomial fit	-0.99201053	0.16985306	-0.008546819	0.00013908698	0.03	0.30	81
Blood urea nitrogen (BUN)	Polynomial fit	-106.12723	17.743768	-0.87579778	0.014072232	1.41	0.41	85

Appendix 2.2c: contd.

Blood parameter	Curve of best fit	a	b	c	d	S	r	n
T1 contd.								
Creatine phosphokinase (CPK)	Polynomial fit	-2688.2718	424.56573	-20.5964	0.3237109	55.58	0.38	80
Globulin	Quadratic fit	-419.27361	53.669811	-1.5558213		4.47	0.65	28
Haemoglobin (HB)	Reciprocal quadratic fit	0.1056509	-0.0040623966	8.5096089E-05		1.11	0.38	80
Lactate dehydrogenase (LDH)	Polynomial fit	-22741.686	3449.1098	-163.32778	2.5214563	284.24	0.42	80
Packed cell volume (PCV)	Sinusoidal fit	0.47733695	0.032291381	0.42783769	-2.6299489	0.04	0.48	87
Red blood cells (RBC)	Sinusoidal fit	4.0700432	0.2673043	0.3883263	-2.7407626	0.32	0.45	87
Reticulocyte count	Quadratic fit	30.840711	-2.3730889	0.050545257		2.10	0.34	79
T2								
BUN:creatinine	Saturation growth rate	-1640572	-341669650			0.04	0.76	80
Blood urea nitrogen (BUN)	Sinusoidal fit	10.638612	1.0814339	0.59251367	-2.1513103	1.38	0.46	84
Haemoglobin (HB)	Vapour pressure model	7.5033242	-26.928673	-1.1083983		1.08	0.40	79
Packed cell volume (PCV)	Polynomial fit	-2.4382895	0.37481556	-0.015967768	0.00022684842	0.04	0.53	86
Red blood cells (RBC)	Polynomial fit	-29.294241	4.4000574	-0.19199167	0.0027856958	0.30	0.55	86
Reticulocyte count	Logisitic model	3.6630157	-2594.6224	0.51389383		2.08	0.37	78
Total bilirubin	Log fit	10.095267	-2.4244695			1.34	0.26	75
T3								
BUN:creatinine	Saturation growth rate model	-4491733.9	-941246210			0.04	0.76	81
Blood urea nitrogen (BUN)	Saturation growth rate model	8.1231161	-5.163377			1.43	0.36	85
Haemoglobin (HB)	Power fit	11.750115	0.121216117			1.14	0.26	80
Packed cell volume (PCV)	Polynomial fit	-0.97982355	0.18231261	-0.0076008972	0.00010676895	0.04	0.41	87
Red blood cells (RBC)	Logistic model	4.2417282	1521.369	0.55996165		0.33	0.43	87
Reticulocyte count	Linear fit	8.2459152	-0.18467362			2.14	0.27	79
D1								
ALP:creatinine	Geometric fit	1.4035499	0.0031709205			1.53	0.41	65
BUN:creatinine	Logistic model	0.12095974	6016131.9	0.32321685		0.02	0.69	64
CPK:creatinine	Polynomial fit	-48.571216	2.2052089	-0.032090902	0.00015470178	0.59	0.60	64
Creatine phosphokinase (CPK)	Geometric fit	66.320025	0.0028279549			45.49	0.51	65
Creatinine	Polynomial fit	1746.2273	-69.800605	0.97030883	-0.0044322552	14.53	0.66	63
Haemoglobin (HB)	Linear fit	20.79144	-0.063368794			0.82	0.66	65
LDH:creatinine	Logistic model	11.039339	1205553.2	0.28015225		2.31	0.73	64
Lactate dehydrogenase (LDH)	Polynomial fit	-22930.779	1049.8753	-15.085339	0.071325033	242.09	0.55	68
Packed cell volume (PCV)	Polynomial fit	1.6558218	-0.054476344	0.00085303801	-4.5247839E-06	0.03	0.71	69

Appendix 2.2c: contd.

Blood parameter	Curve of best fit	a	b	c	d	S	r	n
D1 contd.								
Red blood cells (RBC)	Polynomial fit	8.0568668	-0.2222321	0.0042169406	-2.6383671E-05	0.24	0.71	69
Sodium	Sinusoidal fit	155.10117	2.5875509	0.20944666	-10.752177	2.58	0.56	68
Sodium:creatinine	Logistic model	1.5994515	236738870	0.40325868		0.20	0.73	64
E1								
BUN:creatinine	Heat capacity model	0.065739909	1.3803394E-06	-5765821.4		0.02	0.52	67
Blood urea nitrogen (BUN)	Sinusoidal fit	11.639287	1.1980006	0.00014508475	-0.84911848	1.23	0.56	71
CPK:creatinine	Polynomial fit	-2.5218388	0.00036981039	-1.284871E-08	1.5500285E-13	0.60	0.60	66
Creatine phosphokinase (CPK)	Polynomial fit	-122.9437	0.029123894	-1.101937E-06	2.368488E-11	44.90	0.63	67
Creatinine	Linear fit	139.92603	-0.0010217475			17.21	0.42	66
Haemoglobin (HB)	Polynomial fit	8.5703482	0.00087512524	2.6891769E-08	2.4442637E-13	0.98	0.49	68
Lactate dehydrogenase (LDH)	Polynomial fit	-520.26447	0.13108335	-3.926352E-06	4.0676732E-11	281.07	0.43	70
Packed cell volume (PCV)	Polynomial fit	0.2886103	2.1453819E-05	-6.829788E-10	6.2467697E-15	0.03	0.50	72
Platelets	Quadratic fit	172.98566	-0.0046936075	8.1905287E-08		26.07	0.46	36
Red blood cells (RBC)	Polynomial fit	1.5082567	0.00026292163	-7.938422E-09	7.1422413E-14	0.29	0.49	72
E2								
BUN:creatinine	Polynomial fit	-0.7077063	7.8459415E-05	-2.513373E-09	2.6534894E+14	0.02	0.53	66
Haemoglobin (HB)	Heat capacity model	29.477414	-0.0002875468	-3131449200		0.92	0.60	65
Lactate dehydrogenase (LDH)	Polynomial fit	-2488.9034	0.34720836	-1.158438E-05	1.2741006E-10	259.35	0.35	69
Packed cell volume (PCV)	Polynomial fit	0.31029597	9.6488397E-06	-2.153911E-11	-3.2750469E-15	0.03	0.65	69
Red blood cells (RBC)	Quadratic fit	1.5795943	0.00017909316	-3.04277E-09		0.26	0.63	69
E3								
Blood urea nitrogen (BUN)	Log fit	-11.16382	2.1476569			1.38	0.33	72
Creatine phosphokinase (CPK)	Geometric fit	71.980521	2.0078547E-06			50.64	0.45	68
Haemoglobin (HB)	Polynomial fit	-9.7500122	0.0025239108	-7.476452E-08	6.9295624E-13	0.87	0.64	69
LDH:creatinine	Power fit	0.00043699086	0.95262712			2.82	0.55	66
Packed cell volume (PCV)	Polynomial fit	-0.3513875	7.8572908E-05	-2.325343E-09	2.1487466E-14	0.03	0.63	73
Red blood cells (RBC)	Polynomial fit	-3.8810788	0.00074722523	-2.197491E-08	2.0274023E-13	0.26	0.63	73
E4								
ALP:creatinine	Reciprocal log fit	4.0562339	-0.36082394			1.55	0.43	80
LDH:creatinine	Hyperbolic fit	15.683048	-201768.47			3.20	0.37	76
Platelets	Quadratic fit	322.61959	-0.01518231	2.6025693E-07		25.76	0.47	43

Appendix 2.2d: Curves of best fit, coefficients (a, b, c, d), standard error (S), levels of significance (r) and sample sizes (n) for Simo (T1. Mean water temperature the week prior to the blood sample; T2. Mean water temperature of the month in which blood was sampled; T3. Mean water temperature of the r prior to the blood sample; E1. Energy intake of day prior to blood sample; E2. Mean daily energy intake of week in which blood was sampled; E Mean daily energy intake of week (E3) prior to blood sample).

Blood parameter	Curve of best fit	a	b	c	d	S	r	n
AGE								
ALP:creatinine	Polynomial fit	530.82194	-126.98139	10.136419	-0.26858529	1.09	0.59	50
BUN:creatinine	Polynomial fit	-11.180831	2.8585835	-0.24172898	0.0068205605	0.03	0.54	51
Creatinine	Log fit	340.58842	-94.315738			18.95	0.38	47
Eosinophils	Linear fit	39.49293	-2.5783124			4.44	0.50	46
Lactate dehydrogenase (LDH)	Polynomial fit	-51440.228	14783.256	-1383.3234	42.851711	163.90	0.72	48
Neutrophils	Geometric fit	24.630302	0.031876293			9.67	0.58	52
WEIGHT								
Alkaline phosphotase (ALP)	Sinusoidal fit	305.98015	112.76092	0.21201688	-20.052773	112.60	0.62	38
Corrected index	Linear fit	12.736388	-0.057729574			1.05	0.41	44
Haemoglobin (HB)	Quadratic fit	147.5044	-1.4779292	0.0042276754		0.96	0.42	42
Mean cell volume (MCV)	Sinusoidal fit	112.22254	1.5976132	0.17396679	-22.786718	1.95	0.55	45
Platelets	Linear fit	604.68799	-2.5163199			31.49	0.51	35
Reticulocyte count	Linear fit	22.449537	-0.10171457			2.06	0.37	45
MONTH								
BUN:creatinine	Sinusoidal fit	0.099982506	0.019447876	0.56079297	-4.4330335	0.03	0.46	51
CPK:creatinine	Sinusoidal fit	1.637167	0.59458332	0.50585438	1.8764046	0.59	0.64	50
Creatine phosphokinase (CPK)	Sinusoidal fit	170.50865	35.128297	0.46670503	2.0810412	43.79	0.56	52
Creatinine	Sinusoidal fit	108.1573	16.653544	0.46839956	-0.62611334	16.64	0.61	47
LDH:creatinine	Modified Hoerl model	2.7943143	2.7276412	0.52806383		2.75	0.47	45
Lactate dehydrogenase (LDH)	Modified Hoerl model	504.14452	1.8309729	0.28768974		219.75	0.35	48
Sodium	Saturation growth rate model	158.82246	0.026131654			2.08	0.40	50
Sodium:creatinine	Sinusoidal fit	1.4873493	0.26255691	0.48142011	2.1172833	0.31	0.56	51
White blood cells (WBC)	Polynomial fit	7.4997902	-1.0854581	0.3035146	-0.017722174	1.72	0.58	50
T1								
Alkaline phosphotase (ALP)	Log fit	-1124.8064	468.44327			115.68	0.51	45
BUN:creatinine	Sinusoidal fit	0.10116604	0.020866975	0.42015209	-6.9302561	0.03	0.50	51
Creatine phosphokinase (CPK)	Vapour pressure model	45.987838	-193.96327	-10.350752		42.89	0.57	52
Creatinine	Polynomial fit	1322.5348	-182.39175	8.793978	-0.13672694	16.15	0.64	47
Lactate dehydrogenase (LDH)	Sinusoidal fit	943.88231	147.12873	0.59978003	-5.2375388	206.75	0.49	48

Appendix 2.2d: contd.

