Spatio-temporal factors affecting the growth of cultured silver-lip pearl oyster, *Pinctada maxima* (Jameson) (Mollusca: Pteriidae) in West Papua, Indonesia

Thesis submitted by

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Pinctada maxima oyster
with silver and gold South Sea pearls

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Anne Michelle Lee Date

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Abstract

This thesis addressed various growth aspects of cultivating *P. maxima* in a commercial farm in Indonesia with special emphasis on the influence of the environment. *P. maxima* of three age-classes were grown at three sites (Ganan, Manselo and Batu Terio) and two depths over a period of 18 months and various aspects of somatic growth (linear and weight measurements), gonad growth (visual and histological observations) and factors which influence growth (environmental and biological) were monitored.

Environmental monitoring of the three sites and two depths showed that seawater parameters varied spatially between sites and depths as well as temporally throughout the sampling period. Some of the parameters measured were physico-chemical properties (water temperature, salinity, pH) and particulate matter (suspended particulate matter, particulate organic matter and chlorophyll *a*, *b* and *c*). These environmental descriptors provide the basis for comparison of growth rates of *P. maxima* held at the three sites in subsequent chapters.

Total growth (G_T) and monthly instantaneous growth (G_{30}) of both length and weight were computed as part of the study on growth of *P. maxima.* G_{30} is a better indicator of growth as it is a standardised measure and permits comparison to be made between various age-classes of oysters with different shell sizes as well as at a specific point in time. Growth studies showed age has an inverse relationship on *P. maxima* growth, with both G_T and G_{30} decreasing with increasing age. Multivariate testing showed that growth of all age classes was affected by culture depth, but not culture site. Growth rate at a depth of 5 m was higher than at 15 m. When oysters were partitioned by size before analysis, there was a site effect on growth rate for medium and small oysters. Spatial differences in *P. maxima* growth was shown to be linked to the local culture environment, where variation in somatic grow was influenced by varying pH, salinity and pH levels, and gonad growth was influenced by water temperature, pH, SPM, POM.

Growth of *P. maxima* was fitted into five mathematical models i.e Special Von Bertalanffy Growth Function (VBGF), General VBGF, Gompertz, Richards and Logistic and various growth parameters computed. The criteria used for best fit was low mean residual sum of squares (MRSS), high coefficient of determination (r^2) and low deviation of the asymptotic length (L_∞) from the maximum length (L_{max}). Based on these criteria, the Special VBGF and the General VBGF equally provided the best fit to length-at-age data for all the pearl oysters grown at the area. However, when data was plotted, General VBGF tended to underestimate L_∞. The Special VBGF best described the growth of *P. maxima* cultured at the farm, with growth parameters estimates of L_∞ = 168.38 mm, K = 0.930 y^{-1}, t_0 = 0.126.

Biofouling studies showed that six classes of macro-fouling which settled on the shells of *P. maxima* were invertebrates from the classes Maxillopoda, Polychaeta, Bivalvia, Demospongiae, Foraminifera and Ascidicea. The quantity and diversity of biofouling was found to affect growth in medium and small oysters. The spatial and temporal variation in quantity and diversity of the six classes of biofouling was in turn affected by various environmental parameters. Regression analysis provided information on environmental parameters acting in concert to affect biofouling while principal component analysis showed the interaction between different biofouling taxa and environmental parameter. Together, they allowed examination of the interaction between various parameters, apportionment of environmental factors towards taxa of fouling and the degree a particular environmental variable affects fouling. Chlorophyll levels, pH and salinity were found to have a greater affect on biofouling settlement than SPM, POM and seawater temperature.

Macroscopic investigation of gonads and comparison to histological data in this study support previous reports that gonad colour and appearance may be used to determine sex and stage of development in *P. maxima*. A fundamental difference in the colour and the area occupied by the developing gametes made it possible to distinguish between the gender and various stages of development of *P. maxima* oysters with relative ease. While most of the oysters observed appeared to be of indeterminate sex, enough male and female oysters were observed to show that gametogenesis in cultured *P. maxima* occurred between August to February, with spawning occurring twice during that period; once in October/November and again in February. Sex ratio

in cultured *P. maxima* was overwhelmingly biased towards maleness, with no spatial difference in sex ratio between oysters cultured at various sites and depths. The expression of maleness was weakly correlated to water temperature, pH and rainfall, while there was no correlation between femaleness and environmental descriptors. Size, and not age, was more important in determining the sex of *P. maxima*.

