SYNCHRONISATION OF BREEDING IN POPULATIONS OF THE BROWN MUSSEL *PERNA PERNA* ON THE SOUTH COAST OF SOUTH AFRICA.

Submitted in fulfilment of the requirements for the degree of

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by

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ABSTRACT

The general biology and seasonality of breeding of intertidal populations of the brown mussel *Perna perna* in South Africa are reasonably well known, but we have little information on variability either within or among populations. Synchronous spawning offers adaptive advantages to externally breeding animals. Firstly, it enhances fertilization rates and therefore the species' reproductive fitness. Secondly, spawning can also be timed to coincide with environmental conditions conducive to larval settlement and development. In addition, synchronisation of spawning will influence the synchrony of settlement. Synchronisation of larval settlement, in turn, has implications for population biology, as highly pulsed settlement is likely to lead to density-dependant mortality of recruits and uncoupling of adult/recruit densities, while poorly synchronised settlement will not.

Generally, sea temperature and food availability are considered the key factors underlying the initiation and the duration of the breeding cycle of mussels. However, there are proximate local cues that trigger the proliferation, maturation and release of gametes. In this study, the hypothesis tested is that factors that control food availability affect gonad development and so influence synchrony among populations. Much of the published work on spawning is based on observations of the presence of larvae in the plankton, or on settlement. A more reliable method correlates the sequence of gonad development throughout the year with changes in length-weight relationships, using histology. This study is also designed to investigate temporal differences in the timing of the breeding cycle between sheltered and exposed sites along the south coast of South Africa by histological analysis of the reproductive tissue (the gonad) and by dry weight/shell length regressions. To do this, these two techniques were applied to six mussel populations at three

ocalities that were separated on scales of about 10-20km. Within each locality, two study sites were identified. One was exposed to strong wave action and one was sheltered. A few hundred meters separated these sites. The first technique used length-weight regressions as an indication of mussel condition. Abrupt decreases in the dry body weight of a hypothetical standard animal were taken to indicate periods of spawning. Regressions were assessed for samples of 40 mussels taken from each site at intervals of 4 weeks over 13 months. The results were analysed using a 3-way ANCOVA, with dry weight as the dependent variable, shell length as a covariate, and site, exposure and month, as independent variables.

The second approach used the more reliable and detailed method of assessing the annual reproductive cycle using histological sections of the gonad. Histological sections of gonads from thirty female mussels, sampled monthly from each site, were examined in the laboratory. Each gonad was categorized into one of six arbitrary developmental stages based on ovary morphology. Synchrony in spawning was examined by comparison of gonad developmental stages of individuals within and among populations. The data were analysed by 3-way nested ANOVA with mean gonad index for each population as the dependent variable, month as an independent variable and exposure nested in site.

The results obtained from both techniques showed strong synchronisation among different populations, regardless of the scales at which they were separated. The data also indicated good synchrony within populations and, again among populations, regardless of the degree of exposure. The results also indicated that the gonad condition varied significantly at each site, exposure level and month. However, there were significant interactions among these three factors. This means that

on a broader **seasonal** scale, the six mussel populations were reasonably synchronised, but on finer **monthly** scales, there were temporal differences in duration of gametogenic events. The implication is that ovary development is cued by environmental factor(s) that operate over scales of at least 7-20km, rather than more localized events that may affect food availability either through aggregation of food (local hydrography at different localities) or food delivery to the shore (degree of wave action at different sites).

DEDICATION

This work is dedicated to my parents, Mr and Mrs Ben Samente (and also Sikelelwa and Sivuyile Ndzipa), whom I regard as the pillars of my success, in gratitude for their support and efforts towards my education and above all, for their prayers and immeasurable love.

DECLARATION

This dissertation is my own unaided work and is being submitted for the degree of Master of Science in the Zoology and Entomology Department, Rhodes University, Grahamstown. It has not been previously submitted in whole or in part for any degree or examination in any other university.

Victoria Ndzipa

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Fieldwork was not easier, but for the fear of sounding like an unenthusiastic scientist with a serious lack of motivation, I will say nothing more. Anyway, I remember how I used to literally drag my feet to the beach every month I had to go out sampling mussels for my research. Thanks to those people who made these field trips less dreadful. Some by driving me to the beach (NJ, Lungelo Nabo, Patience Nganwa, Xoli Muleka) or walking along the long stretch of the beach to my sampling sites (Fiona Paterson, Fleur Theophilus, Andrew van der Spuy,). Others like Bhayi, Nono, Nombasa and Arran Stibbe) had sometimes braved the strong tides and helped me collect mussels.

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The list is almost endless. If I've left anyone out, a thousand apologies, and many thanks!!!

CHAPTER 1

GENERAL INTRODUCTION

Chapter 1 : General introduction

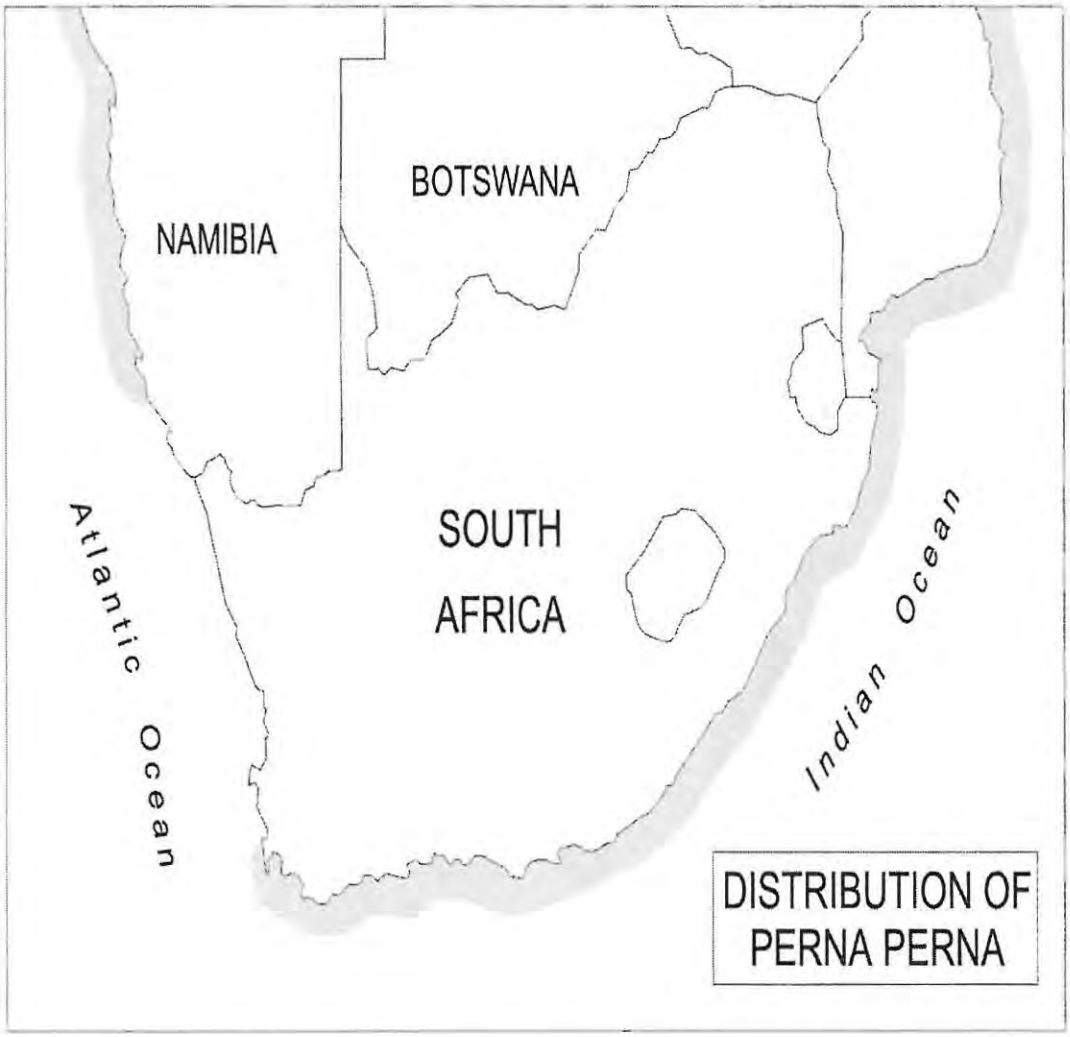
1.1 Introduction

The brown mussel *Perna perna* (Linn.) is widely distributed in tropical and subtropical regions of the Indo-Atlantic (Berry, 1978; Siddall 1980). On the coast of southern African *P. perna* has been recorded from Barra Falsa ($22^0$55'S) in central Mozambique down to False Bay ($34^0$18'E) in the Cape of Good Hope (Berry, 1978; van Erkom Schurink & Griffiths, 1990). *Perna perna* is very rare in the cooler waters of the west coast, becoming more abundant again in northern Namibia (Fig.1). Isolated individuals occur throughout the west coast but do not form dense beds in the area between False Bay and central Namibia. Although *Mytilus galloprovincialis* forms multi-layered beds on the west coast, on the east coast of South Africa, *Perna* forms single-layered beds (Harris *et al.*, 1998).

Of the four most common mussels in South Africa, (*Aulacomya ater*, *Perna perna*, *Choromytilus meridionalis* and *Mytilus galloprovincialis)*, the brown mussel is the most abundant in the warm waters of the south and east coasts (van Erkom Schurink & Griffiths, 1990). It is usually associated with wave-exposed conditions, often forming dense aggregations on rocky shores from the lower balanoid zone to a depth of 5m (Berry, 1978; Lasiak, 1986; van Erkom Schurink, 1990). *P. perna*, *C. meridionalis* and *M.galloprovincialis* co-exist in mussel assemblages on the south coast of South Africa (van Erkom Schurink and Griffiths, 1990).

The mytilids demonstrate a great deal of variation in morphological features and these are of great taxonomic importance. The existence of physiological races and a wide range of ecological

Fig 1.1 Distribution of *Perna perna* (shaded area) along the southern African coast (from Branch *et al.*, 1994).



morphologies in the Mytilidae created a taxonomic challenge for researchers (Siddall, 1980). *Perna perna* was first described by Soot-Ryen, 1952 (see Siddall, 1980) and this was followed by an intensive survey of the historical development of the genus, involving name changes. *Perna perna* has a smooth brown shell, which is broad, yet elongate. Internally the mature gonad is not a discrete organ but is inextricably integrated with the visceral mass (Griffiths & Griffiths, 1987; Pekkarinnen, 1991b; Seed & Suchanek, 1992). *P.perna* is dioecious, but the sexes are distinguishable only after examination of the gonad tissue (Brousseau, 1981; Pipe, 1985, 1987; Pekkarinnen, 1991a; Seed & Suchanek, 1992). It is possible to determine the sex of mussels from the gonad colour once the animal has reached a certain initial state of gonad maturity. At this stage, the female mantle assumes a definite bright orange hue while the male mantle is cream or slightly yellow (Brousseau, 1983). This intense coloration is due to the accumulation of carotenoids in the gonads at maturation (Campbell, 1969; Brousseau, 1982, 1983) and it is for this reason that females were chosen in this study instead of male where gonad tissue can be easily identified. The growth rates in intertidal and sublittoral populations of the brown mussel have been recorded from Durban (Berry, 1978) and in intertidal populations on the Transkei coast (Lasiak & Dye, 1989) and the southern Cape (Crawford & Bower, 1983; McQuaid & Lindsay, 2000). These data indicate a westward trend of declining water temperatures accompanied by a pronounced reduction in growth rate (Harris *et al.*, 1998).

Mussels are recognised as key species in structuring communities on temperate intertidal rocky shores worldwide (Suchanek, 1985; Harris *et al.* 1998). In many parts of the world heavy exploitation of the mussels has important implications for rocky-shore community structure, and is

thus significant in the conservation of biodiversity (Tomalin, 1997). Disturbance of mussel beds may also impact negatively on mussel bed-associated fauna. Knowledge about the factors that influence the abundance of mussels is therefore crucial for the recovery of over-exploited populations and effective management strategies. Archaeological records and recent studies in southern Africa show that *Perna perna* has been used as food by man for several thousand years. It is still heavily exploited as a staple food by indigenous communities in the Transkei coast (Hockey & van Erkom Schurink, 1992; Lasiak & Dye, 1989) and in some areas on the Zululand coast (Berry, 1978; Tomalin, 1997). Because of heavy exploitation pressure, extensive mussel beds in Transkei are found only in nature conservation areas or on inaccessible rock platforms. Experimental analyses of the ecological impact of exploitation have shown that algae usually replace mussels following disturbance and that recovery may take more than eight years (Dye *et al.*, 1997; Lasiak & Dye, 1989). As mussels tend to recruit preferentially into already existing mussel beds, exploitation not only affects reproductive output but also reduces the preferred settlement habitat (Lasiak & Barnard, 1995; Dye *et al.*, 1997; Tomalin, 1997). In addition, survival of this indigenous species also seems to be threatened by an alien mussel species, *Mytilus galloprovincialis,* which has become invasive on the Southern African coast (Griffiths *et al.*, 1992; Hockey & van Erkom Schurink, 1992).

Generally, mussels are becoming economically important in the shellfish industry. Hecht and Britz (1988) reported an unprecedented upsurge in South African aquaculture in the 1980's with a tenfold rise in total production from 1980 to 1988. Mussels, which had only been cultured for five years then, accounted for slightly over half of the total production in 1988. *Perna perna* features prominently in the list of shellfish harvested for human consumption (Hecht and Britz, 1988).

Mussels are most preferred by humans for consumption when they are in the "full" condition. When "full" the animal is plump and laden with germ cells, becoming flaccid after the discharge of these gonad products (Battle, 1932). Studies on the timing and intensity of spawning events are of particular importance and can be used to predict subsequent spat settlement. Gamete release also has an immediate and important effect on the market value of the adult stock since over half of the total wet weight can be lost in a single spawning event (Rodhouse *et al.*, 1984; van Erkom Schurink and Griffiths, 1991). From an ecological perspective, mussel gametes can serve as an important energy source for pelagic and benthic filter feeders. Also, since mussels are major space-occupiers, they have a profound influence on their communities with effects including the build-up of fouling layers, displacement or facilitation of other sedentary species and provision of food for a variety of predators. Therefore, mussels deserve attention not only in terms of fisheries management, but also as complex ecological systems that are home to a wide variety of species (van Erkom Schurink & Griffiths, 1991). Mussel beds have been considered as microhabitats enhancing the establishment of assemblages of different sessile and motile organisms (Alvarado & Castilla, 1996). Mussel beds harbour a large number of invertebrate animal species (Suchanek, 1980; Griffiths *et. al*, 1992; Iwasaki, 1995) presumably because of their great structural complexity, which results from their extensive shell surface and the byssus threads. Population size structure, density and biomass give important baseline information about mussel populations. Comparisons of mussel populations are confounded by variations over time and space and the degree of interaction that occurs between recruitment, size, growth, density and biomass (Lindsay, 1998). In studies of population energetics, the proportion of the total energy budget allocated to reproductive processes, i.e. gamete production is of fundamental interest (Griffiths, 1980; Kautsky,

1982). An understanding of mussel biology (especially of reproductive patterns) is also vital for the management of species in over-exploited intertidal populations.

The reproductive biology of bivalves also has relevance to larval behaviour and recruitment (Todd & Doyle, 1981; Suchanek, 1985; Phillips, 1994). As it is difficult to follow a cohort of planktonic larvae in the field for example, observed peaks of spawning and recruitment have often been used in studies of population dynamics. It seems logical that the quantity of gametes released must be reflected in the numbers settling, unless otherwise masked by over-riding hydrographic or planktonic factors. Although studies on reproduction of bivalves are numerous (Chipperfield 1953; Giese, 1959; Sastry, 1968; Seed, 1969a; Sastry, 1970; Seed, 1976), gamete production has received little attention (Thompson, 1979). Commonly used definitions of production most frequently take into account only somatic growth, whereas reproductive output is an essential component of total production (Seed & Suchanek, 1992).