Blood parameter	Curve of best fit	a	b	c	d	S	r	n
T1 contd.								
Mean cell volume (MCV)	Reciprocal log fit	0.007909976	0.00033269			2.20	0.29	49
Neutrophils	Polynomial fit	-573.79345	97.111261	-4.7815567	0.076297909	11.01	0.42	52
Sodium	Hoerl model	92.399814	0.98467024	0.28352567		2.00	0.49	51
Sodium:creatinine	Vapour pressure model	14.678344	-60.000144	-3.744004		0.32	0.52	51
White blood cells (WBC)	Sinusoidal fit	7.8618165	1.7652886	0.5737889	-4.6721328	1.56	0.68	50
T2								
Alkaline phosphotase (ALP)	Polynomial fit	15258.873	-2219.9633	107.3683	-1.6939972	107.76	0.59	44
BUN:creatinine	Polynomial fit	1.7559587	-0.20942788	0.0088397055	-0.0001254051	0.03	0.53	50
Creatinine	Modified exponential fit	226.37305	-15.915194			16.61	0.58	47
Mean cell volume (MCV)	Modified geometric fit	105.22532	0.44091909			2.16	0.32	48
Neutrophils	Polynomial fit	-951.70359	153.25714	-7.5522579	0.12151745	11.27	0.36	51
Sodium	Gaussian model	158.56946	19.780888	33.340559		1.99	0.45	49
Sodium:creatinine	Reciprocal log fit	-1.1259074	0.59013926			0.30	0.55	50
White blood cells (WBC)	Polynomial fit	-399.39006	57.886304	-2.6972772	0.041224218	1.66	0.64	49
T3								
Alkaline phosphotase (ALP)	Polynomial fit	15898.101	-2260.2111	106.8432	-1.6493405	111.25	0.59	45
BUN:creatinine	Polynomial fit	-4.7630827	0.68631976	-0.031695935	0.0004795663	0.03	0.57	51
Creatinine	Vapour pressure model	-33.356568	186.97963	9.5224352		15.47	0.66	47
Mean cell volume (MCV)	Hoerl model	57.963129	0.98168429	0.34574059		2.15	0.38	49
Neutrophils	Heat capacity model	276.50787	-6.9296515	-27350.732		11.00	0.40	52
Sodium:creatinine	Polynomial fit	-40.991014	6.1362055	-0.28902638	0.0044451196	0.31	0.58	51
White blood cells (WBC)	Vapour pressure model	51.068078	-237.88411	-12.328448		1.55	0.68	50
E1								
Lactate dehydrogenase (LDH)	Quadratic fit	13500.754	-0.61839299	7.5541791E-06		194.34	0.58	42
E2								
Eosinophils	Log fit	-218.23831	21.498065			4.63	0.37	35
Lactate dehydrogenase (LDH)	Polynomial fit	-47416.113	4.1687518	-0.0001174414	1.0841184E-09	168.11	0.65	36
White blood cells (WBC)	Polynomial fit	-685.92508	0.056443942	-1.513987E-06	1.3393718E-11	1.44	0.61	38
E3								
Eosinophils	Hyperbolic fit	34.907019	-1015189.7			4.33	0.49	37
Neutrophils	Saturation growth rate model	37.145977	-15817.792			10.11	0.39	42

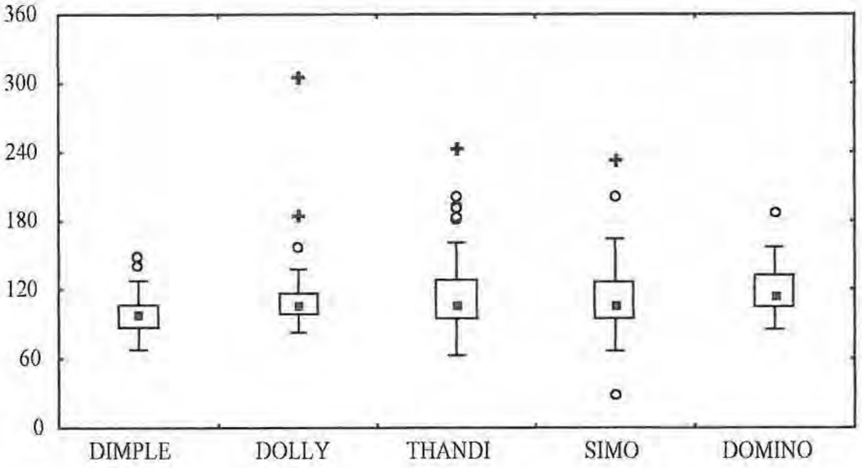
Appendix 2.2e: Curves of best fit, coefficients (a, b, c, d), standard error (S), levels of significance (r) and sample sizes (n) for Domino (T1. Mean water temperature of the week prior to the blood sample; T2. Mean water temperature of the month in which blood was sampled; T3. Mean water temperature of the mont prior to the blood sample; D1. Total food consumed (kg) in week prior to blood sample; E1. Energy intake of day prior to blood sample; E2. Mean dai energy intake of week in which blood was sampled; E3. Mean daily energy intake of week and month (E4) prior to blood sample.

Blood parameter	Curve of best fit	a	b	c	d	S	r	n
AGE								
BUN:creatinine	Heat capacity model	0.076521298	0.0017914809	-0.07501223		0.01	0.67	35
Blood urea nitrogen (BUN)	Reciprocal model	-0.0027171355	0.12332355			0.53	0.37	37
Corrected index	Sinusoidal fit	2.7416065	0.6322791	1.6303564	2.1258134	0.96	0.46	38
Creatinine	Linear fit	144.96835	-8.5299586			12.61	0.54	33
LDH:creatinine	Polynomial fit	-19.370974	26.246581	-8.3313908	0.86332383	0.72	0.76	27
Lactate dehydrogenase (LDH)	MMF model	875.12353	935071490000	1089.675	815073	105.96	0.69	37
Platelets	Geometric fit	225.87887	-0.041980716			19.21	0.62	22
Reticulocyte count	Geometric fit	6.5875134	-0.062883875			1.95	0.34	38
White blood cells (WBC	Weibull model	8.1688675	1.0438716	3866.0072	-7.5312393	0.98	0.41	39
WEIGHT								
BUN:creatinine	Vapour pressure model	-57.673664	1129.0707	9.5185016		0.01	0.44	33
Blood urea nitrogen (BUN)	Vapour pressure model	-29.723275	688.43703	5.4603018		0.52	0.48	35
Mean cell volume (MCV)	Gaussian model	114.52239	146.86237	147.47408		1.38	0.47	36
Platelets	Linear fit	460.58583	-2.0596467			19.50	0.59	21
Total bilirubin	Reciprocal model	-0.0077136037	1.4804258			1.11	0.39	31
BLUBBER								
BUN:creatinine	Bleasdale model	454306.14	-16720.19	4.4361964		0.01	0.68	12
CPK:creatinine	Reciprocal model	-0.061712728	1.8735724			0.37	0.80	13
Creatine phosphokinase (CPK)	Reciprocal model	-0.000295771	0.010873675			31.95	0.71	11
Haemoglobin (HB)	Linear fit	7.3536392	0.48125386			1.24	0.69	12
Lactate dehydrogenase (LDH)	Heat capacity model	-7447.8145	289.76507	1018785.4		62.06	0.94	13
T1								
Neutrophils	Sinusoidal fit	55.828576	9.6907112	0.46818398	-7.4266252	9.59	0.56	39
Packed cell volume (PCV)	Polynomial fit	-1.7102825	0.29625787	-0.012889537	0.00018104162	0.03	0.58	35
T2								
Creatinine	Polynomial fit	-2163.6644	309.91338	-13.938136	0.20772475	12.08	0.56	33
Neutrophils	Sinusoidal fit	54.812328	9.0388965	0.50463051	-8.3751386	9.76	0.54	39
Packed cell volume (PCV)	Sinusoidal fit	0.48650831	0.020807379	0.51801622	-3.937008	0.03	0.54	35
Total bilirubin	Saturation growth rate	1.0362922	-11.384337			1.15	0.39	33

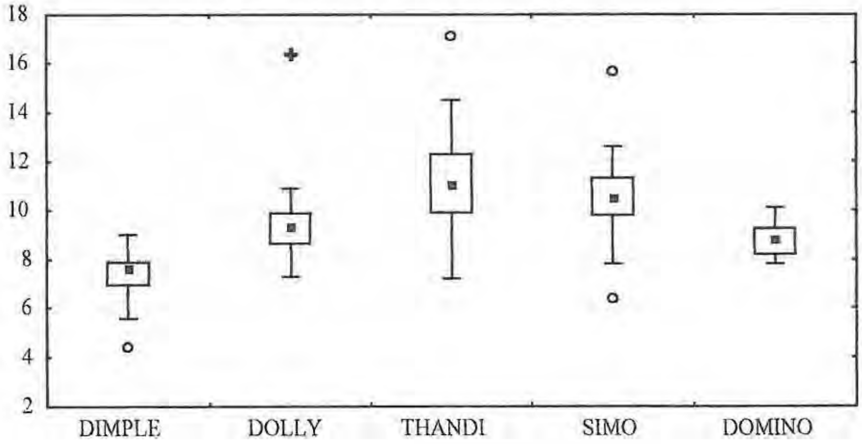
Appendix 2.2e: contd.

Blood parameter	Curve of best fit	a	b	c	d	S	r	n
T3								
Neutrophils	Heat capacity model	-140.88306	5.7240889	31396.191		10.17	0.46	39
D1								
Alkaline phosphotase (ALP)	Sinusoidal fit	1044.4824	213.43764	0.25893252	-7.8832177	232.18	0.57	33
BUN:creatinine	Reciprocal quadratic fit	19.194494	0.020352467	-0.0040575416		0.01	0.67	32
Creatinine	Quadratic fit	43.717054	5.6328013	-0.094959292		12.31	0.60	30
LDH:creatinine	Polynomial fit	-39.471972	4.0911051	-0.11945204	0.0011618247	0.85	0.60	24
Sodium:creatinine	Bleasdale model	0.75154116	-0.01496962	6.7341555		0.15	0.57	32
E1								
Blood urea nitrogen (BUN)	Saturation growth rate model	11.593627	9027.8489			0.53	0.46	34
Creatinine	Weibull model	121.45175	170869420	1281398.4	-1.0824321	13.47	0.51	30
LDH:creatinine	Quadratic fit	23.257101	-0.0013230978	2.6641204E-08		0.70	0.74	24
E2								
Alkaline phosphotase (ALP)	Quadratic fit	8415.5692	-0.47686398	7.5344459E-06		223.96	0.59	33
BUN:creatinine	Bleasdale model	13548.427	-0.34671245	3.0635001		0.10	0.72	32
Creatine phosphokinase (CPK)	Polynomial fit	-7999.5561	0.89043958	-3.16285E-05	3.6888537E-10	38.55	0.50	32
Lactate dehydrogenase (LDH)	Modified power fit	628.57428	1.0000151			136.57	0.36	34
Sodium:creatinine	Polynomial fit	-31.010603	0.0035294587	-1.28067E-07	1.5419319E-12	0.15	0.60	32
E3								
Alkaline phosphotase (ALP)	Power fit	4416242.5	-0.1997622			250.88	0.43	32
Blood urea nitrogen (BUN)	Hyperbolic fit	10.389365	-45338.505			0.54	0.41	33
Creatinine	Weibull model	124.51391	155376650	394561.83	-0.97157519	11.22	0.68	29
LDH:creatinine	Vapour pressure model	-64.251729	146773.51	5.9542254		0.77	0.68	24
E4								
ALP:creatinine	Saturation Growth Rate model	460.79431	-14986.56			235.28	0.51	33
Creatine phosphokinase (CPK)	Polynomial fit	-18316.408	2.0077458	-7.159128E-05	8.4086116E-10	30.16	0.73	32
Creatinine	Polynomial fit	3338.6399	-0.34807986	1.2515919E-05	-1.496484E-10	11.68	0.68	30
LDH:creatinine	Polynomial fit	-122.56884	0.014410653	-5.351739E-07	6.6452748E-12	0.72	0.73	24
Platelets	Saturation Growth Rate model	122.6853	-10416.786			21.00	0.59	19

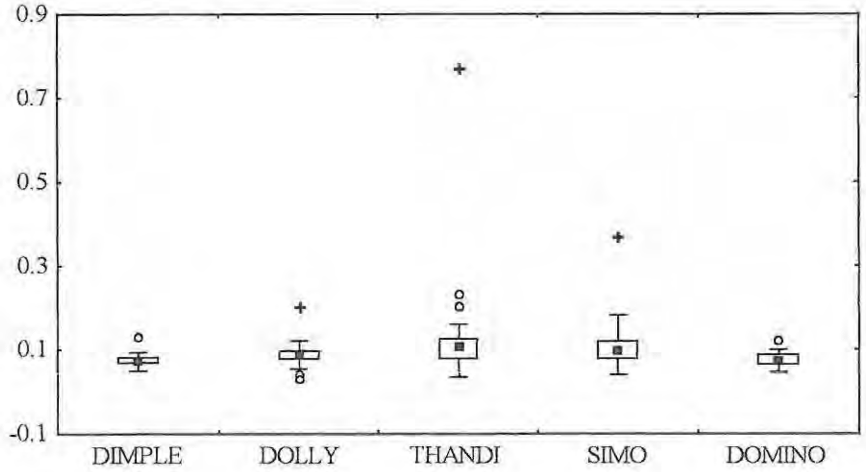
A. Creatinine (µmol/l)