In summary, this research presented new data on growth of different age classes of *P. maxima* cultured in a farm situation in Indonesia. It has added to our knowledge the importance of various environmental factors and biofouling on somatic and gonadal growth of *P. maxima.* This information can be utilised to improve farming management practices through judicious selection of future culture sites. It is hoped that this will form a basis for further study into grow-out of *P. maxima* in the pearling industry in Indonesia and South-East Asia and lead to further improvement and expansion in the industry for the future

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CHAPTER 1

A brief introduction to pearl oyster culture, with emphasis on *Pinctada maxima*

1.1 Introduction

Pearls are produced through biological processes unlike physical methods that create other gems. The origin of pearls has been the source of many romantic notions in the past - the Chinese believed moonlight made pearls grow, while the Greeks held that pearls were dew from the moon collected by oysters (Strack, 2006). Today, cultured pearls are created in a far less mysterious fashion in pearl farms around the world.

1.2 Pearls in history

One of the oldest written references to pearls dates from 2206 BC (Kunz and Stevenson, 1908). They were also referred to in the Vedas, Bible, Koran and Talmud. The veneration of pearls spread to Europe with the campaign of Alexander the Great who linked the Orient with the Occident and paved the way for goods, crafts and cultures (Müller, 1997).

The importance of pearls continued throughout the ages well into the 20th century. By the turn of this century, pearls were one of the most popular jewels in the world. Today, pearls remain as popular as they were centuries ago; with a difference – pearl ownership is no longer restricted to royalty and the elite, but has become accessible to more people through their mass cultivation in pearl farms.

1.3 Formation of pearls

Natural pearls form when a foreign object such as a grain of sand or a parasite lodges itself into the soft tissues of a pearl forming mollusc. As the irritant enters the mollusc, some cells from the mantle may become attached to it or dislodged. These mantle cells may grow and divide to form a “pearl-sac” enclosing the particle or “nucleus”. Nacre or “mother-of-pearl” is deposited by the pearl sac to coat the irritant thus forming a pearl.

Spherical pearls are usually found loose within the soft body tissue of the oyster, whereas more irregularly shaped pearls (blister pearls) commonly form on the inner shells of pearl oysters (Taylor and Strack, 2008).

The composition of a cultured pearl is almost indistinguishable from a natural pearl. The difference is that a nucleus is generally much larger and is surgically implanted into the body of the pearl oyster using specialised tools. One or more nuclei, usually spherical, are implanted into the gonad of the pearl oyster together with a piece of mantle tissue from a donor pearl oyster. If the graft is successful, the mantle tissue eventually grows around the nucleus forming a pearl-sac and secretes nacreous deposits to form a pearl (Taylor and Strack, 2008). Half pearls or blisters, called "mabe" pearls are cultured by attaching one or several nuclei onto the inner shells of pearl oysters.

1.4 Brief history of pearl culture

From a commercial point of view cultured pearls first appeared in the 1920s (Strack, 2008). However, the Chinese had used freshwater mussels to coat small objects with a pearly layer as long as 3000 years ago (Farn, 1986). By the 12th century, pearl images of Buddha were produced by attaching carved templates onto the inner surfaces of the valves of freshwater mussels (Gervis and Sims, 1992).

The first patent to produce half pearls was awarded to Kokichi Mikimoto, who in 1896 successfully produced blister pearls in the Japanese akoya oyster, *Pinctada imbricata*[1]. In 1908, joint ownership of the method to produce spherical pearls was awarded to two Japanese, Tokishi Nishikawa and Tatsuhei Mise. However, it has been reported that the Japanese obtained the knowledge of this technique from an Australian, William Saville-Kent, who was believed to have produced the first spherical pearls from the pearl oyster, *Pinctada maxima*, in the 1890s, almost two decades before the Japanese (George, 1978). Regardless of who was the first to produce cultured pearls, Mikimoto went on to

