THE REPRODUCTIVE BIOLOGY OF FOUR SPECIES OF SOUTH AFRICAN DOLPHINS

THESIS

Submitted in fulfilment of the
requirements for the Degree of
MASTER OF SCIENCE
of Rhodes University

by

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January 1996

Dolphins are incestuous,
they like to do relations
I guess that means, scientifically,
they're kinky and cetaceans.

They scan you with a sonic beam,
they're often rather raucous
Sometimes they learn by accident,
but other times, on porpoises (cringe).

By Robert Day

ABSTRACT

Seasonality of reproduction in common dolphins (*Delphinus delphis*), bottlenosed dolphins (*Tursiops aduncus*), striped dolphins (*Stenella coeruleoalba*) and humpbacked dolphins (*Sousa chinensis*) was examined using standard light microscope techniques. In the males four stages of testicular development could be identified. Individuals less than 2.26m in length and 105kg in mass had testes that contained small seminiferous tubules (0.048mm ± 0.01mm in diameter) and were classified either as juveniles (gonocytes present) or inactive (no gonocytes). Adults whose testes contained spermatozoa were said to be in late spermatogenesis while those whose testes contained no spermatids or spermatozoa were referred to as being in early spermatogenesis. In the males of *D. delphis*, *T. aduncus* and *S. chinensis*, late spermatogenesis occurred throughout the year and there was no significant difference in the monthly mean seminiferous tubule diameters ($P > 0.05$) within species. I conclude therefore that in these three species spermatogenesis is aseasonal. No conclusions about seasonality of spermatogenesis could be made for *S. coeruleoalba* owing to the small sample size.

Large Graafian follicles occurred in the ovaries of all four species in most months of the year. However, the presence of Graafian follicles can not be taken as an indication of timing of reproduction since they may be remnants of follicles that have not yet degenerated. In *D. delphis*, *T. aduncus* and *S. coeruleoalba* luteal bodies (corpora albicans or corpora lutea) occurred in most months of the year. Active corpora lutea will be present in all months, in some members of the population, since gestation is approximately 12 months.

The sperm structure of *D. delphis* was examined by scanning electron microscopy. The sperm of *D. delphis* is essentially similar to that described for two other species of Cetacea (*Physeter catodon* and *Tursiops aduncus*), having an ellipsoidally shaped head and a short mid-piece with nine mitochondria. The sperm dimensions for *D. delphis* were head length, 4.4µm; head width, 2.0µm and mid-piece length, 2.4µm.

An analysis of foetal age in *D. delphis* showed that the majority of the foetuses were conceived in January with birth occurring the following summer (December), suggesting that reproduction is seasonal. Neonates of *T. aduncus* were found throughout the year suggesting aseasonal reproduction. Foetal material was not available for *S. coeruleoalba* and *S. chinensis*. Seasonality of reproduction in the four species of dolphins studied has been discussed in relation to feeding, migration and worldwide distribution of the species.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

ACKNOWLEDGEMENTS

I wish to extend my sincere gratitude to the following people and organizations that assisted me in the completion of this project:

To my supervisors, Ric Bernard and Alan Hodgson, for their supervision and provision of funding for this project from their research grants. Their constructive criticism of this manuscript and our discussions proved invaluable in refining my arguments.

To Vic Cockcroft, Debbie Young, Leszek Karczmzarski and staff of the Port Elizabeth Museum for their assistance and provision of specimens and foetal data used in this project.

To Shirley Pinchuck and Robin Cross for their assistance with the electron microscopy work.

To Martin Villet for his friendship, proof-reading of this manuscript and helping format this manuscript for final printing.

To Anu Idicula and Clinton Boyd for helping organise the samples at the Port Elizabeth Museum.

To Michelle Smith for helping prepare histological slides of the ovaries of bottlenosed dolphins.

To Janine Hall for offering many invaluable suggestions on various aspects of spermatogenesis covered in Chapter 2.

A special thanks to the Foundation for Research and Development for their financial support and to Rhodes University Zoology Department and EM unit for the use of their equipment.

To all my friends for their friendship, patience and understanding during my years at Rhodes.

To Richard Chambers for his friendship and inspiring me to do a masters at Rhodes.

Finally, to my parents, Kaycee and Radha Reddy, for without their love, encouragement and financial support, this manuscript would not have been completed.

CHAPTER 1

GENERAL INTRODUCTION

Dolphins and whales, belonging to the mammalian order Cetacea, are regarded as perhaps the most highly evolved of the predators of the open seas (Branch & Branch, 1992). Their mode of feeding has allowed scientists to classify them into two suborders, the suborder Odontoceti (toothed whales) and the suborder Mysticeti (baleen whales). The suborder Odontoceti includes sperm whales, beaked whales, dolphins and porpoises, while the suborder Mysticeti includes right whales, pygmy right whales and rorquals.

Worldwide the order Cetacea comprises about 80 species (May, 1990), of which 37 species have been recorded off southern Africa (Payne & Crawford, 1992), including the largest mammal that ever existed, the blue whale (*Balaenoptera musculus*). Twenty eight species of odontocetes have been recorded on the SE coast of southern Africa (Ross, 1984).

Since the first bottlenosed dolphin (*Tursiops truncatus*) was successfully held in captivity in the United States in 1938, there has been increased interest in the biology of all cetaceans (Robeck, Curry, McBain & Kraemer, 1994). However, as all cetaceans are adapted to an exclusively underwater existence, knowledge of these animals in their natural environment has been either lacking or incompletely available to biologists. Bottlenosed dolphins are the most commonly held dolphins in oceanaria throughout the world as they are readily available close to shore, train well and adapt easily to captive life (Ross, 1977). Hence, bottlenosed dolphins are among the better understood species of cetaceans (Smolker, Richards, Connor & Pepper, 1992).

Most of our knowledge about cetacean reproduction stems from the autopsy of specimens of spotted dolphins (*Stenella attenuata*), striped dolphins (*Stenella coeruleoalba*) and short-finned pilot whales incidentally killed in the fisheries' nets off the Pacific coast of Japan (Marsh & Kasuya, 1984; Miyazaki, 1977; Miyazaki & Dawbin, 1974; Perrin, Coe & Zweifel, 1976), on captive bottlenosed dolphins in South Africa (Cheal & Gales, 1991) and in Florida (Tavolga & Essapian, 1957) and on the sperm whale (*Physeter catadon*) off the west coast of South Africa (Best, 1967, 1969) and Nova Scotia (Mitchell & Kozicki, 1984). Information on the reproductive biology and life history of Hector's (*Cephalorhyncus hectori*) dolphin found in New Zealand waters is provided by Slooten (1991).

Man is known to have a great impact on the Cetacea. The history of whaling is one of tragic overexploitation of one species of whale after another (Bronson, 1989). Large whales, mainly the mysticetes, have been fished from the eastern Canadian shores since the 1890s (Mitchell & Kozicki, 1984). The mid-1970s saw the dramatic expansion of Latin America's fishing industry, particularly in the eastern Pacific and local populations of several species of small cetaceans have been affected, mainly by the increased use of monofilament gill nets (May, 1990). A further development in recent years has been the intentional killing of small cetaceans to provide bait for commercial fisheries (May, 1990). The Beiji or Yangtze river dolphin (*Lipotes vexillifer*) which is considered the most endangered of all cetacean species is threatened by overcrowding of the Yangtze river (May, 1990). Pei-Xun, Ren-Jun and Ke-Jie (1984) provide information on the reproductive biology of the Yangtze river dolphin.

Whaling in South Africa ceased at the end of 1975 after 184 virtually uninterrupted years (Payne & Crawford, 1992). The use of gillnets in the tuna fishing industry has been outlawed in many parts of the world and South Africa has been particularly vociferous in condemning them. In Natal, South Africa, the practice of deploying inshore shark-nets to protect bathers is controversial (Dudley & Cliff, 1992). There is no conclusive evidence that shark nets are capturing any species at a non-sustainable rate, although there is a cause for concern about two species of dolphin (*Tursiops aduncus* and *Sousa chinensis*). Marine mammalogists from the Port Elizabeth Museum believe that the shark nets pose no threat to the well-being of the common dolphin (*Delphinus delphis*) which occurs in large numbers (in excess of 15 000) and frequent Natal only in mid-winter, from June to September, during the annual sardine run. However, it is feared that the catch of the humpback dolphin (*S. chinensis*) may be greater than the local population can sustain and also that the Natal population of bottlenosed dolphins may be unable to sustain the losses to the nets (Dudley & Cliff, 1992; Cockcroft, Cliff & Ross, 1989).

Knowledge of the reproductive biology of dolphins has important implications for management of these mammals, as well as for predicting the effects of dolphin catches in shark nets. In particular, detailed information on the reproductive biology of females is crucial to an understanding of the likelihood of a species's survival (Cockcroft & Ross, 1990). Several species of dolphins inhabit the coastal waters of the south east Indian Ocean. Much of the dolphin research in South Africa focuses on the three most common species: the bottlenosed and common dolphins, and the endangered humpback dolphin. Other lesser-known species studied include the striped and Risso's dolphin (*Grampus griseus*). Much of this research is carried out at the Centre for Dolphin Studies (CDS) based at the Port Elizabeth

Museum which aims to expand existing knowledge of southern African coastal and pelagic marine mammals.

Although some information on the reproductive biology of the bottlenosed dolphin (*Tursiops aduncus*), the common dolphin (*Delphinus delphis*), the humpback dolphin (*Sousa chinensis*) and the striped dolphin (*Stenella coeruleoalba*) exists (Ross, 1984; Collet & Girons, 1984; Saayman & Taylor, 1979), detailed studies of gametogenesis have not been done. Such studies are necessary to examine seasonality of reproduction in these dolphins. The aim of this study is therefore to provide more detail on the reproductive biology of *D. delphis*, *T. aduncus*, *S. coeruleoalba* and *S. chinensis*. Chapter two describes both spermatogenesis and the general structure of spermatozoa in *D. delphis*. Chapter three examines seasonality of reproduction in *D. delphis* by assessing both male and female reproductive activity and describing foetal growth. Chapter four examines seasonality of reproduction in *T. aduncus*, *S. coeruleoalba* and *S. chinensis* by assessing both male and female reproductive activity and looking at the monthly capture of *T. aduncus* neonates. Finally, Chapter five provides a general discussion on the seasonality of reproduction in marine mammals.

CHAPTER 2

SPERMATOGENESIS AND SPERM MORPHOLOGY IN THE COMMON DOLPHIN, *DELPHINUS DELPHIS* (LINNAEUS, 1758).

2.1. INTRODUCTION

Spermatozoa were first observed in 1677 by Johan Ham, a medical student who brought them to the attention of the distinguished Dutch microscopist Antony van Leeuwenhoek (Bishop & Walton, 1960). The microscopes available to van Leeuwenhoek and early workers were primitive by modern standards and consequently the early drawings of spermatozoa are rather crude. Fortunately, with the development of new kinds of microscopy (polarising, ultra-violet, electron, phase-contrast, interference-contrast and fluorescence), our knowledge of the nature of the spermatozoon has expanded. There exists a vast amount of information on the morphology of mammalian spermatozoa (Fawcett, 1965, 1970, 1975) but very little information on the spermatozoa of cetaceans (Flemming, Yanagimachi & Yanagimachi, 1981).

The mammalian spermatozoon consists of two major parts, the head and the tail (Bishop & Walton, 1960; Fawcett, 1965). The principal component of the head is the nucleus which is covered anteriorly by a characteristic cap-like structure, the acrosome and posteriorly by a cytoplasmic sheath, the post-acrosomal cap (Bishop & Walton, 1960). In most mammalian spermatozoa the shape of the head is largely determined by the shape of the condensed nucleus (Fawcett, 1965) and the nucleus acquires a shape characteristic of each species (Fawcett, 1975). Since nuclear shape may be species specific, it can be used as an additional tool in phylogenetic studies (Bernard, Hodgson & Campbell, 1991). Since the early phylogeny of Cetacea is uncertain (May, 1990; Young, 1981) a study of sperm structure may aid in our understanding of the origin of the Cetacea.

Epididymal spermatozoa have been reported present in cetaceans (Brownell, 1984; Fisher & Harrison, 1970; Harrison, Boice & Brownell; 1969; Perrin, Coe & Zweifel, 1976) and have only been described for the sperm whale by scanning electron microscopy (SEM) (Montano, *et al.*, quoted by Flemming, *et al.*, 1981) and for the Atlantic bottlenosed dolphin, *Tursiops truncatus* by SEM and transmission electron microscopy (TEM) (Flemming, *et al.*, 1981).

Reproduction in dolphins has been the subject of numerous studies (Harrison, Boice & Brownell, 1969; Hohn, Chivers & Barlow, 1985; Miyazaki, 1984) but very few authors (Collet & Girons, 1984) provide detailed studies on the testicular activity in dolphins. Collet & Girons (1984) discuss the state of spermatogenic activity in adult male common dolphins, *D. delphis*, by describing three developmental stages: immature, prepubescent and mature. An understanding of spermatogenesis in dolphins is important for determining the animal's state of reproductive activity and stage of sexual maturity.

This study describes both spermatogenesis and the general structure of spermatozoa of the common dolphin *Delphinus delphis*.

2.2. MATERIALS AND METHODS

2.2.1. SPERMATOGENESIS

The gonads of adult male dolphins collected from strandings and shark nets, between 1970 and 1993, by the Port Elizabeth Museum and Natal Sharks Board, were fixed in 4% formalin and stored in 70% ethanol. Representative tissue samples from the testes were dehydrated in a graded ethanol series and embedded in Paraplast via xylene. 5µm thick sections were cut on a microtome and stained with Mallory's trichrome.

The state of spermatogenic activity in adult male dolphins was assessed qualitatively by examining the seminiferous tubules and describing the developmental stages (Bernard, *et al.*, 1994; Vergouwen, *et al.*, 1993). The mean diameter of 20 seminiferous tubules, measured using an optical micrometer, was used as a measure of tubule diameter (Miyazaki, 1984). Representative examples of the stages of spermatogenesis were photographed using an Olympus BX50 microscope and camera.

2.2.2. SPERM MORPHOLOGY-SEM

Sample of testes (1mm^3 blocks) were fixed with 2.5% glutaraldehyde in 0.2M-cacodylate buffer (pH 7) for 4 hours at room temperature, and washed twice with 0.2M-cacodylate buffer for 10 minutes. The samples were dehydrated through an ethanol series (30% to 100%) then infiltrated by an amyl acetate series (25% to 100%) and were critical point dried. The preparations were coated with gold in a SEM coating unit E5100 (Polaron Equipment Ltd.) and observed at 10kV with a JEOL JSM 840 (scanning electron microscope).

2.3. RESULTS

2.3.1. SPERMATOGENESIS

Four developmental stages of spermatogenic activity in males could be recognised and are described below.

Stage 1 (Juveniles): seminiferous epithelium comprising one cell layer of Sertoli cells (small cells, often elongated with their longitudinal axis generally orientated perpendicular to the basement membrane and containing a basally located nucleus). Centrally located gonocytes (large round cells with darkly-stained nucleus and a cytoplasm that is light) (Figure 2.1) are also present. The seminiferous tubules are narrow (0.038mm-0.051mm in diameter) and surrounded by abundant interstitial tissue.

Stage 2 (Inactive): seminiferous epithelium comprising one cell layer of spermatogonia (cells of an irregular shape that are smaller than gonocytes and contain a nucleus that stands out only slightly against the cytoplasm) and Sertoli cells, but no gonocytes (Figure 2.2). The seminiferous tubules are narrow (0.045mm-0.057mm in diameter) and embedded in abundant interstitial tissue.

Stage 3 (Early spermatogenesis): seminiferous epithelium comprising 2-4 cell layers of spermatogonia and spermatocytes, but no spermatids (Figure 2.3). The diameter of the seminiferous tubules range from 0.078mm to 0.093mm. The interstitial tissue occupies little space between the seminiferous tubules.

Stage 4 (Late spermatogenesis): seminiferous epithelium comprising 3 or more cell layers of spermatogonia, spermatocytes and spermatids (Figure 2.4). Spermatozoa are present in the lumina of the seminiferous tubules. The diameter of the seminiferous tubules range from 0.104mm to 0.262mm. The interstitial tissue occupies very little space between the seminiferous tubules.

2.3.2. SPERM MORPHOLOGY

The overall structure of the spermatozoa of *D. delphis* is typical of eutherian mammals (see Fawcett, 1975 for review). The head of the spermatozoon is dorso-ventrally compressed and in surface view has an elongated ellipsoid shape (Figure 2.5a). The sperm dimensions for *D. delphis* (n=1) are head length, 4.4µm; maximum head width, 2.0µm and middle piece length, 2.4µm (Figure 2.5a). The lengths of the acrosome and post-acrosomal cap are 2.3µm and 2.1µm, respectively. There are nine mitochondria in the middle piece (Figure 2.5a).

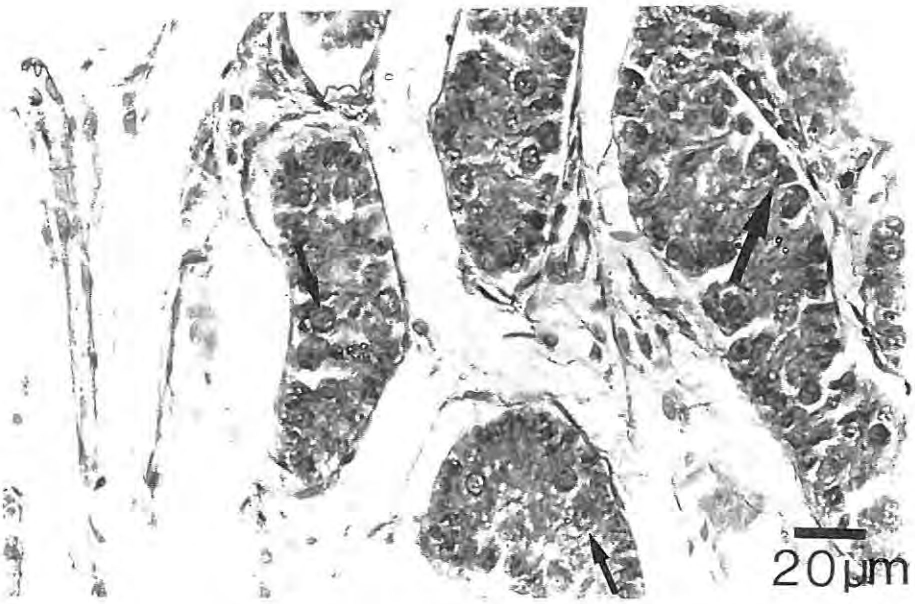

Figure 2.1. Photomicrograph of a section of testis from a juvenile dolphin. → gonocyte; → Sertoli cell.