B. Blood urea nitrogen (BUN) (mmol/l)

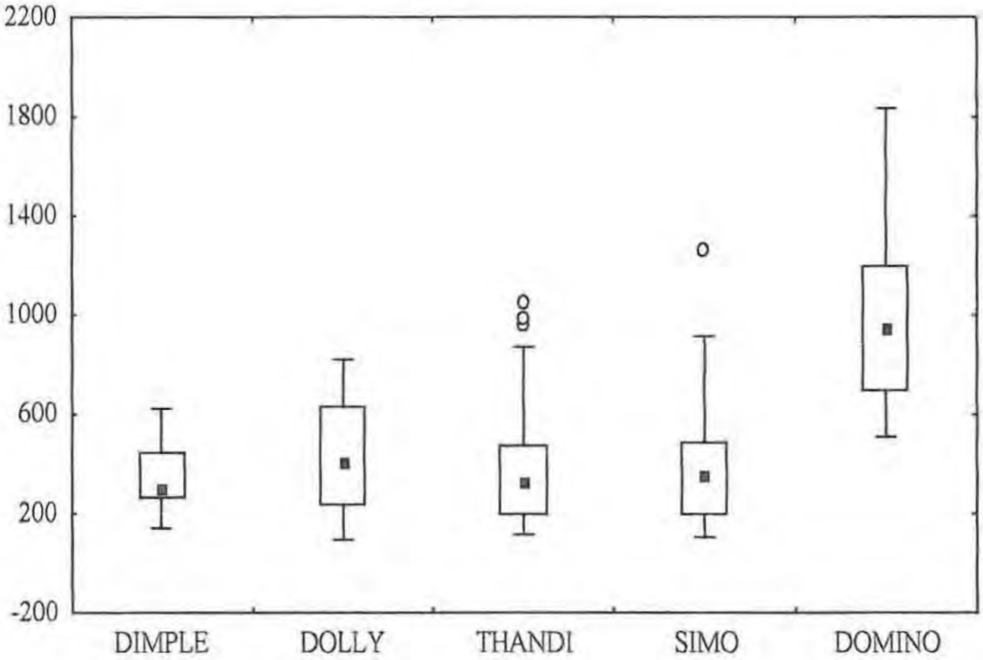


C. BUN: creatinine

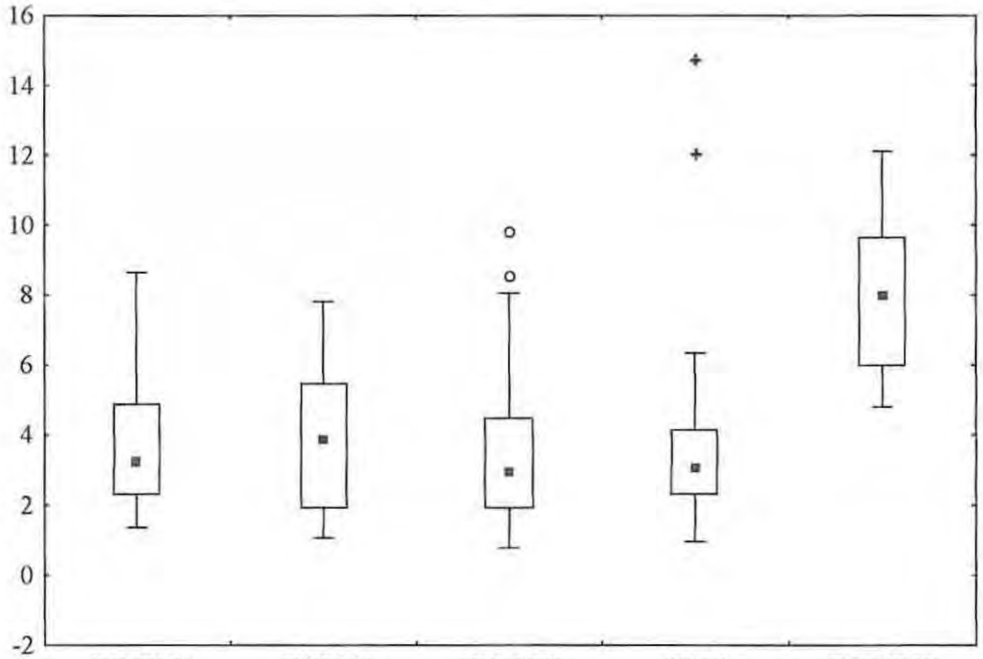


Appendix 3.1: Box-whisker plots for serum creatinine, blood urea nitrogen (BUN) and the BUN:creatinine ratio measured in captive dolphins.Plots include the median, 50 % range, minimum, maximum and outlying (o) and/or extreme (+) values.

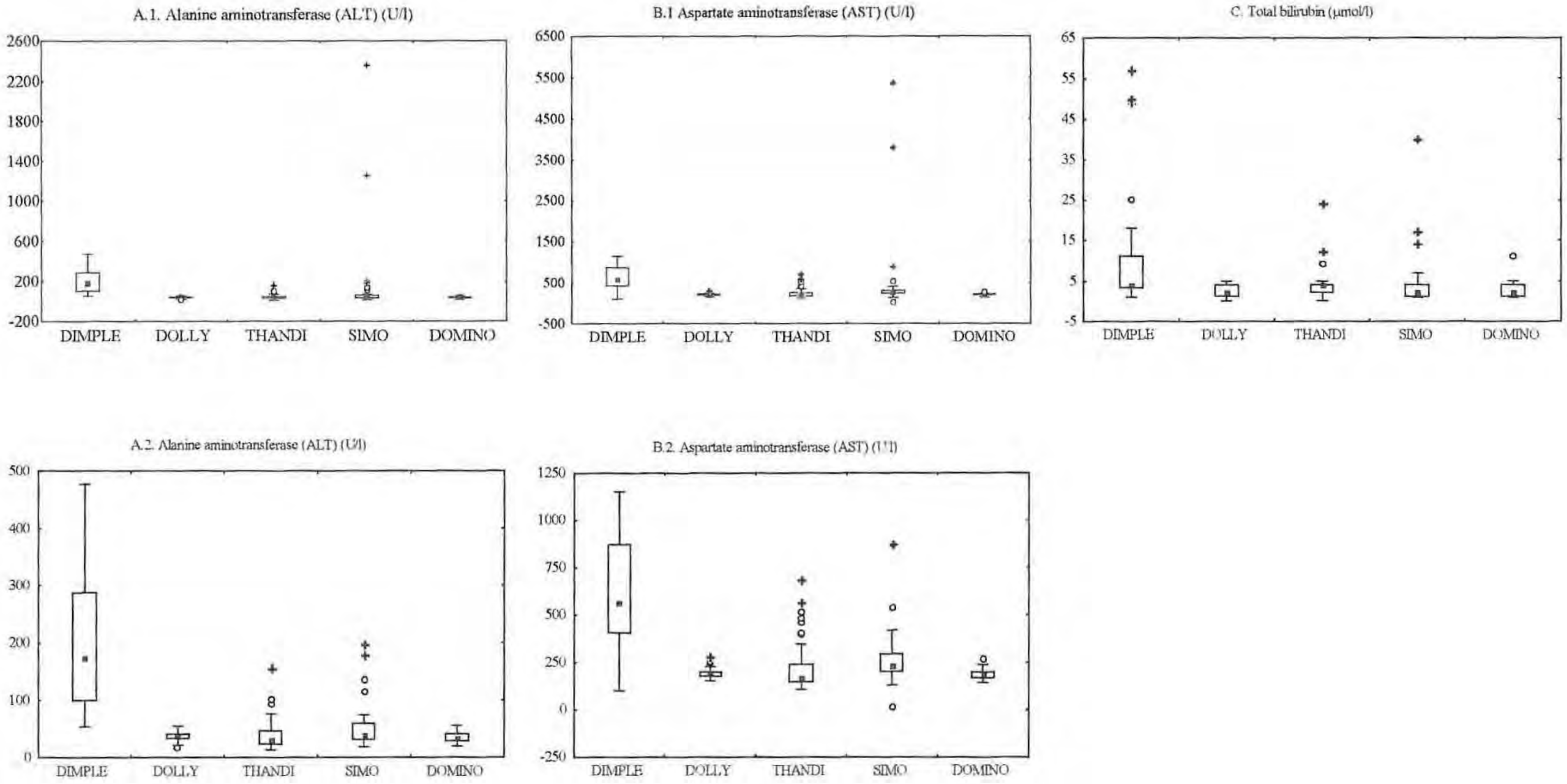
A. Alkaline phosphotase (ALP) (U/l)



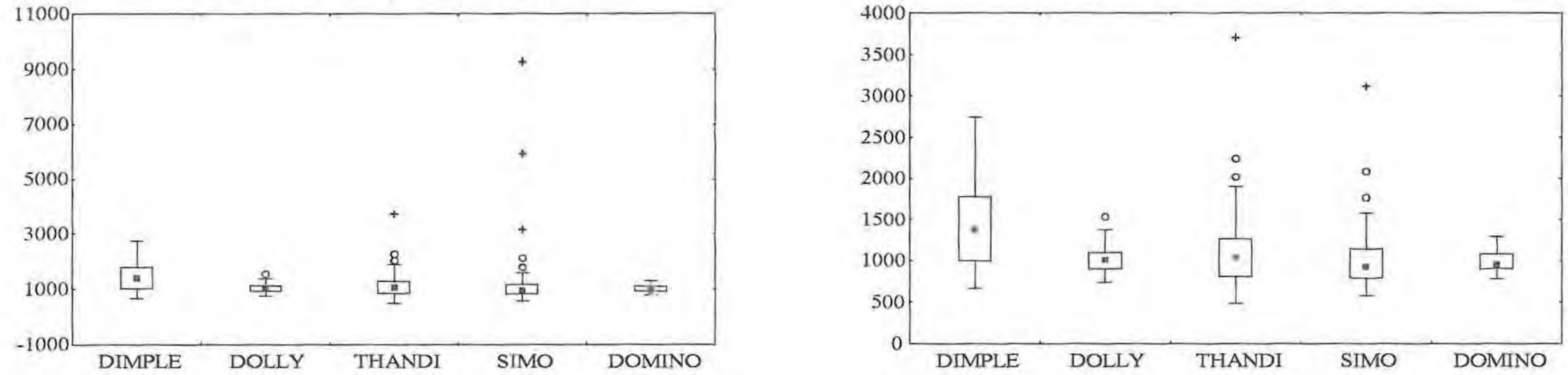
B. ALP: creatinine



Appendix 3.2: Box-whisker plots for serum alkaline phosphotase (ALP) and the ALP:creatini ratio measured in captive dolphins. Plots include the median, 50 % range, minimu maximum and outlying (o) and/or extreme (+) values.