[1] Earlier studies distinguished the Japanese akoya pearl oyster *Pinctada fucata martensii*, the Indian oyster *Pinctada fucata* and the eastern lingah shell from the Indian Ocean, *Pinctada vulgaris*, as separate species (Shirai, 1994). While these species have now been proposed to be from one species, *Pinctada imbricata* Röding, 1798 (Shirai, 1994), the complex of *Pinctada fucata-martensii-radiata- imbricata* has not been completely resolved (Wada and Tëmkin, 2008). Within this thesis, the different terminologies used by cited authors will be employed when reference is made to their relevant work. For general discussions, the terms *P. imbricata* or Akoya pearl oyster will be used.

dominate the cultured round pearl industry and brought worldwide acceptance to cultured pearls. Even today, the name Mikimoto is intimately associated with cultured pearls.

1.5 Species and distribution of commercial pearl oysters

It is generally accepted that many species of mollusc, under suitable conditions, are capable of producing pearls, although not necessarily good quality ones. Some species of pearl-bearing marine molluscs include pearl oysters (pteriidae), abalone (haliotidae) , the giant clams (tridacnidae), conch (strombidae) and nautilus (nautilidae) (Landman *et al*., 2001). Although pearls may occur in a variety of mollusc, those of commercial importance, on account of their brilliant lustre, are usually from oysters of the family Pteriidae within the genera of *Pinctada* and *Pteria*.

These two genera of cultivated marine oysters occupy a taxonomic position within:

Phylum:	Mollusca
Class:	Bivalvia
Subclass:	Pterimorphia
Suborder:	Pterioida or Mytiloida
Family:	Pteriidae
Genus:	*Pinctada* and *Pteria*

The commercially important pearl oysters include the silver-lip or gold-lip pearl oyster *Pinctada maxima,* the black-lip pearl oyster *P. margaritifera,* the akoya pearl oyster *P. imbricata*[1] and the winged oysters, *Pteria penguin* and *Pteria sterna.* Less important species include the Indian pearl oyster, *Pinctada fucata,* and the American pearl oyster, *Pinctada mazatlanica* (Southgate *et al.*, 2008). The species which this study is focused on is *P. maxima*.

[1] Earlier studies distinguished the Japanese akoya pearl oyster *Pinctada fucata martensii*, the Indian oyster *Pinctada fucata* and the eastern lingah shell from the Indian Ocean, *Pinctada vulgaris,* as separate species (Shirai, 1994). While these species have now been proposed to be from one species, *Pinctada imbricata* Röding, 1798 (Shirai, 1994), the complex of *Pinctada fucata-martensii-radiata- imbricata* has not been completely resolved (Wada and Tëmkin, 2008). Within this thesis, the different terminologies used by cited authors will be employed when reference is made to their relevant work. For general discussions, the terms *P. imbricata* or Akoya pearl oyster will be used.

1.5.1 Pinctada maxima

Pinctada maxima is the largest and thickest of the pearl oysters, with its shell growing up to 35 cm in length (Landman *et al*., 2001). It is distributed in warm waters over a large geographical range in the Indo-Pacific, from Burma to the Solomon Islands (Fig. 1.1). The range extends north to Hainan Island off the coast of China and south to the northern coastline of Australia (Gervis and Sims, 1992) approximately from the Abrolhos Islands in Western Australia and Harvey Bay on the east coast of Australia. The most prolific shell grounds are to be found in Australia, Papua New Guinea and the Philippines (George, 1978).