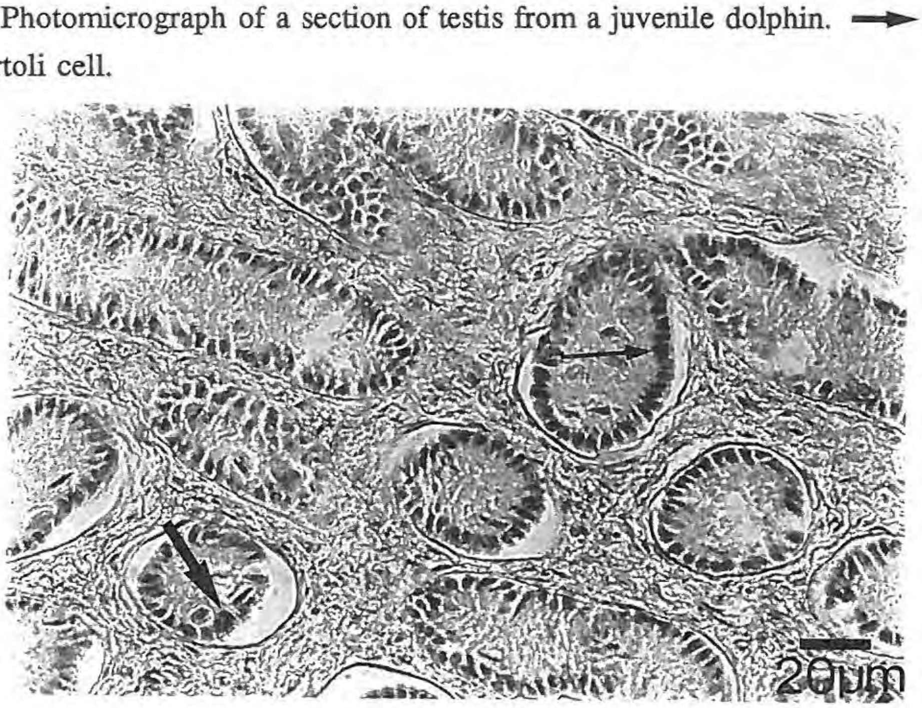

Figure 2.2. Photomicrograph of a section of testis from a reproductively inactive dolphin. The space between the layer of cells and the interstitial tissue is an artifact due to shrinkage of the seminiferous tubules that can occur during embedding process. → spermatogonia; → Sertoli cell.

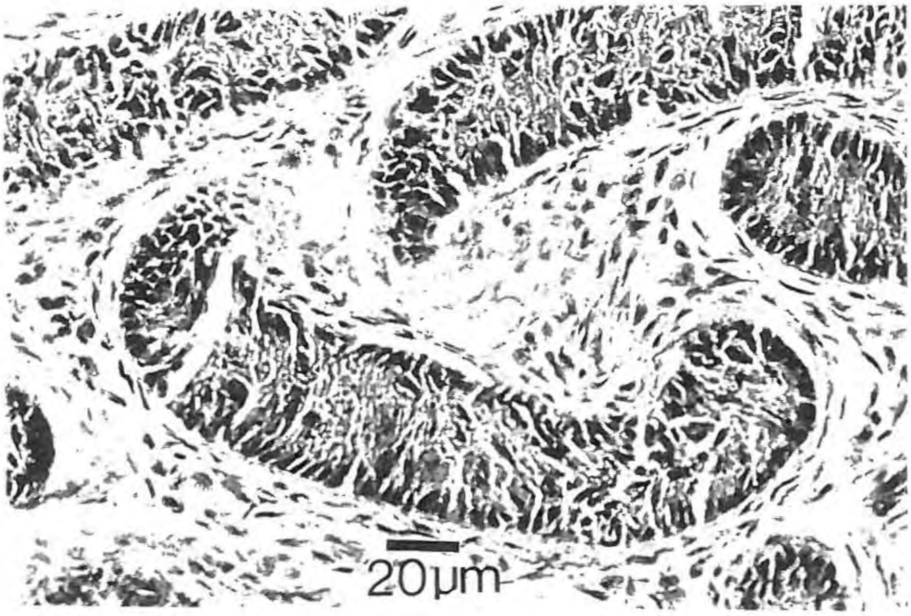

Figure 2.3. Photomicrograph of a section of testis from a dolphin in early spermatogenesis.

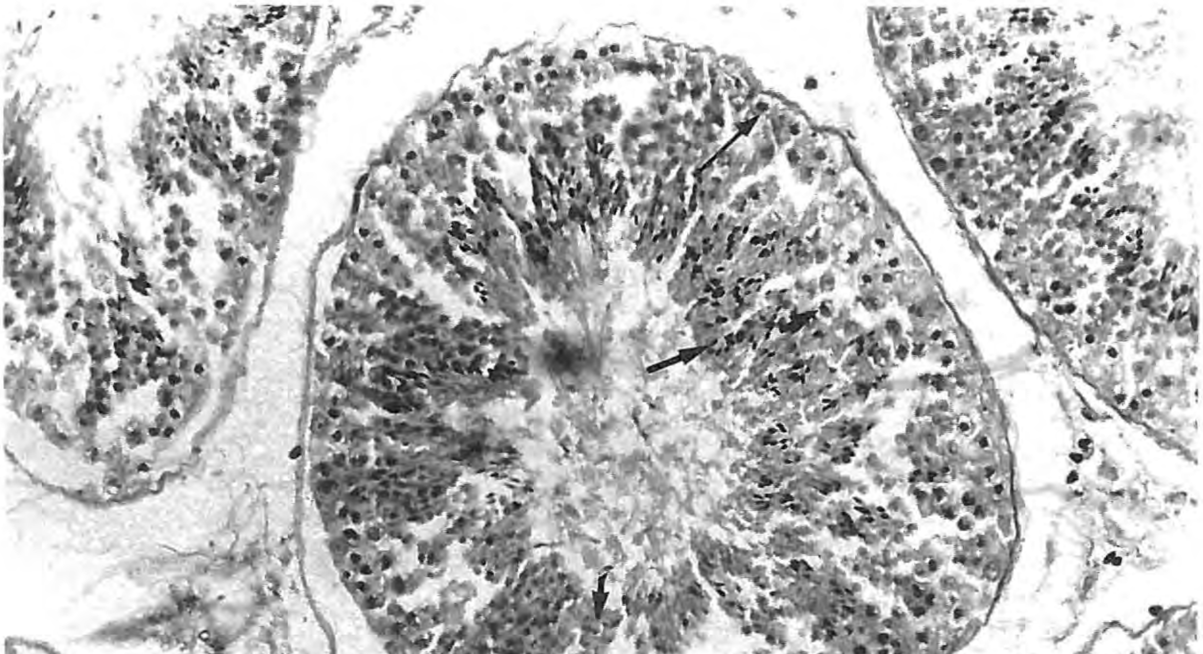

Figure 2.4. Photomicrograph of a section of testis from a dolphin in late spermatogenesis. ⟶ spermatogonia; ➔ spermatocytes; ➡ spermatids.

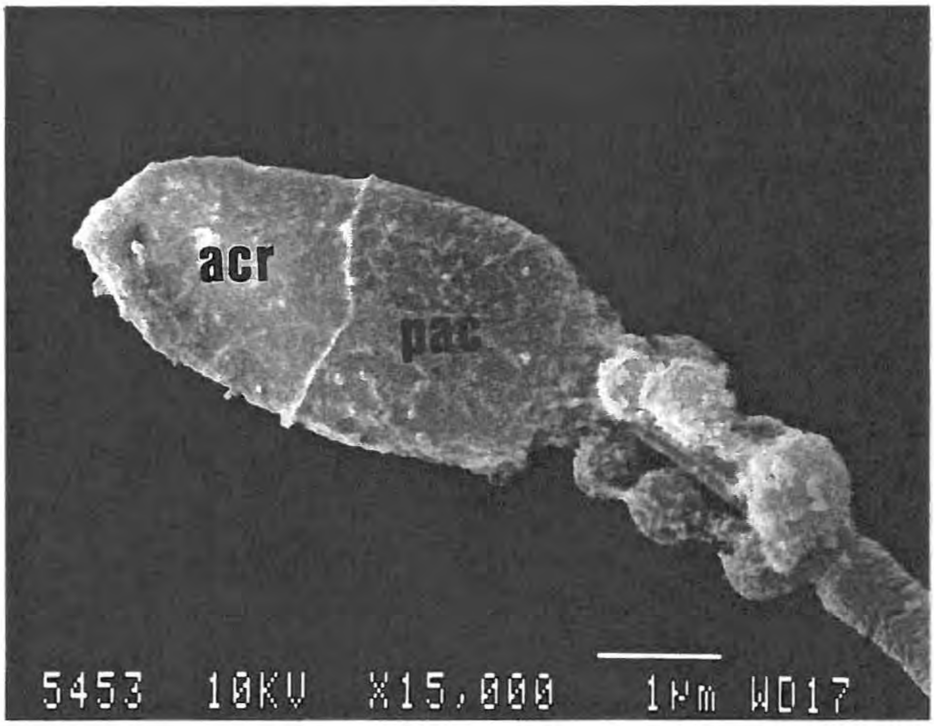

Figure 2.5a. Scanning electron micrograph of the spermatozoon of *D. delphis*. acr = acrosome; pac = post-acrosomal cap.

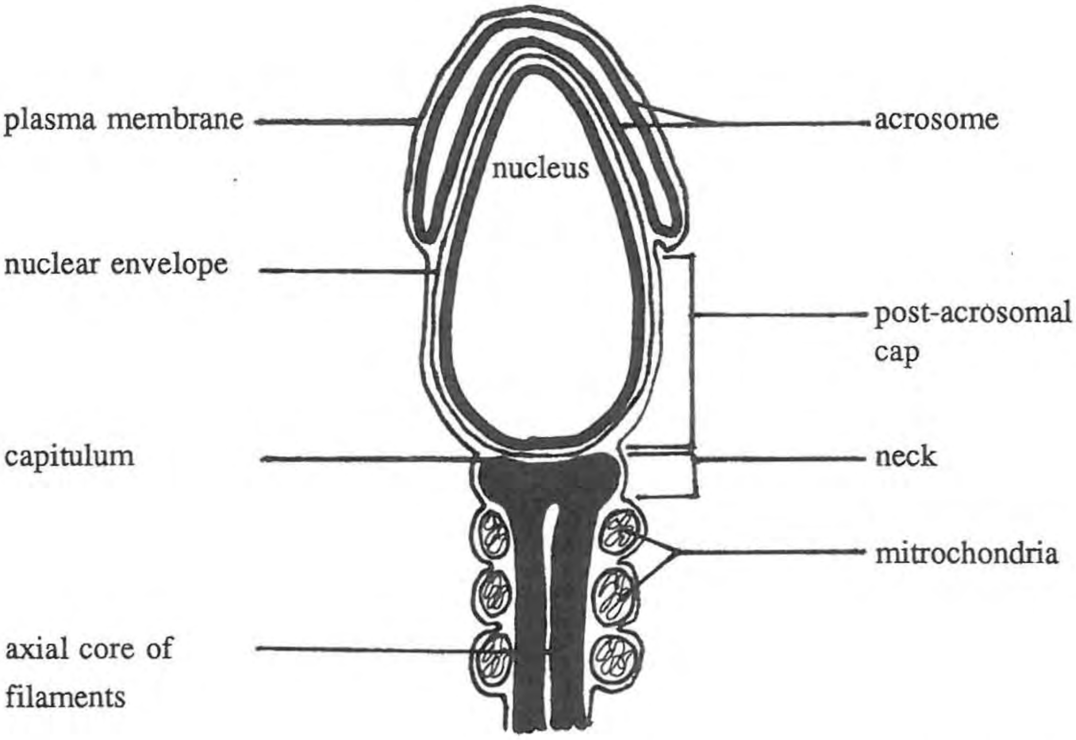

Figure 2.5b. A theoretical sagittal section through the spermatozoon of *D. delphis*.

The sperm of *D. delphis* could not be examined by TEM as there was no fresh material available. However, based on the extensive literature published on the fine structure of mammalian sperm (Fawcett, 1965, 1970, 1975; Bishop & Walton, 1960) and data from the spermatozoon of the bottlenose dolphin (Flemming, *et al.*, 1981) a drawing of the spermatozoa of common dolphins in sagittal section has been produced (Figure 2.5b).

2.4. DISCUSSION

In juveniles of male dolphins the seminiferous epithelium comprises one cell layer of Sertoli cells with centrally located gonocytes. In the present study, the gonocytes were identified using the criteria described by Kluin & de Rooij (1981) and van Haaster, van Eerdenburg & de Rooij (1993). These large, round cells that are centrally located in juveniles are not present at the start of spermatogenesis. Gonocytes have not been reported in cetaceans and previously authors (Collet & Girons, 1984; Sorensen & Kinze, 1994) regarded these centrally located cells as spermatogonia. It is possible that detailed TEM studies would confirm the identity of these cells. Gonocytes divide to form the first spermatogonia in mammalian testes (Setchell, 1978) and in mice gonocytes are present in the centre of the seminiferous tubule while spermatogonia occur along the basal lamina (Vergouwen, *et al.*, 1993). Gonocytes are sometimes referred to as germ cells in mice (Kluin, Kramer & de Rooij, 1984) and either germ cells or prespermatogonia in hamsters (Miething, 1989). In hamsters, the number of gonocytes decreased by day 5 (a day before spermatogenesis began), probably due to the degeneration of gonocytes, the reduction in gonocyte proliferation and division of the gonocytes to form A spermatogonia (van Haaster, *et al.*, 1993).

The immature, prepubescent and mature stages described by Collet & Girons (1984) are equivalent to the inactive, early spermatogenesis and late spermatogenesis stages of the present study, respectively. The process of spermatogenesis in *D. delphis* is essentially similar to that described for mammals (Setchell, 1978).

The dimensions and shape of the head of the spermatozoa of *D. delphis* (present study) are similar to those obtained by Flemming, *et al.* (1981) for the Atlantic bottlenosed dolphin (*Tursiops truncatus*) (head length=4.5µm, head width=2.0µm). However, the length of the middle piece of the spermatozoa of the Atlantic bottlenosed dolphin was greater (4.0µm) in comparison to that of *D. delphis* (2.4µm) suggesting possibly that more mitochondria are present.

In the bottlenosed dolphin, the post-acrosomal region is characterized by 14-16 elevated ridges running parallel to the long axis of the spermatozoa and an examination of transverse sections of the ridges showed distinct globular structures between the plasma membrane and nuclear envelope (Flemming, *et al.*, 1981). The general morphology of the spermatozoa of the common dolphin is similar to that of the bottlenosed dolphin, but like the spermatozoa of the sperm whale *Physeter catodon* (Montano, *et al.*, 1976 quoted by Flemming, *et al.*, 1981), it does not possess the ridges in the post-acrosomal region. The 9 mitochondria found in the common dolphin (present study) are located close to the base of the sperm head and the neck of the spermatozoon is extremely short. However, in the bottlenosed dolphin the sperm neck is fairly long and the mitochondria are located about 1.0µm from the base of the sperm head.

Sperm morphology has been used in studies of mammalian taxonomy (Bernard, *et al.*, 1991) and since the origin of Cetacea is unclear, it is possible that sperm morphology may clarify the early origin of the order. For the purpose of phylogenetic studies, the twenty-six eutherian orders have been grouped into four cohorts (Young, 1981). Cetaceans have been distinct since the Eocene era and their affinities are quite obscure, so they have been classed as a separate cohort (Mutica), named by Linnaeus (Young, 1981). The suspected ancestors of the Cetaceans are the Mesonychidae (primitive carnivorous land mammals), which are also believed to be the forerunners of the modern ungulates or hoofed mammals (Colbert & Morales, 1991; May, 1990). The nearest relative to cetaceans are the carnivores and the ungulates, and studies on protein structures of these animals show that cetaceans are closely related to even-toed ungulates (Order: Artiodactyla) for example, cattle, sheep and camels (Slijper, 1962). The length of the midpiece of the spermatozoa of even-toed ungulates is much larger (in relation to sperm head length) than the mid-piece of the spermatozoa of Cetacea (Cummins & Woodall, 1985). The typical fine structure of the sperm of extant even-toed ungulates is not like that of Cetacea and therefore sperm structure is of no value in determining the early origins of the order.

The four developmental stages of testicular activity described in this study for *D. delphis* occurred in the three other species of dolphins *Tursiops aduncus*, *Sousa chinensis* and *Stenella coeruleoalba* examined in this study (Chapter 4). The above mentioned developmental stages can therefore be adopted as a criterion to determine stage of sexual maturity and state of reproductive activity in male dolphins.

CHAPTER 3

THE REPRODUCTIVE SEASONALITY OF *DELPHINUS DELPHIS* (LINNAEUS, 1758).

3.1. INTRODUCTION

Delphinus delphis (Common dolphin) is one of the commonest warm-temperate and tropical species of dolphin (Branch, Griffiths, Branch and Beckley, 1994). It is a small to medium sized dolphin attaining a length of up to 2.75m (Ross, 1984) and a mass of up to 160kg (Collet, 1983). The body is relatively robust with a characteristic hourglass-shaped pattern on its sides (Figure 3.1). A narrow dark stripe is seen ventro-laterally from flipper to the lower jaw. *D. delphis* is gregarious and forms schools of 1000-5000 individuals. It is known to feed opportunistically, mostly on shoaling pelagic fish, and huge schools of these dolphins are seen pursuing the annual Natal sardine run (Ross, 1984).

Figure 3.1. *D. delphis* at the Port Elizabeth Oceanarium, South Africa. Courtesy of The Port Elizabeth Museum.

D. delphis attains sexual maturity at about 5-7 years of age which corresponds to a body length of 1.9m in females and 2m in males (Collet, 1983). The body mass of mature males is 10% greater than that of mature females, and males possess testes that are about 4.2% of their body weight (Cockcroft, 1993). The evolution of large testes has been attributed to high copulatory frequency, sperm production and competition among sperm of different males for the fertilization of the same female (Kenagy & Trombulak, 1986). The estimated duration of pregnancy for *D. delphis* is 11 months with a period of lactation of at least 10 months and an interbirth interval of approximately 2 years (Collet, 1983). The length of calves at birth is about 0.90m (Collet, 1983) and obligate nursing appears to cease at a length of about 1.5m (Ross, 1984) and age of about 10 months (Collet, 1983).

D. delphis occurs in the eastern Cape waters during the summer months and migrates into the Natal waters during the winter months. This type of seasonal movement appear to be associated with feeding and breeding (Bronson, 1989; Collet, 1983).

Prior to 1969, only scattered information existed on the reproduction of *D. delphis*. Harrison, Boice and Brownell (1969), provided a comprehensive summary of all earlier studies on reproduction in wild and captive *D. delphis* in California. Their results indicated that testes of adult dolphins were active during most of the year, births occurred in winter from December to March and the corpus luteum of pregnancy persisted until near term. By contrast Collet and Girons (1984) undertook a preliminary study of the male reproductive cycle of *D. delphis* in the Eastern North Atlantic. They described the different stages of spermatogenesis in relation to body length, looked at spermatogenic activity of 11 animals and inferred that spermatogenesis followed a seasonal pattern. Ivashin (1984) reviewed literature on the differences in macroscopic and histologic structures of two types of corpora albicans in *D.*

delphis as described by Soviet scientists in the 1950s and 1960s. It is important to distinguish between the corpus albicans of pregnancy and corpus albicans of ovulation (in the absence of fertilization and pregnancy) in order to estimate the total number of births. Ovulation is believed to be spontaneous (Benirschke, Johnson and Benirschke, 1980; Kirby & Ridgway, 1984). However, these authors could draw no conclusion about the seasonality of reproduction.