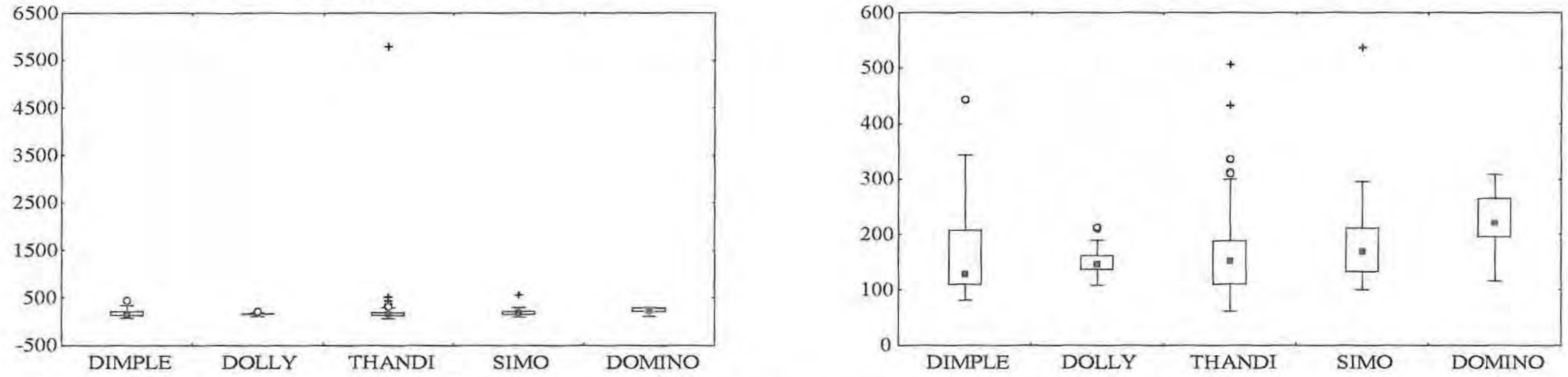


Appendix 3.3: Box-whisker plots for liver-associated enzymes and total bilirubin measured in captive dolphins. Plots include the median, 50 % range, minimum, maximum and outlying (o) and/or extreme (+) values.



A.1. Lactate dehydrogenase (LDH) (U/l)

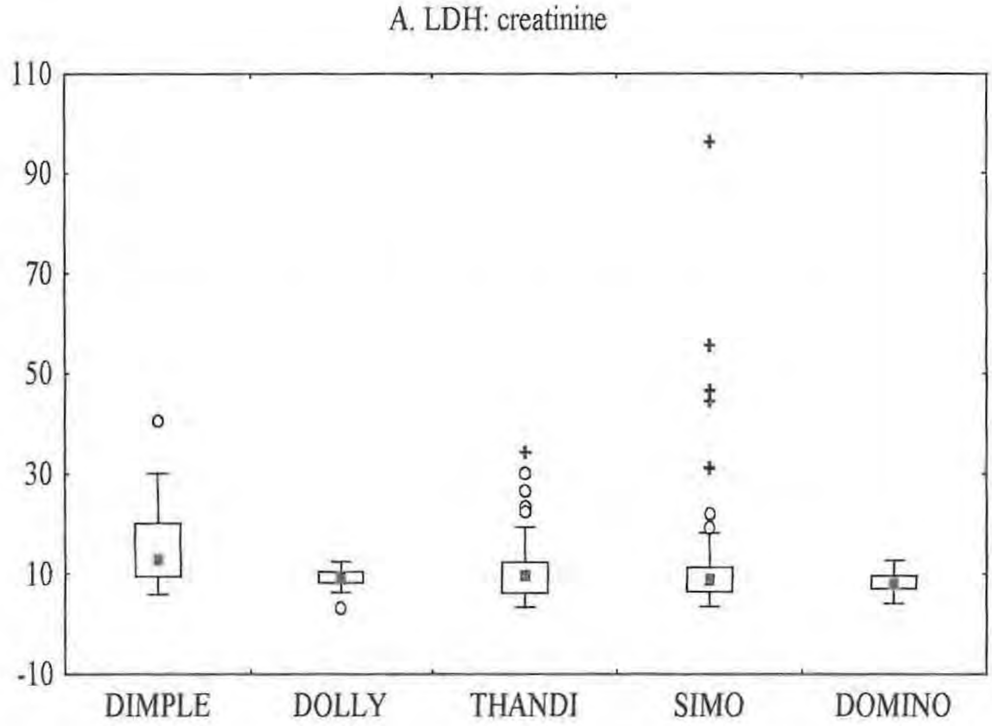
A.2. Lactate dehydrogenase (LDH) (U/l)

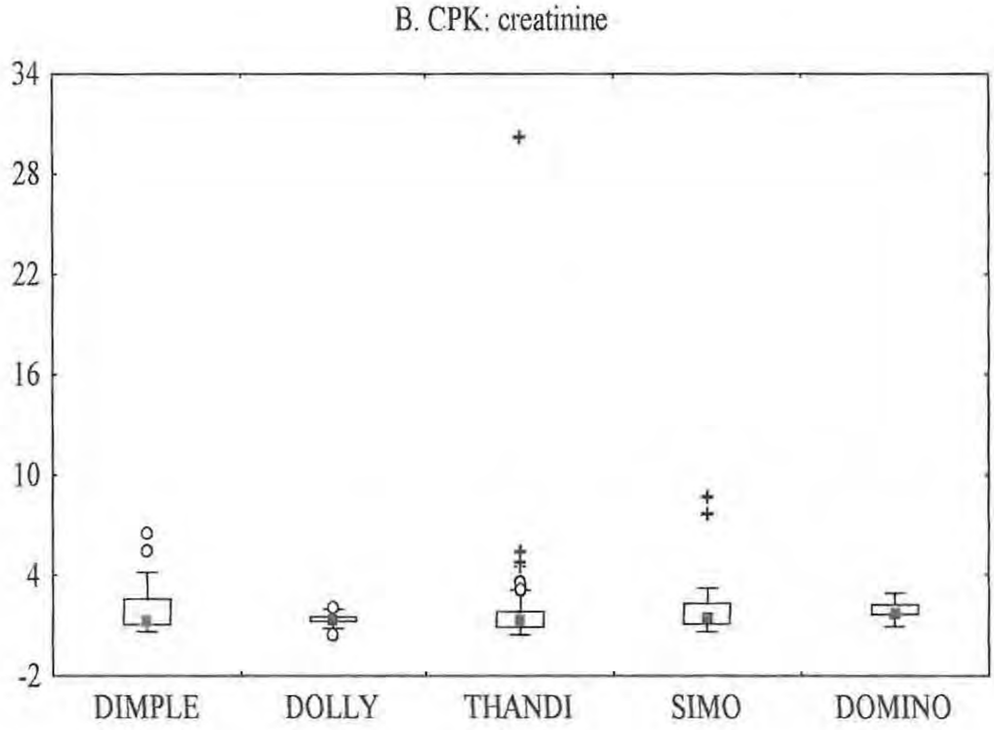


B. 1. Creatine phosphokinase (CPK) (U/l)

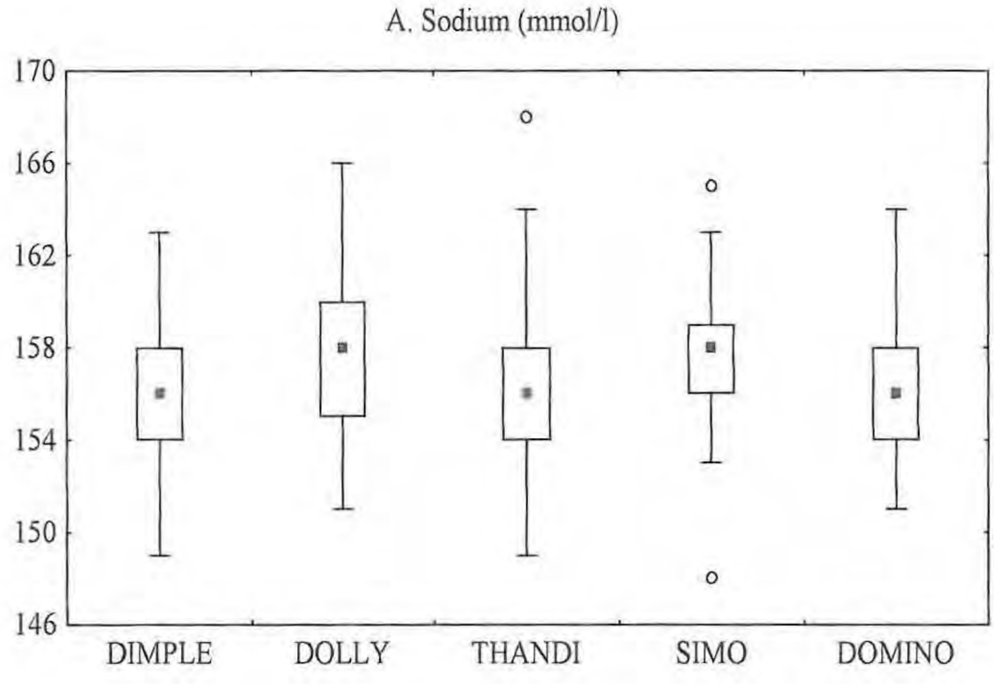
B. 2. Creatine phosphokinase (CPK) (U/l)

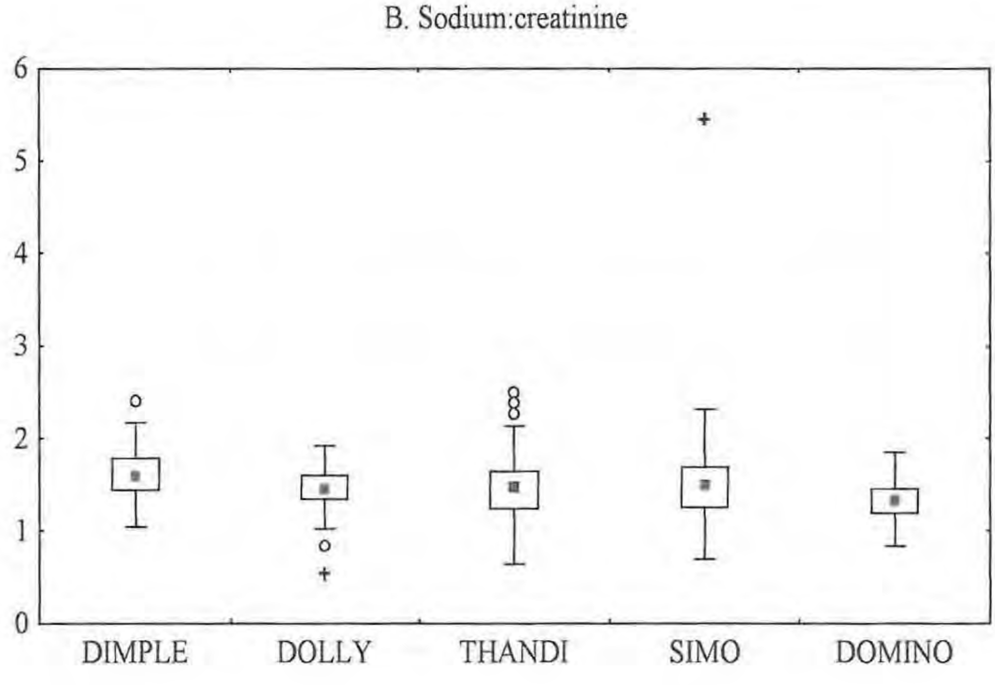
Appendix 3.4: Box-whisker plots for serum lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) measured in captive dolphins. Plots include the median, 50 % range, minimum, maximum and outlying (o) and/or extreme (+) values.