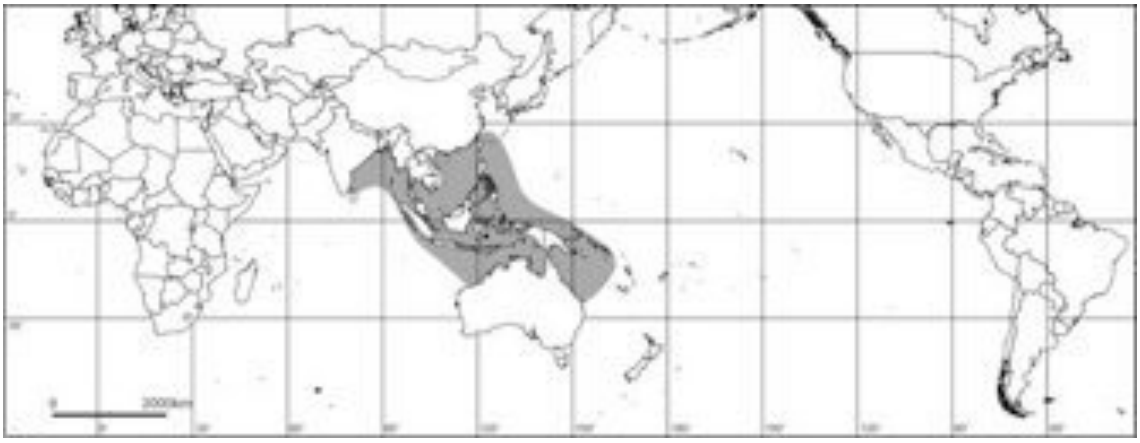

Fig. 1.1 Geographic distribution of *Pinctada maxima* (From Wada and Tëmkin, 2008).

Pearls from *P. maxima,* also known as South Sea pearls, are much sought after on account of their size and thick nacre, with pearls growing up 15 mm in diameter (Strack, 2006). Their colour range from silver and white to a deep gold, which are very rare and the most valuable of South Sea pearls. Cultivation of South Sea pearls is carried out through much of their natural geographical range, with Australia, Indonesia and the Philippines producing over 90% of the world's supply (Table 1.1).

Table 1.1 Production of cultured South Sea pearls from *P. maxima* in 2005 (Source: Henricus-Prematilleke, 2005)

Country	Volume (kg)	Value (US$ millions)
Indonesia	3750	85
Australia	3000	123
Philippines	1875	25
Myanmar	563	13
Malaysia	75	2
Papua New Guinea	75	Unknown
TOTAL	9338	248 million

1.6 Life cycle of pearl oysters

The life cycle of the pearl oyster is similar to that of other bivalves. External fertilisation takes place when eggs and sperm are discharged into the seawater. The unfertilised eggs are irregular in shape and become spherical when fertilised. The larval stages of a pearl oyster vary from 16 to 30 days depending on the species, water temperature, and availability of food and settlement substrate (Gervis and Sims, 1992). The larva grows through the straight-hinge or D-shape veliger, umbo, eyespot and pediveliger stages in the pelagic phase (Rose and Baker, 1994) before settling onto a suitable substratum as a sessile spat (Fig. 1.2). The larval stages of different species of pearl oysters have been studied in detail (Alagarswami *et. al*., 1983a, 1983c; Alagarswami *et. al*., 1989; Rose and Baker, 1994) and are compared in Table 1.2.

Juveniles attach to suitable surfaces by secreting thread-like tufts of byssal fibres. Byssus which are severed or damaged are renewed by fresh secretions (Farn, 1986). The juvenile stage lasts from 6 months to 2 years, depending on the species, after which the oysters mature as males. Pearl oysters are protandrous hermaphrodites with the ratio of

males to females tending to 1:1 with increasing age (Gervis and Sims, 1992). *P. maxima* mature as males during the first year when their shell height exceed 110 mm (Rose *et. al.*, 1990) while gonadal maturity in *P. margaritifera* is reached in the second year (Tranter, 1958a; Reed, 1966). Tranter (1959) reported that *P. fucata* reached sexual maturity 6 months after settlement, when their dorsoventral shell length measured between 2.6 cm and 3 cm.

Table 1.2 Timing of larval development for *P. maxima*, *P. margaritifera* and *P. fucata martensii* (Adapted from Gervis and Sims, 1992).
h = hours, m = minutes, d = day

	P. maxima[1]	*P. margaritifera*[2,3]	*P. fucata martensii*[4]
Egg spherical	0 h	0 h	0 h
Straight hinge or D shape	24 h	24 h	20 h 40 min
Early Umbo	d 6	d 9	-
Umbo	d 8	d 12	d 10 – d 12
Eye Spot	d 15	d 16	d 15
Pediveliger	d 18	d 20	d 20
Plantigrade	d 18 – d 25	d 20	d 22