A considerable amount of research has been done on the reproductive biology of the four common southern African mussels (Griffiths 1977; Berry, 1978; Lasiak, 1986. Lasiak & Dye, 1989; van Erkom Schurink & Griffiths, 1990). In the case of *Perna perna,* reproductive cycles of populations from the Durban area (Berry, 1978) and Transkei (Lasiak, 1986) have been documented. van Erkom Schurink & Griffiths (1991) compared the reproductive cycles and reproductive output of all four common South African mussel species. The gametogenic cycle of the sand mussel, *Donax serra*, on the western coast of South Africa has also been reported by Birkett & Cook (1979) and De Villiers (1973). While substantial information exists about stock sizes, growth rates and the physiology of *P.perna* (van Erkom Schurink and Griffiths 1990 and 1992) on the south east coast of South Africa, the reproductive biology of the species has been based on incomplete observations

restricted to the examination of the spawning period in the studies of Phillips (1994) and Hop (1990), (see van Erkom Schurink & Griffiths, 1991). Furthermore, most studies tend to concentrate on specific sites, or are conducted over a short period, limiting their usefulness in understanding large-scale variation (Harris *et al.*, 1998). Although the reproductive biology of mussels on the South African coasts is fairly well documented, there is still little knowledge of temporal and spatial variation in breeding patterns of the species. Complex interactions between intrinsic and extrinsic variables (e.g. food availability, salinity, temperature, nutrient reserves, hormones, genotypes, etc.) determine the initiation and duration of the various phases of the reproductive cycle. These thus ensure synchrony of gamete development within a population (Newell *et al.*, 1982). This has great significance for a dioecious broadcast spawning species with external fertilisation, as synchronised liberation of gametes is essential.

It is common for ecologists to concentrate on synchronisation of the reproductive behaviour with external environmental conditions, e.g. temperature, food availability, photoperiods, lunar periods, etc. (Bayne, 1976). Attempts to correlate breeding cycles with environmental factors are numerous, since it is obviously advantageous to synchronise the release of gamete between sexes (Levitan & Petersen, 1995). This also offers the species the adaptive value of matching gametogenesis and larval growth to optimal environmental conditions (Bayne, 1976) and increases fertilisation success (Levitan & Petersen, 1995). Coordination of reproductive events with the environment also serves to maximise reproductive success (Newell *et al.*, 1982). It is often possible to recognise that spawning occurs under conditions that are advantageous for the development of the larvae. High levels of food are required not only by adults so that they can spawn successfully, but also by planktotrophic larvae (Page & Hubbard, 1987; Rodhouse *et al.*,

1984). There is also evidence that biogeographic population limits are set not by a failure to reproduce, but by a failure to recruit (McQuaid & Payne, 1998). Thus, the predictability of recruitment largely determines the stability of the population. Synchronisation therefore also has implications for population biology, as highly pulsed settlement is likely to lead to density-dependent mortality of recruits and uncoupling of adult/recruit densities, while poorly synchronised settlement will not (Connell, 1985). McQuaid & Payne (1998) recorded that most intertidal species on South African shores exhibit non-synchronised trickle spawning. Also recruitment is highly erratic and free space is available on many shores. Breeding synchronisation has profound influences on larval biology and hence on population dynamics. Furthermore, an understanding of reproductive periodicity in mussel populations forms a cornerstone of models used to set harvest levels (Harris *et al.*, 1998).

Three stages in the breeding cycle are amenable to environmental synchronisation or entrainment, namely the onset of the proliferation of the gametes from the germinal epithelium; maturation of the gametes and the initiation of spawning (Bayne, 1976). Clarke (1965) discussed some consequences of synchronisation operating at the initiation of the gamete production compared with synchronisation of spawning. In the first case, variable rates of oocyte growth resulting from short-term variation of food supply might result in a prolonged period of spawning. If maturation and spawning are synchronised, ova of similar maturity could be released within a brief period of spawning. However, other workers have argued that asynchronous, intermittent spawning may be an adaptation to life in unpredictable environments. Newell *et al.* (1982) pointed out that continuous dribble spawning ensures that, in the event of catastrophic effects which could harm

larvae or prevent larval settlement, only a small proportion of the potential recruits would be lost (Brousseau, 1983; Lasiak, 1986).

Generally, temperature and food availability are considered the key factors influencing breeding patterns of marine invertebrates. It has been established that temperature is the most important factor controlling the reproduction of marine mussels (Giese, 1959; Hrs-Brenko, 1967; Sprung, 1983). However, attention has been drawn to the lower limits or critical temperatures at which gonadal activity is initiated. In a study of reproduction in *Modiolus modiolus*, Brown (1984) concluded that seasonal variability in temperature played an important role in determining the extent and duration of gametogenesis. On other hand, spawning was more dependent upon attainment of a limited range of minimum temperatures. Two spawning events were observed at higher latitudes, France and Spain (Sprung, 1983) and in British waters (Campbell, 1969; Seed & Brown 1978; Lowe *et al.,* 1982), where a marked annual cycle existed. The fact that these populations spawned twice was mainly attributed to the fact that the critical sea temperature was passed twice during the breeding cycle (Sprung, 1983).

Newell *et al.* (1982) recorded that *Mytilus edulis* normally depends on nutrient reserves accumulated when food is abundant to provide energy for gametogenesis and catabolism in winter. Spawning then is usually timed such that both larvae and adults have access to abundant food supplies, thus maximising the probability of successful recruitment and the rate of energy acquisition by adults for the following reproductive season. (Bayne, 1976; Sastry, 1970; Newell *et al.*, 1982). It is therefore evident that environmental variation between habitats, resulting in different levels of food availability, could alter nutrient storage cycles and therefore the timing of

gametogenic events. Although feeding time did not show any significant effect on gonad weight in *Choromytilus meridionalis*, the growth rate was significantly affected (Griffiths & Buffenstein, 1981). Numerous studies have shown that suspension feeders have a remarkable capacity to filter the water column such that they are food-limited at high culture densities (Navarro *et al.*, 1991). Phytoplankton standing stock or production may be compared to ingestion requirements of bivalve populations over daily, seasonal or other time scales to determine the biomass of shellfish that can be sustained in a given system (Carver & Mallet, 1990). Gradients of intertidal primary production around the coast of South Africa are related to consumer biomass (Bustamante *et al.*, 1995) and a positive relationship between primary production and consumer biomass has been established. Food quantity and temperature can give a good inference of both growth and reproductive output of a species, and can thus be used in studies assessing carrying capacity of mussel populations Grant & Tyler, (1983a and b).

Of the many physical aspects that shape the intertidal biome, the degree of wave exposure is considered to be one of the most significant (Denny & Gaines, 1990; van Erkom Schurink & Griffiths, 1993). The effect of wave action on individuals as well as on populations is varied and has been documented for several species of filter-feeders (reviewed by Lindsay, 1998). Animals that feed by filtering food particles from water are advantaged by strong wave-action because waves continually replace the water from which they draw their food (Branch & Branch, 1981). Kautsky (1982) has observed a rapid maturation of the reproductive tissue of *Mytilus edulis* during the spring phytoplankton bloom, when food supply is increased. The feeding time in intertidal filter-feeding species is not only governed by the rate of bulk movement of water over the individuals, but also by turbulence of the water flow, which generally enhances the rate of

diffusion (Bell & Denny, 1994). The increased splash and spray at wave exposed sites is thought to push zones further upshore by increasing the period of submersion during which animals at higher levels can feed (Underwood, 1981). Numerous studies have established that exposure to wave action affects almost all aspects of the life histories of marine organisms and can thereby have an important role in the structuring of marine communities (Lindsay, 1998). Biological processes like larval recruitment, competition, herbivory, predation, etc. are strongly influenced by the degree of wave exposure of the habitat (Denny, 1987). Exposure to wave action also affects population densities and species distribution (Underwood, 1981; Swinbanks, 1982; Denny & Gaines, 1990; Menge, 1991; Petraitis, 1991; Franz, 1993; Bell & Denny, 1994; Alvarado & Castilla, 1996). Wave-induced water motion also has a direct effect on the rate at which planktonic larvae are transported, and can therefore indirectly control the dynamics of population structure and available space (Denny, 1987). At seven West coast sites, rocky shore communities varied in structure and composition of intertidal organisms over scales of hundreds and thousands of metres, particularly in response to differences in wave action (Emanuel *et al.*, 1992). Petraitis (1991) has observed that extensive mussel beds of *Mytilus edulis* are most common on wave-swept shores because waves tend to hamper activities of their predators. Studies on six rocky shores of varying wave exposure, in Hong Kong (Kaehler & Williams, 1996), revealed that of the eight recorded common species of encrusted algae, the greatest abundance of algae was observed on shores of intermediate exposure. McQuaid & Branch (1984, 1985) have also recorded that the balance between different trophic compartments in intertidal communities is largely controlled by the degree of exposure to wave action. This leads to differences in the species that dominate exposed and sheltered shores, in particular biomass of filter-feeders is dramatically increased under exposed conditions.

Physical features of some rocky shores make them more exposed to strong wave-action than others, resulting in the so-called 'exposed' and 'sheltered' shores. There is however, a continuum between the two extremes. It has been hypothesised that factors that control food availability, such as strong wave action, may affect the rate of gonad development and so influence synchrony among populations. Furthermore, variability in microhabitats may affect gonad development and so influence synchrony among different populations. This study aims at determining whether: 1. populations of *Perna perna* separated on different spatial scales exhibit temporal variation in the breeding cycle; 2. the breeding cycle is synchronised within a population; 3. exposed and sheltered populations exhibit variations in the timing of the breeding cycle. Two techniques were employed to compare the reproductive condition of six mussel populations namely, 1. dry body weight / shell length regression and 2. histological examination of sections of the reproductive tissue.

1.2 Study sites

South Africa has a long wave-exposed coastline extending 2570 km from the Namibian border (28^0S, 16^0E) in the west, to the Mocambique border (26^0S, 32^0E) in the east (Calvo Ugarteburu, 1997). The warm Agulhas Current on the east, and the cold Benguela Current on the west largely determine sea temperatures on the South African coast. Based on these two currents, the South African coastline can be divided into three biogeographic regions: a subtropical east coast region extending southward along the Natal coast to East London; a warm temperate south coast region reaching from East London to Cape Agulhas; and a cold temperate west coast region extending northward into Namibia from Cape Agulhas (van Erkom Schurink & Griffiths, 1990; Emanuel *et*

al., 1992). These coasts also differ in terms of species of mussels that predominate (van Erkom Schurink & Griffiths, 1993; Harris *et al.*, 1998).

On a global scale, the South African coastline is generally regarded as wave-exposed (van Erkom Schurink & Griffiths, 1991). However, because of significant variations in wave action on a micro-scale, rocky shores can be further subjectively classified as exposed or sheltered relative to the local or regional wave conditions (Lindsay, 1998). The south coast consists of long sandy beaches, which are interspersed with rocky stretches. Even within these stretches, rocks are intermittent, so that mussel beds are encountered on a scale of metres (usually 100 m) rather than kilometres (Lindsay, 1998).

Three study locations were established on the open coast at three aeolian dune-rock shores: Three Sisters, Diaz Cross and High Rocks (Fig 1.2). Once more, on a smaller geographic scale, the sites were subjectively classified, *a priori,* as wave-exposed or wave-sheltered based on aspect to the predominant swells, protection by offshore reefs and the nature of wave impact on the shore. Exposed shores experienced waves breaking directly on the shore, whilst sheltered shores had waves breaking offshore and reaching the shore as white water. At each of the three locations, a sheltered and an exposed site were identified for the study, resulting in six different mussel populations. These were Three Sisters Exposed (TSE) and Sheltered (TSS); Diaz Cross Exposed (DXE) and Sheltered (DXS); High Rocks Exposed (HRE) and Sheltered (HRS). These sites are shown in Plate 1. Note that the pictures were taken on the same day. Therefore the observable differences between wave exposed and wave sheltered sites within the same locality are due to

prevalent wave conditions and not differences in conditions on different sampling days. All samples were taken from the same tidal height (mid-mussel zone).

Fig 1.2 Three study locations established on the open coast along the south coast of South Africa are Diaz Cross (1), High Rocks (2) and Three Sisters (3). Each location includes a wave-exposed and a wave-sheltered site, separated by at least 100 m.

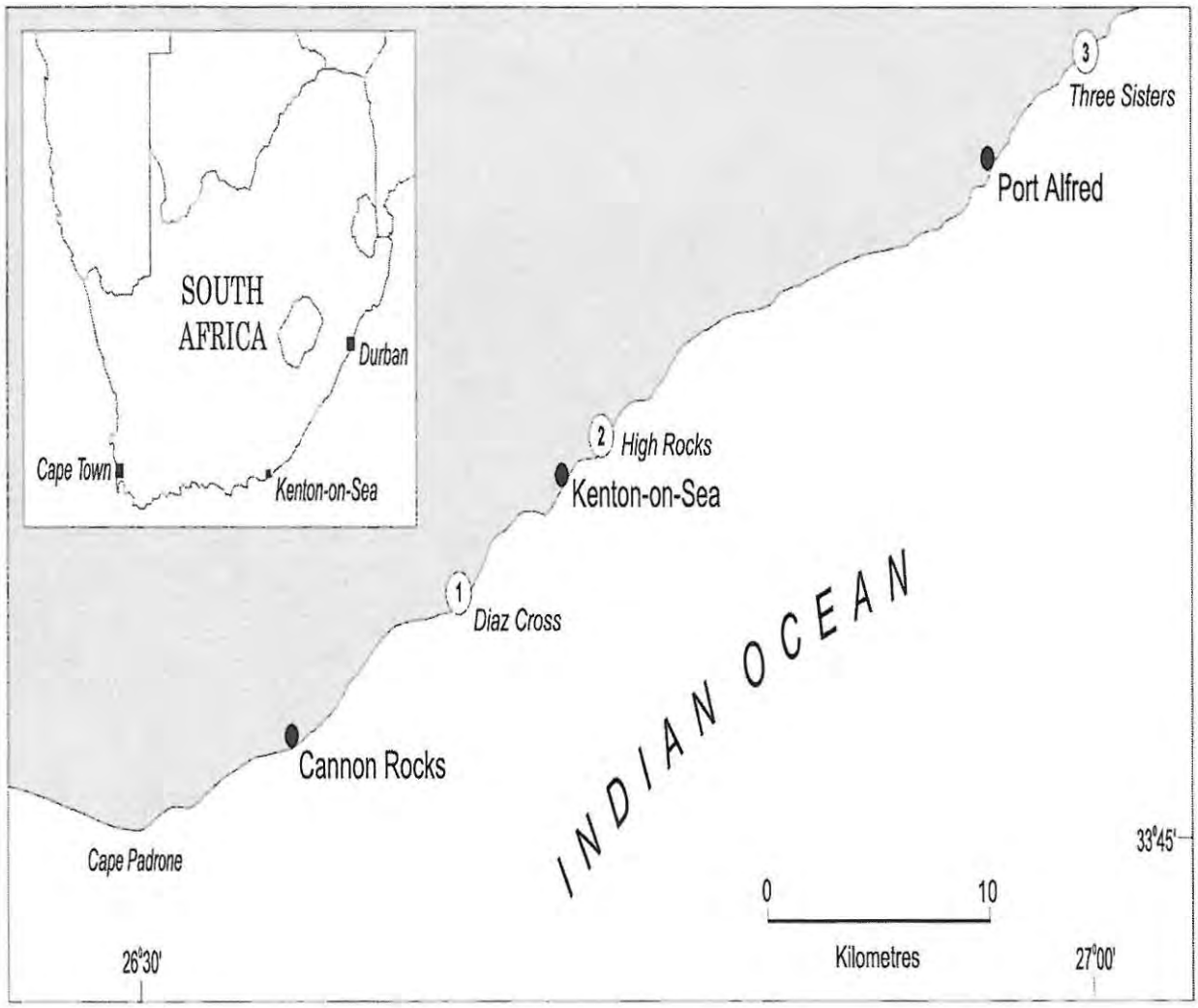


Plate 1.1. Three Sisters Exposed

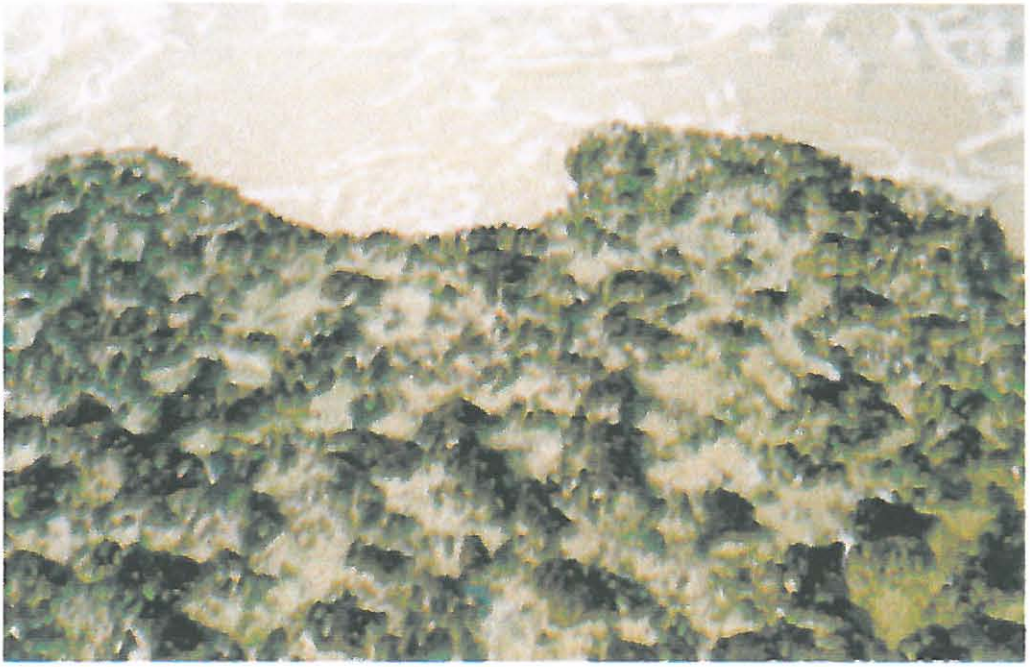
Plate 1.2. Three Sisters Sheltered

Plate 1.3. High Rocks Exposed

Plate 1.4. High Rocks Sheltered

Plate 1.5. Diaz Cross Exposed

Plate 1.6. Diaz Cross Sheltered



1.2.1 Diaz Cross

Diaz Cross is located about 7 km south of Kenton-on-Sea. The wave-exposed site is situated behind a miniature headland, where the waves break. Mussels are patchily distributed in clumps of less than 15 individuals. The wave-sheltered site is about 200 m farther south, with mussel beds occurring along the seaward edge and on the surface of the wave-cut dune-rock platform.