There have however been no detailed studies on gametogenesis although such studies are necessary to establish whether reproduction is seasonal. Knowledge of the timing of reproduction has important implications for management of these mammals, particularly for predicting the effects of dolphin catches in shark nets and on population dynamics.

The aim therefore of this study is to provide more detail on the reproductive biology of these dolphins by describing spermatogenesis, oogenesis and foetal growth. This information will then be related to the general biology of the species. This chapter will also examine the seasonal patterns of reproduction in *D. delphis* from the south east coast of South Africa.

3.2. MATERIALS AND METHODS

The gonads of adult dolphins collected from strandings and shark nets, between 1970 and 1993, by the Port Elizabeth Museum and Natal Sharks Board, were fixed in 4% formalin and stored in 70% ethanol. Representative tissue samples from the testes and ovaries were dehydrated in a graded ethanol series and embedded in Paraplast via xylene. 5µm thick sections were cut on a microtome and stained with Mallory's trichrome.

A sample size of 32 males (2.05-2.58m in length), 17 females (2.07-2.34m in length) and 39 foetuses (0.026- 1.18m in length) collected monthly over a period of 24 years was studied (Table 3.1). The large sample sizes observed for the months of July and August can be attributed to the larger number of dolphins caught in shark nets during the annual Natal sardine run.

Table 3.1. Monthly sample sizes for males, females and foetuses of the common dolphin.

	J	F	M	A	M	J	J	A	S	O	N	D
Males	1	1		1	1	2	12	6	5	2	1	
Females					2	1	5	4	3	1		
Foetuses				1	2	1	15	10	3			7

3.2.1. MALE REPRODUCTIVE ACTIVITY

The state of spermatogenic activity in adult males was assessed both qualitatively and quantitatively. One hundred seminiferous tubules were examined for each dolphin and the animals categorized under one of four developmental stages (Bernard, *et al.*, 1994; Vergouwen, *et al.*, 1993): juvenile (seminiferous epithelium comprising one cell layer of Sertoli cells, and the presence of gonocytes), inactive (seminiferous epithelium comprising one cell layer of spermatogonia and Sertoli cells, but no gonocytes), early spermatogenesis (seminiferous epithelium comprising 2-4 cell layers, but no spermatids), late spermatogenesis (seminiferous epithelium comprising 3 or more cell layers and the presence of spermatids).

The mean diameter of 20 seminiferous tubules was used as an index of tubule diameter (Miyazaki, 1984) and was measured using an optical micrometer. A one-way-analysis of variance (ANOVA) was used to determine whether the mean seminiferous tubule diameters differed significantly for each month. Reproductively inactive male dolphins are referred to as juveniles.

3.2.2. FEMALE REPRODUCTIVE ACTIVITY

Sections of ovaries were examined for the presence and number of both small-medium (< 4mm in diameter) and large (> 4mm in diameter) Graafian follicles and luteal bodies (corpora albicantia and corpora lutea). Unfortunately, separation of corpora albicantia from corpora lutea was not possible due to the inadequate preservation of tissue. Females in which the ovaries lacked growing follicles and corpora were considered immature (Harrison & Brownell, 1971; Perrin & Donovan, 1984). The presence of one or more corpora in one or both ovaries was used as the criterion for female sexual maturity (Brownell, 1984). Data on whether the females were lactating and/or pregnant was provided by the Port Elizabeth Museum.

3.2.3. FOETAL GROWTH CURVES

The relationship between mammalian foetal weight and conception age has been described by Huggett and Widdas (1951). The foetal age of 39 foetuses collected monthly over a number of years was calculated using known foetal weights and the following general formula given by Huggett and Widdas (1951):

$$W^{1/3} = a(t-t_0)$$

where $W^{1/3}$ = cube root of foetal mass in grams

a = specific foetal growth velocity

t = age of foetus in days

t_0 = total gestation x f

$f = 0.2$ for mammals with gestation between 100-400 days (Hugget & Widdas, 1951)

The length of *D. delphis* at birth is about 0.90m (Collet, 1983) which corresponds to an average mass of 9000g. The estimated pregnancy of *D. delphis* is 11 months (330 days).

Using the above formula and data from Collet (1983), the specific foetal growth velocity (a) for *D. delphis* was calculated as follows:

w = mass at birth = 9000g

t = 330 days

$t_0 = 330 \times 0.2 = 66$.

Therefore: $a = w^{1/3} / t - t_0$

$= 9000^{1/3} / 330 - 66$

$a = 0.079$

Hence, the specific foetal growth velocity for *D. delphis* was 0.079. With specific foetal growth velocity and foetal mass (w) known, foetal age (t) was calculated from the following equation:

$$t = \frac{w^{1/3}}{0.079} + 66$$

3.3. RESULTS

3.3.1. MALE REPRODUCTIVE ACTIVITY

Of the 32 males collected 5 were juveniles, 2 were spermatogenically inactive, 3 were in early spermatogenesis and 22 were in late spermatogenesis. In most months, except January and October, 50% or more of all adult males were producing spermatozoa (Figure 3.2). The seminiferous tubule diameters of the juveniles were small and showed little variation (0.048mm to 0.051mm) during the year (Figure 3.3). By contrast, the mean monthly diameters

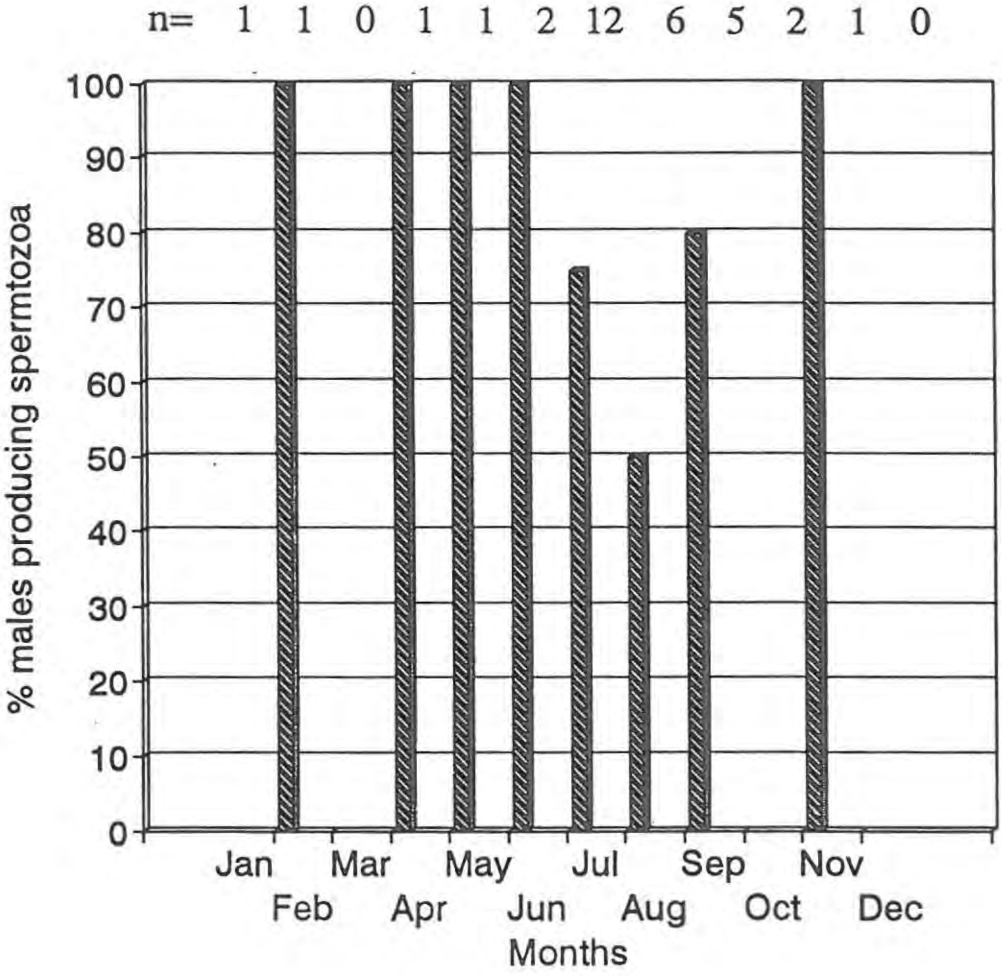

Figure 3.2. The percentage of adult *D. delphis* males producing spermatozoa during the year.

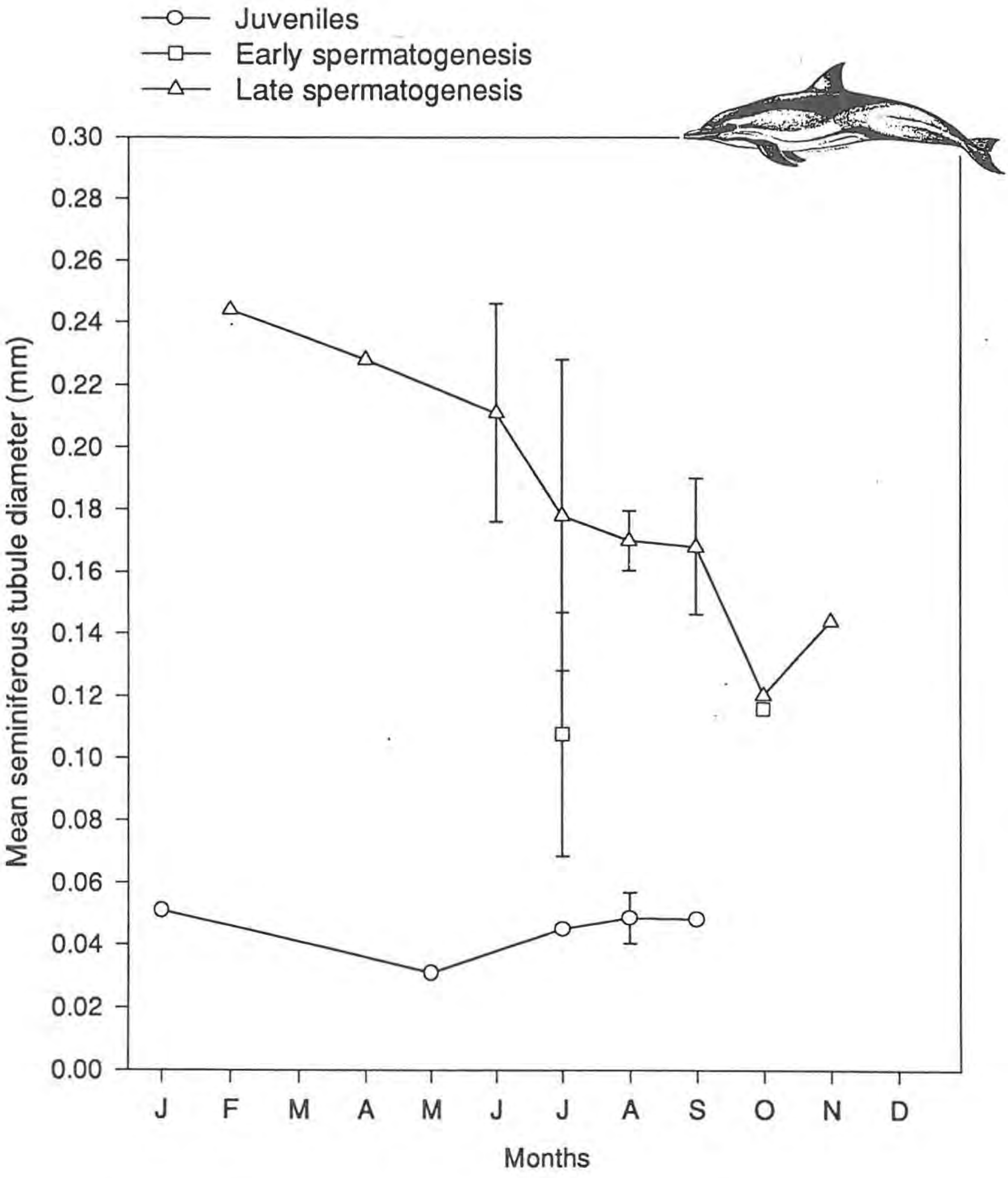

Figure 3.3. A monthly plot of seminiferous tubule diameter ($\overline{x}$ ± SD) for the 3 different stages of male *D. delphis* sexual maturity.

of those in late spermatogenesis ranged from 0.104mm to 0.262mm and decreased from February to November although this was not statistically significant ($P<0.05$). There is great variation in the size of the seminiferous tubule diameter of specimens in late spermatogenesis (Figure 3.4a-c). In spite of the variation in seminiferous tubule diameter, in all animals that were producing spermatozoa, more than 82% of all seminiferous tubules were in late spermatogenesis (Appendix 1).

An analysis of seminiferous tubule diameters, and lengths and weights of dolphins measured at different stages of maturity indicates that *D. delphis* probably does not attain sexual maturity at a mass of less than 94kg and a length of less than 2.15m (Table 3.2).

Table 3.2. Measurements of seminiferous tubule diameters (STD) (mm), lengths (m) and weights (kg) of the dolphins at different stages of male maturity in *D. delphis*.

Stage of Maturity	Range	Mean ± SD
Juveniles & Inactive		
STD	0.038-0.058	0.048 ± 0.01
Length	2.14-2.38	2.26 ± 0.09
Weight	90-110	105 ± 7.0
Early Spermatogenesis		
STD	0.078-0.093	0.097 ± 0.02
Length	2.27-2.29	2.34 ± 0.1
Weight	116-131	126.3 ± 8.9
Late Spermatogenesis		
STD	0.104-0.262	0181 ± 0.04
Length	2.15-2.55	2.38 ± 0.1
Weight	94-163.3	139.7 ± 21.1

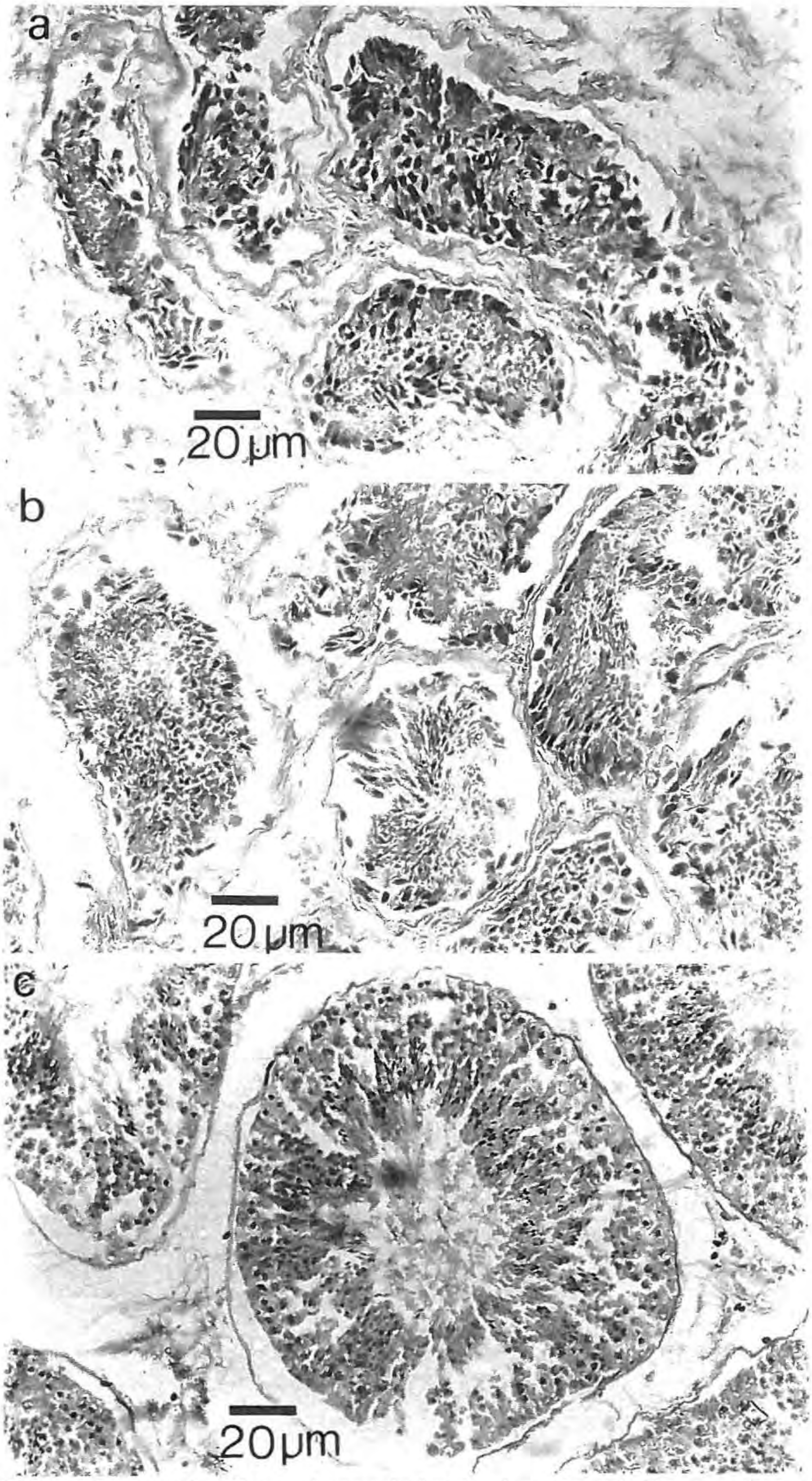

Figure 3.4a-c. Photomicrographs illustrating variation in size of seminiferous tubule diameters of specimens of *D. delphis* in late spermatogenesis. **a** mean seminiferous tubule diameter = 0.104mm; **b** mean seminiferous tubule diameter = 0.121mm and **c** mean seminiferous tubule diameter = 0.224mm.

3.3.2. FEMALE REPRODUCTIVE ACTIVITY

Of the 16 females caught, 12 were reproductively active, and of these 8 were visibly pregnant and 4 were lactating. In all months for which data are available, more than 30% of the animals had at least one luteal body in one of the ovaries and more than 50% of the animals had large (> 4mm in diameter) Graafian follicles (Figure 3.5, p26). When all the animals are analyzed together (n=16), 50% possessed luteal bodies and 69% possessed large Graafian follicles. Of the 16 females, only 4 females, did not possess either large Graafian follicles or luteal bodies and were thus considered reproductively inactive. All four females were greater than 2m in length and thus could not be considered as juveniles.

The quality of fixation of the ovarian tissue precluded any detailed study of follicular development or the structure of the corpus luteum. It was not possible to distinguish the corpus luteum from the corpus albicans. A typical ovary contained one corpus (characterized by a solid spheroidal mass of large glandular cells and connective tissue septa) (Figure 3.6) and two to five large Graafian follicles.