[1]Rose and Baker (1994)
[2]Alagarswami *et al*. (1989)
[3]Doroudi and Southgate (2000)
[4]Alagarswami *et al*. (1983b)

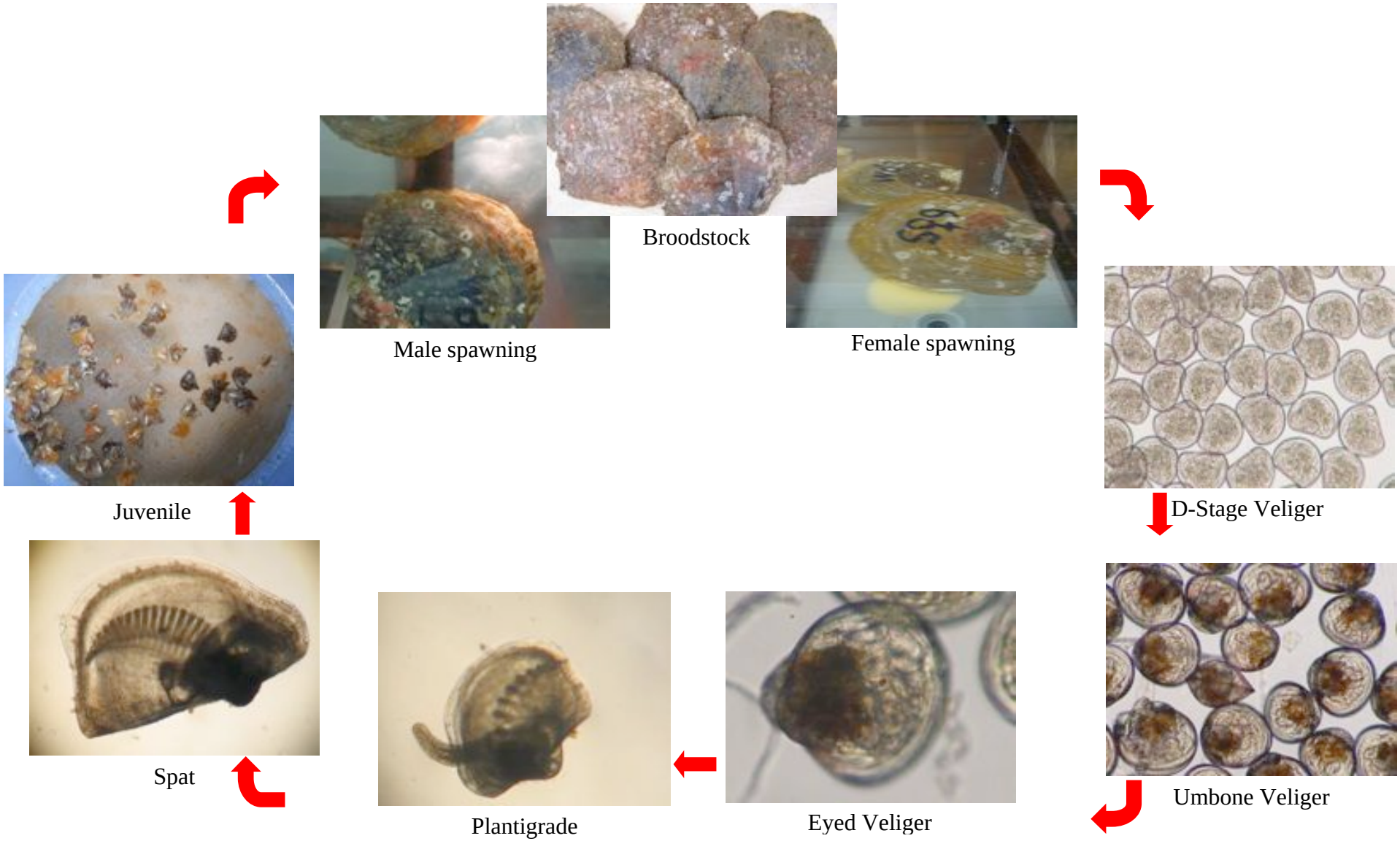

Fig. 1.2 Various stages in the life cycle of *P. maxima* (Photos courtesy of Dr Jens Knauer, PearlAutore).