1.2.2 High Rocks

High Rocks is located about 10 km north of Diaz Cross. Mussels on the exposed site are greatly scattered over the surface of the sloping dune-rock shore. Only smaller individuals form aggregates and larger animals occur almost singly, half sunk into the substratum. This site is characterised by massive growth of the foliose green algae, *Ulva*, which sometimes hides the mussels. The sheltered site, which has less scattered individuals, is located on a flat dune-rock shore about 100m away to the north. The sheltered site is characterised by extensive growth of the foliose red algae *Gelidium pristoides*.

1.2.3 Three Sisters

Three Sisters is about 20 km North of High Rocks.The mussel bed at the exposed site is found on the rock reef. It is subjected to strong wave action with waves breaking directly onto a sea-facing rock before rolling over the mussel bed on the right. About 100 m to the north, the sheltered site is

splashed by bulk flow of broken waters also coming sideways from the sea. Both sites are characterised by extremely extensive mussel beds.

CHAPTER 2

HISTOLOGICAL ANALYSIS

Chapter 2 : Determination of synchrony by histological analysis of the gonad

2.1 Introduction

Several methods have been used to examine the course of reproduction in bivalves, as reviewed by Seed and Suchanek (1992). In one way, the reproductive period can be inferred from the appearance of larvae in the plankton, or juvenile mussels in the population. Generally, maximum abundance of larvae at any given stage may be indicative of a recent major spawning event. The difficulty in studying larvae is that one knows where the survivors appear, but not where they come from (McQuaid & Phillips, 2000). Most benthic species have pelagic larvae, which adds to the complexity and magnitude of temporal and spatial variation in recruitment (Harris *et al.*, 1998). The dispersive larval phase results in uncoupling of local recruitment from local reproduction (McQuaid & Phillips, 2000). Seed (1976) noted that, although there was reasonable agreement in spawning periods within populations of *Mytilus edulis*, there were often considerable differences in spawning periods between nearby populations. Thus monitoring larval settlement to assess the timing of a recent spawning event may sometimes be erroneous, as it does not take into account immigration and emigration of larvae (Suchanek, 1985). Observations of the reproductive cycle from such indirect methods may not reflect the true breeding pattern for the area studied since larvae and spat (juvenile mussels) may not come from the same source. Larvae may have been transported considerable distances by currents from parental stocks living under quite different hydrographic conditions (Widdows, 1991; Seed & Suchanek, 1992). Owing to water currents, larvae from different localities may mix, producing heterogeneous populations of larvae, which are of different ages (Kautsky, 1982). Despite heavy spawning, Wilson & Seed (1974), found relatively few mussel plantigrades in algae or on rope

substrata, and even those that appeared 2-4 weeks after spawning were of a relatively larger size. This suggests that settlement was due to movement of plantigrades from other sites of primary attachment. Spatial variation in larval settlement is a result of passive (e.g. hydrodynamics) and active (including behavioural) processes operating at various scales (Hunt & Scheibling, 1996). Extended periods of settlement can also be due to the incursion of foreign larvae. On the other hand, the duration of planktonic life can be influenced by the availability of suitable substrata (Wilson & Seed, 1974; Seed & Suchanek, 1992). Active habitat selection occurs at a small scale as a response to various biotic (e.g. microbial film, conspecifics, etc) and abiotic (surface texture and chemistry) cues associated with the substratum (Hunt & Scheibling, 1996). Variability in both space and time in the recruitment of sessile invertebrates and algae is a result of many factors (predation, competition, wave action, etc.) other than spawning (Menge, 1991). Considering the number of perilous steps involved in larval recruitment to adult stocks, caution should be exercised when using settlement solely to study the course of reproductive cycles.

Studies of larval abundance and settlement can nevertheless serve as valuable checks on data obtained by more direct methods (Seed, 1976). Even in studies of ecologically similar species, there may be constraints on which method(s) is possible. In this case, at least two methods of assessment of gonad development must be used together so that a meaningful re-construction of the gametogenic cycle of an animal can be described. Usually, the most reliable and detailed information regarding the reproductive cycle of bivalves is that obtained from histological preparations of the reproductive tissue. Such observations are made at regular intervals throughout the year to identify the progressive development of oocytes and the subsequent changes in morphology of the gonad tissue over time (Seed, 1976; Seed & Suchanek, 1992).

Compared to other techniques, it offers the advantage of definite and precise determination of the sex of the studied organism, as well as its reproductive condition (Grant & Tyler, 1983a and b). However, since animals are sacrificed, continuous observations cannot be made on the same individual. This method also gives an immediate indication of gonad condition at each sampling period without the need to compare between sampling occasions (as in the use of length/weight analyses). Furthermore, histological analysis enables detection of changes in gonad structure, which may affect weight, e.g. accumulation of nutritional reserves, or the presence of parasites, (Griffiths, 1977). To analyse the development of gonads more carefully, several authors have divided the development of the gonads into a number of arbitrary stages (Chipperfield, 1953; Lammens, 1967; Moore & Reisch, 1968; Sastry, 1968; Seed, 1969a; Barkati & Ahmed, 1990), depending on gonad morphology. The technique of histological assessment of the gonad maturity condition used in this research has been adapted from Barkati & Ahmed (1990). The general reproductive condition of the population can be determined by calculating the mean gonad maturity index of all individuals in the sample. Gametogenesis leads to an increase in this index, while a decrease denotes spawning (Wilson & Seed, 1974; Seed & Brown, 1978; Sunila, 1981; Kautsky, 1982).

2.2. Materials and Methods

The breeding cycles of wild populations of the brown mussel *Perna perna* were studied for 13 months from July 1997 to July 1998. In an effort to clarify the reproductive biology of *Perna perna*, this study was designed to define and categorise the sequence of gametogenic development based on microscopic examination of the gonad tissue, and also to determine the frequency and duration of spawning cycles in the natural environment. Sampling was conducted at monthly intervals from July 1997 and July 1998, inclusive. Samples were stored in a freezer at - 5^0 C until examined. Every month twenty female mussels, covering the entire adult size range (a little less than 20mm to about 70 mm), were selected from each of the six mussel populations surveyed. Only sexually mature individuals were selected for the study. In previous studies, no differences were found between rates of gametogenesis in mature animals of different sizes in *Mytilus edulis* (Wilson & Seed, 1974; Seed, 1976). Seed (1969a) showed that the gonad index in samples including very small mussels was usually somewhat lower at any given time due to the presence of virgin animals, or those with rudimentary genital systems. Chipperfield (1953) found no difference in the time of maturation of gametes in mussels of different sizes. These specimens were used for histological assessment of the gonad. A portion of the gonad tissue was cut from the middle region of the mantle lobe and fixed in Bouins' aqueous solution. The tissues were then dehydrated in ascending concentrations of alcohol, washed in xylene and then embedded in Paraplast (55^0C Melting Point). Paraplast tissue blocks were later cut into 5μm sections using a Leica Microtome. No significant differences were observed in the gonadal development in sections of the gonad tissue of *M. edulis* (Brousseau, 1982; Lowe *et al.*, 1982). Likewise, gonad development was homogenous throughout the reproductive tissue of *Perna perna* (pers.

observation). Therefore, sections were cut at random positions of the embedded tissue. Mounted slides of these sections (one slide per individual for 20 animals by 6 populations per month for 13 months were stained in Haemotoxylin and counterstained in Eosin and examined using light microscopy. Comparisons of gonad development condition were made among individuals within the same population, and also among different populations. Examination of the annual cycles is facilitated by the use of the gonad condition index. By giving the percentage of individuals in each stage an arbitrary ranking, each sample can be assigned a mean value indicative of the degree of gonad development. This value is the gonad maturity index or GI (Wilson & Seed, 1974). The morphological condition of the reproductive tissue of each individual was determined according to prevalent gonad condition. Gonads were classified into six arbitrary stages (Table 2.1), according to the progressive stages of ovary development, following Barkati & Ahmed (1990). Each stage of gonad condition was assigned a numerical ranking, which roughly correlates with the actual weight of the gonad (Barkati & Ahmed 1990). In each month, a population gonad index was calculated from the individual scores for gonad condition, to give a sample mean gonad index (mean GI), according to the equation:

Mean GI = gn/ N, where

g = rank of the stage,

n = number of individuals assigned to a stage and

N = sample size

Table 2.1 Gonad classification in *Perna perna,* based on histological slides.

Stage	Rank	Condition	Description
B	2	Developing	Initial stages of gamete formation (gametogenesis); connective tissue is abundant; size of follicles small; ripe gametes absent
C	3	Maturing	Considerable increase in gonadal mass observed; developing gametes restricted to the periphery of follicle, lumen of follicles occupied by maturing gametes
D	3	Mature	Follicles fully occupied by ripe gametes; oocytes assume roundish or polygonal shapes
E	2	Partial spawning	Reduction in density of follicular content prominent in some follicles; unspawned ripe gametes loosely fill the follicles
F	1	Heavy spawning	Few residual eggs present in follicles; partially or completely empty
S	0	Spent	All gametes shed; follicles collapsed, or narrow and small

The criteria for separating these arbitrary stages in a continuous process are undeniably subjective and intermediate stages inevitably occur. Thus, only by following the entire annual cycle does it become possible to recognize each stage with any measure of confidence (Seed, 1976). Where partial spawning is followed by further gametogenesis, it is difficult to distinguish spawning and re-development stages without exercising subjectivity. The mean gonad index of a population will be a summation of all individuals in a sample in their respective maturity stages, divided by the total number of individuals in that population (normally 20). In effect, the mean gonad maturity index for a sample (GI) ranges from 0 –3 corresponding to when the whole population is spent (0) and when it has attained maximum reproductive condition (3), respectively. The data were analysed using a 3-way nested analysis of variance (3-way nested ANOVA), using Statistica, to test for significant differences in mean GI in different sampling periods (months) in each sampling area (site) or population. The independent variables were site, exposure and month and gonad maturity index (GI) was the dependent variable. Exposure was nested within site. Frequency histograms were drawn for each population over the sampling period.

2.3 RESULTS

Seasonal variation in the mean gonad index (GI) for the six mussel populations are shown in Table 2.2 and illustrated in Figure 2.1 (raw data of population monthly GI values are in Appendix 1). Monthly comparisons of gonad morphology among individuals within the same populations (as proportions of individuals in each stage) are shown in Figures 2.3.1. – 2.3.6.

2.3.1 Synchrony among populations

The results of the statistical analysis (Table 2.2) show that all factors i.e. site (S), exposure (E) and month (M) had significant effects on gonad maturity index (GI). These effects can be interpreted as follows:

Site : the are significant differences in GI among sites

Exposure: different exposures show significant differences in GI

Month : GI varied significantly throughout the sampling period

Interactions: significant interactions between site and month and between exposure and month were observed, indicating that temporal variation differed among sites and between exposure categories. The site by month by exposure interaction cannot be estimated because the design was nested.

The effects of site, exposure and month on gonad condition were not always clear-cut because there were significant interactions between ***exposure*** and ***month*** and between ***site*** and ***month.*** This

Table 2.2 Three-way nested ANOVA with interactions on the effects of site (S), exposure (E) and month (M) on Gonad Maturity Index (GI)

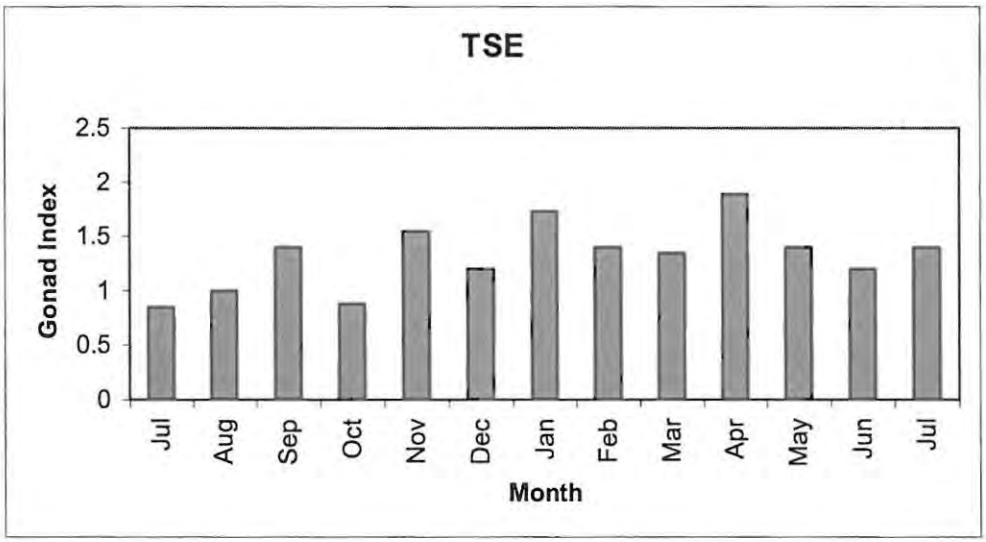
Sources of variation	Df	MS	F-ratio	Significance Levels
Main effects :				
Site	2	2. 444	8. 87646	<0.000147
Exposure (Site)	3	1. 864	6. 077267	<0.000156
Month	12	15. 267	55. 45743	<0.000001
Interactions:				
SM	24	1.654	6.00957	<0.000001
EM	36	0. 880	3.19639	<0.000001
SEM	----		-------	------
Residual	1482			

Table 2.3 Results of Scheffe's Post-hoc Test. Homogeneous groups are indicate by similar letters.

Variable = GI
Main Effect = Site

Site	Code	Mean	Homogeneous groups Alpha = 0.5	
			1	2
Diaz Cross	2	1.2778	a	
Three Sisters	1	1.3634		b
High Rocks	3	1.4161		b

Fig 2.1 Monthly mean gonad index in six populations of *Perna perna*. (TS = Three Sisters, DX = Diaz Cross, HR = High Rocks and E & S = Exposed and Sheltered shores respectively).