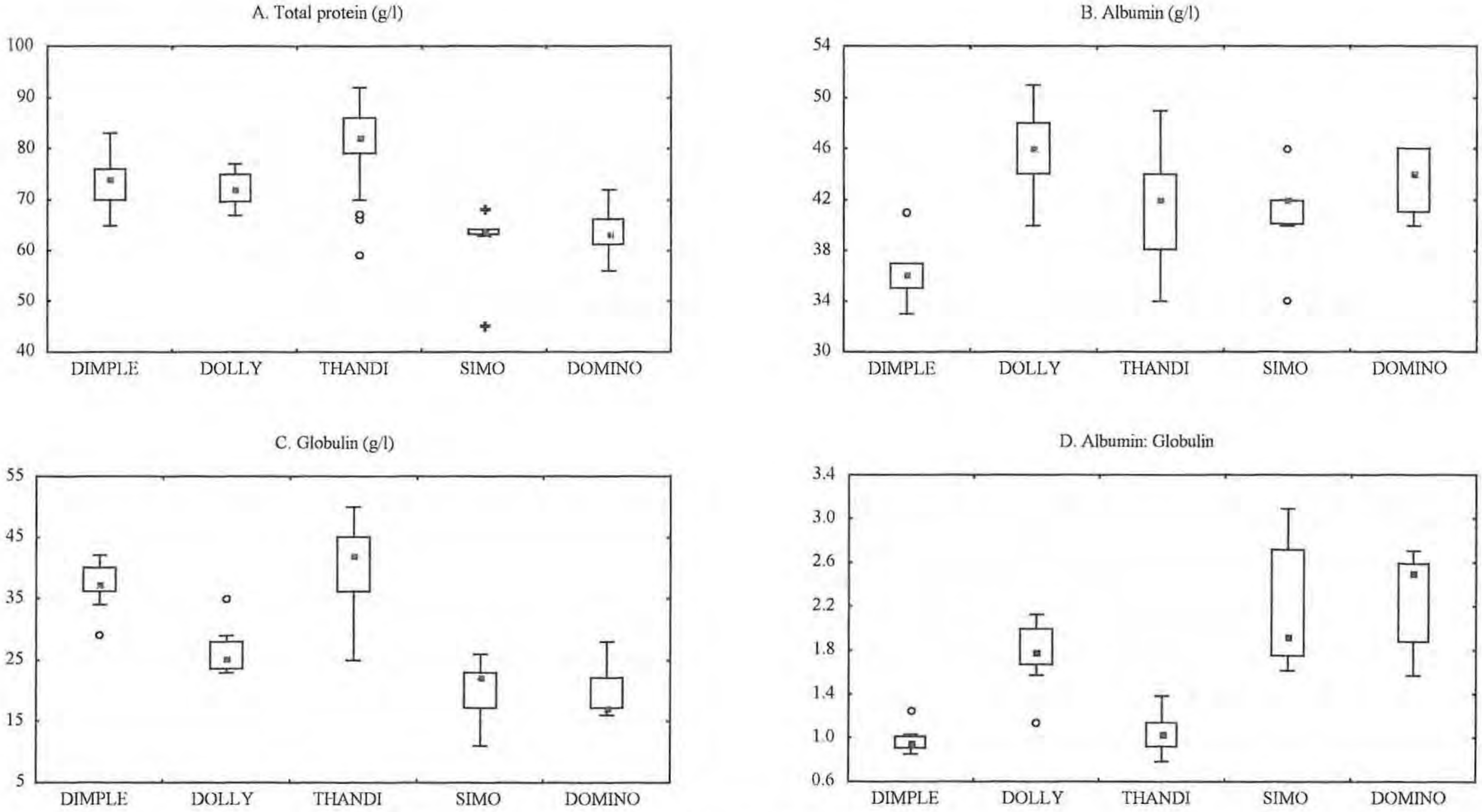


Appendix 3.5: Box-whisker plots for the serum LDH:creatinine and CPK:creatinine ratios measured in captive dolphins. Plots include the median, 50 % range, minimum, maximum and outlying (o) and/or extreme values (+).



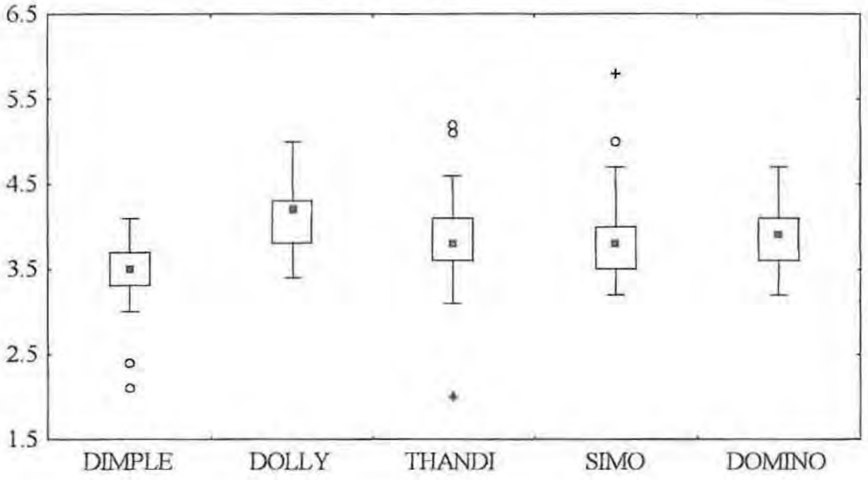


Appendix 3.6: Box-whisker plots for serum sodium and the sodium:creatinine ratio measured in captive dolphins. Plots include the median, 50 % range, minimum, maximum and outlying (o) and/or extreme values (+).

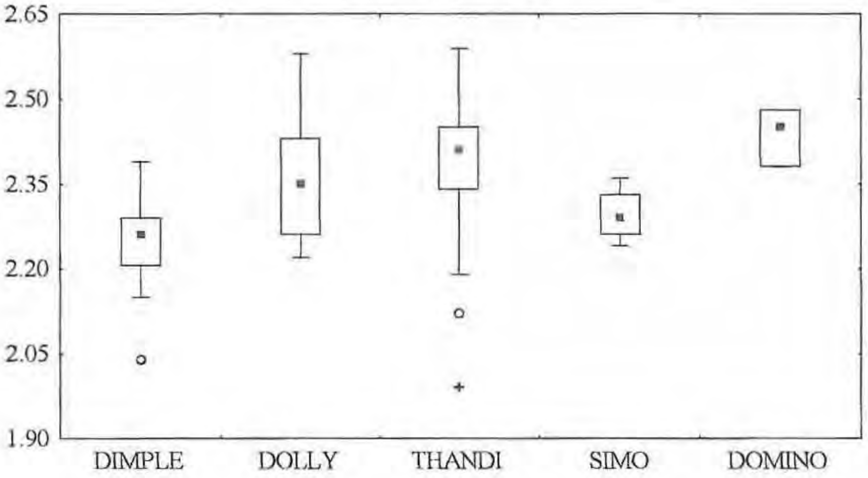


Appendix 3.7: Box-whisker plots for serum total protein, albumin, globulin and the albumin: globulin ratio measured in captive dolphins. Plots include the median, 50 % range, minimum, maximum and outlying (o) and/or extreme (+) values.

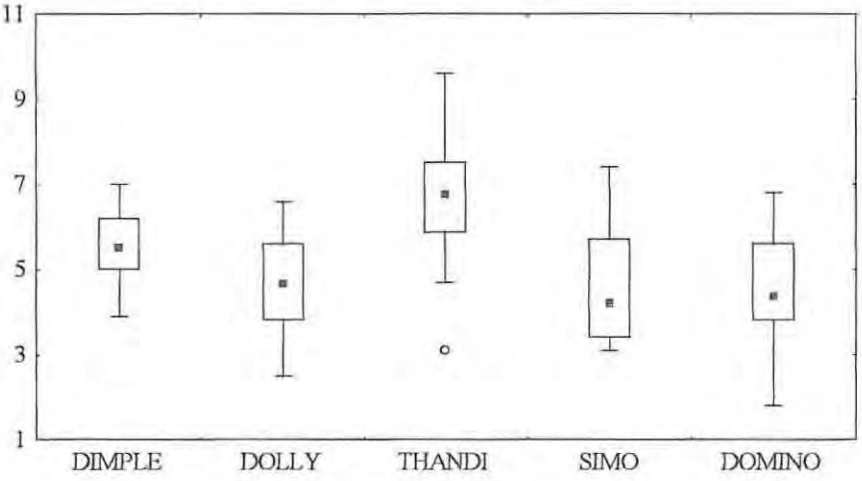
A. Potassium (mmol/l)



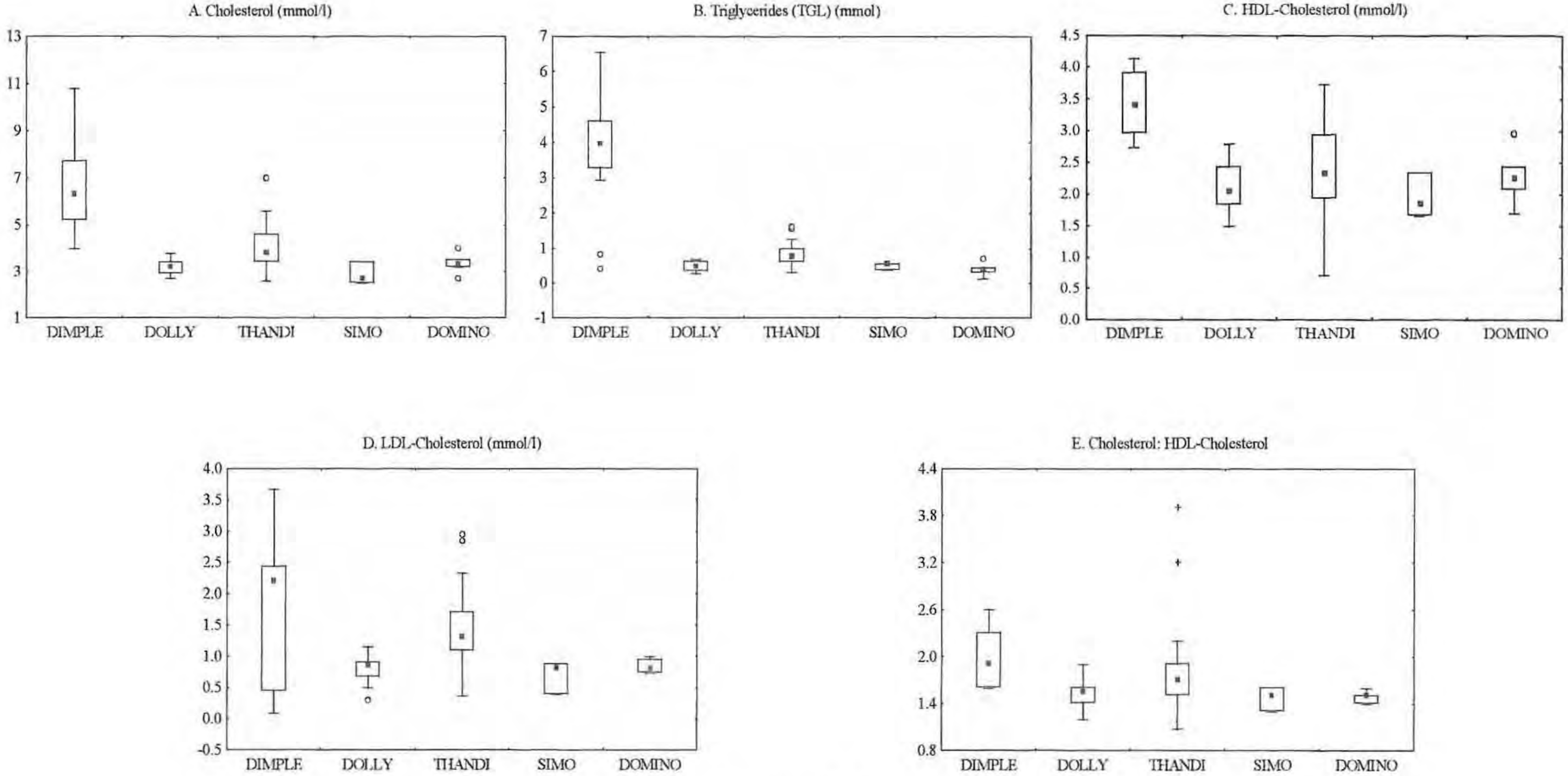
B. Calcium (mmol/l)



C. Glucose (mmol/l)



Appendix 3.8: Box-whisker plots for serum potassium and calcium and plasma glucose measured in captive dolphins. Plots include the median, 50 % range, minimum, maximum and outlying (o) and/or extreme values (+).



Appendix 3.9: Box-whisker plots for serum lipids measured in captive dolphins. Plots include the median, 50 % range, minimum, maximum and outlying (o) and.or extreme values (+).

Appendix 4: Haematological data reported in the literature for other marine mammal species (CP: captive population; NP: natural population; M: male; F: female; A: adult; J: juvenile; Ad: adolescent; At: Atlantic; Pa: Pacific; C: coastal; O: offshore; I: intermediate) Data are present as means and standard deviations ($\bar{x}$ (SD)) or ranges (min-max).