1.7 Pearl oyster aquaculture

Mikimoto established the first pearl farm in 1888 on the Shinmei inlet in Shima, Japan (Strack, 2006). Wild stocks of pearl oysters were collected, implanted with spherical beads of mother-of-pearl, and placed in bamboo baskets moored at sea (Strack, 2006). He examined them every few months and successfully produced cultured blister pearls. This marked the beginning of pearl and pearl oyster cultivation. The methods devised by Mikimoto have since been modified and improved upon in the last hundred years.

Pearl culture operations can be divided into three phases; collection, on-growing and pearl culture. Today, the new category of hatchery production is becoming more widespread and an increasingly important source of oysters for pearl culture (Southgate, 2008). The sequence of pearl oyster cultivation will briefly be discussed here, with the on-growing of pearl oysters discussed in detail later.

1.7.1 Spat collection

Until the 1980s, cultured pearl production depended on a plentiful supply of mature oysters which were collected and used directly for pearl production (Gervis and Sims, 1992). With the depletion of wild stocks, restrictions to the collection of mature oysters have been put in place. The oysters are allowed to spawn in the wild and a percentage of the oyster spat produced are collected and grown. Collectors are placed in the sea during the settlement stage of the larvae of target oyster species. Materials used for spat collection vary, according to the location, species to be collected and available material in the area (Southgate, 2008).

1.7.2 Hatchery Production

Hatchery production of pearl oysters is becoming more widespread and assuming greater significance to the industry as it ensures a continual supply of juveniles allows for selective breeding and avoids exclusive reliance on wild stock. Broodstock are collected from the wild and spawning is induced by a variety of methods (Southgate, 2008). These include chemical induction (Alagarswami *et. al.,* 1983b), using filtered ultra violet sterilised seawater (Rose and Baker, 1989), ammoniated seawater (Wada, 1942; Kuwatani 1965; Tanaka and Kumeta, 1981) and temperature variation (Tanaka and Kumeta, 1981; Rose *et. al.,* 1990; Rose and Baker, 1994). Temperature induced

spawnings usually result in higher fertilisation and larval survival rates (Tanaka and Kumeta, 1981; Rose and Baker, 1989; Rose *et. al.*, 1990).

Techniques for larval rearing have been described for *P. maxima* (Minaur, 1969; Rose and Baker, 1994), *P. fucata* (Alagarswami *et. al.*, 1983a and 1983c), *P. fucata martensii* (Wada, 1973; Hayashi and Seko, 1986) and *P. margaritifera* (Tanaka *et. al.*, 1970; Southgate and Beer, 1997). These techniques rely on good food quality, with lipid contents of the microalgal food being of prime importance (Brown and Jeffreys, 1992) along with clean water and low larval stocking densities. Larvae are collected on spat collectors which are placed into culture tanks to provide settlement substrate (Southgate, 2008).

1.7.3 Grow-out of pearl oysters

The grow-out or on-growing of pearl oysters begins when the spat on collectors are large enough to be removed from their point of attachment, graded and placed in secondary culture systems, they may either be collected from natural spat fall or hatchery-produced.

Various systems of on-growing are used for pearl oysters. These include rafts, long-line and fence-lines, trestles and ear-hanging (Gervis and Sims, 1992; Southgate, 2008) and the choice of system depends on various factors such as the environment in which the oysters are reared and operations costs.

1.7.4 Pearl Culture

Oysters need to reach a minimum size before pearl production, which in the case of *P. maxima* is 12 cm (Strack, 2006). The implantation of nuclei into the gonad of a mature pearl oyster together with a piece of mantle tissue varies slightly with different species of commercial oysters. Oysters are conditioned before operation to weaken musculature so as not to expel the implanted nuclei and to rid the gonad of gametes to create more space for the nuclei to be inserted (Gervis and Sims, 1992; Taylor and Strack, 2008). Highly skilled technicians seed the pearl oyster with nuclei usually originating from freshwater mussels of the family Unionidae (Alagarswami, 1970). Following the insertion of nuclei, a pearl-sac forms and completely encloses the nucleus within seven

days (Kawakami, 1952a and 1952b). Secretion of periostracal material, prismatic and nacreous layers follow and a pearl is gradually formed. Formation of the pearl-sac following nucleus insertion in *P. maxima* was described in detail by Scoones (1990). The culture period of a pearl depends on the species of pearl oyster, the size of the nucleus, the physiological condition of the individual oyster and environmental factors such as water temperature and salinity. In *P. maxima*, this takes approximately two years (Strack, 2006)