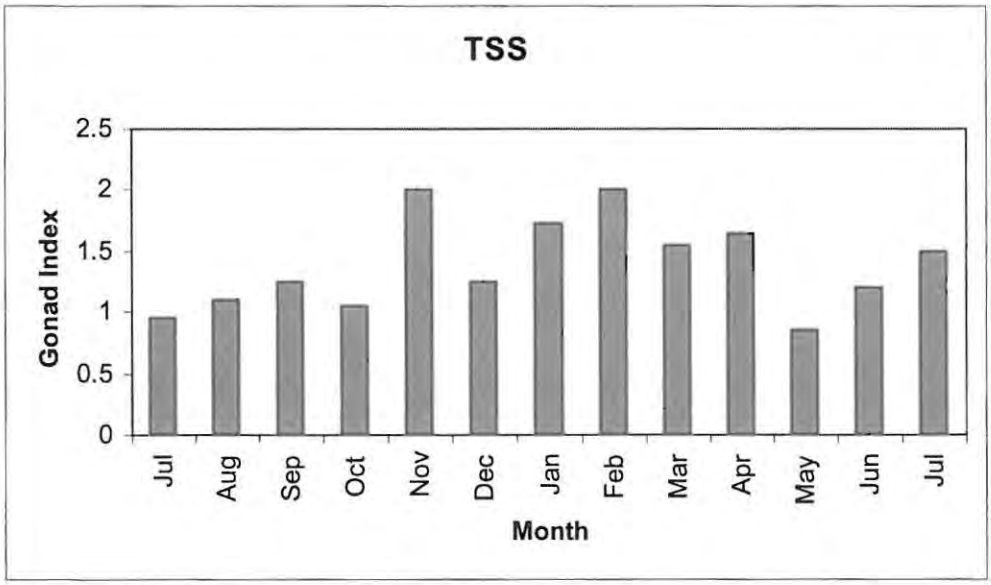
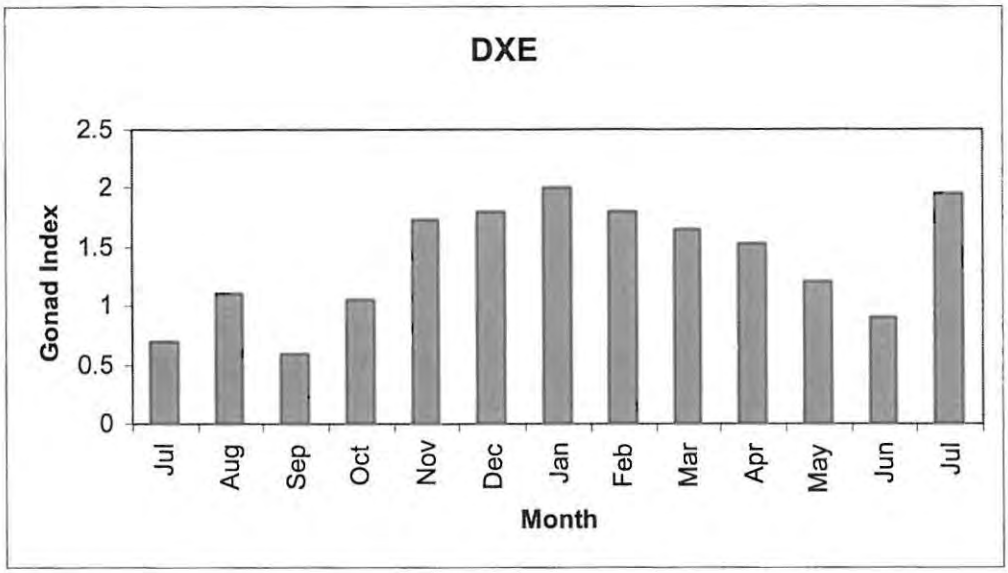


Fig 2.1 Monthly mean gonad index continued.



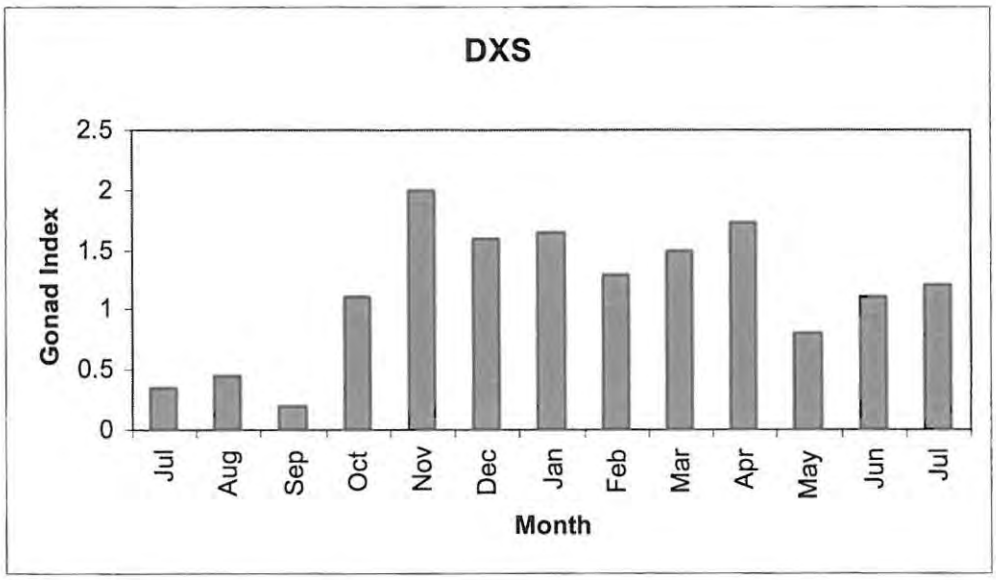
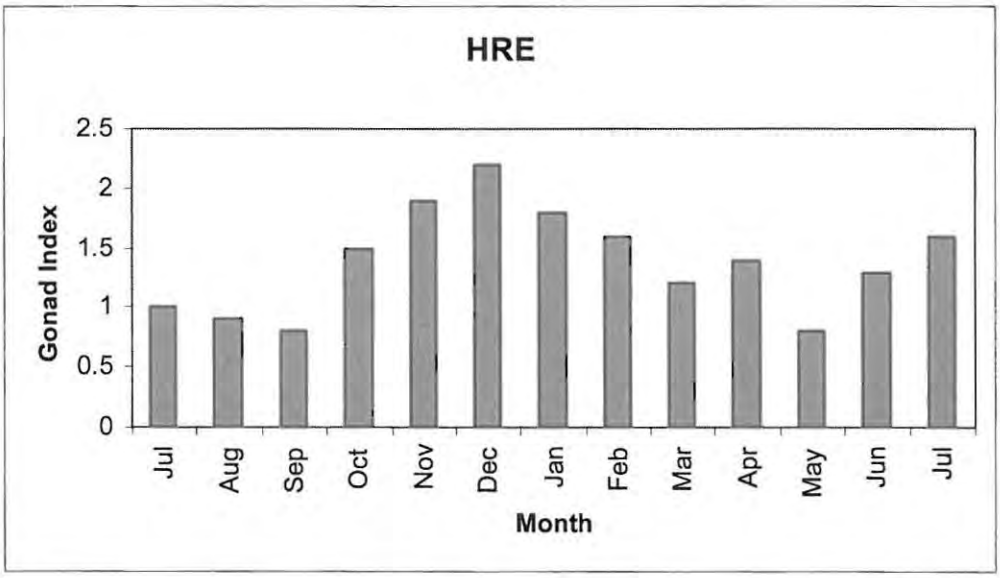
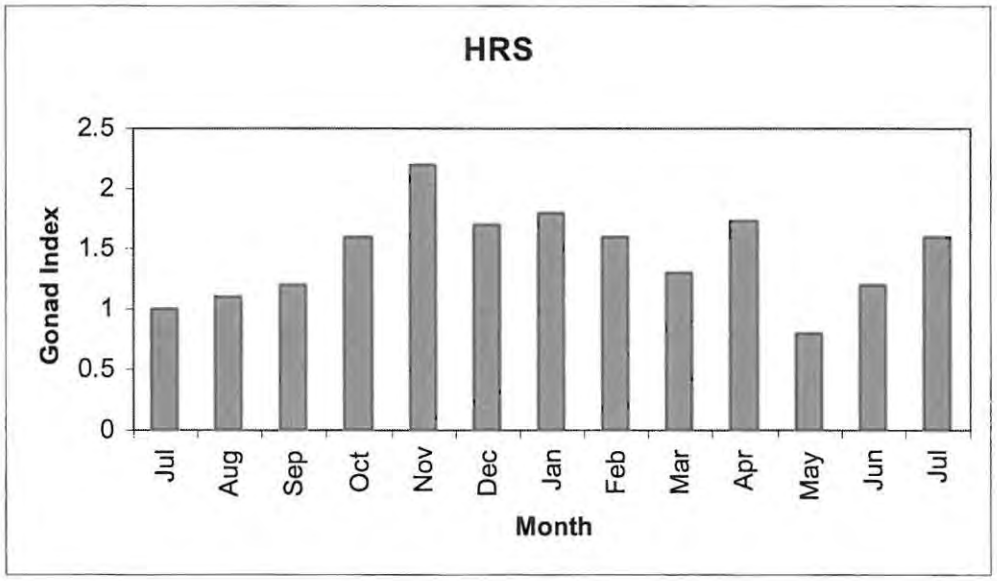


Fig 2.1 Monthly mean gonad index continued.

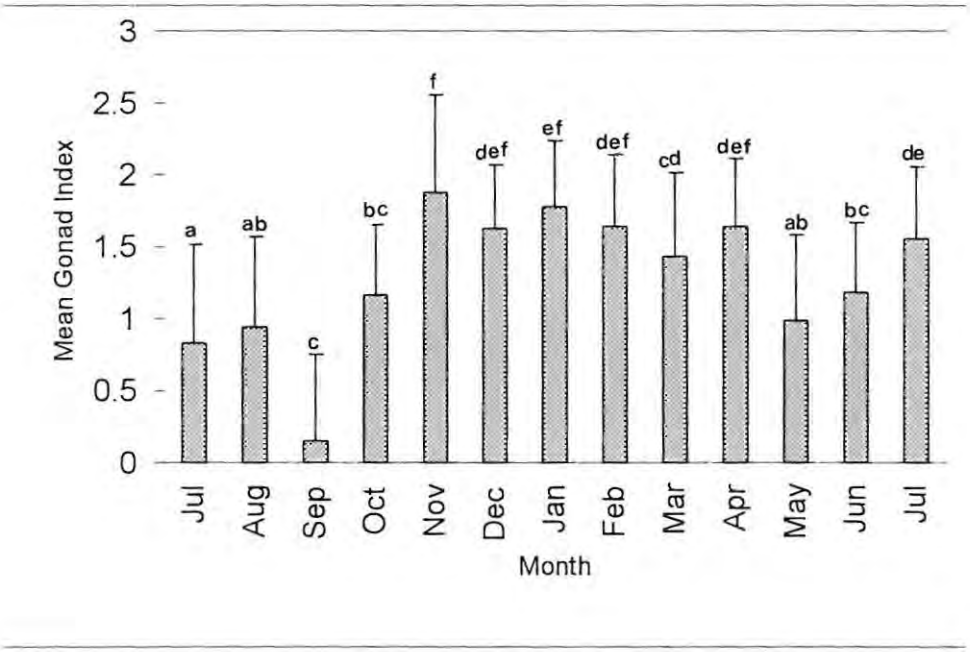




suggests that variability in gonad condition is not a function of a single factor but is a result of complex interactions between these factors and that none of these factors had an overriding influence on gonad index. All populations showed a well-defined reproductive cycle. On a broader, annual scale (Fig. 2.1) all sites had a similar pattern in the breeding cycle, indicating that all populations are synchronised on a seasonal basis. The maximum rises and declines in reproductive condition were well co-ordinated in time among the different populations. All populations showed a maximum in reproductive condition (maximum GI) between the months Nov – Apr/ May, which is indicative of summer gametogenesis. The minimum gonad condition (minimum GI) was observed between Jul – Oct the previous year, suggesting winter spawning. The same trend was repeated in Apr/ May – Jul 1998, when the magnitude of the GI dropped dramatically. However, the intensity of spawning in 1998 was lower than that observed in the previous year, as shown by a weaker decrease in GI levels in 1998. The spawning event was extended in 1997 compared to the short duration in 1998.

The seasonality of reproduction comes out rather clearly in post-hoc tests (Fig. 2.2). Significant interactions between month and both exposure and site indicate different temporal patterns in different places. Nevertheless, the results of Scheffe's post-hoc test conducted on the single effect "month" (Fig 2.2) illustrate a clear overall pattern. Essentially, samples taken during winter (Jul - Oct 1997; May – Jun 1998) form a group with low GI values. Samples collected in summer (Nov 1997 – Apr 1998) form a group with high GI values. Of course, individual sites may not conform to this broad pattern. The grouping of the July '98 sample with the samples showing high GI values indicates the extent of inter-annual variability in the timing of the onset of gametogenesis.

Fig 2.2 Relationship between mean gonad index (+ SD) and month. Data were pooled for all populations for each month. Samples showing the same letter (a – f) belong to the same homogenous group.



Despite the broad similarity in the breeding cycle observed among these populations, the significant interaction between month and site indicates that the temporal patterns did vary among sites. In effect, this interaction suggests that, while all sites seem to conform to a broad seasonal pattern, on a smaller monthly scale, populations exhibited differences in timing of the breeding cycle. The breeding pattern observed at the Three Sisters Exposed site (TSE) looked slightly different from those observed in the rest of other populations (see Fig 2.1). Scheffe's Post–hoc Test on the single factor 'site' (Table 2.2) was used to determine whether the significant differences among sites was due to the fact that Three Sisters was different from the other two locations (Diaz Cross and High Rocks), but the result showed that Three Sisters was in fact similar to High Rocks. The significant interaction between exposure and month suggests that exposure to wave action did not have a clear effect on the timing of the breeding cycle. The magnitude of the gonad index in any given month did not depend solely on whether the site was sheltered or exposed. Synchrony among populations therefore did not depend on the degree of exposure.

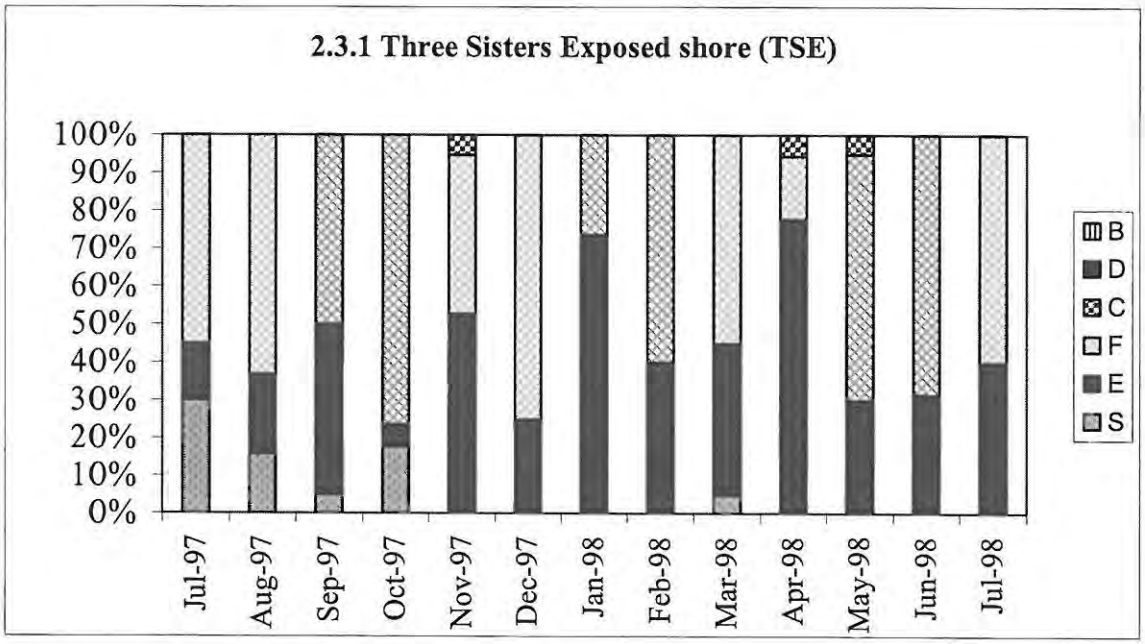
Thus, contrary to the proposed hypothesis, wave action did not have any overriding influence on the timing of the breeding cycle, while on an annual scale mussel populations at different locations were relatively synchronised. Furthermore, interaction between site and month suggests that synchrony did not depend on proximity of site.

2.3.2 Synchrony within populations

Histological sections of reproductive tissues were further analysed to compare the gonad condition among individuals of the same population. Individual gonads were examined and were each allocated to one of the six arbitrary stages, based on the prevailing condition of the ovary. In all populations, the gonad maturity condition varied considerably among individuals (Fig. 2.3.1 – 2.3.6). Different stages of gonad condition were encountered within each population at any given month. In fact, oocytes at different stages of development were frequently found within the same individuals. The stages did not differ haphazardly among individuals but were always within the same range of development (i.e. stages encountered in one month were sequential with respect to cyclic gametogenic events), and no more than three different stages were found in a given month. Spawning individuals were found throughout the sampling period. In addition, an individual could attain maximum gonad condition and shed some gametes at the same time. But at no time were any individuals in a population just full (mature), ready for the next spawning event. Despite continuous shedding of gametes, a clear overall annual pattern of gametogenesis and spawning pattern was observed in all populations. For example, the majority of individuals in the TSS population were releasing gametes between July – Oct in 1997 and May-Jul 1998 (predominant gonad stage was F = heavily spawning). Between the spawning peaks, gametogenesis progressed with small incidents of 'trickle' spawning by almost all individuals in the population. Maximum gonad development was observed between Nov – Dec 1997 and in Feb 1998, although the tissues still showed traces of gamete shedding even during these periods. The intensity of spawning observed in these two years was different, with the 1997 event being more prolonged (extending from Jul to Oct in most populations) than in 1998. In

addition to the heavily spawning individuals, the proportion of spent individuals was higher in 1997 compared to 1998 during the spawning period (see Appendix 2). No post-spawning resting phase (i.e. reproductive quiescent phase) was observed, but within the population as a whole, spawning was followed by a rapid regeneration of the gonad.