Toothed whales	**WBC** (x10^9/l)	**Neutrophils** (%)	**Eosinophils** (%)	**RBC** (x10^12/l)	**HB** (g/dl)	**PCV** (l/l)	**MCV** (fl)	**Reference**
Bottlenose dolphin								
(Tursiops truncatus)								
				6.89	20.30	0.52	75.50	Joliet 1902 in De Monte and Pilleri 1988
				10.00	26.50			Sudzuki 1924 in De Monte and Pilleri 1988
				6.85	14.20			Morimoto et al. 1921 in De Monte and Pilleri 1988
CP				3.49 (2.87)	14.20 (1.10)	0.41 (0.03)	117.10 (6.60)	Medway and Geraci 1964
CP -At	9.00	45.00-75.00	5.00-30.00		14.40	0.45		Ridgway 1965*
CP -Pa	7.00	40.00-70.00	5.00-30.00		17.00	0.53		
				3.64	14.20	0.45	116.70	Boice et al. 1967 in De Monte and Pilleri 1988
CP-F				3.76		0.42		Horvath et al. 1968
CP-M						0.47		
CP?	8.70			4.16	14.80	0.44	105.00	Harkness and Grayson 1969
				3.98	15.90	0.43	108.10	Lenfant 1969
CP-M	10.68 (4.86)	61.00 (13.00)	13.00 (9.00)	4.14 (0.50)	15.10 (1.50)	0.45 (0.04)		Ridgeway et al. 1970
CP-F	9.78 (3.09)	61.00 (13.00)	15.00 (9.00)	3.97 (0.40)	14.40 (1.40)	0.43 (0.04)		
				4.48	18.90	0.47	107.00	Korzhuev & Glazova 1971
CP	19.30			3.58	15.00	0.38		Geraci and Medway 1973
				3.11	15.48	0.42	135.90	Rhodes 1976 in De Monte and Pilleri 1988
CP	6.00-12.00	55.00-65.00	6.00-27.00	3.50-4.00	14.00-16.00	0.41-0.49	113.00-124.00	Medway and Geraci 1978**, Clarke 1990
	4.10-10.80	49.00-77.00	7.00-37.00	2.90-5.40	12.70-18.10	0.36-0.51	101.00-143.00	Bossart and Dierauf 1990
CP			12.00 (5.00)				120.00 (6.00)	Asper et al. 1990
CP-C				3.52 (0.20)	14.60 (0.60)	0.42 (0.02)		
CP-I				3.73 (0.20)	16.10 (0.20)	0.45 (0.01)		
CP-O				4.48 (0.20)	18.60 (0.90)	0.50 (0.01)		
CP-A	6.40 (1.10)	67.00 (6.00)						
CP-J	8.00 (1.50)	60.00 (8.00)						
NP?				4.48	18.90	0.47		Korzhuev and Bulatova 1952 in Korzhuev and Glazova 1973**
NP	6.51 (0.47)	57.60 (4.30)	17.30 (2.60)	5.01 (0.29)				Bogdanova and Lebedev, 1973
AtC					14.50	0.42		Ridgway 1972, Duffield et al. 1983 in Ridgway 1986
PaO					18.40	0.52		
NP		47.00 (11.00)	30.00 (11.00)				114.00 (5.00)	Asper et al. 1990
NP-C				3.56 (0.20)	14.30 (0.90)	0.41 (0.03)		
NP-I				3.96 (0.10)	16.40 (0.50)	0.47 (0.03)		

Appendix 4: contd.

	WBC	**Neutrophils**	**Eosinophils**	**RBC**	**HB**	**PCV**	**MCV**	**Reference**
	(x10^9/l)	(%)	(%)	(x10^12/l)	(g/dl)	(l/l)	(fl)	
(Tursiops truncatus)								
NP-A	11.00 (3.00)							Asper et al. 1990
NP-J	9.66 (3.00)							
NP	10.30 (2.94)	45.26 (6.57)	30.70 (7.98)	3.53 (0.31)	13.77 (0.98)	0.42 (0.03)	119.84 (7.23)	Rhinehart et al. 1991
NP	10.06 (2.33)	45.74 (9.12)	1.94 (1.73)	3.49 (0.27)	14.16 (0.88)	0.43 (0.03)	123.41 (6.82)	Rhinehart et al. 1991
NP	9.90 (2.60)	48.00 (9.00)	29.00 (8.50)	3.57 (0.28)	13.90 (0.76)	0.42 (0.03)	118.00 (6.80)	Rhinehart et al. 1992
(Tursiops gilli)				4.69	18.20	0.51	107.50	Lenfant 1969
Common dolphin								
(Delphinus delphis)								
NP?				5.65	19.10	0.48		Korzhuev and Bulatova 1952 in Korzhuev and Glazova 1973
NP	8.31 (1.54)	65.70 (3.92)	11.40 (2.18)	4.86 (0.85)				Bogdanova and Lebedev 1973
NP?-F				5.30		0.47		Horvath et al. 1968***
Risso's dolphin								
(Grampus griseus)								
				4.92	21.40	0.54	110.70	Kenney 1967 in Lenfant 1969
	6.00-29.00	54.00-95.00	0.00-18.00	3.80-5.40	14.70-20.30	0.42-0.58	77.00-152.00	Bossart and Dierauf 1990
Tucuxi								
(Sotalia fluvitalis)								
CP	9.16	56.50	13.50	4.27	13.40	40.50	94.00	Van Foreest 1980^
Pacific white-sided dolphin								
(Lagenorhynchus obliquidens)								
				5.36	16.60	0.49	95.00	Boice et al. 1967 in De Monte and Pilleri 1988
CP-M				6.15		0.47(0.50)		Horvath et al. 1968
CP-F				5.58		0.50		
				5.86	18.70	0.51	87.30	Lenfant 1969
CP-M	7.92 (2.29)	43.00 (15.00)	21.00 (11.00)	5.31 (0.50)	17.80 (1.60)	0.50 (0.05)		Ridgway et al. 1970
CP-F	6.67 (1.30)	41.00 (11.00)	24.00 (10.00)	5.83 (0.50)	19.80 (1.90)	0.54 (0.03)		
CP	5.00-8.00	35.00-50.00	11.00-34.00	5.00-6.00	17.00-21.00	0.49-0.56		Medway and Geraci 1978
CP	8.00	57.00	20.00	5.50	18.00	0.49	89.00	Engelhardt 1979
	6.50-9.50	54.00-60.00	17.00-23.00	5.30-5.80	18.00	0.49	85.00-93.00	Bossart and Dierauf 1990

Appendix 4: contd.

	WBC (x10^9/l)	Neutrophils (%)	Eosinophils (%)	RBC (x10^12/l)	HB (g/dl)	PCV (l/l)	MCV (fl)	Reference
Dusky dolphin								
(L. obscurus)								
CP-F	5.10	34.00	10.00	6.20	19.90	0.57		Fothergill and Jogessar 1986""
CP-M	3.80	45.00	9.00	5.50	18.80	0.52		
(Lagenorhynchus sp.)					17.00	0.53		Ridgway and Johnston 1966 in Ridgway 1986
Pygmy sperm whale								
(Kogia breviceps)								
NP					15.70	0.50		Boice et al. 1967 in De Monte and Pilleri 1988
Pilot whale								
(Globicephala sp.)								
NP	11.50 (3.16)	60.10 (11.90)	7.00 (9.20)	3.71 (0.38)	15.8 (1.20)	0.45 (0.04)	123.00 (8.80)	Medaway and Moldovan 1966
CP	11.45 (1.55)	66.10 (10.00)	7.00 (7.00)	3.87 (0.40)	16.50 (1.90)	0.46 (0.04)		Ridgway et al. 1970
CP	10.00	50.00-70.00	2.00-20.00					Ridgway 1965**
CP	9.00-14.00	55.00-75.00	1.00-15.00	3.00-4.00	15.00-18.00	0.42-0.49	114.00-132.00	Medway and Geraci 1978**
Beluga								
(Delphinapterus leucas)								
				3.34	19.30	0.46	134.13	Dhindsa et al. 1974 in De Monte and Pilleri 1988
				3.31	18.00	0.49	151.00	MacNeill 1975 in De Monte and Pilleri 1988
CP	8.00-12.00	43.00-56.00	10.00-21.00	3.00-4.00	17.00-19.00	0.42-0.48	118.00-147.00	Medway and Geraci 1978
CP	7.50	66.00	5.00	3.20	21.00	0.54	169.00	Engelhardt 1979
CP-M					20.90 (0.90)	0.53 (0.04)		Ridgway et al. 1984^^^
CP-F					20.30 (0.20)	0.52 (0.03)		
	7.60-11.00	46.00-74.00	2.00-21.00	2.90-3.60	19.00-23.00	0.48-0.62	161.00-182.00	Bossart and Dierauf 1990
Pygmy killer whale								
(Feresa attenuata)								
				4.00	14.90	0.43	106.00	White 1976 in De Monte and Pilleri 1988
Killer whale								
(Orcinus orca)								
				3.95	16.00	0.44	111.50	Lenfant 1969
CP	10.38 (3.80)	78.00 (12.00)	2.00 (1.00)	4.00 (0.30)	16.20 (0.90)	0.45 (0.06)		Ridgway et al. 1970

Appendix 4: contd.

	WBC (x10^9/l)	Neutrophils (%)	Eosinophils (%)	RBC (x10^12/l)	HB (g/dl)	PCV (l/l)	MCV (fl)	Reference
(Orcinus orca)				4.34	16.30	0.40	94.47	Dhindsa et al. 1974 in De Monte and Pilleri 1988
				4.06	16.30	0.45	111.00	MacNeill 1975 in De Monte & Pilleri 1988
CP	6.00-9.00	50.00-75.00	2.00-8.00	~4.00	15.00-17.00	0.43-0.49	111.00-119.00	Medway and Geraci 1978
CP	4.50-11.00	54.00-86.00	0.00-8.00				112.00	Cornell 1983 (medians used as estimate of central tendency)
CP-O?				3.80-5.00	16.00-19.00	0.44-0.55		
CP-C?				3.20-4.30	13.00-16.00	0.37-0.49		
	4.50-11.00	54.00-86.00	0.00-8.00	3.80-5.00	16.00-19.00	0.44-0.55	94.00-123	Bossart and Dierauf 1990
Narwhal								
(Monodon monodon)								
NP					22.50	0.58		Vogl and Fisher 1982 in Ridgway 1986
NP					20.00	0.58		MacNeill 1975 in Ridgway 1986
Sperm whale								
(Physeter catadon)								
NP				2.10	15.10	0.42	195.00	Lenfant 1969
Dall's porpoise								
(Phocoena dalli)								
CP	5.51 (1.94)	48.00 (19.00)	5.00 (3.00)	5.50 (0.60)	19.70 (1.80)	0.53 (0.04)		Ridgway et al. 1970
CP	6.50	60.00-85.00	2.00-5.00		20.50	0.57		Ridgway 1965
CP-M						0.53		Horvath et al. 1968
NP-M				7.48		0.58		
Harbour porpoise								
(Phocoena phocoena)								
				5.00	17.40	0.50	100.00	Anderson 1966
CP	7.00 (4.70)	47.00 (17.00)	7.00 (5.00)	5.20 (0.80)		0.45 (0.08)	86.00 (7.00)	Nielsen and Andersen 1982*^
NP-FJ	11.30	76.00	8.00	5.10	10.30	0.52		Kastelein et al. 1990^^
NP-MA	8.80	73.00	2.00	6.50	11.80	0.56		
NP-MAd	6.00	55.00	13.00	6.80	12.40	0.62		
NP	7.39 (0.96)	52.70 (5.40)	10.20 (3.80)	5.14 (0.20)				Bogdanova and Lebedev 1973
	2.00-23.30	4.00-94.00	1.00-23.00	1.60-6.40	2.70-13.10	0.14-0.56	73.00-127.00	Bossart and Dierauf 1990
NP					19.76 (1.41)	0.52 (0.03)		Koopman et al. 1995
					20.30	0.57		Ridgway and Johnston 1966 in Ridgway 1986

Appendix 4: contd.