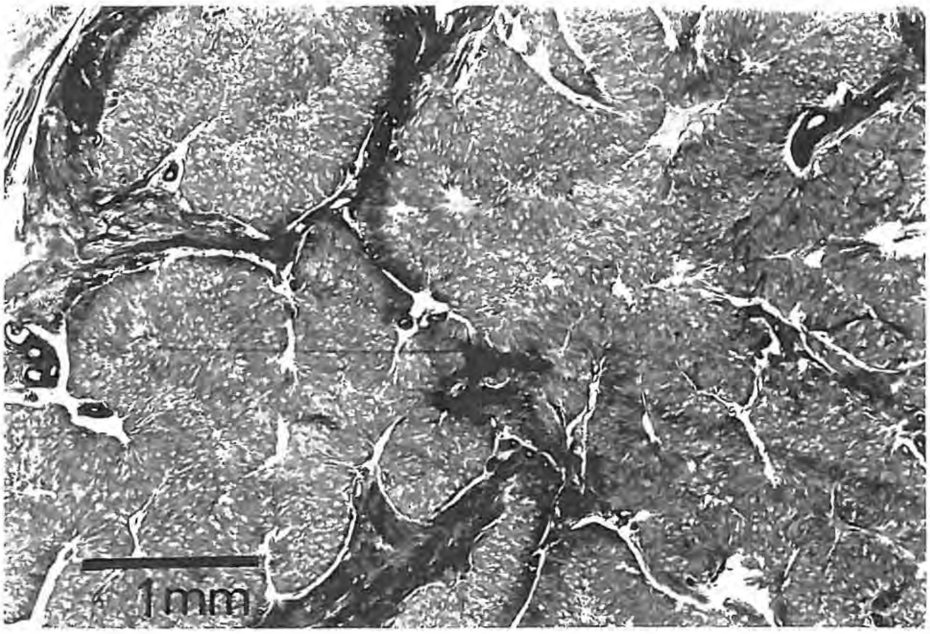

Figure 3.6. Photomicrograph of a section of an ovarian corpus in *D. delphis*.

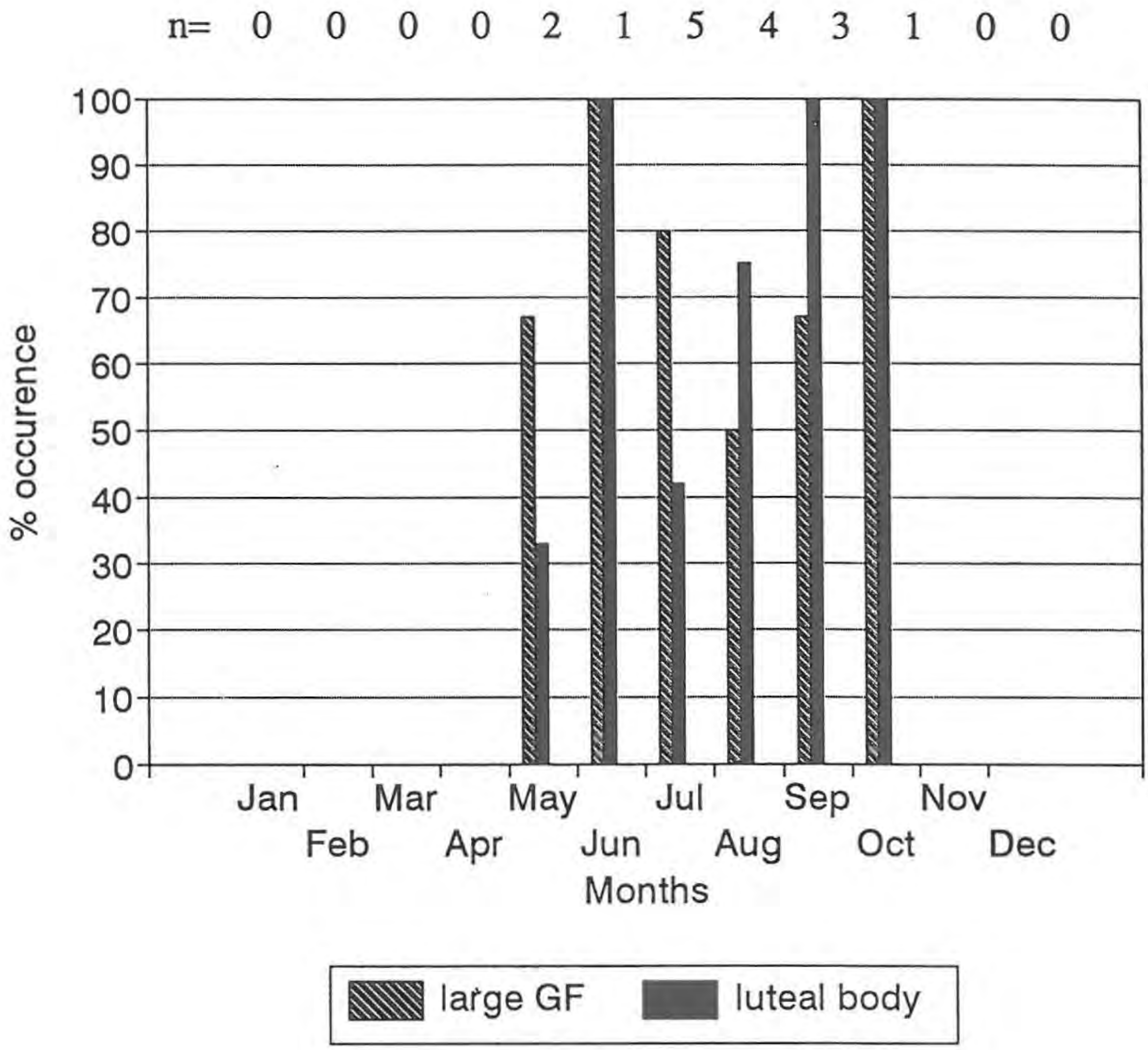

Figure 3.5. The percentage occurence of large Graafian follicles (GF) and luteal bodies throughout the year.

3.3.3. FOETAL GROWTH

As expected foetal mass increased exponentially with foetal age (Figure 3.7a). It should be noted that the accuracy of this curve remains untested in the absence of any known age foetuses. When foetal age is plotted against the month in which the mother was caught (Figure 3.7b), there is a marked increase in foetal age from April through to December. The predicted conception dates and birth dates for these foetuses were calculated by counting backwards (conception) and forward (birth) (Table 3.3). It is calculated that fifty percent of the foetuses were conceived in January and fifty percent of the births would have occurred in December.

Table 3.3. Percentage foetal conception and occurrence of birth during different months, calculated from foetal size data (appendix 3) using Hugget & Widdas's (1951) formula.

	J	F	M	A	M	J	J	A	S	O	N	D
Conceptions	50	13	18	13	2	2	2					
Births	13	18	13	2	2	2						50

3.4. DISCUSSION

In the present study, late spermatogenesis was found to occur throughout the year in *D. delphis* from the Indian Ocean and there is a continuous production of spermatozoa. This supports the findings of Harrison, *et al.* (1969) who concluded that spermatogenesis in wild and captive *D. delphis* was continuous. By contrast Collet and Girons (1984) found great variation in testis size among mature Common dolphins in the North East Atlantic which led them to consider the existence of a cycle of testis activity. Furthermore, histological examination of the gonads of 11 individuals showed different stages of spermatogenesis. This led them to hypothesize a season of testicular activity between December and June-July.

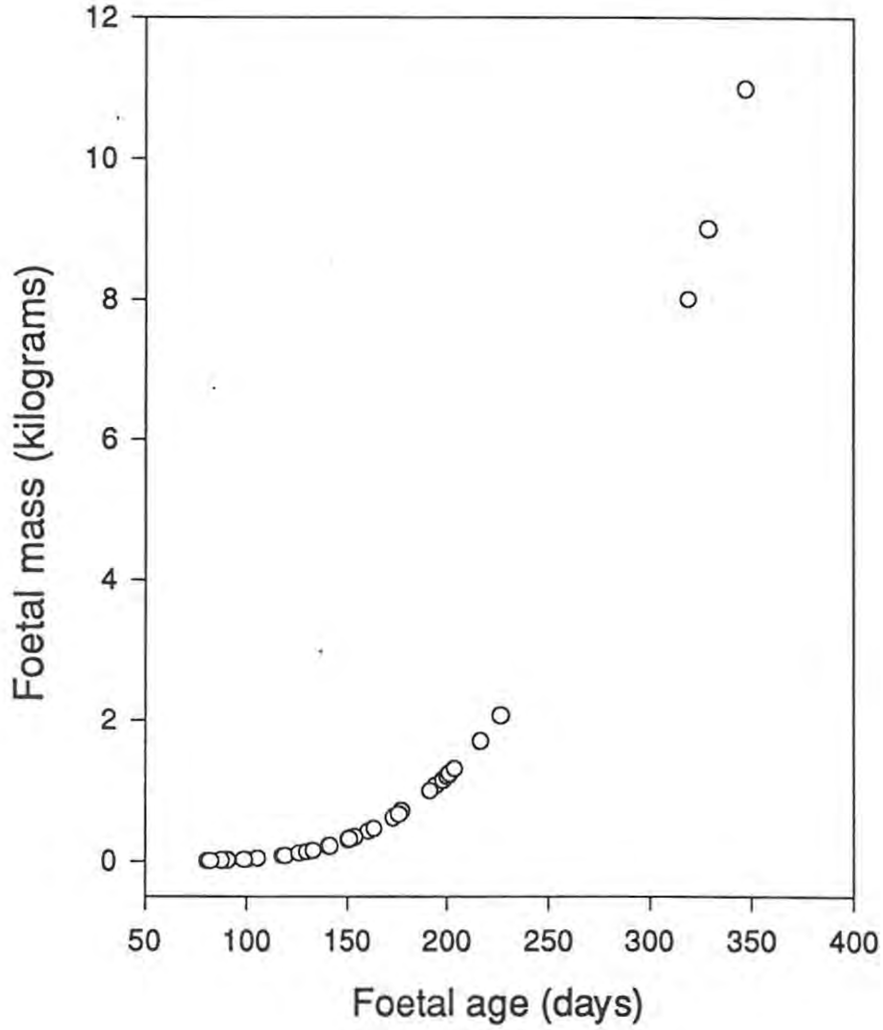

Figure 3.7a. Calculated foetal growth curve for *D. delphis*.

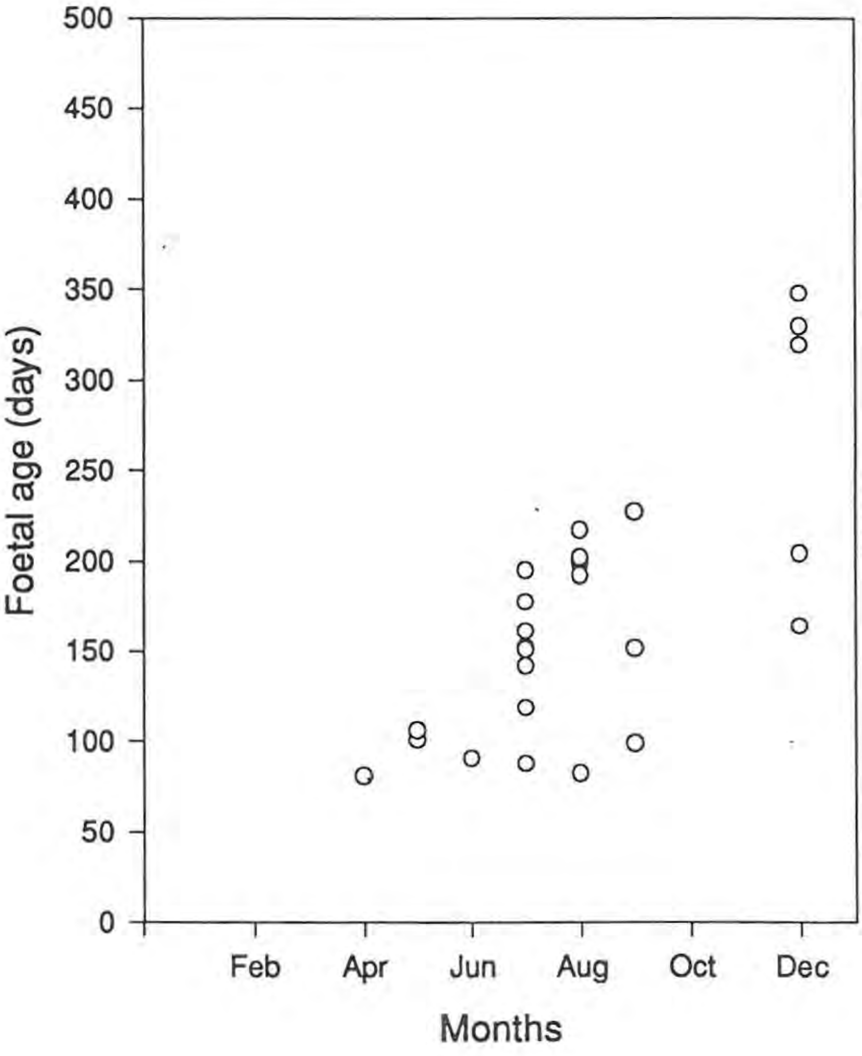

Figure 3.7b. Monthly plot of calculated foetal age for *D. delphis*.

However, their findings are not based on sexually mature *D. delphis* males as no specimen contained spermatozoa either in the seminiferous tubules or epididymis.

In the present study there is a great variation in the size of the seminiferous tubules of specimens in late spermatogenesis (0.104mm - 0.262mm) and similar results have been reported by Miyazaki (1984) for striped dolphins. He found that the diameter of the seminiferous tubules in mature males (testes containing spermatozoa) ranged from 0.045 to 0.214mm and increased rapidly during the period from 7 to 15 years of age after which it remained constant. Collet & Girons (1984) found that the smallest seminiferous tubules in mature male *D. delphis* had an average diameter of 0.1mm, while some attained 0.2 to 0.25mm. In view of Miyazaki's (1984) results, it seems likely that the difference in the diameter of the seminiferous tubules of *D. delphis* (present study) may be related to the age of the dolphin or may be due to the quality of fixation.

Although spermatogenesis was continuous, fifty percent of the foetuses were conceived in January and 94 percent of the births in *D. delphis* would have occurred in summer (from December to March) with a few births in early winter. These results are similar to those of Harrison *et al.* (1969) for *D. delphis* for the Northern hemisphere (Californiia) where births occur in winter from December to March and the testes of adult dolphins are active during most of the year.

D. delphis inhabits the coastal waters of the eastern Cape during the warm summer months and feeds mainly on cephalopods (Ross, 1984; Young & Cockcroft, in press) which are abundant in these waters (Young & Cockcroft, in press). The high water content in cephalopods (Cooper, 1979) may be beneficial for lactating females during this calving period and this is supported by the fact that lactating females of *D. delphis* consume more squid than pregnant females (Young & Cockcroft, 1994). A sample of 15 ml of milk collected from one lactating *D. delphis* contained 57.7 % water (Ross, 1984). *D. delphis* migrates to the Natal waters during winter (June and July), probably in response to a similar movement by large shoals of fish, notably pilchard, and/or as a response of adult females moving into the warmer Natal waters to continue lactating. Calves start to take fish after 4 months (Hare & Papastavrou, 1993) and this period (June/July) coincides with the annual Natal sardine run. This ensures not only an adequate supply of fish for the lactating females but also a large number of small fish for calves being weaned.

The seasonal pattern in foetal growth suggests the existence of a synchronized period of ovulation and conception in January and this is supported by Sorensen & Kinze (1994) for Harbour porpoises (*Phocoena phocoena*). Unfortunately, due to the lack of data for females for November to April, this period of ovulation was not observed. Corpora lutea involute to form corpora albicantia and in cetaceans they are believed to persist until death (Bryden, Harrison and Gemmell, 1984; Perrin & Donovan, 1984). This may explain the high percentage of luteal bodies in the ovaries of females occurring in most months. Active corpora will always be present throughout the year as pregnancy lasts for approximately 11 months.

The presence of large Graafian follicles from May through to October is peculiar since if ovulation is synchronized in January (summer) then the development of follicles at other times would appear unnecessary. However, prior to ovulation, many follicles develop at the same time, with only one follicle rupturing during ovulation (Harrison & Bryden, 1988), and the others may be remnants that have not yet degenerated. This may explain the presence of large Graafian follicles from May through to October. Importantly, therefore, the presence of Graafian follicles can not be taken as an indication of timing of ovulation.

In conclusion, the seasonal variation in foetal size and the calculated seasonal peaks of conception and births in *D. delphis* provides strong evidence for seasonality of reproduction in this species. It is suggested that the births in summer are timed so that females have access to squid while in early lactation and so that calves can utilize the increase in abundance of fish that occurs during the sardine run. It appears that the annual migration may be intimately linked to the timing of reproduction.

CHAPTER 4

REPRODUCTIVE SEASONALITY OF *TURSIOPS ADUNCUS* (EHRENBERG, 1832), *STENELLA COERULEOALBA* (MEYEN, 1833) and *SOUSA CHINENSIS* (OSBECK, 1757).

4.1. INTRODUCTION

Bottlenosed dolphins belonging to the genus *Tursiops* are widely distributed in cool temperate to tropical waters of the world and four species have been described of which the taxonomic status is still in considerable doubt. The taxonomy of the bottlenosed dolphin in South African waters has been described fully by Ross (1977, 1984). In Southern African waters there exists two distinct species of *Tursiops*, namely *T. aduncus* (Indian ocean bottlenosed dolphin) and *T. truncatus* (Atlantic bottlenosed dolphin). *T. aduncus* inhabits the coastal waters of the south east coast while *T. truncatus* occurs inshore (and possibly offshore) on the west coast, but mainly inhabits offshore waters on the south and south east coasts (Ross, 1984) (Figure. 4.1). Although the distributions of *T. aduncus* and *T. truncatus* overlap along the SE coast, there is no evidence of interbreeding (Ross, 1984).

Bottlenosed dolphins are characterised as having a snout which is abruptly narrower than the head and hence roughly 'bottlenosed' (Ross, 1984) (Figure 4.2a). Externally, *T. aduncus* resembles *T. truncatus* in the relative robustness of the body. However, *T. aduncus* has a melon which is less convex in profile and a longer and narrower snout than *T. truncatus*, and hence *T. aduncus* appears to have a more slender profile (Ross, 1984). *T. aduncus* is generally a smaller dolphin with males and females attaining lengths up to 2.54m and 2.52m, respectively (Ross, 1977). *T. truncatus* is larger with males and females attaining lengths up to 3.65m and 2.93m (Skinner & Smithers, 1990), respectively.