Fig 2.3 Stages of gonad development of individuals within populations. Shading indicates different stages as indicated on insert. Stages defined in Table 2.1.



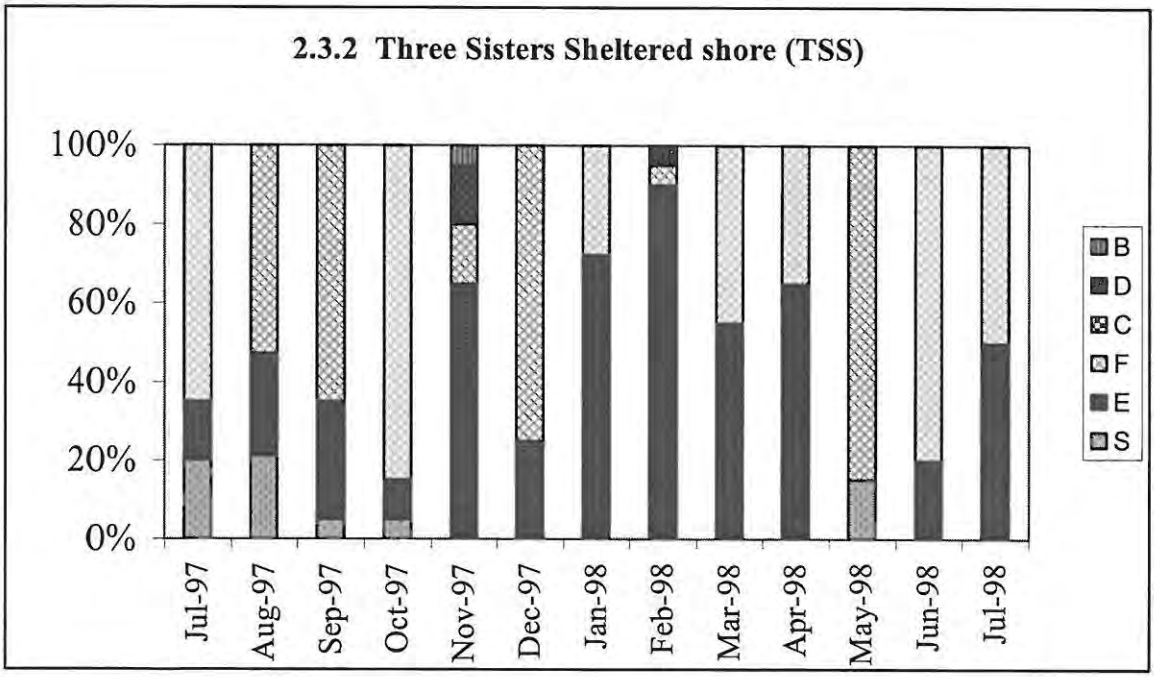
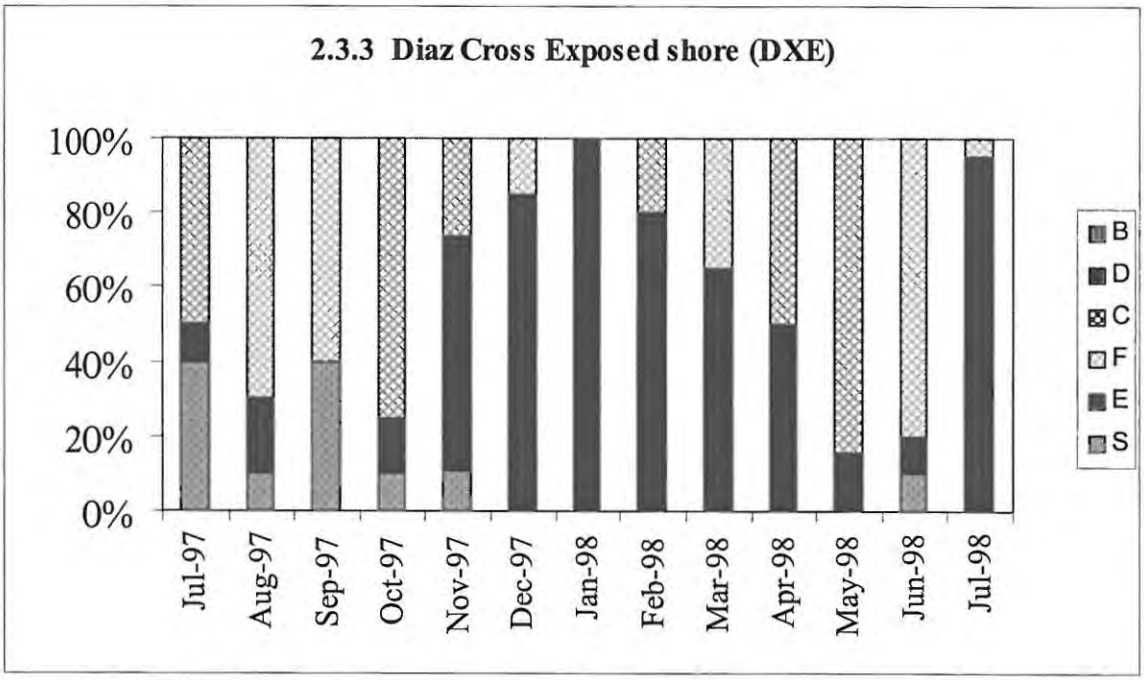


Fig 2.3 Stages of gonad development continued.



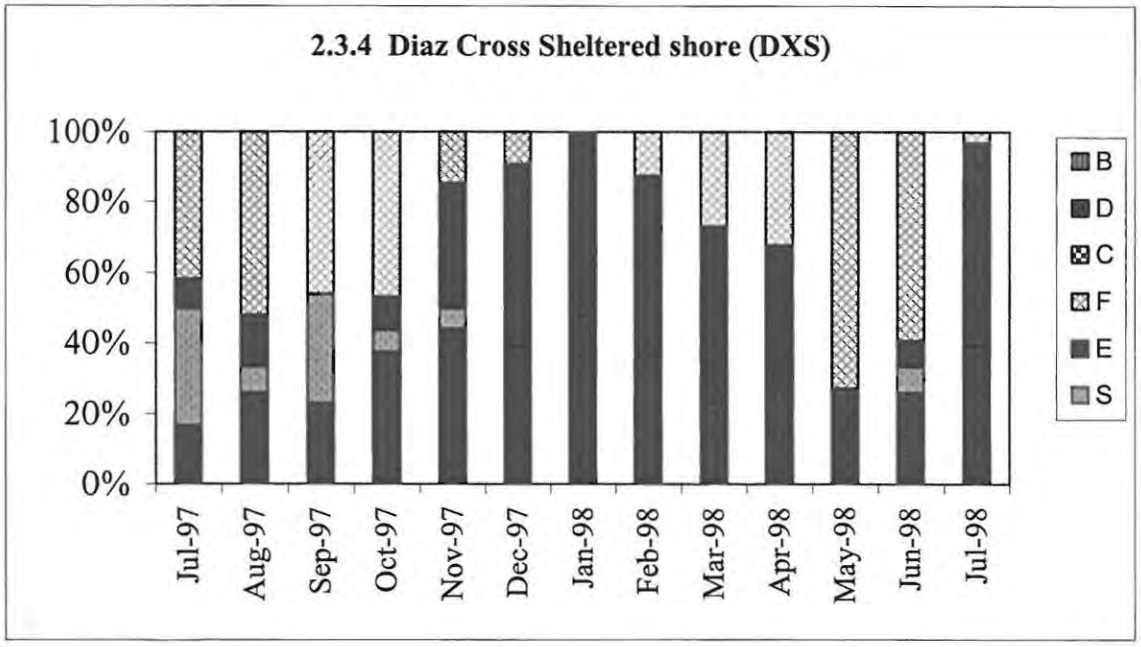
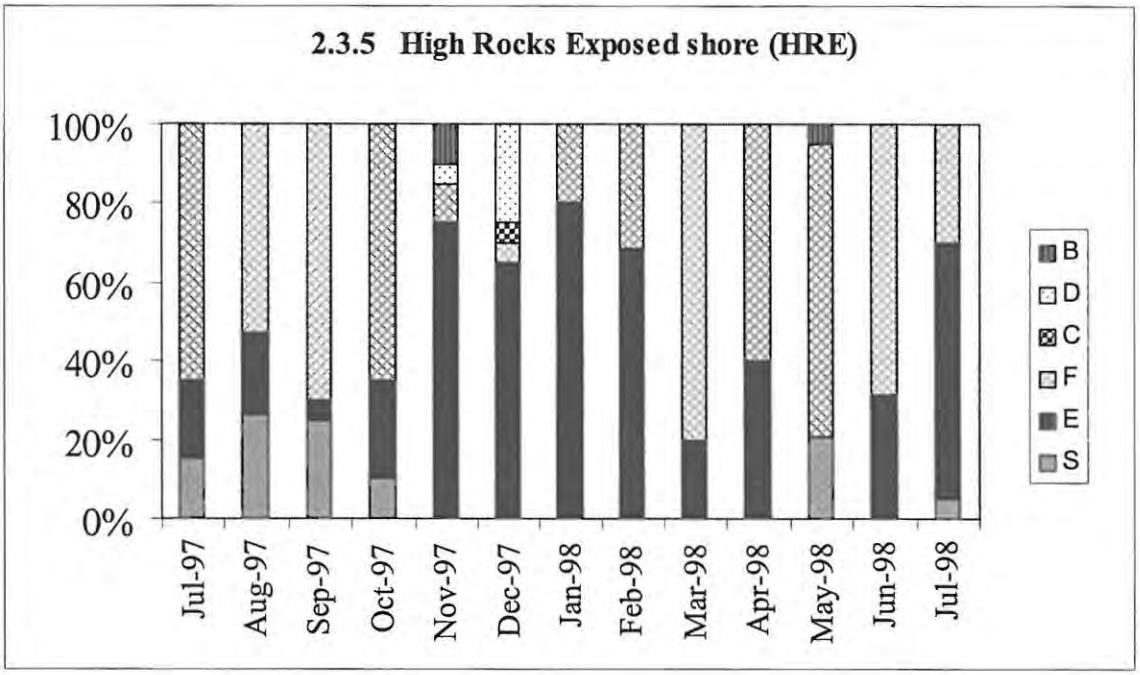
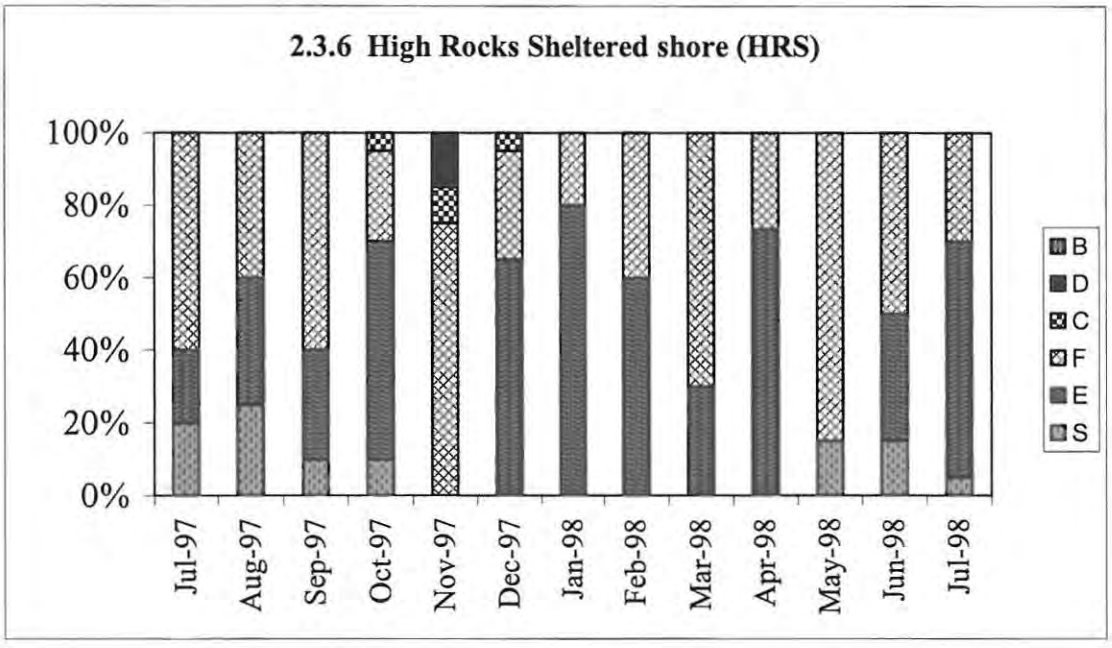


Fig 2.3 Stages of gonad development continued.





2.4 Discussion

Reproductive cycles of *Perna perna* have previously been recorded in different regions of South Africa. On the Natal coast (Berry, 1978), two conspicuous spawning events were observed between May and October, based on both histological and dry body weight analyses. Protracted spawning with a major peak in Apr-July was observed in Transkei (Lasiak, 1986), based on histological sections of the gonad. In False Bay in the western Cape, a different pattern was observed (van Erkom Schurink & Griffiths, 1991), with an intense spawning event between Apr-Aug followed by minor spring or summer events. The results of the histological analysis in this study show that on the south coast, *P. perna* spawns once, in winter (Jul – Sep 1997 and Apr – Jun 1998), with trickle spawning throughout the rest of the year. All six populations showed clear annual cycles, and were synhcronised on a seasonal basis. Within season, there was variation in the timing of gametogenic events. Interaction between site (at the same location) and month indicate that synchrony did not depend on proximity between sites. The results of this study do not corroborate the hypothesis that exposure to wave action has added advantages to gonad maturity condition as there did not seem to be a clear trend in the magnitude of reproductive condition with respect to the degree of wave exposure in *P. perna*, nor did exposure have a clear effect on timing of gametogenic events as shown by the month on exposure interaction. Wave action has many very important effects on the biology of intertidal organisms, such as growth, reproduction, larval settlement, feeding, etc. Nevertheless, it is difficult to estimate the extent of the effects on aspects of life history because of complex interactions with other factors. Also, measurement or accurate prediction of the maximal forces required to induce an effect need to be established. The basic similarities in gametogenesis and spawning in the six populations suggest that a regime of environmental conditions common to all locations might

cause the populations to be broadly synchronised, but at finer temporal scales (months), there are differences in timing among populations (as shown by site and month interactions). At even finer temporal scales (within months), there are differences in timing among individuals within the same population. Thus, on a smaller temporal scale there is variability in the state of development, and broader synchronisation at coarser time scales.

CHAPTER 3

LENGTH/WEIGHT REGRESSION

Chapter 3 : Determination of synchrony using weight on length regression

3.1 Introduction

One of the major disadvantages of using the arbitrary classification of gonads as a technique to study reproductive cycles is that it is subjective and does not fully recognise the occurrence of intermediate stages of development. Furthermore, it has the disadvantage of being subjective, in that estimates of developmental stages by different people are unlikely to be the same. Consequently, some authors (Bayne *et al.*, 1978; Lowe *et al.,* 1982; Newell *et al.*, 1982) have used more quantitative methods by measuring the proportion of gonad weight to the total somatic weight. Gonad indices can also be expressed as a function of dry body weight and shell length (Branch, 1974; Griffiths, 1977; Suchanek, 1980; Seed and Suchanek, 1992). This normally involves recording fluctuations in body weight in standard sized individuals from seasonal body length to flesh weight regressions and has been used for a number of South African mussels (Berry, 1978; Griffiths, 1977; Griffiths & King, 1979; van Erkom Schurink & Griffiths, 1991). According to Seed (1976), spawning generally appears to be simultaneous in all sizes of mussels. Mature mussels may lose a large proportion of their body weight during spawning (Bayne, 1976; Griffiths, 1977; Griffiths & King, 1979; Thompson, 1979; Bayne & Worral, 1980; Rodhouse *et al.*, 1984). Since a major proportion of the mature gonad develops within the mantle, weight losses from this tissue may be used as an estimate of both the reproductive condition and reproductive output (Bayne *et al.*, 1982). Size and age are obviously important in the consideration of reproductive output as older individuals of marine invertebrates allocate a very high proportion of their resources to reproduction (Thompson, 1979). One of the advantages of using weight/length analyses to study gonad development is that it is a more rapid method and

also provides a quantitative estimate of gonad production, which may be used in comparing reproduction in populations from different areas (Griffiths, 1977). However, in mussels, it is very difficult to single out the gonad proportion from the total body tissue because the gonads occupy a great part of the mantle lobes and indeed branch throughout the entire visceral mass. Gonads form a considerable proportion of the total body weight before spawning (Sprung, 1983; Rodhouse *et al.*, 1986). Pekkarinnen (1991a) reported a rapid rise in gonad volume fraction (GVF) due to an increase in the volume of developing gametes, which was followed by a dramatic decline during spawning.