	WBC (x10^9/l)	**Neutrophils** (%)	**Eosinophils** (%)	**RBC** (x10^12/l)	**HB** (g/dl)	**PCV** (l/l)	**MCV** (fl)	**Reference**
River dolphins								
(Inia geoffrensis)				4.07	14.30	0.42	103.00	Lenfant 1969
CP-M	14.59 (3.14)	66.00 (16.00)	5.00 (4.00)	3.90 (0.50)	14.40 (1.40)	0.42 (0.03)		Ridgway et al. 1970
CP-F	12.20 (2.10)	67.00 (14.00)	4.00 (3.00)	3.80 (0.30)	13.30 (0.30)	0.40 (0.04)		
NP	8.33	69.88	11.00					De Monte and Pilleri 1972
(Platanista indi)								
NP		85.75	2.25					
(P. gangetica)								
NP		80.25	0.00					
Baleen whales (Mysticeti)								
Gray whale								
(Eschrichtius robustus)								
NP-A				3.30	14.00	0.43	129.00	Lenfant 1969
NP-FJ				2.40	10.00	0.34	128.00	Zettner 1974 in De Monte and Pilleri 1988
CP	5.60	47.04	0.00	3.00	15.00	0.43	143.00	Medway and Cornell 1989^^
				3.30	14.00	0.48	129.00	Bossart and Dierauf 1990
Bowhead whale								
(Balaena mysticetus)								
NP		20.00-76.00	<1.00-1.00					Medway 1983
Sirenids								
Dugongs								
(Dugong dugong)								
CP		61.00-73.00	0.00-3.00	3.20-3.30	12.80-13.00	0.40-0.41		Allen et al. 1976"
Manatees								
(Trichechius sp.)								
CP-A	9.30 (1.28)	56.20 (15.80)	5.20 (0.30)	3.6 (0.2)	14.80 (0.70)	0.47 (0.05)	129.50 (7.80)	White et al. 1976
CP	8.00-10.60	40.00-72.00	5.00-6.00	3.00-4.00	14.00-15.00	0.46-0.47	122.00-138.00	Medway and Geraci 1978
CP	8.81 (1.28)	57.50 (18.00)		3.10 (0.3)	12.93 (1.60)	0.39 (0.04)	125.40 (6.10)	Irvine et al. 1980
CP	6.12 (1.35)	48.30 (7.90)		3.20 (0.20)	13.42 (1.00)	0.41 (0.02)	125.90 (5.60)	
NP	4.50-13.10	30.00-84.00	1.00-6.00	2.30-3.80	9.90-12.60	0.30-0.40	115.00-139.00	Bossart and Dierauf 1990

Appendix 4: contd.

* Recorded as Atlantic and Pacific porpoise
** Specific name not recorded
*** Recorded as *Delphinus delphis bardii*
^ Nine animals from different zoos, tested at different labs
^^ Stranded animal, last sample following rehabilitation
^^^ Captive animals diving in open ocean
*^ Period in captivity ranged from days to 3 years
" Animal known to be ill, died shortly after sample taken
"" Following treatment with Ketocanozole

Appendix 5: Blood chemistry (liver-, muscle- and kidney associated enzymes) data reported in the literature for other marine mammal species (CP: captive population; NP: natural population; M: male; F: female; A: adult; J: juvenile; Ad: adolescent; At: Atlantic; Pa: Pacific; C: coastal; O: offshore; I: intermediate). Data are present as means and standard deviations (x (SD)) or ranges (min-max) (For interpretation of superscripts, refer to Appendix 4).

Toothed whales (Odontoceti)	**Creatinine**	**BUN**	**ALP**	**LDH**	**CPK**	**Reference**
Bottlenose dolphin	(µmol/l)	(mmol/l)	(U/l)	(U/l)	(U/l)	
(Tursiops truncatus)						
CP		4.66-6.66				Ridgway 1965*
CP		9.44 (2.73)				Medway and Geraci 1965
CP-M		8.82 (2.00)	42.00 (27.00) KAU	271.00 (181.00) WU		Ridgway et al. 1970
CP-F		8.33 (2.17)	30.00 (14.00) KAU	234.00 (133.00)		
CP		6.83		116.00	11.00	Geraci and Medway 1973
CP					5.00	Engelhardt 1979
CP		6.83-10.99	33.00-403.00		23.00-190.00	Medway & Geraci 1978**
	97.24-221.00	5.16-12.15	123.00-1050.00	159.00-1393.00	14.00-486.00	Bossart and Dierauf 1990
CP	132.60 (17.68)	8.33 (0.67)				Asper et al. 1990
CP-J			401.00 (113.00)	244.00 (34.00)	80.00 (14.00)	
CP-A			259.00 (127.00)	194.00 (26.00)	60.00 (13.00)	
NP	123.76 (61.88)	10.16 (1.83)				
NP-M				250.00 (62.00)		
NP-F				214.00 (62.00)		
NP-A			203.00 (96.00)		60.00 (20.00)	
NP-J			404.00 (175.00)		81.00 (27.00)	
NP	144.98 (28.29)	9.37 (1.15)	283.29 (178.38)	482.83 (107.50)		Rhinehart et al. 1991
NP	128.18 (26.52)	9.87 (1.61)				Rhinehart et al. 1991
NP	141.44 (22.98)	9.16 (1.32)	306.00 (260.10)	450.00 (62.50)		Rhinehart et al. 1992
Common dolphin						
(Delphinus delphis)						
NP		4.91 (0.03)(U)				De Monte and Pilleri 1977
Striped dolphin						
(Stenella coeruleoalba)						
NP		3.75 (0.56) (U)				De Monte and Pilleri 1977
Tucuxi						
(Sotalia fluvitalis guianensis)						
CP			984.00	771.00	48.00	Van Foreest 1980^

Appendix 5: contd.

	Creatinine (µmol/l)	BUN (mmol/l)	ALP (U/l)	LDH (U/l)	CPK (U/l)	Reference
Pacific white-sided dolphin *(Lagenorhynchus obliquidens)*						
CP					12.00	Engelhardt 1979
CP-M		5.99 (1.33)	23.00 (4.00)	507.00 (193.00)		Ridgway et al. 1970
CP-F		6.49 (1.50)	42.00 (8.00)	372.00 (262.00)		
CP		4.83 (7.66)				Medway and Geraci 1978
					9.00-15.00	Bossart and Dierauf 1990
Dusky dolphin *(L. obscurus)*						
CP			900.00-1 000.00			Fothergill et al. 1991
Risso's dolphin *(Grampus griseus)*						
	123.76-247.52	6.11-11.49	7.00-308.00	74.00-732.00	48.00-157.00	Bossart and Dierauf 1990
Pilot whale *(Globicephala sp.)*						
CP		8.66 (1.83)	42.00 (29.00)	347.00 (100.00)		Ridgway et al. 1970
CP		6.82-12.32				Medway and Geraci 1978**
NP		10.37 (3.61)	19.60 (2.06) S-FU	1018.00 (203.00) B-BU		Medaway and Moldovan 1966
Beluga *(Delphinapterus leucas)*						
CP		8.49-11.16				Medway and Geraci 1978
CP					9.00	Engelhardt 1979
	79.56-185.64	6.33-11.49	54.00-162.00	85.00-200.00	30.00-122.00	Bossart and Dierauf 1990
Killer whale *(Orcinus orca)*						
CP		5.50 (1.33)	38.00 (17.00)	263.00 (178.00)		Ridgway et al. 1970
CP	44.20-176.80	3.33-8.33	50.00-350.00	115.00-240.00	11.00-110.00	Cornell 1983

Appendix 5: contd.

	Creatinine (µmol/l)	**BUN** (mmol/l)	**ALP** (U/l)	**LDH** (U/l)	**CPK** (U/l)	**Reference**
Narwhal						
(Monodon monodon)						
NP	353.60	7.47	15.00	96.00	226.00	De Monte and Pilleri 1983
Dall's porpoise						
(Phocoena dalli)						
CP		5.99-7.66				Ridgway 1965
CP		6.66 (2.00)		573.00 (240.00)		Ridgway et al. 1970
Harbour porpoise						
(Phocoena phocoena)						
CP	74.26 (20.33)	8.25 (2.77)		1434.00 (458.00) WrU		Andersen 1968
CP-A			14.80 (2.60) KAU			
CP-J			50.30 (10.60) KAU			
CP	64.00 (19.00)		291.00 (178.00)	810.00 (469.00)		Nielsen and Andersen 1982*^
	35.36-159.12		9.00-800.00	273.00-2340.00		Bossart and Dierauf 1990
NP-MA	61.00	3.78 (U)	147.00	668.00	120.00	Kastelein et al. 1990^^
NP-MAd	40.00		249.00	1371.00	300.00	
NP-FJ	34.00	6.97 (U)	245.00		279.00	
NP	78.90 (16.80)	6.68 (1.44) (U)	550.40 (294.70)		637.00 (247.00)	Koopman et al. 1995
River dolphins						
(Inia geoffrensis)						
CP-M		6.33 (1.83)	15.00 (8.00)	462.00 (220.00)		Ridgway et al. 1970
CP-F		7.33 (1.67)	21.00 (6.00)	305.00 (96.00)		
Baleen whales (Mysticeti)						
Gray whale						
(Eschrichtius robustus)						
CP	141.44 (44.20)	9.66 (1.63)	50.00 (26.00)	387.00 (273)	96.00 (62.00)	Medway and Cornell 1989^^
Bowhead whale						
(Balaena mysticetus)						
NP	291.72-760.24	8.16-10.99	75.00-444.00			Medway 1983
NP	406.64 (167.96)	10.87 (2.01)	274.70 (178.20)		1744.60 (1297.10)	Heidel et al. 1996

Appendix 5: contd.

	Creatinine (µmol/l)	**BUN** (mmol/l)	**ALP** (U/l)	**LDH** (U/l)	**CPK** (U/l)	**Reference**
Fin whale						
(Balaenoptera physalus)						
	26.52-265.20	9.49-16.32				Bossart and Dierauf 1990
Sirenids						
Dugongs						
(Dugong dugong)						
CP	229.84		10.00 KAU			Allen et al. 1976"
Manatees						
(Trichechius sp.)						
CP		2.33-3.00				Medway and Geraci 1978
CP		2.66 (0.38)				White et al. 1976 (2 adults)
CP	159.12 (53.04)	1.38 (0.55)	118.30 (31.10)	283.60 (105.30)		Irvine et al. 1980
NP	150.28 (70.72)	2.15 (0.62)	132.30 (38.60)	375.00 (353.60)		
CP/NP	144.09 (53.04)	2.36 (0.67)	142.00 (26.00)		284.00 (255.00)	Medway et al. 1982
semi-CP (Columbia)	97.24-238.68	0.08-2.07	0.00-91.50	0.00-324.00	0.00-176.50	Montoya-Ospina 1994
(Puerto Rico)	123.76-194.48	0.00-1.33	19.80-101.80	202.00-306.00	0.00-384.00	

HU: Henry units
SU: Sigma units
RfU: Rf units
IMU: i.m. units
KAU: KA units
S-FU: Sigma-Frankel units
WU: Wracker units
B-BU: Berger-Broida units
WrU: Wroblewski units
(U): converted from urea

Appendix 6: Blood chemistry (liver-associated enzymes and bilirubin) data reported in the literature for other marine mammal species (CP: captive population; NP: natural population; M: male; F: female; A: adult; J: juvenile; Ad: adolescent; At: Atlantic; Pa: Pacific; C: coastal; O: offshore;I: intermediate). Data are present as means and standard deviations ($\bar{x}$ (SD)) or ranges (min-max) (For interpretation of superscripts, refer to Appendix 4).