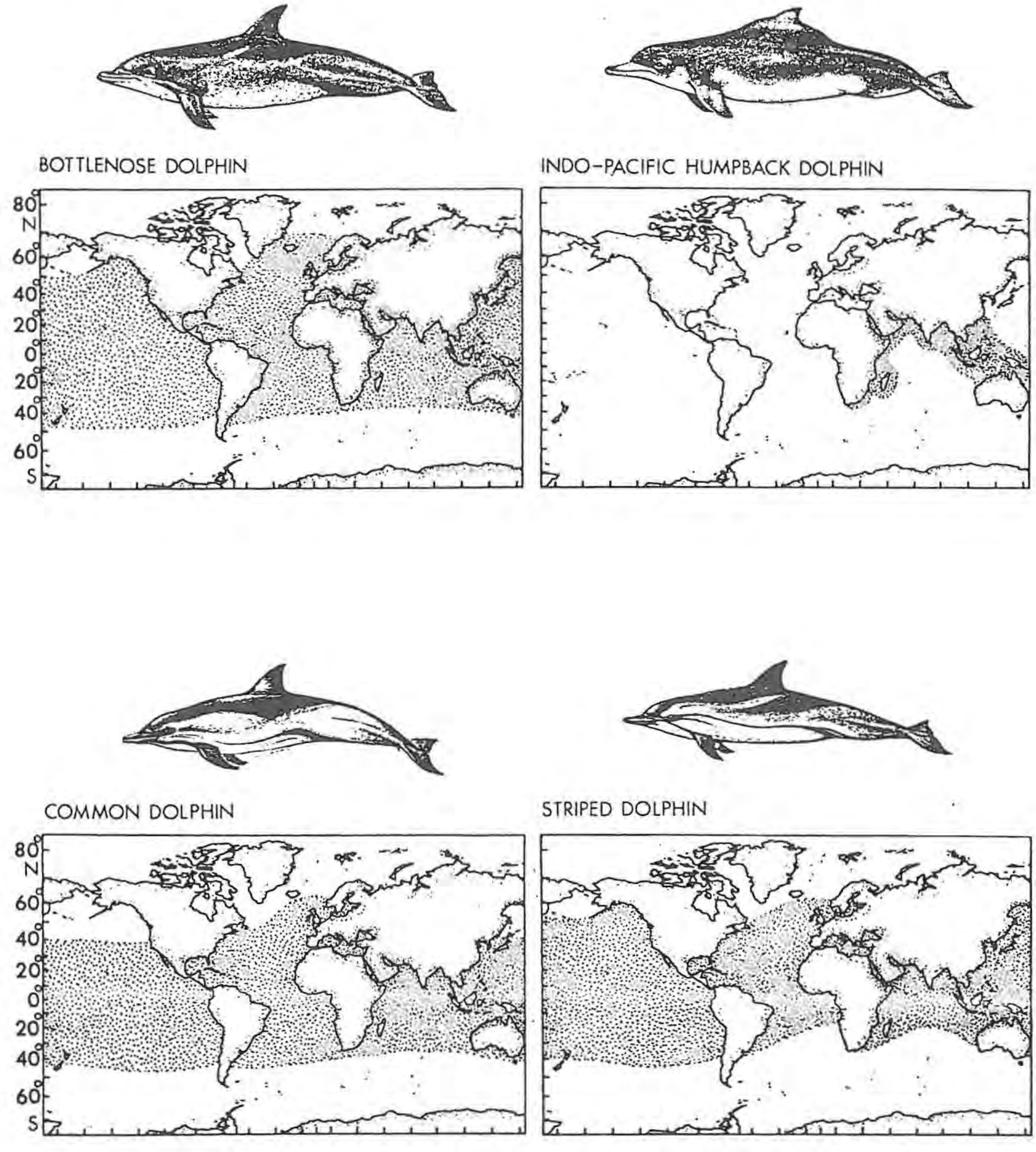

Figure 4.1. Worldwide distribution of *Tursiops* spp., *Sousa chinensis*, *Delphinus delphis* and *Stenella coeruleoalba* (from Payne & Crawford, 1992).

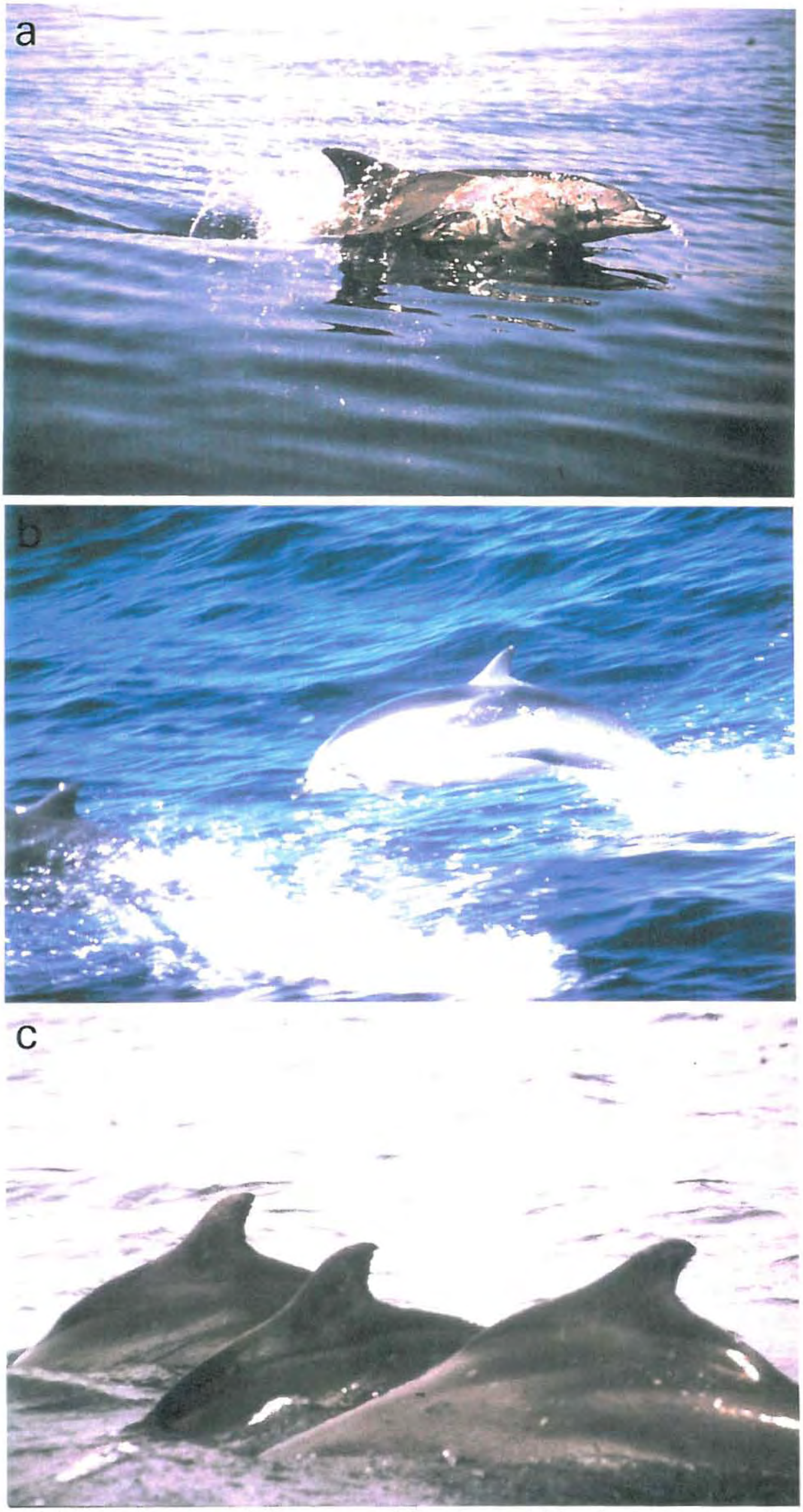

Figure 4.2. Dolphins seen around the coast of Southern Africa include **a** *T. aduncus*, **b** *S. coeruleoalba* and **c** *S. chinensis*. Courtesy of the Port Elizabeth Museum.

T. aduncus has a grey upper body, with a darker grey 'cape' on the back and surrounding area, including the dorsal fin. The belly is off-white, often specked with grey spots (Branch, *et al.*, 1994). Cockcroft and Ross (1990) report that the appearance of ventral speckles in coastal bottlenosed dolphins is correlated with sexual maturity.

Cockcroft and Ross (1990) have found that female bottlenosed dolphins from South African waters attain sexual maturity between 9 and 11 years of age, 2 or 3 years earlier than males. However, Tavolga and Essapian (1957) in Florida found that one of their captive bottlenosed dolphins conceived as early as six years and gave birth a year later. McBride & Kritzler (1951) quoted by Tavolga & Essapian (1957) believed that the female bottlenosed dolphin reaches sexual maturity at 4 years and may bear her infant in the spring of her fifth year. The length at sexual maturity in females lies between 2.13m and 2.30m and between 2.10m and 2.40m for males (Ross, 1984). The length of gestation is about 12 months with a lactation period of about 18 months to two years (Cockcroft & Ross,1990).

T. aduncus occurs in groups ranging from one to five to 300-1000 individuals (Ross,1984). Although *T. aduncus* is frequently regarded primarily as a benthic feeder, Ross (1977, 1984) found that these animals consumed a wide variety of food items, including both benthic and pelagic species.

Bottlenosed dolphins are the most commonly held dolphins in oceanaria throughout the world. Members of the genus *Tursiops* are readily available close inshore, train well and adapt easily to captive life (Ross, 1977). Hence, bottlenosed dolphins are among the better understood species of cetacean (Ross, 1977; Smolker, Richards, Connor & Pepper, 1992). *T. aduncus* is also the most commonly stranded and sighted cetacean on the south east coast of South Africa

(Ross, 1984). However, most work done on bottlenosed dolphins is on the larger Atlantic form, *T. truncatus* (Cheal & Gales, 1991; Cockcroft, Cliff & Ross, 1989; Leatherwood, Deerman & Potter, 1978; Robeck, Curry, McBain & Kraemer, 1994).

The limited data available on *T. aduncus* in South African waters is provided by Ross (1977, 1984). The normal calving interval for *T. aduncus* is unknown (Ross, 1984). Cockcroft & Ross (1990) found that births in *T. truncatus* occurred throughout the year with a peak in summer, between November and February, when over 60% of births occurred. Ross (1977) reported that births in *T. aduncus* also occurred throughout the year but predominately in the late spring and summer months.

Three species of *Stenella* namely, *S. coeruleoalba* (striped dolphin), *S. attenuata* (spotted dolphin) and *S. longirostris* (spinner dolphin) have been recorded from the south east coast of southern Africa (Ross, 1984). *S. coeruleoalba* is widely distributed in tropical and subtropical waters of the Atlantic and Pacific oceans and the Mediterranean seas (Miyazaki, 1984) (Figure 4.1). The warm waters of the Agulhas current allows the striped dolphin with tropical and subtropical distributions to penetrate South Africa's east or south coasts (Payne & Crawford, 1992). *S. coeruleoalba* forms schools of 5-400 individuals (Branch, *et al.*, 1994).

S. coeruleoalba is characterized by three distinct dark lines that run posteriorly from the eye, one to the anus, another to the base of the flipper, and a third between these two which is short and tapering (Ross, 1984) (Figure 4.2b). The striped dolphin is the largest member of the genus *Stenella* and attains a maximum length of 2.7m (May, 1990). The females of *S. coeruleoalba* become sexually mature at 2.1m and males between 2.1m and 2.2m in length

(Ross, 1984). This is consistent with the findings of Miyazaki (1977), who found that males and females in Japanese waters reach sexual maturity at 2.16m and 2.19m, respectively. One reproductive cycle for *S. coeruleoalba* occurs over a period of 3 years and 2 months, with a gestation period of 13 months, 16 months in lactation and 7 months in resting (Miyazaki, 1984). The estimated length of *S. coeruleoalba* at birth is 1m and weaning starts at the age of 3 months (or 1.35 m) (Miyazaki, 1977).

Large-scale studies of *S. coeruleoalba* based on 3000-5000 individuals have been carried out by Japanese scientists (Kasuya, 1972, 1976; Miyazaki, 1977, 1984) as this species is annually caught by the fisheries or hand harpoons in the Pacific coast of Japan (Kasuya, Miyazaki & Dawbin, 1974). Other species of the genus *Stenella* are also caught by this method and are therefore widely studied and their reproductive biology well established (Benirschke, Johnson & Benirschke, 1980; Hester, 1984; Hohn, Chivers & Barlow, 1985; Perrin, Coe & Zweifel, 1976).

Kasuya (1972) reported two mating seasons for *S. coeruleoalba*, one in November and December (winter) and another in May and June (summer), based on age determination by means of dentinal growth layers. Kasuya (1976) reconsidered the life history parameters based on cemental layers but detailed examination of the gonads were not done. Miyazaki (1977) found three mating seasons in a year, from February to May, from July to September, and in December for *S. coeruleoalba* off the Pacific coast of Japan. This study involved both detailed observations of testes and ovaries as well as an analysis of dentinal layers. There is limited data available on the reproductive biology of *S. coeruleoalba* in South African waters (Ross, 1984) and the reproductive seasonality in these dolphins has not yet been established.

The taxonomic status of species of *Sousa* remains unclear. The population living off the West African coast (currently named *Sousa teuszii*) differ slightly from those found in South Africa and the Indian Ocean (currently known as *Sousa chinensis*) (May, 1990). The humpbacked dolphin is most easily recognised by a small, slightly rounded fin on top of a large hump located in the dorsal region (May, 1990) (Figure 4.2c). The Indo-Pacific humpbacked dolphin is a coastal species which is rarely found more than a kilometre offshore around South Africa (Payne & Crawford, 1992) (Figure 4.1) where it forms schools of up to 25 individuals (Branch, *et al.*, 1994). In eastern Cape waters, the observed group size of the humpback dolphin lies between 3-24 animals and the average group size is 7 animals (Karczmarski, pers comm.).

The humpbacked dolphin attains a length of 3.2m in males and 2.44m in females (Skinner & Smithers, 1990). Along the SE coast of South Africa, specimens of up to 2.79m (males) and 2.44m (females) have been recorded (Ross, 1984). Humpbacked dolphins exhibit greatest sexual dimorphism where males are 60% heavier than females and have small testes that average only 0.7% of their body weight (Cockcroft, 1993). The reproductive cycle of the humpbacked dolphin has not yet been established. Ross (1984) assumed that the length at birth of the humpbacked dolphin is 1m. This measurement was obtained from Saayman & Taylor (1979) for only two stranded neonates. The total population of humpback dolphins in Southern Africa is small (Branch, *et al.*, 1994) and is considered endangered. The total population of humpbacked dolphins in the eastern Cape waters is approximately 150-200 animals (Karczmarski, pers comm.). For this reason, the humpbacked dolphin is one of the lesser understood dolphin species. Ross (1984) provides some data based on the testes of only four male specimens and a single foetus. At Plettenberg Bay, Saayman & Taylor (1979) observed small calves throughout the year, with peaks in summer.

Although some information on the reproductive biology of *T. aduncus*, *S. coeruleoalba* and *S. chinensis* exists (Ross, 1984; Saayman & Taylor, 1979; Skinner & Smithers, 1990), detailed studies of gametogenesis have not been done. Such studies are necessary to examine seasonality of reproduction in these dolphins. Knowledge of the reproductive seasonality of dolphins has important implications for management of these cetaceans, as well as predicting the effects of dolphin catches in shark nets.

This chapter examines the reproductive seasonality of 2 species of oceanic dolphin, *Tursiops aduncus* (Indian Ocean bottlenosed dolphin) and *Stenella coeruleoalba* (striped dolphin) and 1 species of coastal dolphin, *Sousa chinensis* (Indo-Pacific humpbacked dolphin).

The aim of this study therefore is to provide more detail on the reproductive biology of these dolphins by describing spermatogenesis and oogenesis. This information will then be related to the general biology of the species.

4.2. MATERIALS AND METHODS

The gonads of adult dolphins collected by the Port Elizabeth Museum and Natal Sharks Board, between 1970 and 1993, from strandings and shark nets, were fixed in 4% formalin and stored in 70% ethanol. Representative tissue samples from the testes and ovaries were dehydrated in a graded ethanol series and embedded in Paraplast via xylene. 5µm thick sections were stained with Mallory's trichrome.

The monthly sample sizes of both males and females of *T. aduncus*, *S. coeruleoalba* and *S. chinensis* collected over a period of 24 years is given in Tables 4.1. & 4.2. The large sample sizes obtained for the months of May to September for *T. aduncus* can be attributed to the larger number of dolphins caught in shark nets during the annual Natal sardine run.

Table 4.1. Sample sizes of males of *T. aduncus*, *S. chinensis* and *S. coeruleoalba* for each month of the year.

Species	J	F	M	A	M	J	J	A	S	O	N	D
T. aduncus	2	4	1	3	7	3	7	7	1	2		
S. chinensis	3	2	1	1	2		1	1		1	2	3
S. coeruleoalba	1	1					1		1		2	1

Table 4.2. Sample sizes of females of *T. aduncus*, *S. chinensis* and *S. coeruleoalba* for each month of the year.

Species	J	F	M	A	M	J	J	A	S	O	N	D
T. aduncus	2	1			5	6	1	6	7	2	1	1
S. chinensis	1	1	1	1			4	2	1	4		1
S. coeruleoalba	1	2	2		2	1	2	1		2	1	1

Due to the unavailability of foetal specimens for either species, foetal growth curves for *S. chinensis* and *S. coeruleoalba* were not obtained. However, data for the monthly capture of neonates of *T. aduncus* have been provided by the Port Elizabeth Museum, South Africa.

4.2.1. MALE REPRODUCTIVE ACTIVITY

The state of spermatogenic activity in adult males was assessed both qualitatively and quantitatively. Cross sections of one hundred seminiferous tubule were examined for each dolphin and the animals categorized into one of four developmental stages (Bernard, *et al.*, 1994; Vergouwen, *et al.*,1993): juvenile (seminiferous epithelium comprising one cell layer of Sertoli cells, and the presence of gonocytes); inactive (seminiferous epithelium comprising one cell layer of spermatogonia and Sertoli cells but no gonocytes); early spermatogenesis (seminiferous epithelium comprising 2-4 cell layers, but no spermatids); and late spermatogenesis (seminiferous epithelium comprising 3 or more cell layers and the presence of spermatids).

The mean diameter of 20 seminiferous tubules, measured using an optical micrometer, was used as an measure of tubule diameter (Miyazaki, 1984). A one-way-analysis of variance (ANOVA) was used to determine whether the mean seminiferous tubule diameter differed significantly for each month.

Reproductively inactive male dolphins are referred to as juveniles.

4.2.2. FEMALE REPRODUCTIVE ACTIVITY

Sections of ovaries were examined for the presence and number of both small-medium (< 4mm in diameter) and large Graafian follicles (> 4mm in diameter) and luteal bodies (Corpora albicantia and corpora lutea). Unfortunately separation of corpora albicantia from corpora lutea was not possible due to the inadequate preservation of tissue. Females in which the ovaries lacked growing follicles and corpora were considered immature (Harrison & Brownell, 1971; Perrin & Donovan, 1984). The presence of one or more corpora in one or both ovaries was used as the criterion of female sexual maturity and probable ovulation (Brownell, 1984). Data on whether the females were lactating were provided by the Port Elizabeth Museum.