Caution should be exercised when using dry weight/shell length regression because of the need to observe periodic variations in the amount of reserves other than gametes. Energy derived from food and destined to be used in gametogenesis may be stored and used at a later date, resulting in a 'biochemical cycle' that is related to the gametogenic cycle (Bayne, 1976; Zandee *et al.*, 1980). Thompson (1984a and b) observed a cyclic increase in carbohydrates and lipids reserved in the digestive gland in mussels. These reserves are transferred to the gonad during gametogenesis. Bayne and Worral (1980) have also reported that glycogen stored in various body tissues is later used for energy for the process of gametogenesis and/or converted into lipids in developing gametes. Seasonal changes in PCBs (Polychlorinated Biphenyls) and lipid content of the mantle have been studied (Hummel *et al.*, 1989) in relation to the reproductive cycle of *Mytilus edulis,* and the results show that the development of the gonad tissues is the cause of the relative increase in mantle weight. A strong decline in PCBs and fats during spring coincides with spawning of gametes. Gametes have a relatively high fat concentration and these fats are shed with the gametes during spring. The observed increase in carbohydrate content of the body was

accompanied by gamete synthesis and these carbohydrates rapidly declined during spawning (Thompson, 1984a). Nutrient reserves are thus accumulated when food is abundant and are used, in part, to meet the energy demands of the animal when food is scarce and are also used in the production of gametes (Bayne, 1976). In populations of the bay scallop *Placopecten magellanicus*, somatic weight declined as gamete development commenced, but increased again during the periods of low gametogenic activity, suggesting a close relationship between the energy available for growth and the reproductive cycle (MacDonald & Thompson, 1986). In this chapter, seasonal changes in length/weight regression have been used to compare breeding cycles in six populations of the brown mussel *Perna perna.*

3.2 Materials and methods

The diffuse nature of the reproductive organs of mussels precludes any measure of absolute growth of the reproductive tissue during gametogenesis. Consequently, an allometric relation of the total body weight to shell length was computed for wild populations of the brown mussel *Perna perna.* It has been observed that values of gonad/total weight ratio are not always independent of body size and it is therefore important to examine whether there is such dependence by including body length as a covariate in the analysis of variance (Grant & Tyler, 1983a). This provides estimates of mean total body weight, compensated for differences in animal sizes. Thus, the aim of measuring the shell length in calculating the gonad index is to standardise body weight in order to produce an indicator of the state of gonad development (GI), which is independent of animal size. The analysis of the GI can be performed on the logarithm of body weight with log(body length) as a covariate. In this analysis the relationship between body

weight and body length can be estimated in the form: body weight = a (body length)^b which also gives an indication of how reproductive effort increases with size.

From each of the six mussel populations surveyed in this study, monthly collections were made of 40 individuals covering the entire adult size range (approximately 20 – 70 mm shell length). The shell length was measured (maximum anterior-posterior dimension), using vernier callipers to the nearest 0.01 millimetres. The flesh was removed and dried at 55^{0}C for 48 hours. Drying the animal tissue eliminates possible errors due to any variations in water content of different individuals and different reserve organs (Grant and Tyler, 1983a). Samples were cooled in desiccators for at least 30 minutes prior to weighing. Dry body weight was measured to the nearest 0.01g. On a monthly basis, dry body weights were related to shell lengths for each population separately, using the allometric equation:

$W = a\ L^b$, where

L = shell length, and

W = dry body weight

The values of the dry weight and shell length were log-transformed ($\log W = a.\ \log L^b$). Dry weight was regressed on shell length and the data were analysed by a 3-way analysis of covariance (3-Way ANCOVA), with shell length as a covariate. Site, exposure and month were the independent variables and dry weight was the dependent variable. To determine seasonal changes in gonad condition the dry body weight of a standard animal was plotted against month. Comparisons were made both among and within populations.

3.3 Results

The results of the analysis of co-variance are recorded in Table 3.1 below. Data passed both Cochran's test for homogeneity of variance (P = 0.05231) and normality of distribution.

Summary of all effects

The data showed significant effects ($P < 0.01$) of the co-variate (shell length) and of all three factors, site, exposures and month on body weight. However, there were significant interactions among the different factors.

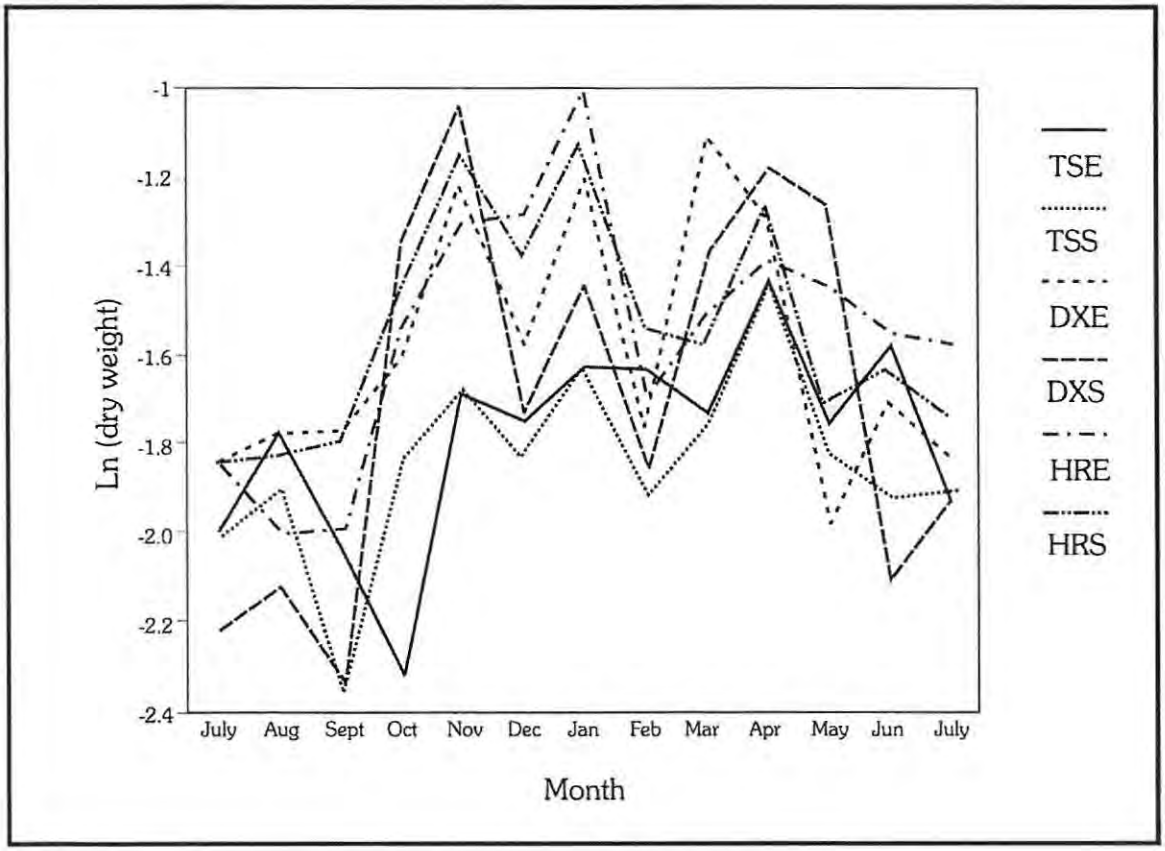
Again, the fundamental reproductive cycles of the six mussel populations were broadly similar (Fig 3.1). The general seasonal pattern of weight change was the same for all populations. The timing of major decreases and increases in magnitude of dry body weight was reasonably similar in all populations, i.e. five of the six populations tended to be synchronised with respect to the timing of maximum and minimum values of dry body weight. However, such maxima and minima in the dry weight values were not clearly marked in both Three Sisters Exposed and Sheltered (TSE and TSS, respectively) populations. This is mainly because of intermittent spawning, and as a result the gonad weight never reached very high levels.

The results also show two major spawning events at most sites between Jul - Sep/Oct 1997 and May- Jul 1998. Also two partial spawning events were observed in December 1997 and February 1998. Despite the recorded similarity in the fundamental pattern of the breeding cycles, there were significant differences in the magnitude of dry body weight among the different sites on a

Table 3.1 Summary of all effects

Sources of variation	df	F-ratios	Significant Levels
Co-variate :			
shell length	1	12 194.36	<0.0001
Main effects :			
Site (S)	2	401.38	<0.0001
Exposure (E)	1	51.06	<0.0001
Month (M)	12	251.12	<0.0001
Interactions:			
SE	2	52.14	<0.0001
SM	24	29.42	<0.0001
EM	12	20.10	<0.0001
SEM	24	8.65	<0.0001
Residual	3040		

Fig 3.1 Comparison of monthly dry body weight of a standard animal among six mussel populations at Three Sisters (TS), Diaz Cross (DX) and High Rocks (HR) at exposed (E) and sheltered (S) sites between July 1997 and July 1998.

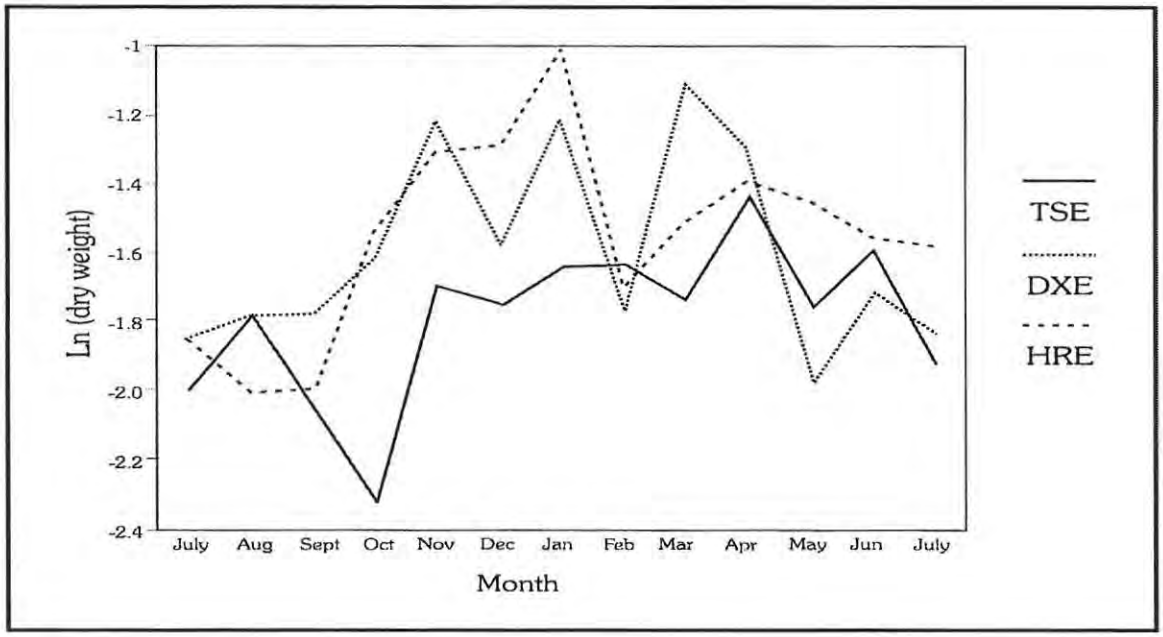


finer monthly scale. The six populations did not show consistent ranking by dry body weight, as shown by the interaction between site and month. When the three different locations were compared at the same exposure (Fig 3. 2), it was apparent that there was no clear hierarchical trend in values of dry weight among the populations, although Three Sisters tended to show the lowest values in both exposed and sheltered comparisons. There was a tendency for one population to show the highest value of dry body weight in one month and the least value in another. The similarity in the basic pattern of the breeding cycle did not depend on the proximity of the populations either. Site and exposure did not always show coinciding peaks.

The significant interaction between site and exposure implies that the effect of exposure on body weight differed at different sites (Fig.3.3). There was no hierarchical trend between the sheltered and exposed populations in magnitude of the dry weight among the three locations. When comparing the different sites at the same exposure and sampling period, they exhibited different magnitudes in dry weight values. Variability in the magnitude of dry weight values was independent of the degree of wave exposure. The interaction between site and exposure thus, indicates that the degree of exposure to wave action did not have a consistent effect on magnitude of the gonad index.

Lastly, there was also a significant interaction between site, exposure and month. This indicates that body weight was significantly affected by month, site and exposure in a complex fashion. The exact timing of weight increases and decreases differed among sites and between exposures, when considered on a monthly scale.

Fig 3.2 Comparison of monthly dry body weight of a standard animal among wave exposed and wave sheltered shores at Three Sisters (TS), Diaz Cross (DX) and High Rocks (HR) between July 1997 and July 1998.



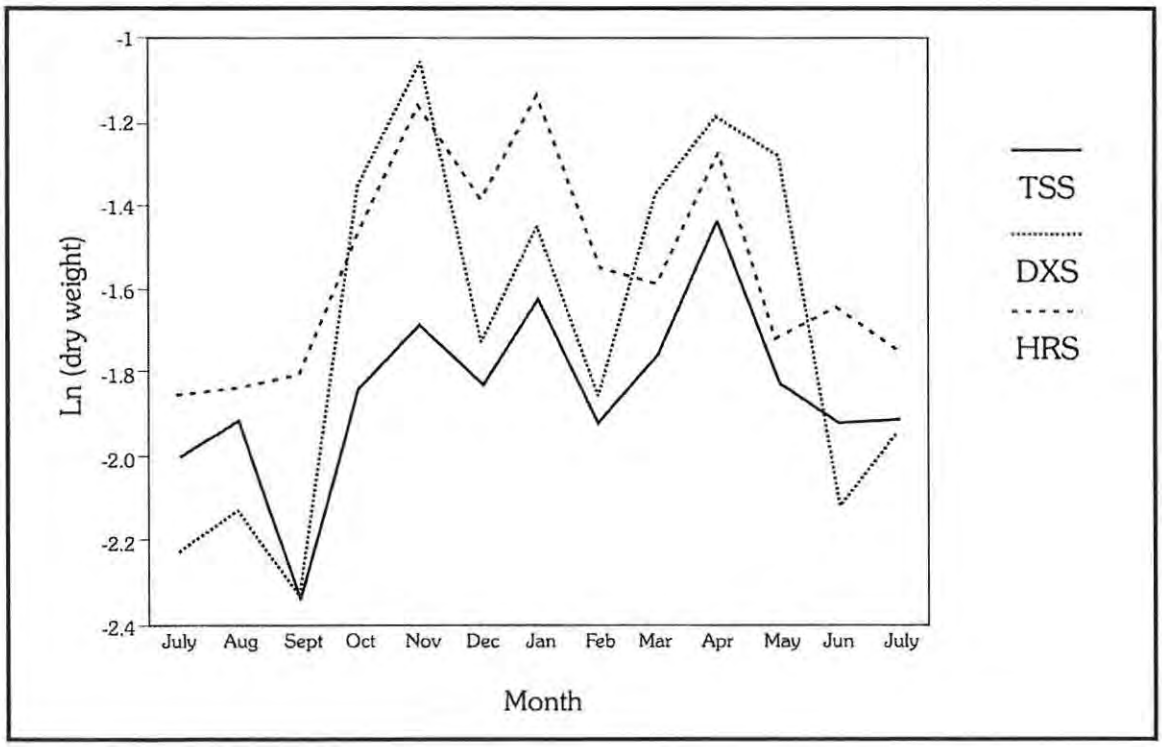
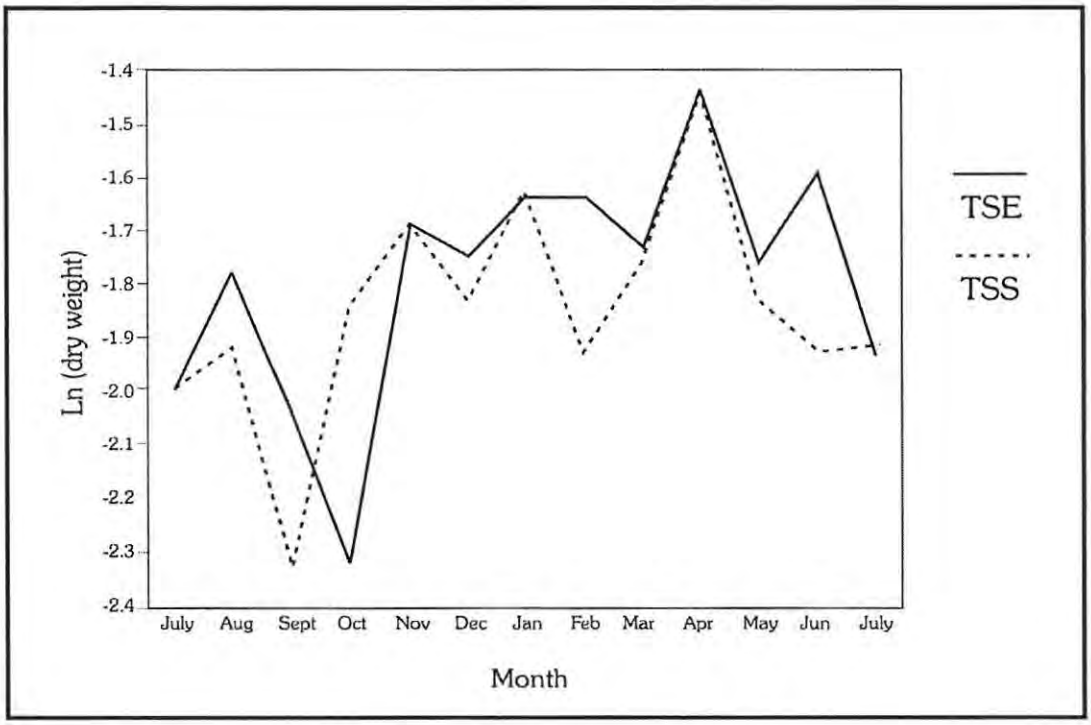


Fig 3.3 Within-location comparisons of monthly dry body weight at Three Sisters, Diaz Cross and High Rocks between July 1997 and July 1998.