Toothed whales	SGOT/AST	SGPT/ALT	Total bilirubin	Reference
Bottlenose dolphin	(U/l)	(U/l)	(µmol/l)	
(Tursiops truncatus)				
CP-M	225.00 (109.00) HU	40.00 (24.00) HU	8.55 (8.55)	Ridgway et al. 1970
CP-F	188.00 (89.00)	38.00 (18.00)	5.13 (3.42)	
CP	95.00	8.00	3.42	Geraci and Medway 1973
CP		28.00		Engelhardt 1979
CP	33.00-190.00	7.00-19.00		Medway and Geraci 1978**
	48.00-250.00	8.00-47.00	0.00-1.71	Bossart and Dierauf 1990
CP			3.93 (16.07)	Asper et al. 1990
CP-J	140.00 (23.00)	32.00 (6.00)		
CP-A	115.00 (18.00)	25.00 (5.00)		
NP	139.00 (76.00)	31.00 (18.00)	3.42 (1.71)	
NP	226.92 (58.72)	21.74 (12.23)	3.59 (1.71)	Rhinehart et al. 1991
NP			6.33 (4.79)	Rhinehart et al. 1991
NP	241.00 (60.90)	23.00 (9.90)	3.42 (1.37)	Rhinehart et al. 1992
Common dolphin				
(Delphinus delphis)				
NP	123.10 (20.80) (U/ml	66.00 (11.80) (U/ml)		De Monte and Pilleri 1977
Striped dolphin				
(Stenella coeruleoalba)				
NP	78.80 (38.40) (U/ml)	58.70 (19.10) (U/ml)		De Monte and Pilleri 1977
Tucuxi				
(Sotalia fluvitalis)				
CP	85.00	16.00		Van Foreest 1980^
Pacific white-sided dolphin				
(Lagenorhynchus obliquidens)				
CP		44.00		Engelhardt 1979
CP-M	255.00 (115.00)	99.00 (33.00)	10.26 (10.26	Ridgway et al. 1970
CP-F	193.00 (63.00)	88.00 (22.00)	3.42 (1.71)	
		40.00-48.00		Bossart and Dierauf 1990
Risso's dolphin				
(Grampus griseus)				
	184.00-516.00	51.00-214.00	0.00-1.71	Bossart and Dierauf 1990
Pilot whale				
(Globicephala sp.)				
CP	199.00 (52.00)	27.00 (17.00)		Ridgway et al. 1970
NP	144.80 (17.30) SU	38.00 (11.00) SU		Medaway and Moldovan 1966
Beluga				
(Delphinapterus leucas)				
CP		13.00		Engelhardt 1979
	30.00-87.00	3.00-18.00	3.42-10.26	Bossart and Dierauf 1990
Killer whale				
(Orcinus orca)				
CP	61.00 (11.00)	12.50	3.42 (1.71)	Ridgway et al. 1970
CP	14.00-36.00	3.00-18.00	1.71-6.84	Cornell 1983
Narwhal				
(Monodon monodon)				
NP	27.00	25.00		De Monte and Pilleri 1983

Appendix 6: contd.

	SGOT/AST	SGPT/ALT	Total bilirubin	Reference
	(U/l)	(U/l)	(μmol/l)	
Dall's porpoise				
(Phocoena dalli)				
CP	406.00 (181.00)	107.00 (15.00)		Ridgway et al. 1970
Harbour porpoise				
(Phocoena phocoena)				
CP	19.00 (6.40) IMU	44.40 (22.50) IMU	<5.13	Andersen 1968
CP	195.00 (128.00)	93.00 (54.00)		Nielsen and Andersen 1982*^
	2.00-665.00	18.00-220.00	1.20-10.60	Bossart and Dierauf 1990
NP-MA	300.00	276.00		Kastelein et al. 1990^^
NP-MAd	23.00	270.00		
NP-FJ	299.00	304.00		
NP	293.80 (63.40)	80.00 (40.00)	1.50 (1.01)	Koopman et al. 1995
River dolphins				
(Inia geoffrensis)				
CP-M	175.00 (101.00)	37.00 (23.00)	1.71	Ridgway et al. 1970
CP-F	150.00 (62.00)	20.00 (9.00)	5.13	
Baleen whales (Mysticeti)				
Gray whale				
(Eschrichtius robustus)				
CP	64.00 (35.00)	14.00 (6.00)	18.10 (6.84)	Medway and Cornell 1989^^
Bowhead whale				
(Balaena mysticetus)				
NP	48.00-139.00	12.00-43.00	6.84-34.20	Medway 1983
NP	212.20 (195.90)		11.97 (6.84)	Heidel et al. 1996
Sirenids				
Dugongs				
(Dugong dugong)				
CP	15.00 RfU	16.00 RfU	3.59	Allen et al. 1976"
Manatees				
(Trichechius sp.)				
CP	22.80 (11.00)			White et al. 1976 (2 adults)
CP	8.00 (7.4)	6.40 (3.10)		Irvine et al. 1980
NP	14.60 (8.40)	19.90 (9.60)		
CP/NP	7.00 (3.00)	49.00 (14.00)		Medway et al. 1982
semi-CP (Columbia)	0.00-7.00	13.00-37.00	0.00-5.13	Montoya-Ospina 1994
(Puerto Rico)	0.00-12.00	0.50-12.50	0.00-13.68	

* Recorded as Atlantic and Pacific porpoise
** Specific name not recorded
^ Nine animals from different zoos, tested at different labs
^^ Stranded animal, last sample following rehabilitation
*^ Period in captivity ranged from days to 3 years
" Animal known to be ill, died shortly after sample taken
HU: Henry units
SU: Sigma units
RfU: Rf units
IMU: i.m. units
KAU: KA units
S-FU: Sigma-Frankel units
WU: Wracker units
B-BU: Berger-Broida units
WrU: Wroblewski units
(U): converted from urea

Appendix 7: Blood chemistry (proteins and electrolytes) data reported in the literature for other marine mammal species (CP: captive population; NP: natural population; M: male; F: female; A: adult; J: juvenile; Ad: adolescent; At: Atlantic; Pa: Pacific; C: coastal; O: offshore; I: intermediate). Data are present as means and standard deviations ($\overline{x}$ (SD)) or ranges (min-max) (For interpretation of superscripts, refer to Appendix 4).

Toothed whales (Odontoceti)	**Albumin** (g/l)	**Globulin** (g/l)	**Albumin: globulin**	**Sodium** (mmol/l)	**Calcium** (mmol/l)	**Reference**
Bottlenose dolphin						
(Tursiops truncatus)						
CP-At				118.00-153.00		Ridgway 1965*
CP-Pa				120.00-140.00		
CP	50.70 (5.40)		2.38 (0.40)	165.80 (7.90)	2.18 (0.10)	Medway and Geraci 1965
CP-M				153.00 (7.00)	1.25 (0.20)	Ridgway et al. 1970
CP-F				155.00 (7.00)	1.23 (0.28)	
CP				156.00	2.25	Geraci and Medway, 1973
CP				159.00		Engelhardt, 1979
CP	30.00-50.00		2.00-3.00	150.00-161.00	1.75-2.00	Medway & Geraci 1978**
	28.00-56.00	11.00-39.00		141.00-168.00	1.93-2.43	Bossart and Dierauf 1990
CP	46.00 (3.00)	22.00 (4.00)			2.30 (0.08)	Asper et al. 1990
NP	39.00 (8.00)	37.00 (9.00)			9.60 (0.70) mg/dl	
NP	31.30 (3.61)	40.90 (8.54)	0.82 (0.33)	155.92 (3.08)	8.86 (0.54) mg/ml	Rhinehardt et al. 1991a
NP	44.20 (6.19)	29.20 (6.28)	1.61 (0.53)	152.70 (5.82)	8.97 (0.70) mg/ml	Rhinehardt et al. 1991b
NP	33.00 (2.00)	43.00 (7.10)	0.80 (0.15)	155.00 (1.90)	8.90 (0.40) mg/ml	Rhinehart et al. 1992
Tucuxi						
(Sotalia fluvitalis guianensis)						
CP				150.00		Van Foreest 1980^
Pacific white-sided dolphin						
(Lagenorhynchus obliquidens)						
CP				162.00		Engelhardt 1979
CP-M				157.00 (9.00)	5.00 (0.80) mEq/l)	Ridgway et al. 1970
CP-F				153.00 (7.00)	5.20 (0.80) mEq/l	
CP	20.00-60.00			145.00-161.00	4.00-6.00	Medway and Geraci 1978
				161.00-162.00		Bossart and Dierauf 1990
Risso's dolphin						
(Grampus griseus)						
	35.00-49.00	24.00-42.00		139.00-159.00	1.90-2.50	Bossart and Dierauf 1990

Appendix 7: contd.

	Albumin (g/l)	Globulin (g/l)	Albumin: globulin	Sodium (mmol/l)	Calcium (mmol/l)	Reference
Pilot whale						
(Globicephala sp.)						
CP				149.00 (4.00)	4.90 (0.30)	Ridgway et al. 1970
CP	~40.00		1.00-2.00	149.00-157.00	5.00-7.00	Medway & Geraci 1978**
NP	44.80 (3.30)		1.43 (0.24)	157.30 (3.20)	7.40 (1.40) mg/dl	Medaway and Moldovan 1966
Beluga						
(Delphinapterus leucas)						
CP	42.00-47.00		0.80-1.20	161.00-165.00	11.00-12.00	Medway and Geraci 1978
CP				162.00		Engelhardt 1979
	35.00-52.00	15.00-38.00			2.05-2.70	Bossart and Dierauf 1990
Killer whale						
(Orcinus orca)						
CP				155.00 (8.00)	5.40 (1.50)	Ridgway et al. 1970
CP	~40.00		0.90-1.00	149.00-158.00	8.00-9.00	Medway and Geraci 1978
CP	25.00-44.00	20.00-60.00		139.00-157.00	6.80-9.50 mg/dl	Cornell 1983
Narwhal						
(Monodon monodon)						
NP	38.50	37.60	1.02			De Monte and Pilleri 1983
Dall's porpoise						
(Phocoena dalli)						
CP				129.00-148.00		Ridgway 1965
CP				155.00 (9.00)	4.80(0.80)	Ridgway et al. 1970
Harbour porpoise						
(Phocoena phocoena)						
CP	45.60 (9.70)			153.50 (3.70)	10.60 (0.81) mg/dl	Andersen 1968
CP	36.00 (5.00)			151.00 (6.00)	2.57 (0.31)	Nielsen and Andersen 1982*^
	21.00-46.00			138.00-179.00	0.80-2.78	Bossart and Dierauf 1990
NP-MA				157.00	2.41	Kastelein et al. 1990^^
NP-MAd				155.00	2.56	
NP-FJ				155.00	2.31	
NP	39.40 (3.80)	34.70 (9.20)	1.22 (0.33)	156.60 (7.70)	2.41 (0.16)	Koopman et al. 1995

Appendix 7: contd.

	Albumin (g/l)	**Globulin** (g/l)	**Albumin: globulin**	**Sodium** (mmol/l)	**Calcium** (mmol/l)	**Reference**
River dolphins						
(Inia geoffrensis)						
CP-M				144.00 (5.00)	4.50 (0.40)	Ridgway et al. 1970
CP-F				142.00 (5.00)	4.60 (0.50)	
NP	30.45		0.48			De Monte and Pilleri 1972
(Platanista indi)						
NP	44.34		0.96			
(P. gangetica)						
NP	53.80		1.43			
Baleen whales (Mysticeti)						
Gray whale						
(Eschrichtius robustus)						
CP	48.00	35.00	1.40	141.00 (6.60)	10.00 (1.20)	Medway and Cornell 1989^^
Bowhead whale						
(Balaena mysticetus)						
NP	12.00-42.00	17.00-30.00	0.70-1.90	148.00-172.00	2.58-3.10	Medway 1983
NP	48.00 (5.00)	~30.00	~1.60	183.80 (8.90)	11.30 (1.80)	Heidel et al. 1996
Fin whale						
(Balaenoptera physalus)						
	28.00-48.00	9.00-41.00		135.00-188.00	2.18-3.23	Bossart and Dierauf 1990
Sirenids						
Dugongs						
(Dugong dugong)						
CP	48.00	33.00				Allen et al. 1976"
Manatees						
(Trichechius sp.)						
CP			1.00	143.00-145.00	~10.00	Medway and Geraci 1978
CP-A				143.80 (1.20)	10.10 (0.70)	White et al. 1976
CP				148.40 (1.27)	10.30 (0.30) mg/dl	Irvine et al. 1980
NP	57.00 (2.00)	28.00 (3.00)		149.80 (6.40)	10.30 (0.40) mg/dl	
CP/NP	48.50 (4.00)	34.30 (3.00)	1.42 (0.20)	151.00 (4.30)	9.90 mg/dl	Medway et al. 1982
semi-CP (Columbia)	38.00-62.00			118.00-150.00	1.63-2.63	Montoya-Ospina 1994
(Puerto Rico)				143.80-169.80	1.85-3.65	