4.3. RESULTS

4.3.1. MALE REPRODUCTIVE ACTIVITY

Of the 37 males of *T. aduncus* collected, 7 were juveniles, 4 were spermatogenically inactive, 5 were in early spermatogenesis and 21 were in late spermatogenesis. In all sampled months, except for March, June, September and October, more than 50% of all adult males were producing spermatozoa (Figure 4.3). The seminiferous tubule diameters of juveniles were small and showed little variation (0.038mm - 0.068mm) during the year (Figure 4.4). The mean seminiferous tubule diameters of those in late spermatogenesis were much larger and ranged from 0.148mm - 0.20mm (Figure 4.4) and did not differ significantly ($P>0.05$).

Of the 7 males of *S. coeruleoalba* analyzed, 1 was a juvenile, 1 was spermatogenically inactive, 3 were in early spermatogenesis and 2 were in late spermatogenesis. A monthly plot of the mean seminiferous tubule diameter is shown in figure 4.5. No statistical test was performed due to insufficient data for each month.

Of the 17 males of *S. chinensis* collected, 2 were juveniles, 1 was inactive, 8 were in early spermatogenesis and 6 were in late spermatogenesis. Males in late spermatogenesis occurred in most months of the year (Figure 4.6). A monthly plot of the seminiferous tubule diameter is shown in figure 4.6. No statistical test was performed due to insufficient data for each month.

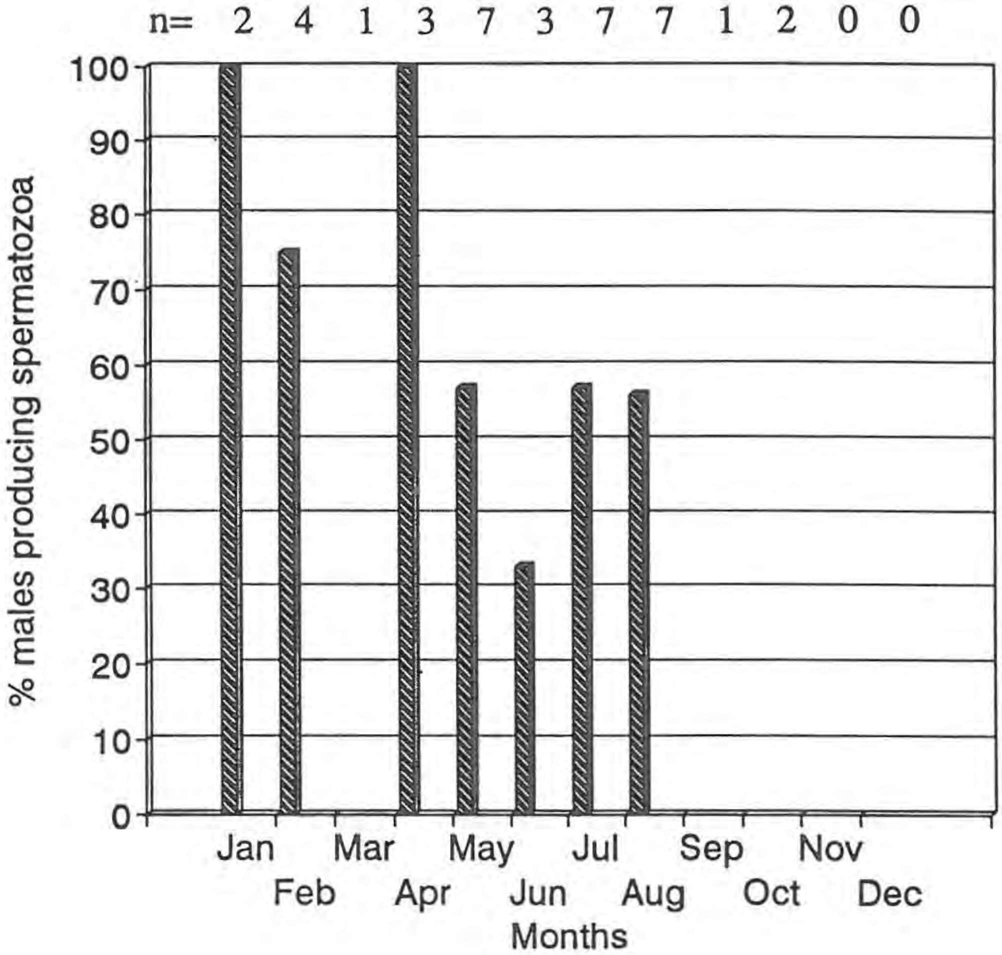

Figure 4.3. The percentage of adult *T. aduncus* males producing spermatozoa.

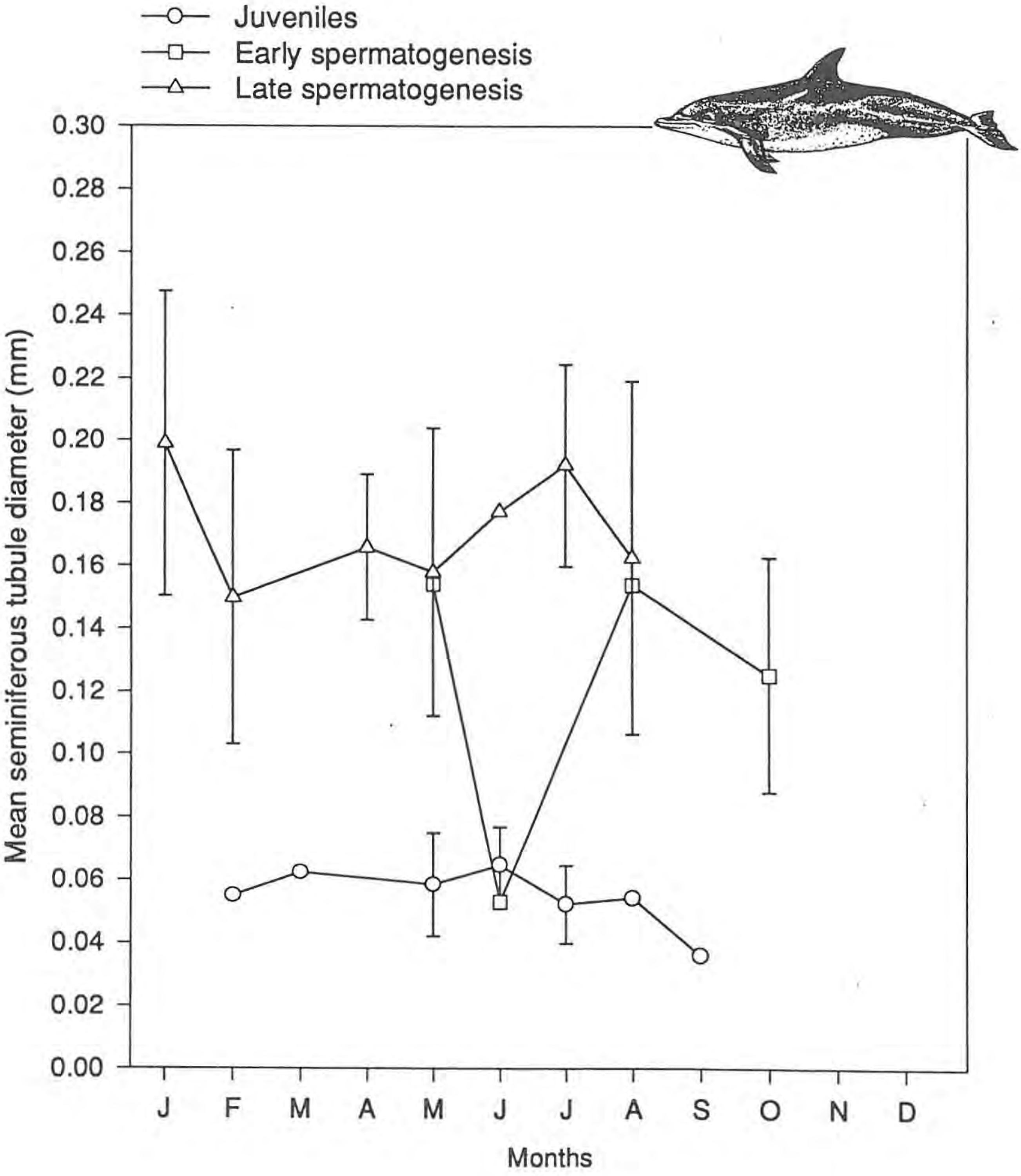

Figure 4.4. A monthly plot of seminiferous tubule diameter ($\bar{x} \pm$ SD) for the 3 different stages of male *T. aduncus* sexual maturity.

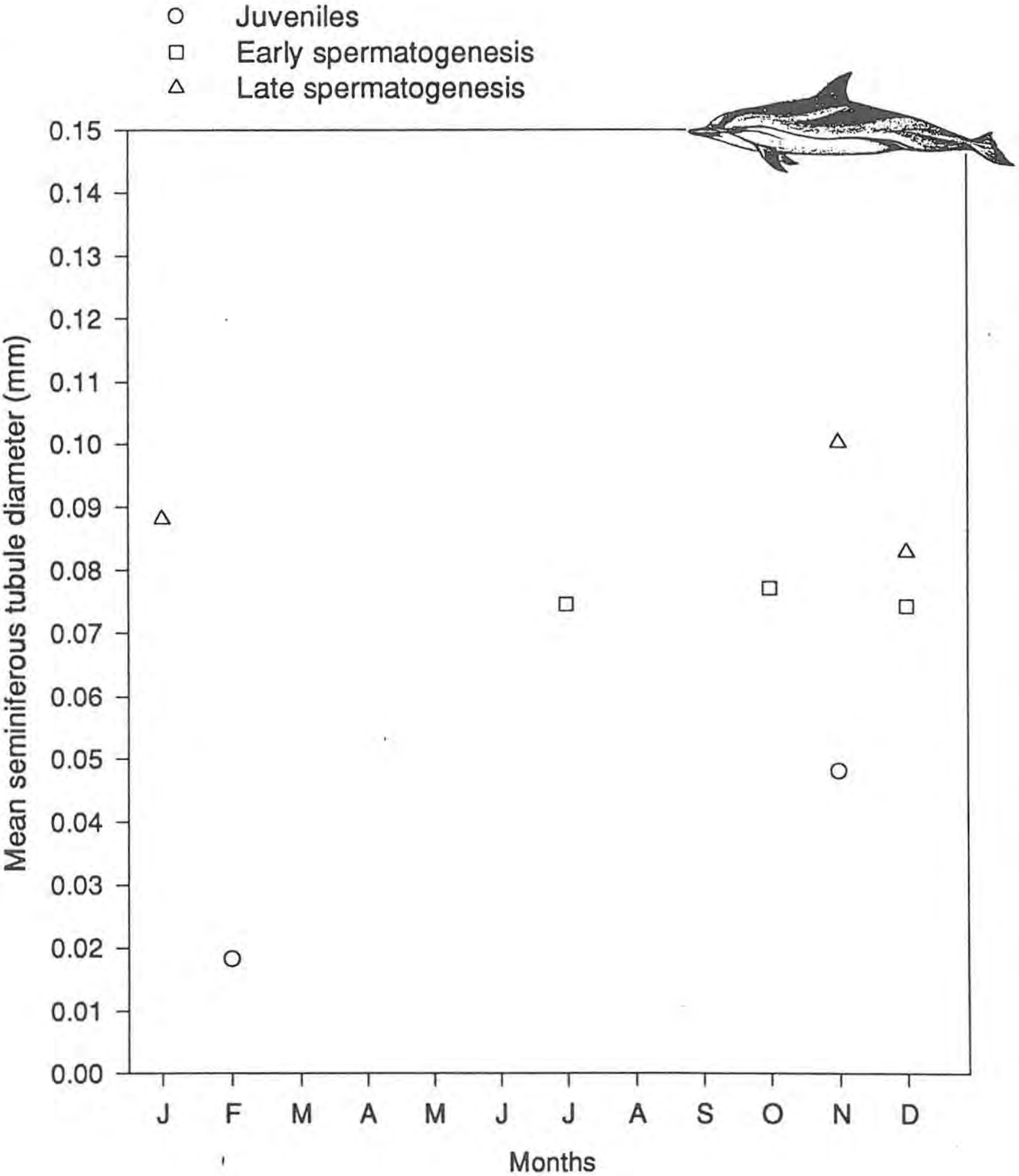

Figure 4.5. A monthly plot of seminiferous tubule diameter for the 3 different stages of male *S. coeruleoalba* sexual maturity.

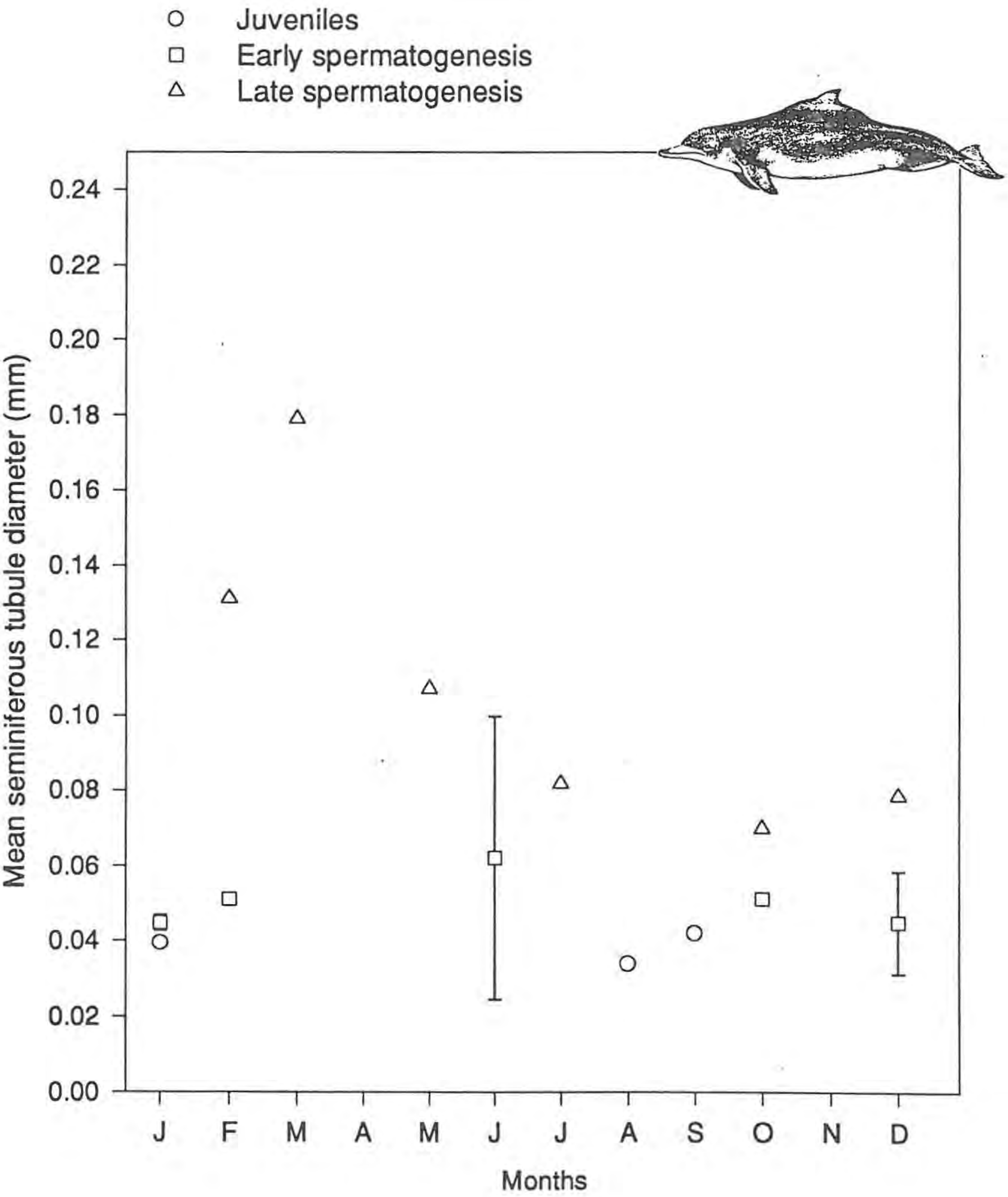

Figure 4.6. A monthly plot of seminiferous tubule diameter for the 3 different stages of male *S. chinensis* sexual maturity.

4.3.2. FEMALE REPRODUCTIVE ACTIVITY

Of the 32 females of *T. aduncus* caught, 27 were reproductively active, and only two of these 27 were visibly pregnant; one in June and one in September. Lactating females occurred throughout the year. In all months for which data are available, more than 30% of the animals had at least one luteal body in one of their ovaries, except for July, November and December and 50% and more had large (> 4mm in diameter) Graafian follicles, except in July and October (Figure 4.7). When all the animals were analyzed together (n=32), 47% possessed luteal bodies and 75% possessed large Graafian follicles. Of the 32 females, only 5 females did not possess either large Graafian follicles or luteal bodies and were thus considered reproductively inactive. All 5 females were greater than 2m in length, too large to be considered juveniles.

Of the 15 females of *S. coeruleoalba* caught, all were reproductively active. There are very little reproductive data for *S. coeruleoalba* and the occurrence of pregnancy in the females was not recorded. In all months for which data are available, 50% or more of the animals had at least one luteal body in one of the ovaries, except in May, August and December, and 100% of the animals had large (> 4mm in diameter) Graafian follicles (Figure 4.8). When all the animals were analyzed together (n=15), 53% possessed luteal bodies and 100% possessed large Graafian follicles.