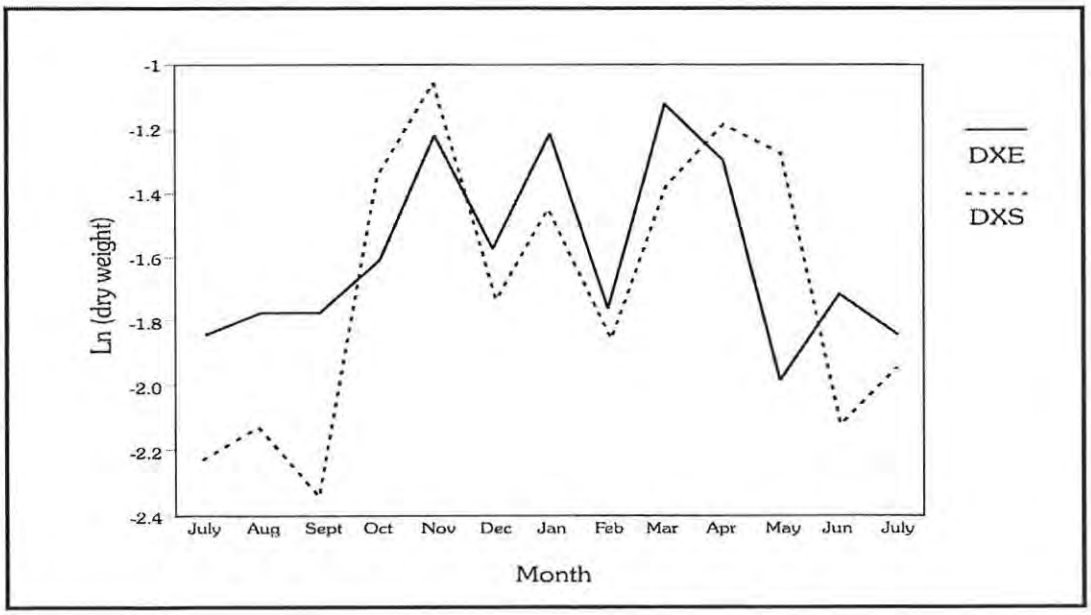
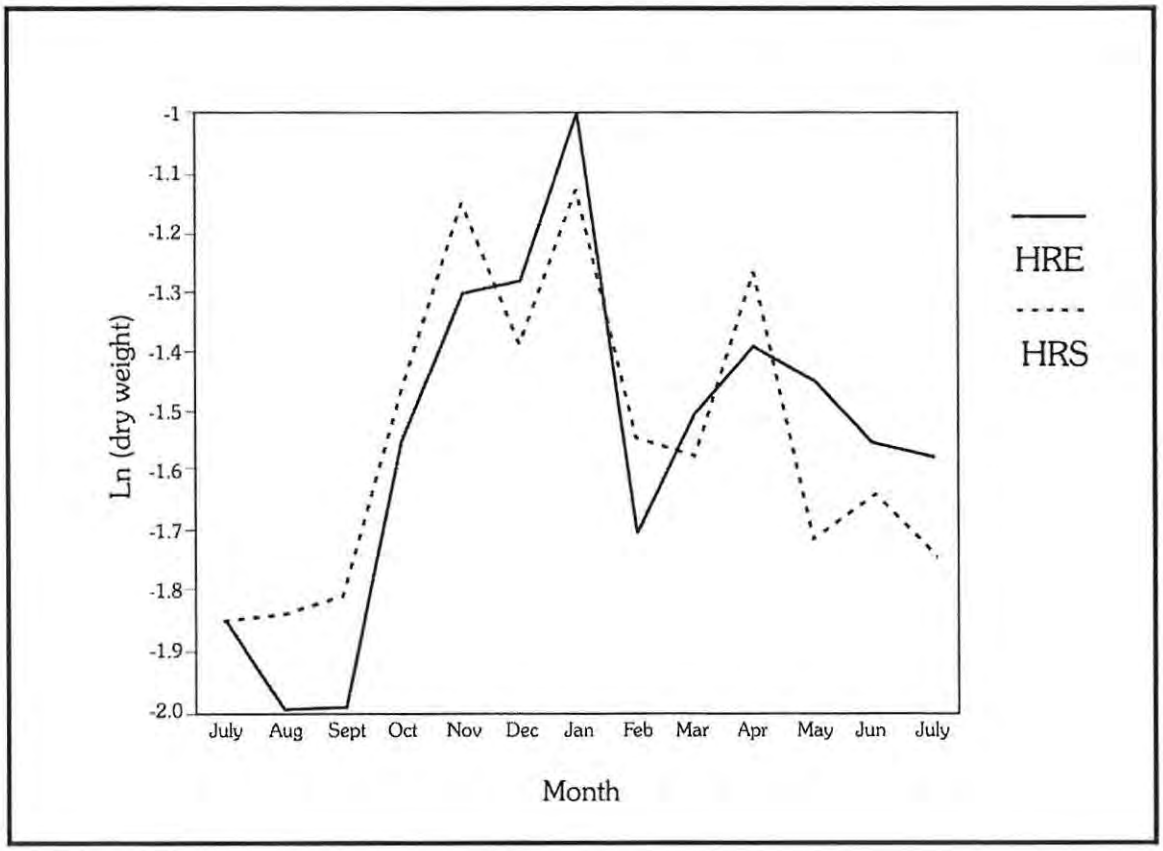


Fig 3.3 Within-location comparisons continued.



3.4 Discussion

The basic spawning pattern was similar in all populations. The overall timing of the maximum losses and increases in the dry body weight was reasonably similar in all populations, i.e. at least five of the six population tended to be synchronised with respect to the major drops and increases in dry body weight. However, Three Sisters populations seemed to exhibit an intermittent spawning pattern, as indicated by the absence of well-marked weight maxima and minima.

There were only two distinct spawning peaks observed over the 13 months of sampling. Both incidents of spawning occurred at the same period (in winter) in each year (i.e. Jul - Oct in 1997 and Apr - Jul in 1998). However, the spawning period was rather extended in 1997. *Perna perna* therefore has one major spawning period, in winter, followed by minor spawning activities in summer (December 1997 and February 1998). The lowest values of dry body weight were recorded following spawning during winter in most populations. The results of the histological sections in chapter 2 revealed that there was no quiescent stage in the development of the gonad in *P. perna*. The gonad is in a continuous state of development, and new eggs develop as the mature ones are released. The minor spawning activities observed in December 1997 and February 1998 are a result of the subsequent release of the unspawned residual eggs.

Although there were significant effects of all three factors (site, exposure and month) on dry body weight, there were significant interactions between these factors. There were temporal differences in the reproductive events on a finer monthly scale, and between adjacent mussel populations. This implies that there were no habitat-specific differences among the breeding

cycles of the populations. Moore and Reisch (1968) also noted temporal variation in the reproductive cycles of adjacent populations in populations of *Mytilus edulis*. Newell *et al.*, (1982) also recorded the largest differences in reproductive cycles in two mussel populations that were closest to each other. It is unlikely that breeding cycle patterns of these populations are governed by the degree of wave action *per se*, for exposure did not have a clear effect on the dry body weight, or on the timing of spawning. Thus, the results of this study do not corroborate the broad generalization that exposure to wave action has an overriding effect on timing of reproductive events. Although site, exposure and month had significant effects on dry body weight, the factors that controlled the overall pattern of the breeding cycles were common over the geographic range of the six populations and affected the timing of spawning irrespective of the degree of wave exposure.

There have been numerous attempts to correlate reproductive cycles with prevailing environmental conditions (as reviewed by Newell *et al.*, 1982). Whilst a variety of stimuli, including mechanical shock, temperature, salinity, tidal height, food availability, etc. have been suggested as being important in inducing spawning, the results have been confusing as no clear patterns have emerged. Perhaps some of the confusion has arisen in the search for a single triggering factor. A complex process such as the breeding cycle involves interaction of both exogenous and endogenous factors.

CHAPTER 4

SYNTHESIS

Chapter 4 : Synthesis

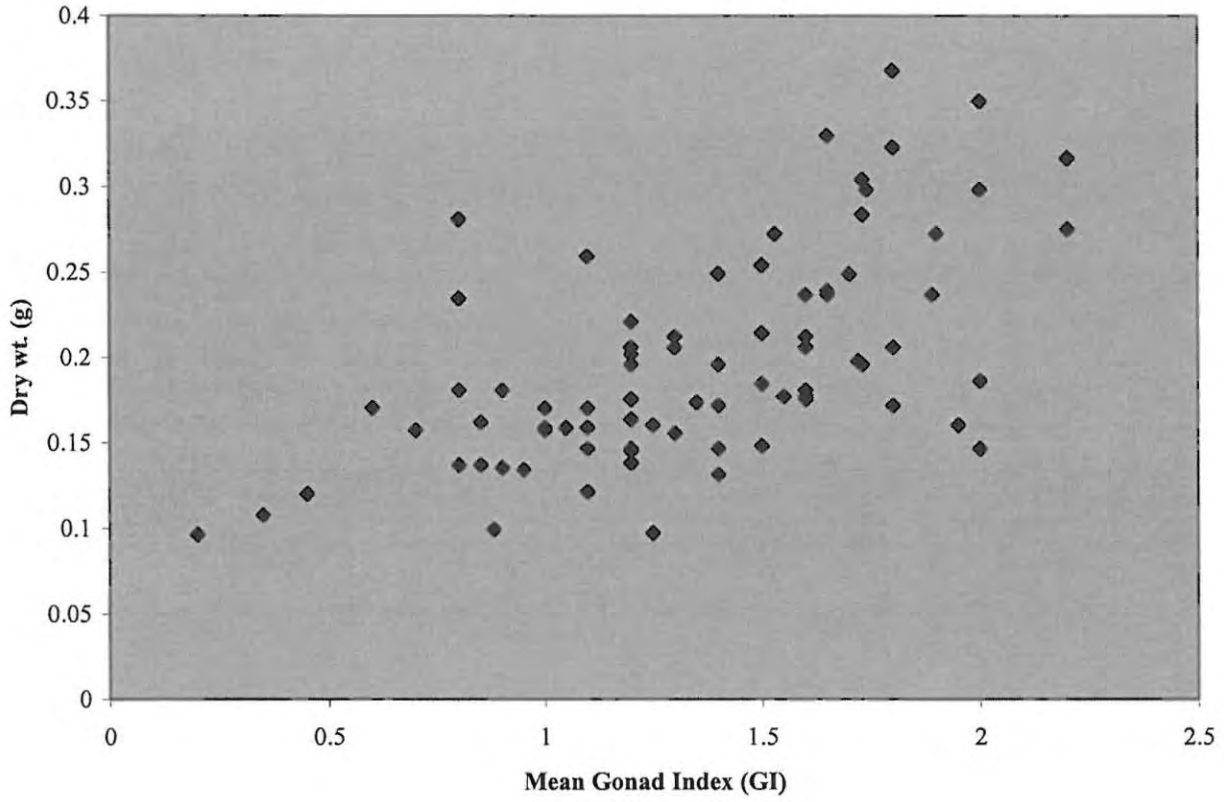
Studies of invertebrate reproduction are relatively expensive in terms of time, effort and money. Regular sampling should be carried over at least one year, and this involves a considerable amount of intensive and demanding laboratory work. Appropriate statistical methods should be applied to the resultant data, methods that will allow the objective examination of reproductive cycles. More than one technique should be used to obtain the best estimate of the reproductive pattern, since different techniques may each have disadvantages. The assumption that loss of body weight of an animal was due to gamete product loss seems reasonable, considering that the substantial losses in dry weight recorded in chapter 3 also often coincided with the spawning season identified in chapter 2. The spawning period was seen from the graphs as a marked drop in gonad maturity index (GI) in chapter 2, or as a major drop in dry body weight (ln dry weight) in chapter 3. Therefore, a positive correlation between the two data sets could also indicate the validity of the results obtained from the two protocols.

The results obtained from shell length/dry body weight regression and those obtained from histological sections of the reproductive tissue, complement each other well. The fundamental pattern of the breeding cycles of the six populations, as revealed by the two different techniques, was similar. Maximum spawning intensity was identified as being achieved roughly at the same time (in winter) with both techniques (i.e. at about Jul – Oct 1997 and May – Jul 1998). Although the exact timing of the stages of the gametogenic cycle was slightly different among the different populations, the broad annual cycle was consistently similar in all population as observed in the overall results of both techniques. Furthermore, a significant positive correlation

($r = 0.605$; $n = 78$ and $P < 0.0001$, Fig. 4.1) was established between the two data sets using weight and GI data for the same populations each month. Gonads regressed and reached minimum values between July and September 1997 as well as between April and July in 1998. However, the duration of spawning activity was shorter in 1998 than was the previous spawning event (1997). Thompson (1979) has previously noted annual variations in maximum reproductive condition in a population of *Mytilus edulis*. Griffiths (1977) attributed such variations in South African populations of *Choromytilus meridionalis* to variable food availability. Yet, there was no consistent effect of exposure on body weight observed in chapter 3, although one would expect mussels on exposed shores to have more food and growth rates of *Perna perna* are significantly higher under exposed conditions (McQuaid & Lindsay, 2000). On the Transkei coast, *P. perna* has an extended breeding season characterised by low levels of spawning activity from February to September (Lasiak, 1986). The results of Berry's study (1978) on the Natal coast on *P.perna* show a spawning pattern closer to the findings of this study. Two well-defined spawning peaks were observed in winter (May – August) and in spring (September- October) (Berry, 1978). However, Berry also noted that spawning activity tended to take place over an extended period, with 25% of the population breeding for three to six months.

The results obtained from both techniques consistently showed significant interactions between at least site/month and month/exposure. Because exposure was nested in site (chapter 2), interactions between site, exposure and month, (SEM) could not be estimated. All the same, reproduction at all the sites was relatively synchronised on a seasonal basis, but within seasons, 1. there was variation in timing; 2. synchrony didn't depend on proximity of sites, furthermore, 3. exposure did not have a consistent effect on the reproductive cycle.

Fig 4.1 Correlation between dry weight of a standard animal and mean population gonad index (GI). r = 0.605; n = 78 and P < 0.0001.



Temporal variation in the duration of different stages of the reproductive cycle of adjacent mussel populations, as observed in this study has previously been recorded elsewhere. Wilson and Hodgkin (1967), and Berry (1978) compared the reproductive cycles of different populations of *Perna perna* and concluded that temperature controls broader aspects of the reproductive cycle (i.e. duration and season of gametogenic activity). Differences in finer details (i.e. spawning season and number of spawning peaks) are controlled by unknown factors besides temperature. In an attempt to link sea temperatures with the breeding pattern in limpets, Gray & Hodgson (1997) observed that there was no significant correlation between sea temperatures and spawning, but that an increase in gonad index did correspond to an increase sea temperatures. These findings suggest that temperature is a chief determining factor in regulation of the broader aspects of the reproductive cycles. But, the restriction of spawning to a part of the of reproduction season indicates that factors besides optimal temperatures play an important role in determining the actual spawning period within the reproductive season. For example, Starr *et al.* (1989) recorded a direct coupling of marine invertebrate spawning with phytoplankton blooms. The results of this study do not corroborate the generalization that, because of its influence on food availability, wave exposure has a consistent effect on the gonad condition. Griffiths (1981) observed that there were no significant weight differences between sublittoral and upper-shore zone populations of *Choromytilus meridionalis*, and concluded that reduced feeding time affected growth rate but not flesh or gonad weight. This indicates that species living under conditions of limited food resources may adjust various components of the energy budget to maintain a positive energy balance or scope for growth (Griffiths, 1981).