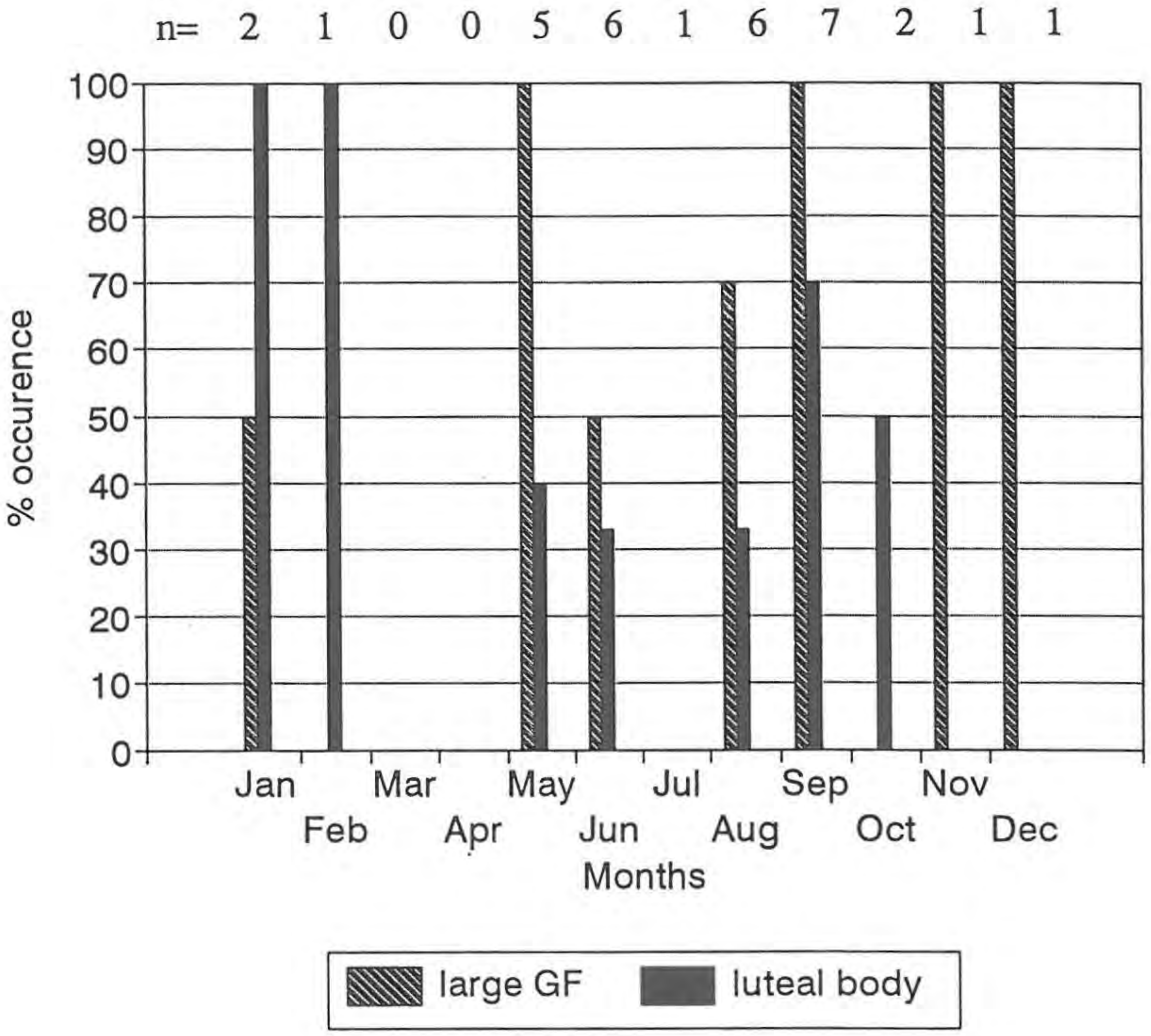

Figure 4.7. The percentage occurence of large Graafian follicles (GF) and luteal bodies in *T. aduncus* throughout the year.

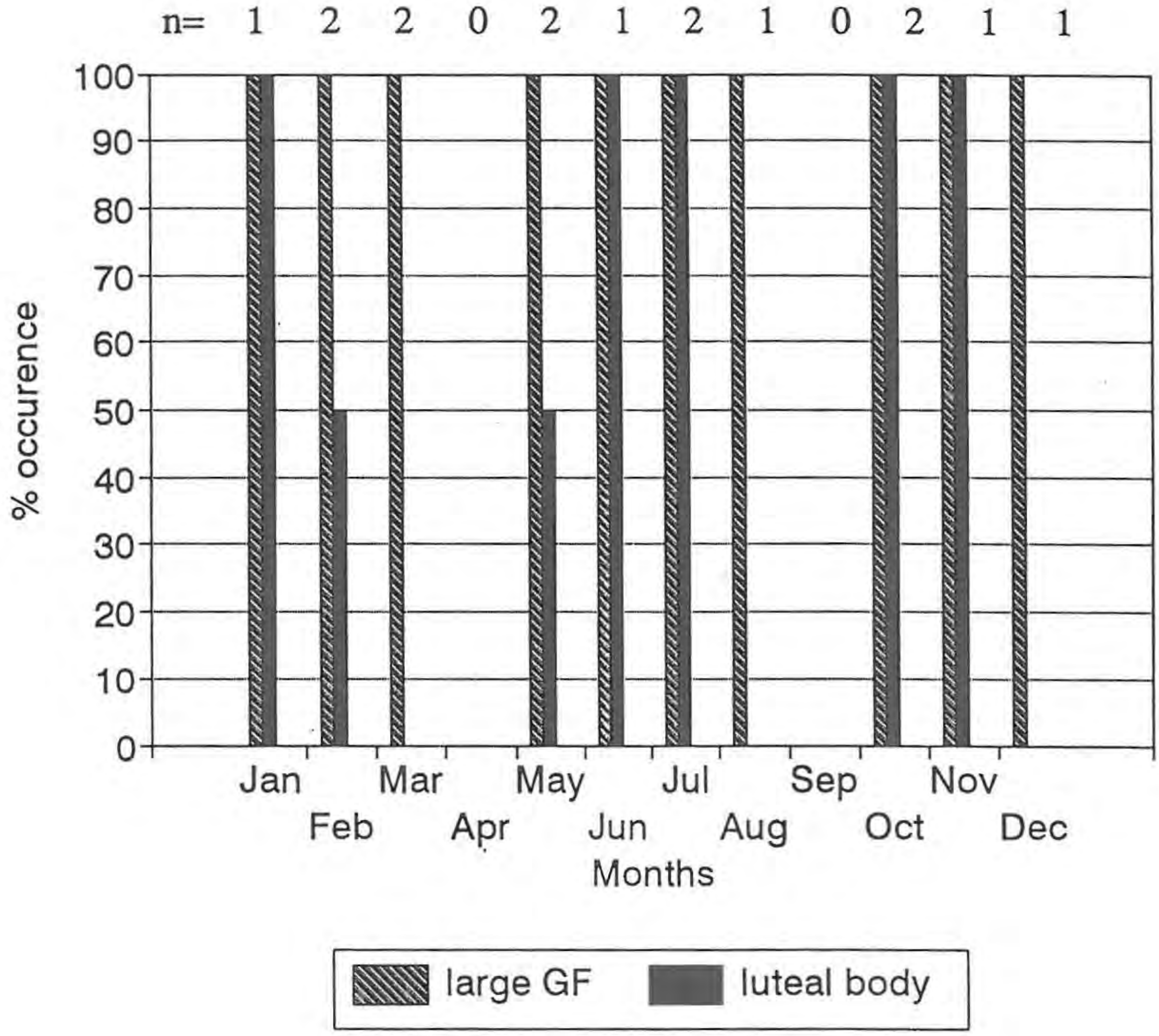

Figure 4.8. The percentage occurence of large Graafian follicles (GF) and luteal bodies in *S. coeruleoalba* throughout the year.

Of the 16 females of *S. chinensis* caught, 15 were reproductively active, and only one was visibly pregnant and 4 were lactating. In all months for which data are available, more than 70% of the animals had large (> 4mm in diameter) Graafian follicles and more than 20% of the animals contained at least one luteal body in their ovaries in January, July and October (Figure 4.9). When all the animals were analyzed together (n=16), 94% possessed large Graafian follicles and 31% possessed luteal bodies. Of the 16, only one did not possess either large Graafian follicles or luteal bodies and is thus considered reproductively inactive. This female was greater than 2m in length, too large to be considered a juvenile.

The quality of fixation of ovarian tissue precluded any detailed study of follicular development or the structure of the corpus luteum. It was not possible to distinguish the corpus luteum from the corpus albicans. A typical ovary of all three species studied is similar to that of *D. delphis* in that it contains one corpus (characterized by a solid spheroidal mass of large glandular cells and connective tissue septa) (refer to figure 3.6) and two to five large Graafian follicles.

4.3.3. NEONATE CAPTURE

The monthly capture of *T. aduncus* neonates is shown in table 4.3. Neonates are newly born calves that are attached to their mothers by an umbilical cord (Cockcroft, pers comm.) Neonates were found in most months of the year suggesting aseasonal reproduction. No similar data are available for the other species.

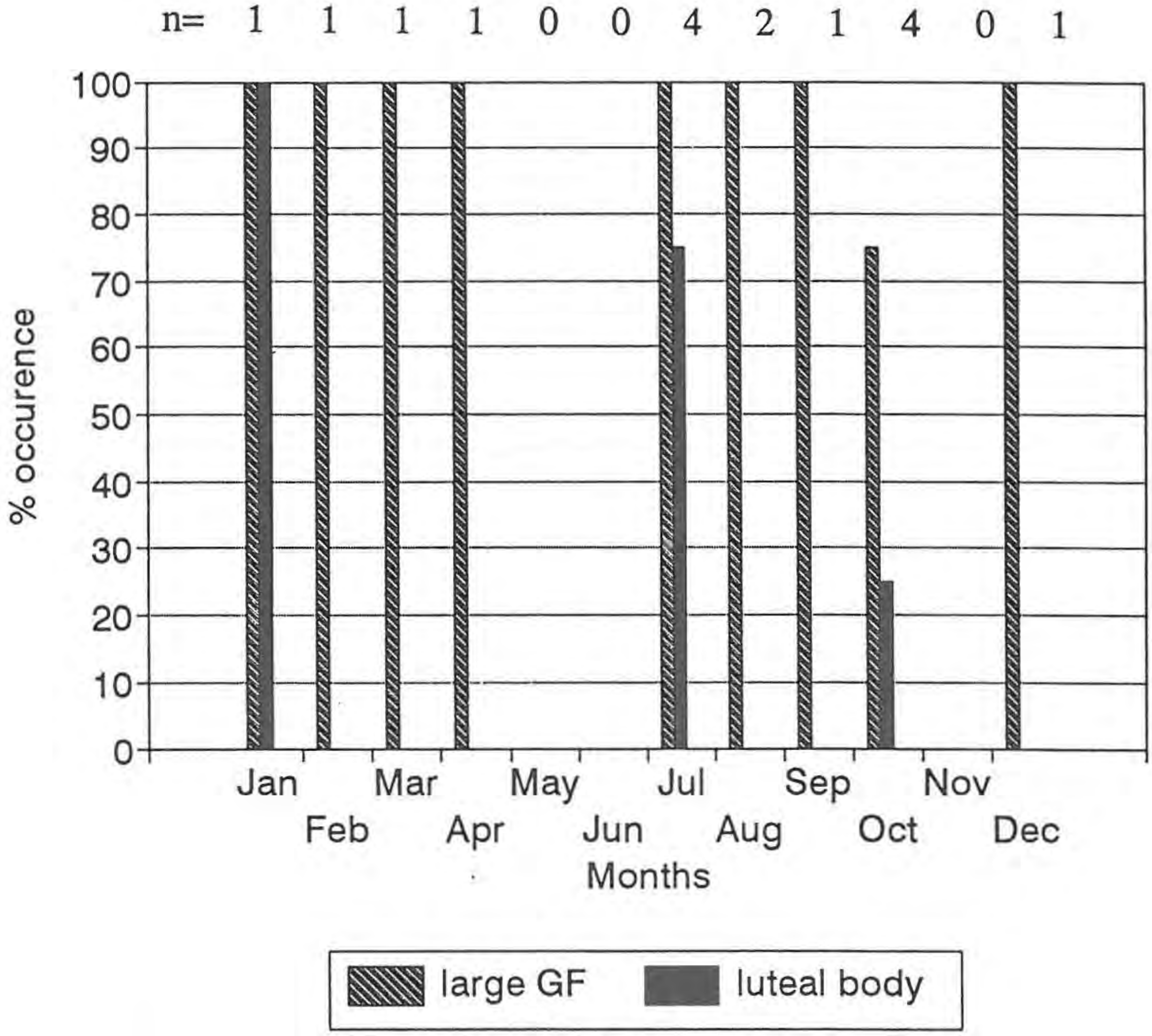

Figure 4.9. The percentage occurence of large Graafian follicles (GF) and luteal bodies in *S. chinensis* throughout the year.

Table 4.3. Number of neonates of *T. aduncus* caught per month.

J	F	M	A	M	J	J	A	S	O	N	D
	2	3	2			3	2		5	3	3

4.4. DISCUSSION

Seasonality of reproduction in dolphins in the past has been based on many different criteria: 1) seasonal variation of the size of the testes (Perrin, *et al.*, 1976; Hohn, et al., 1985; Collet & Girons, 1984); 2) seasonal changes in the seminiferous tubule diameter (Miyazaki, 1977); 3) seasonal change of reproductive activity in the testis (Collet, 1983; Miyazaki, 1977); 4) seasonal peaks in conception and births (Miyazaki, 1977; Saayman & Taylor, 1979); and 5) seasonal changes in hormone levels (Kirby & Ridgway, 1984).

Males in late spermatogenesis occurred throughout the year for *T. aduncus* and *S. chinensis* suggesting that spermatogenesis is aseasonal. Due to insufficient data, no conclusions can be made about spermatogenesis in *S. coeruleoalba.* Females of all 3 species contained large Graafian follicles in their ovaries in most months of the year and this may indicate that ovulation occurs throughout the year. However, prior to ovulation, many follicles develop at the same time, with only one rupturing at ovulation (Harrison & Bryden, 1988); the others may be remnants that have not yet degenerated. This may explain the presence of large Graafian follicles for most months. Importantly, therefore, the presence of Graafian follicles can not be taken as an indication of the timing of ovulation. I have shown that in *Delphinus delphis* (common dolphins) (Chapter 3) where reproduction is seasonal, that Graafian follicles and luteal bodies are present in all the months (May to October) for which data is available.

Corpora lutea involute to form corpora albicantia and in cetaceans they are believed to persist until death (Bryden, Harrison, & Gemmel, 1984; Harrison & Bryden, 1988; Perrin & Donovan, 1984). This may explain the presence of luteal bodies in the females of *T. aduncus* and *S. coeruleoalba* for most months. Also, active corpora will be present throughout the year as pregnancy lasts for about a year. For these reasons, it is difficult to establish seasonal cycles in females without information on the timing of births.

Aseasonal reproduction in only one of the three species of dolphins studied has been confirmed by the capture of neonates. Neonates of *T. aduncus* have been caught throughout the year which confirms Cockcroft & Ross's (1990) observations that births occur during most months of the year. Cockcroft & Ross (1990) found that births in the Atlantic bottlenosed dolphin (*T. truncatus*) occurred throughout the year with a peak in summer. However, my results show no peak in the capture of neonates of *T. aduncus*. *T. aduncus* occurs in the eastern Cape and Natal waters throughout the year (Ross, 1984). There is no evidence to suggest that this species migrates from the eastern Cape to Natal waters in winter like common dolphins.

Miyazaki (1977, 1984) found that *S. coeruleoalba* in Japanese waters reproduces seasonally through detailed studies of both the testes and ovaries and a study of foetal growth. Unfortunately, due to the lack of foetal material for *S. coeruleoalba*, no evidence can be provided for seasonal reproduction of this species in South African waters. Similarly, it remains unclear whether or not *S. chinensis* breeds seasonally. The data obtained in this study shows no seasonality of gametogenesis. However, this does not suggest aseasonal reproduction in *S. coeruleoalba* and *S. chinensis* as other factors such as migration and feeding (availability of food) have been known to influence the timing of births (Bronson, 1989). Ross (1984) provides stranding records of *S. coeruleoalba* which might suggest the seasonal occurrence of this species in the eastern Cape water during the warmer months. Observations of *S. chinensis* at Algoa Bay and Plettenberg Bay suggest that this species occurs in these waters throughout the year (Saayman & Taylor, 1979). Therefore, it can be said that migration might have an influence on the timing of births in *S. coeruleoalba.*

In conclusion, spermatogenesis in both *T. aduncus* and *S. chinensis* is aseasonal. The capture of neonates of *T. aduncus* throughout the year provides further evidence for aseasonal reproduction in this species. Due to the lack of both reproductive and foetal material for *S. chinensis* and *S. coeruleoalba*, it remains unclear whether or not these species breed seasonally.

CHAPTER 5

GENERAL DISCUSSION

Most information about the reproduction of cetaceans is derived from gross or histological examination of gonads from dead animals incidentally recovered from fisheries or strandings. An assessment of the reproductive condition of the gonads of sexually mature dolphins may be used to determine seasonal reproduction (Collet & Girons, 1984; Miyazaki, 1977, 1984). Perrin & Donovan (1984) provide a comprehensive report on the various criteria adopted by different authors to define sexual maturity in cetaceans. The most accepted definition of female sexual maturity in dolphins and whales is that the animal should have ovulated at least once, as indicated by the presence of at least one corpus luteum or corpus albicans in the ovaries (Perrin & Reilly, 1984). It is assumed that the scars of ovulation (whether or not followed by pregnancy) persist indefinitely in cetaceans (Harrison & Bryden, 1988; Marsh & Kasuya, 1984; Perrin & Donovan, 1984). A further assumption is that the presence of any scar on the ovary indicates that ovulation has occurred (Perrin, Coe & Zweifel, 1976). However, it has been suggested that some scars are a result of non-ovulatory events such as the lutealization of Graafian follicles that have not erupted (Perrin & Donovan, 1984). Also pubescent females may ovulate more than once before conceiving (Perrin & Donovan, 1984). It has therefore been concluded that the following three criteria be used to ascertain female sexual maturity (Perrin & Donovan, 1984): first ovulation, first conception and first lactation.

An assessment of male sexual maturity has been based on several criteria. Increase in the weights of testes (Fisher & Harrison, 1970; Kasuya, Miyazaki & Dawbin, 1974; Slooten, 1991), the presence of spermatozoa in the centre of the seminiferous tubule (Brownell, 1984;

Collet & Girons, 1984; Kasuya, *et al.*, 1974), the presence of spermatozoa in the epididymis (Fisher & Harrison, 1970; Perrin, *et al.*, 1976) and rapid change in the diameter of seminiferous tubules (Fisher & Harrison, 1970; Miyazaki, 1977, 1984). An assumption made when using the presence of spermatozoa in the epididymis as a criterion for sexual maturity is that adult males continuously produce spermatozoa (Perrin & Reilly, 1984). However, Collet & Girons (1984) found that in common dolphins (*Delphinus delphis*) sexually mature males may enter a resting phase during which the testis size decreases and the epididymis contains no spermatozoa.

Seasonal reproduction has been reported for several species of Cetacea. Various approaches have been adopted to examine seasonal reproduction in these animals. Collet & Girons (1984) used variation in testis size among mature common dolphins of similar size as an indication of the existence of a cycle of testicular activity. Miyazaki (1977) also reported a monthly fluctuation in the weights of the testes among mature striped dolphins (*Stenella coeruleoalba*) which is attributed to seasonal changes in testis activity. Seasonality of reproduction in the male spotted dolphin (*Stenella attenuata*) was reported based on changes in testis and epididymis weight, and changes in the relative quantity of spermatids and spermatozoa in the seminiferous tubules (Hohn, Chivers & Barlow, 1985). Indications of seasonal testicular activity for dolphins are also available from studies of reproductive physiology. Wells (1984) found an apparent cycle of testosterone production in male Hawaiian spinner dolphins (*Stenella longirostris*) which appeared to be closely linked to the frequencies of occurrence of two behavioural patterns. Genital-to-genital contact and mutual ventral presentations occurred most frequently in *S. longirostris* when the male's testosterone concentrations were high, but no histology of the gonads has been done to determine seasonal reproduction in these dolphins.

An histological analysis of the testes of *D. delphis*, *Tursiops aduncus* (Indian Ocean bottlenosed dolphin) and *Sousa chinensis* (Indo-Pacific humpbacked dolphin) shows that spermatogenesis is aseasonal (Chapter 3 & 4). However, no conclusions about seasonality of reproduction can be drawn from either the gross or histological analysis of the ovaries of *D. delphis*, *T. aduncus*, *S. chinensis* and *S. coeruleoalba* (Chapter 3 & 4). Since reproductive events are hormonally mediated, monitoring hormonal levels in cetaceans has proven to be an effective way of describing ovulatory cycles (Kirby & Ridgway, 1984). Kirby & Ridgway (1984) found that female Atlantic bottlenosed dolphins (*Tursiops truncatus*) had spring oestrous cycles by monitoring progesterone and estrogen levels.

Peaks in calving in dolphins observed through behavioural studies is another method widely used to ascertain reproductive seasonality in these animals (Saayman & Taylor, 1979). By estimating the conception date using foetal lengths, Miyazaki (1984) found that the striped dolphin has two mating seasons.

In general, most cetaceans breed seasonally, sometimes in association with long migrations from one feeding area to another (Bronson, 1989). The common porpoise (*Phocoena phocoena*) of the North Atlantic moves into the waters just outside the entrances to Passamaquoddy Bay from June to August to mate, returning after a gestation period of 9 months to give birth (Fisher & Harrison, 1970). Similarly, whales (for example the southern right whale, *Balaena glacialis* and the humpback whale, *Megaptera novaengliae*) that feed in the Antarctic during the short summer months migrate to the warmer waters of the South Atlantic or the Indian Ocean to mate and give birth after an approximately one year gestation period (Bronson, 1989). Seasonal migration of common dolphins from the coastal waters of the eastern Cape during summer to the warmer waters of Natal in winter may be a response to a similar movement by large shoals of fish (notably pilchard) (Ross, 1984). It appears that the annual migration of common dolphins may also be intimately linked to the timing of reproduction (as discussed in Chapter 3).

However, there appears to be a stronger correlation between habitat and reproduction, than migration and reproduction in cetaceans (Table 5.1). Both aseasonal and seasonal reproduction occurs within the coastal species (Table 5.1) and there appears to be no correlation between reproduction and migration. Seasonal reproduction predominates among the oceanic species (all ocean and northern ocean species) with the exception of *T. aduncus* which breeds aseasonally (Table 5.1). However, in southern African waters, *T. aduncus* inhabits the inshore (coastal) waters of the south east coast region (Ross, 1984) and is thus not a typical oceanic form. It appears that all temperate and tropical species migrate and breed seasonally (Table 5.1). Almost all estuarine and riverine species of dolphins are seasonal breeders. Migration occurs only in the Franciscana dolphin (*Pontoporia blainvillei*) which is actually a coastal dolphin, restricted to the coastal waters of Brazil, Uruguay, Argentina and the La Plata estuary, while the other three are riverine species (May, 1990). It can be concluded that generally all dolphins breed seasonally with the exception of coastal species that may breed either seasonally or aseasonally.

Table 5.1. A summary of reproductive seasonality of cetaceans in relation to migration. The different species are presented in groups according to their distribution as described by May (1990). The data presented in this table have been summarized from May (1990); Skinner & Smithers (1990); Sylvestre (1993) and this study.

Species Name	Common Name	Migrate	Seasonal reproduction
ALL OCEAN SPECIES			
Orcinus orca	Killer whale	Yes	Yes
Globicephala melas	Long-Finned Pilot whale	Yes	Yes
Globicephala macrorhynchus	Short-Finned Pilot whale	Yes	Yes
Delphinus delphis	Common dolphin	Yes	Yes
Tursiops aduncus	Indian Ocean bottlenosed dolphin	No	No
Tursiops truncatus	Atlantic bottlenosed dolphin	No	Yes
ARCTIC SPECIES			
Delphinapterus leucas	Beluga whale	Yes	Yes
Monodon monoceros	Narwhal whale	Yes	Yes
NORTHERN OCEAN SPECIES			
Lagenorhynchus acutus	Atlantic white-sided dolphin	No	Yes
Lagenorhynchus obliquidens	Pacific white-sided dolphin	Yes	Yes
Lagenorhynchus albirostris	White-beaked dolphin	Yes	Yes
TEMPERATE + TROPICAL SPECIES			
Grampus griseus	Risso's dolphin	Yes	Yes
Peponcephala electra	Melon-headed whale	Yes	Yes

Species Name	Common Name	Migrate	Seasonal reproduction
Pseudorca crassidens	False Killer whale	Yes	Yes
Feresa attenuata	Pygmy Killer whale	Yes	Yes
Laegnorhynchus obscurus	Dusky dolphin	Yes	Yes
Stenella coeruleoalba	Striped dolphin	Yes	Yes

COASTAL SPECIES

Cephalorhynchus hectori	Hector's dolphin	Yes	Yes
Orcaeella brevirostris	Irrawaddy dolphin	No	Yes
Sotalia fluviatilis	Tucuxi dolphin	No	Yes
Phocoenoides dalli	Dall's porpoise	Yes	Yes (Japan) No (USA)
Phocoena phocoena	Harbor porpoise	Yes	Yes
Cephaloorhynchus heavisidii	Heaviside's dolphin	No	No
Neophocaena phocaeniides	Finless porpoise	No	Yes
Sousa chinensis	Indo-Pacific humpbacked dolphin	No	Yes
Sousa teuszii	Atlantic humpbacked dolphin	No	No
Cephalorhynchus commersonni	Commerson's dolphin	Yes	Yes

ESTUARINE + RIVER SPECIES

Pontoporia blainvillei	Franciscana dolphin	Yes	Yes
Platinista gangatica	Ganges river dolphin	No	Yes
Platinista minor	Indus River dolphin	No	Yes
Lipotes vexillifer	Yangtze river dolphin	No	Yes

The results obtained in this study have important implications for the management of both the common and bottlenose dolphins, in regard to use of gill nets in Natal waters. One of the most controversial issues related to the Natal Sharks Board shark nets is the annual mortality of some 80 dolphins (Dudley & Cliff, 1992). As discussed in Chapter 1, captures of bottlenose and humpback dolphins in these nets threaten the survival of both species in Natal waters within the next few decades. It is believed that the shark nets pose no threat to the wellbeing of the common dolphin as it occurs in large numbers (in excess of 15000 individuals) and can sustain the losses to the nets (Dudley & Cliff, 1992). Whatever the environmental consequence of shark-netting, it is obviously desirable to minimise or even eliminate dolphin mortalities in these nets.

The common dolphin is known to frequent the Natal waters during the annual sardine run, when catches of dolphins and sharks in these gill nets are high (Dudley & Cliff, 1992). From the present study, it has been concluded that common dolphins breed seasonally (Chapter 3), and if more than 50% of the births occur during December (as calculated) in the eastern Cape waters, the majority of the calves will be approximately six months old during the annual sardine run (around June). More than 50% of conceptions in common dolphins occur in January (Chapter 3) and therefore most of the females will be about five months pregnant. Therefore, the calves and pregnant females of common dolphins caught in the Natal shark nets are likely to be six months old and five months pregnant, respectively. Thus, the Natal shark nets may pose a threat to the structure of the population of common dolphins, in spite of its large population size.

The Natal population of bottlenosed dolphins (*Tursiops aduncus*) breed throughout the year (Chapter 4), and therefore the shark nets may have a lesser effect on their population structure. However, it is already feared that continued mortality of both the humpback and bottlenose dolphins in the Natal inshore waters throughout the year may deplete populations (Cockcroft, Cliff & Ross, 1989).

As long as the shark nets are in use, management of dolphins in South Africa, especially in Natal, is limited. In order to ensure the survival of certain dolphin species, alternative methods or improvements to shark nets must be employed. Since the early 1980s the nets have been removed whenever possible during the annual sardine run, but there has been a conflict of interest as the July school holidays coincide with the sardine run (Dudley & Cliff, 1992). The Natal Sharks Board has been conducting an experiment whereby they increased the mesh size of the nets, in hope that the juvenile dolphins will escape, but still capture the large, potentially dangerous sharks.

In conclusion, an understanding of seasonal reproduction in marine mammals requires a detailed study of both the male and female reproductive cycles that includes monitoring of hormone levels. Foetal growth and field observations of calving and migratory patterns should also be incorporated in the study. Our knowledge of cetology is still deficient, and it is therefore vital that research in this field continues. Our knowledge of the reproductive biology of dolphins has important implications for the management of these mammals, as well as for predicting the effects of dolphin catches in shark nets.

APPENDICES

Appendix 1: Data for *D. delphis* males.

Qual. Anal. = Qualitative analysis of 100 seminiferous tubules expressed as a percentage under the appropriate category: J (Juveniles), I (Inactive), ES (Early spermatogenesis) or LS (Late spermatogenesis).

Quan. Anal. = Quantitative analysis = The mean diameter of 20 seminiferous tubules.

Museum No.	Month died	Length (m)	Weight (kg)	Qual. Anal. (%)				Quan. Anal.
				J	I	ES	LS	(mm)
1015	01	2.24	106	100				0.051
267	02	2.50	163.3				100	0.224
1150	04	2.53	146				100	0.228
545	05	2.15	106.1	100				0.031
1170	05	2.45	132				100	0.205
410	06	2.29	118.6			3	97	0.186
737	06	2.35	152				100	0.235
535	07	2.30	114			9	91	0.104
539	07	2.27	116			85	15	0.078
1210	07	2.35	150			6	94	0.187
1212	07	2.38	110	45	55			0.045
731	07	2.29	131			92	8	0.093
532	07	2.43	145			9	91	0.121
504	07	2.36	153			18	82	0.176
1198	07	2.30	124			3	97	0.152

Museum No.	Month died	Length (m)	Weight (kg)	Qual. Anal. (%)				Quan. Anal.
				J	I	ES	LS	(mm)
499	07	2.55	162			15	85	0.187
417	07	2.49	151.1			2	98	0.170
418	07	2.40	161.9				100	0.262
1194	07	2.15	94				100	0.217
484	08	2.36	104	100				0.049
202	08	2.24	109	40	60			0.057
720	08	2.41	152			2	98	0.180
718	08	2.38	168			3	97	0.170
712	08	2.38	138			5	95	0.161
1420	08	2.14	90	60	40			0.038
472	09	2.38	133			2	98	0.150
469	09	2.41	137				100	0.192
470	09	2.26	103			4	96	0.180
463	09	2.45	163			6	94	0.147
491	09	2.30	110	100				0.049
1170	10	2.45	132			100		0.120
420	11	2.28	112				100	0.144

Appendix 2: Data for *D. delphis* females.

Museum No.	Month died	The presence of		
		Graafian follicles		luteal bodies
		small	large	
1366	May	x		
1088	May	x	x	
1411	June		x	
1186	July		x	
743	July	x	x	x
509	July		x	x
734	July	x		
507	July	x	x	
1625	Aug.	x	x	x
490	Aug.	x		
789	Aug.	x	x	x
1611	Aug.	x		
468	Sept.	x	x	x
1223	Sept.		x	x
1293	Sept.	x		x
549	Oct.	x	x	x

x = present

Appendix 3: Data for foetuses of *D. delphis*.

Museum No.	Month died	Month conceived	Length (mm)	Weight (grams)	Age (days)
312	April	Jan.	26	1.6	80.8
1362	May	Feb.	89.5	20	100.3
1366	May	Jan.	110	31	105.7
1154	June	March	76	7.3	90.5
1361	July	March	149	70	118.15
745	July	Jan.	370	700	177.4
1294	July	April	300	421.9	160.8
1186	July	Jan.	450	1060	194.98
1194	July	Feb.	290	315.4	152.0
1193	July	April	65	5.2	87.8
1204	July	Feb.	330	301.3	150.8
505	July	Feb.	250	211	141.3
1524	July	March	195	74.9	119.4
1525	July	March	215	110	126.6
1550	July	Jan.	390	611	173.3
1622	July	Jan.	300	458.1	163.6
1735	July	Feb.	240	211.8	141.4
1526	July	March	220	109	126.5
1912	July	Jan.	340	660	176.25
1930	July	Feb.	330	422.5	160.9
1386	Aug.	Jan.	510	1700	217.0
1398	Aug.	Jan.	440	1200	200.5

Museum No.	Month died	Month conceived	Length (mm)	Weight (grams)	Age (days)
1434	Aug.	Jan.	430	1150	198.5
723	Aug.	Jan.	400	1200	200.55
724	Aug.	April	54	2.1	82.2
1290	Aug.	Jan.	450	1234	201.8
1096	Aug.	Jan.	430	988	192.07
1612	Aug.	April	220	132	130.4
1905	Aug.	March	287	149	133.0
(Twins)	Aug.	March	326	336	153.9
1393	Sept.	Jan.	570	2060	227.0
1223	Sept.	June	91	17.3	98.8
1616	Sept.	April	320	307.3	151.4
1812	Dec.	June	320	459.4	163.7
1813	Dec.	May	410	1300	204.1
1815	Dec.	Jan.	950	9000	329.3
1817	Dec.	Jan.	1180	11000	347.5
1818	Dec.	Jan.	900	8000	319.2
1820	Dec.	Jan.	950	9000	329.3
1823	Dec.	Jan.	950	8000	319.16

Appendix 4: Data for *T. aduncus* males.

Qual. Anal. = Qualitative analysis of 100 seminiferous tubules expressed as a percentage under the appropriate category: J (Juveniles), I (Inactive), ES (Early spermatogenesis) or LS (Late spermatogenesis).

Quan. Anal. = Quantitative analysis = The mean diameter of 20 seminiferous tubules.

Museum No.	Month died	Length (m)	Weight (kg)	Qual. Anal. (%)				Quan. Anal.
				J	I	ES	LS	(mm)
942	01	2.38	187				100	0.251
944	01	2.41	162			2	98	0.191
1152	02	2.43				2	98	0.156
847	02	1.89	69	100				0.055
1034	02	2.36	152				100	0.193
945	02	2.23	138			25	75	0.100
1591	03	2.05		100				0.062
358	04	2.34	178			4	96	0.188
1501	04	2.24	154				100	0.142
1506	04	2.32	162			40	60	0.167
356	05	2.48	183			100		0.154
355	05	2.32	165			2	98	0.179
1375	05	2.52	156			6	94	0.145
359	05	2.37	164				100	0.101
967	05	2.33			100			0.070
361	05	2.42					100	0.207
1063	05	2.11	110		100			0.047

Museum No.	Month died	Length (m)	Weight (kg)	Qual. Anal. (%)				Quan. Anal.
				J	I	ES	LS	(mm)
942	01	2.38	187				100	0.251
402	06	2.04	101			100		0.053
1183	06	2.57	178			2	98	0.177
1052	06	2.09	108	100				0.065
860	07	2.00	89	83	17			0.038
857	07	2.37	175			2	98	0.136
858	07	2.40	201			4	96	0.194
282	07	2.30		100				0.064
859	07	2.00	90		100			0.057
253	07	2.41	157				100	0.170
248	07	2.43	168			17	83	0.210
1100	08	2.00	81	27	73			0.041
970	08	2.47	175			85	15	0.154
965	08	2.12	114	100				0.066
998	08	2.23	142			7	93	0.119
1304	08	2.16	118			2	98	0.177
1	08	2.40	194				100	0.234
856	08	2.48	204				100	0.239
379	09	2.07		100				0.036
907	10	2.30	163			100		0.099
784	10	2.50			3	97		0.146

Appendix 5: Data for *T. aduncus* females.

Museum No.	Month died	The presence of		
		Graafian follicles		luteal bodies
		small	large	
941	Jan	x	x	x
845	Jan	x		x
846	Feb	x		x
1376	May	x	x	x
360	May	x	x	
213	May		x	
1151	May	x	x	
966	May		x	x
398	June		x	x
1758	June	x		
1282	June	x	x	
403	June	x		
401	June	x	x	x
399	June	x		
543	July	x		
1460	Aug		x	x
222	Aug	x	x	
2	Aug	x	x	
1303	Aug	x	x	
1107	Aug	x	x	x
969	Aug	x	x	

Museum No.	Month died	The presence of		
		Graafian follicles		luteal bodies
		small	large	
1309	Sept	x	x	
1308	Sept		x	x
1235	Sept		x	
1232	Sept	x	x	x
1111	Sept	x	x	x
815	Sept	x	x	x
481	Sept	x	x	x
913	Oct	x		
134	Oct	x		x
1255	Nov	x	x	
814	Dec	x	x	

x = present

Appendix 6: Data for *S. coeruleoalba* males.

Qual. Anal. = Qualitative analysis of 100 seminiferous tubules expressed as a percentage under the appropriate category: J (Juveniles), I (Inactive), ES (Early spermatogenesis) or LS (Late spermatogenesis).

Quan. Anal. = Quantitative analysis = The mean diameter of 20 seminiferous tubules.

Museum No.	Month died	Length (m)	Weight (kg)	Qual. Anal. (%)				Quan. Anal.
				J	I	ES	LS	(mm)
1119	01	2.44	157				100	0.088
1569	02	2.44		97	3			0.018
1053	07	2.33	114			91	9	0.075
781	10	2.07	91			98	2	0.077
1650	11	2.20	110				100	0.100
289	11	2.44			100			0.048
263	12	2.50				100		0.043

Appendix 7: Data for *S. coeruleoalba* females.

Museum No.	Month died	The presence of		
		Graafian follicles		luteal bodies
		small	large	
1652	Jan	x	x	x
1585	Feb	x	x	x
288	Feb	x	x	
936	Mar	x	x	
210	Mar		x	
2040	May	x	x	
1378	May	x	x	x
708	June	x	x	x
849	July	x	x	x
448	July	x	x	x
184	Aug	x	x	
1246	Oct	x	x	x
1993	Nov	x	x	x
264	Dec	x	x	

x = present

Appendix 8: Data for *S. chinensis* males.

Qual. Anal. = Qualitative analysis of 100 seminiferous tubules expressed as a percentage under the appropriate category: J (Juveniles), I (Inactive), ES (Early spermatogenesis) or LS (Late spermatogenesis).

Quan. Anal. = Quantitative analysis = The mean diameter of 20 seminiferous tubules.

Museum No.	Month died	Length (m)	Weight (kg)	Qual. Anal. (%)				Quan. Anal.
				J	I	ES	LS	(mm)
825	01	215	113		5	95		0.043
1036	01	210	102	10	90			0.039
1579	01	215	103			100		0.046
826	02	248	205				100	0.131
1271	02	197	103		4	96		0.051
1038	03	261	250				100	0.179
1364	04	233	134				100	0.107
1498	05	215	118			100		0.036
1179	05	269	245			100		0.089
2090	07	225	128			2	98	0.082
801	08	209	111	98	2			0.034
486	10	211	114	100				0.042
1825	11	223	131				100	0.070
1473	11	211	97			100		0.051
1267	12	219	120			100		0.035
1121	12	267	262			2	98	0.079
1578	12	206	96			100		0.054

Appendix 9: Data for *S. chinensis* females.

Museum No.	Month died	The presence of		
		Graafian follicles		luteal bodies
		small	large	
1978	Jan	x	x	x
1033	Feb		x	
1684	Mar	x	x	
1408	April	x	x	
1934	July		x	x
1778	July	x	x	
1950	July	x	x	x
805	July	x	x	x
1630	Aug	x	x	
971	Aug	x	x	
1314	Sept	x	x	
1086	Oct	x	x	x
487	Oct	x		
1577	Oct	x	x	
544	Oct	x	x	
1266	Dec	x	x	

x = present

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