Of the predictable events underlying synchronous spawning, no single environmental parameter measured was entirely responsible for controlling the timing of spawning in this study. The implication is that ovary development is cued by environmental factor(s) that operate over scales of at least 10-20km, rather than more localized events that may affect food availability either through aggregation of food (local hydrography at different localities) or food delivery to the shore (degree of wave action at different sites). The literature on predictable synchronous spawning events (reviewed by Watson *et al.*, 2000) also suggests that no single environmental factor is wholly responsible for spawning. Although lunar periodicity accurately predicted the spawning date in Palolo worms, temporal variation in time of the solar day at which spawning occurred in different localities were not accounted for (Watson *et al.*, 2000). It is therefore important that environmental cues triggering spawning must be translated by the organism's endogenous control mechanisms, which initiate endocrine changes that finally trigger spawning. More frequent sampling coupled with continuous environmental monitoring is needed to clarify the apparent relationship between environmental factors and spawning activity in the mussel *Perna perna*.

APPENDIX

Appendix 1. Mean Gonad Maturity Index (GI) for six populations of *Perna perna*.

Month	Site	Exposure	Mean GI
Jul-97	TS	E	0.85
Jul-97	TS	S	0.95
Jul-97	DX	E	0.7
Jul-97	DX	S	0.35
Jul-97	HR	E	1
Jul-97	HR	S	1
Aug-97	TS	E	1
Aug-97	TS	S	1.1
Aug-97	DX	E	1.1
Aug-97	DX	S	0.45
Aug-97	HR	E	0.9
Aug-97	HR	S	1.1
Sep-97	TS	E	1.4
Sep-97	TS	S	1.25
Sep-97	DX	E	0.6
Sep-97	DX	S	0.2
Sep-97	HR	E	0.8
Sep-97	HR	S	1.2
Oct-97	TS	E	0.88
Oct-97	TS	S	1.05
Oct-97	DX	E	1.05
Oct-97	DX	S	1.1
Oct-97	HR	E	1.5
Oct-97	HR	S	1.6
Nov-97	TS	E	1.55
Nov-97	TS	S	2

Month	Site	Exposure	Mean GI
Dec-97	DX	E	1.8
Dec-97	DX	S	1.6
Dec-97	HR	E	2.2
Dec-97	HR	S	1.7
Jan-98	TS	E	1.73
Jan-98	TS	S	1.72
Jan-98	DX	E	2
Jan-98	DX	S	1.65
Jan-98	HR	E	1.8
Jan-98	HR	S	1.8
Feb-98	TS	E	1.4
Feb-98	TS	S	2
Feb-98	DX	E	1.8
Feb-98	DX	S	1.3
Feb-98	HR	E	1.6
Feb-98	HR	S	1.6
Mar-98	TS	E	1.35
Mar-98	TS	S	1.55
Mar-98	DX	E	1.65
Mar-98	DX	S	1.5
Mar-98	HR	E	1.2
Mar-98	HR	S	1.3
Apr-98	TS	E	1.89
Apr-98	TS	S	1.65
Apr-98	DX	E	1.53
Apr-98	DX	S	1.73
Apr-98	HR	E	1.4
Apr-98	HR	S	1.73
May-98	TS	E	1.4
May-98	TS	S	0.85
May-98	DX	E	1.2

Month	Site	Exposure	Mean GI
May-98	DX	S	0.8
May-98	HR	E	0.8
May-98	HR	S	0.8
Jun-98	TS	E	1.2
Jun-98	TS	S	1.2
Jun-98	DX	E	0.9
Jun-98	DX	S	1.1
Jun-98	HR	E	1.3
Jun-98	HR	S	1.2
Jul-98	TS	E	1.4
Jul-98	TS	S	1.5
Jul-98	DX	E	1.95
Jul-98	DX	S	1.2
Jul-98	HR	E	1.6
Jul-98	HR	S	1.6

Appendix 2a. Categories of gonad development of individuals at Three Sisters Exposed site (TSE) throughout sampling period. ***Note: different animals were used in each sampling month.***

Indiv	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
1	F	F	F	F	F	E	E	F	F	E	F	E	E
2	F	E	E		C	F	E	F	F	E	F	E	F
3	F		E		E	E	F	F	F	E	F	F	E
4	S	S	F	F	E	F		E	E	D	F	F	E
5	S	S	E	S	E	F	F	F	E	F	E	F	F
6	F	S	E	F	F	E	E	E	E	F	E	F	E
7	F	E	F	F	E	F	E	F	E	F	E	F	F
8	F	F	F	F	S	F	E	F	S	E	E	F	F
9	F	F	E	F	S	E	F	E	E	E	F	F	F
10	E	F	F		F	F	F	F	E	E	F	F	E
11	F	E	E	S	F	F	E	F	F	E	C		F
12	S	F	E	E	E	F	E	E	F	E	E	F	F
13	E	E	F	F	E	F	E	F	E	E	F	F	E
14	S	F	F	F	E	F	E	F	F		F	E	F
15	F	F	F	F	F	E	E	E	E	E	F	F	F
16	F	F	E	S	E	F	E	E	F	E	F	F	E
17	S	F	F	F	F	F	E	F	F	E	F	F	F
18	E	F	E	F	F	F	E	E	F	E	F	E	F
19	S	F	F	F	F	F	E	B	F	E	E	E	F
20	F	F	S	F	E	F	F	F	F		F	E	E

Appendix 2b. Categories of gonad development of individuals at Three Sisters Sheltered site (TSS) throughout sampling period.

Indiv	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
1	F	F	F	F	E	E	F	E	E	E	F	E	F
2	F	S	E	F	E	E	E	E	E	F	S	E	E
3	F		F	F	F	E		E	E	E	F	F	E
4	E	S	E	F	E	F	E	E	F	E	F	F	F
5	S	F	E	F	E	F	E	E	E	E	S	F	F
6	F	F	F	F	D	E	E	E	F	F	F	F	E
7	S	S	S	F	E	F	E	E	F	E	F	F	E
8	F	F	F	F	E	F	E	D	E	E	F	F	F
9	F	F	F	E	E	F	E	E	E	F	F	F	E
10	F	F	F	F	E	F	E	E	F	F	F	F	E
11	E	E	F	F	F	F	F	E	E	E	F	F	F
12	F	E	F	S	F	F	E	E	E	F	F	F	F
13	F	E	F	F	B	F	F	E	E	E	F	F	F
14	F	E	F	F	E	F	E	E	F	E	S	F	F
15	F	F	F	F	E	F	F	E	F	E	F	F	E
16	S	F	E	F	E	E	E	E	E	E	F	E	F
17	E	F	E	F	E	F	E	F	F	F	F	E	E
18	S	S	F	F	E	F	E	E	F	F	F	F	E
19	F	F	F	E	D	F	F	E	E	E	F	F	E
20	F	E	E	F	D	F		E	F	E	F	F	F

Appendix 2c. Categories of gonad development of individuals at Diaz Cross Exposed site (DXE) throughout sampling period.

Indiv.	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
1	S	F	F	F	F	E	E	F	F	E	F	F	E
2	F	F	F	F	E	E	E	E	F	E	E	F	E
3	F	F	F	E	E	E	E	E	F	F	F	E	E
4	F	E	S	F	E	E		E	E	F	F	F	E
5	F	F	S	E	E	E	E	F	E	F	F	F	E
6	E	F	S	F	B	F	E	E	E	F	E	E	E
7	S	F	S	F	E	E	E	F	E	F	F	F	E
8	S	E	S	F		E	E	E	E		F	F	E
9	F	F	S	F	E	E		F	E	F	F	F	E
10	F	S	F	F	E	E	E	E	E	E	E	F	E
11	S	E	F	S	E	E		E	E	E	F	F	E
12	F	F	S	E	E	F	E	E	E	E	F	F	E
13	F	F	F	F	E	E	E	E	E	E	F	F	E
14	F	E	F	F	F	E	E	E	F	F	F	F	E
15	S	F	F	S	E	E	E	E	E	E	F	S	E
16	F	F	F	F	F	E	E	E	F	E	F	F	E
17	S	F	F	F	E	E	E	E	E	E	F	S	F
18	E	F	F	F	F	E	E	E	F	F	B	F	E
19	S	S	F	F	B	E	E	E	E	E	F	F	E
20	S	F	S	F	F	F	E	E	F	F	F	F	E

Appendix 2d. Categories of gonad development of individuals at Diaz Cross Sheltered site (DXS) throughout sampling period.

Indiv	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
1	S	F	F	F	E	E	E	F	E	E	F	F	F
2	S	S	S	E	E	F	F	E	F	E	F	F	E
3	S	F	F	F	E	F	F	F	F	F	F	F	F
4	F	F	F	E	E	E	F	F	E	E	F	F	F
5	F	F	F	F	E	E	E	E	E	E	F	E	E
6	S	F	S	F	E	F	F	F	E	E	F	E	F
7	S	F	S	F	E	E	F	E	F	F	F	F	E
8	F	S	S	E	E	F	E	E	E	E	S	F	F
9	S	S	S		E	E	E	F	F	F	F	F	E
10	F	S	S	F	E	F	E	F	E	E	F	E	E
11	S	F		F	F	F	E	F	F		S	F	F
12	F	S		F	E	E	E	F	E	F	F	E	F
13	F	S	S	F	C	E	F	E	F		F	E	F
14	S	S		F	E	F	E	E	F		F	S	F
15		F		E	E	F	E	F	F	E	F	S	F
16	S	S		F	E	E	E	F	F	E	F	E	F
17		S	S	F	E	E	E	F	E	E	F	S	F
18	S	F		S	E	F	F	E	F	E	F	S	F
19		S	S	F	E	E	E	F	E		S	F	F
20	S	S	S	E	E	E	E	F	E		F	F	F

Appendix 2e. Categories of gonad development of individuals at High Rocks Exposed site (HRE) throughout sampling period.

Indiv	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
1	F	S	F	S	B	E	F	E	F	F	F	F	E
2	F	F	F	F	D	E	E	E	F	F	F	F	F
3	F		F	S	B	E	F	E	F	F	F	F	E
4	F	F	S	F	E	E	E	E	F	E	F	F	E
5	S	F	F	E	E	C	E	E	F	F	S	F	E
6	F	F	F	F	E	D	E	E	F	F	B	F	E
7	F	S	F	F	E	D	E	E	F	E	F	E	E
8	F	E	F	E	E	D	F	E	E	F		E	F
9	F	E	F	F	E	E	E	E	F	F	F	F	F
10	F	S	E	F	F	D	E	F	E	E	F	F	F
11	E	E	S	F	E	E	E	F	E	E	F	E	F
12	E	F	F	F	F	D	E	E	F	F	F	E	E
13	F	S	F	F	E	E	F	F	E	F	F		F
14	F	E	S	F	E	E	E	E	F	F	S	F	E
15	S	S	S	F	E	E	F	F	F	F	S	F	E
16	E	F	F	E	E	F	E	F	F	E	S	F	E
17	E	F	F	E	E	E	E	F	F	E	F	F	E
18	S	F	S	F	E	E	E	E	F	E	F	E	E
19	F	F	F	E	E	E	E		F	E	F	F	E
20	F	F	F	F	E	E	E	E	F	F	F	E	S

Appendix 2f. Categories of gonad development of individuals at High Rocks (HRS) throughout sampling period.

Indiv	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
1	S	E	E	E	E	F	F	F	F	E	S	F	F
2	E	E	F	E	E	C	E	E	F	E	S	F	E
3	S	F	F	F	C	E	E	E	E	E	F	F	E
4	E	F	F	F	E	E	F	E	E	F	F	F	F
5	S	E	E	E	E	E	E	F	F	E	F	F	E
6	F	F	E	E	D	F	E	E	F		F	E	F
7	F	F	S	E	E	E	F	F	F		F	E	E
8	F	F	F	F	E	E	E	F	F	F	F	E	E
9	F	F	F	F	E	E	E	E	E	E	F	F	E
10	F	S	E	E	E	F	E	F	F	E	F	F	S
11	E	S	F	C	E	E	E	E	F	E	S	F	E
12	F	F	F	E	E	E	E	E	F	E	F	E	E
13	F	E	E	E	E	F	E	E	E	E	F	E	F
14	F	S	S	E	E	E	E	E	F		F	F	F
15	F	E	F	E	C	E	E	E	F	F	F	F	E
16	E	S	F	E	E	F	E	E	F	E	F	E	F
17	F	S	F	E	D	F	F	F	F		F	E	E
18	F	E	F	S	E	E	E	E	E		F	S	E
19	F	F	F	F	D	E	E	F	F	E	F	S	E
20	F	E	E	S	E	E	E	F	E	F	F	S	E

Appendix 3. Correlation between monthly gonad maturity index (GI) and dry body weights (ln dry weight) of a standard animal in the six sampled sites.

Site	Exposure	Month	ln Dry Wt.	Mean GI
TS	E	Jul-97	-1.99349	0.95
TS	S	Jul-97	-2.00621	0.85
DX	E	Jul-97	-1.84649	0.7
DX	S	Jul-97	-2.22561	0.35
HR	E	Jul-97	-1.84362	1
HR	S	Jul-97	-1.84854	1
TS	E	Aug-97	-1.77554	1.1
TS	S	Aug-97	-1.91602	1
DX	E	Aug-97	-1.77478	1.1
DX	S	Aug-97	-2.12283	0.45
HR	E	Aug-97	-1.99825	0.9
HR	S	Aug-97	-1.83766	1.1
TS	E	Sep-97	-2.03484	1.25
TS	S	Sep-97	-2.3263	1.4
DX	E	Sep-97	-1.77478	0.6
DX	S	Sep-97	-2.34311	0.2
HR	E	Sep-97	-1.99163	0.8
HR	S	Sep-97	-1.80706	1.2
TS	E	Oct-97	-2.3164	1.05
TS	S	Oct-97	-1.83814	0.88
DX	E	Oct-97	-1.60495	1.2
DX	S	Oct-97	-1.35157	1.1
HR	E	Oct-97	-1.5457	1.5
HR	S	Oct-97	-1.44511	1.6
TS	E	Nov-97	-1.69175	2
TS	S	Nov-97	-1.68212	1.5
DX	E	Nov-97	-1.2157	1.74
DX	S	Nov-97	-1.04955	2
HR	E	Nov-97	-1.30502	1.9
HR	S	Nov-97	-1.15385	2.2
TS	E	Dec-97	-1.7451	1.25
TS	S	Dec-97	-1.83011	1.2
DX	E	Dec-97	-1.58224	1.8
DX	S	Dec-97	-1.73001	1.6

HR	E	Dec-97	-1.28663	2.2
HR	S	Dec-97	-1.39233	1.7
TS	E	Jan-98	-1.63391	1.72
TS	S	Jan-98	-1.62266	1.73
DX	E	Jan-98	-1.20985	2
DX	S	Jan-98	-1.44535	1.65
HR	E	Jan-98	-1.00134	1.8
HR	S	Jan-98	-1.12602	1.8
TS	E	Feb-98	-1.63391	2
TS	S	Feb-98	-1.92417	1.4
DX	E	Feb-98	-1.76399	1.8
DX	S	Feb-98	-1.85866	1.3
HR	E	Feb-98	-1.70744	1.6
HR	S	Feb-98	-1.54613	1.6
TS	E	Mar-98	-1.73203	1.35
TS	S	Mar-98	-1.75344	1.55
DX	E	Mar-98	-1.11159	1.65
DX	S	Mar-98	-1.37899	1.5
HR	E	Mar-98	-1.51259	1.2
HR	S	Mar-98	-1.57893	1.3
TS	E	Apr-98	-1.43658	1.65
TS	S	Apr-98	-1.43252	1.89
DX	E	Apr-98	-1.29761	1.53
DX	S	Apr-98	-1.1875	1.73
HR	E	Apr-98	-1.39506	1.4
HR	S	Apr-98	-1.26542	1.73
TS	E	May-98	-1.75877	0.85
TS	S	May-98	-1.8223	1.4
DX	E	May-98	-1.98104	1.2
DX	S	May-98	-1.27534	0.8
HR	E	May-98	-1.45521	0.8
HR	S	May-98	-1.71679	0.8
TS	E	Jun-98	-1.5841	1.2
TS	S	Jun-98	-1.9196	1.2
DX	E	Jun-98	-1.70926	0.9
DX	S	Jun-98	-2.11414	1.1
HR	E	Jun-98	-1.55529	1.3
HR	S	Jun-98	-1.63847	1.2
TS	E	Jul-98	-1.92455	1.5

TS	S	Jul-98	-1.9092	1.4
DX	E	Jul-98	-1.83207	1.95
DX	S	Jul-98	-1.93581	1.2
HR	E	Jul-98	-1.57756	1.6
HR	S	Jul-98	-1.73719	1.6

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