1.8 Pearl production in Indonesia

Cultured pearls are produced throughout the Indo-Pacific region (Gervis and Sims, 1992). The production estimates for 2005 for South Sea pearls were approximately 3.2 tonnes in Australia, 3.8 tonnes in Indonesia, and 1.7 tonnes in the Philippines (Comtrade, 2005). Pearl production in Indonesia was estimated to have an approximate value of US$60 million (Poernomo, 2005).

However, while Indonesia is now producing larger quantities of *P. maxima* pearls, the value of pearls from Australia is still higher (Table 1.1). This is largely due to the lower quality of pearls produced in Indonesia. Until recently, information and technological advances in the farming of silver-lip oysters have been largely unavailable. Given Indonesia's status as a major producer of these pearls, there is a surprising paucity of information on growth of this species in Indonesia and this lack of information provides the basis for this study. This thesis addresses various aspects of the growth of farmed *P. maxima* in Indonesia with special emphasis on the influence of the environment.

CHAPTER 2

Overview of factors affecting growth of marine bivalves

2.1 Introduction

This study focuses on the growth of *P. maxima* and the environmental factors that influence it. The following section presents a general overview of bivalve growth and the methods used to measure it as background information for this thesis.

2.2 Bivalve growth

From an aquaculture and economic point of view, rapid growth of cultured bivalves is desirable. This has lead to numerous studies on the growth of several important commercial marine bivalve species encompassing larval development and survival (Alargarswami *et al.,* 1989; Pechenik *et al.,* 1990; Cochard and Devauchelle, 1993) to bivalve physiology (Videla *et al.,* 1998; Sobral and Widdows, 1997), bioenergetics (Bayne and Newell, 1983; Grant, 1996) and shell morphometrics (Beaumont and Khamdan, 1991; López *et al.,* 1995; Brown and Hartwick, 1988b).

Ultimately the study of growth should encompass the rates of chemical reactions that govern body composition and dimensions. However, most studies on bivalve growth at this stage examine less detailed aspects such as changes in body dimension, increase in mass and changes in chemical constituents in tissues (Wilbur and Owen, 1964) as they are easier to measure and more relevant to the aquaculturist.

2.3 Measuring growth of bivalves

Growth in molluscs consists of both increases in the shell and the soft body with the former being easier to measure. The more widely used methods to quantify growth include measuring annual growth rings (Stevenson and Dickie, 1954; Chalfant *et al.,* 1980; Quayle and Newkirk, 1989), successive recording of the dimensions of marked individuals (Loosanoff, 1954; Lutz and Hess, 1979; Estacion and Braley, 1988), comparison of successive length frequencies of a large random sample (Quayle, 1951;

Haskin, 1954) and acetate peels of cut shells (Lutz, 1976; Quayle and Newkirk, 1989). Other methods include X- ray and radioisotope measurements and using tetracycline as a marker (Wilbur and Owen, 1964).

2.3.1 Linear measurements

As there is a direct relationship between the linear dimensions of the shell and mass (Quayle and Newkirk, 1989), changes in these dimensions are often used to monitor growth. Some of the more common expressions used to describe the linear dimensions of bivalves are listed below.

2.3.1.1 Dorso-ventral measurement (DVM)

The DVM is measured as the greatest distance from the umbo to the furthest shell margin, or from a line drawn perpendicular to the hinge line across the greatest dorsoventral distance (Fig. 2.1). It is often referred to as the shell height (1988; Pouvreau *et al.,* 1999, Yukihira *et al.*, 2006), although it has also been termed shell length (Chellam, 1988; Nayar and Al-Rumaidh, 1993; Hart and Joll, 2006) and shell width (Nicholls, 1931). There is sometimes confusion between the lay term and anatomical term. DVM is more commonly used to determine growth in pearl oysters (Saucedo and Southgate, 2008) and in this study is referred to as shell height.

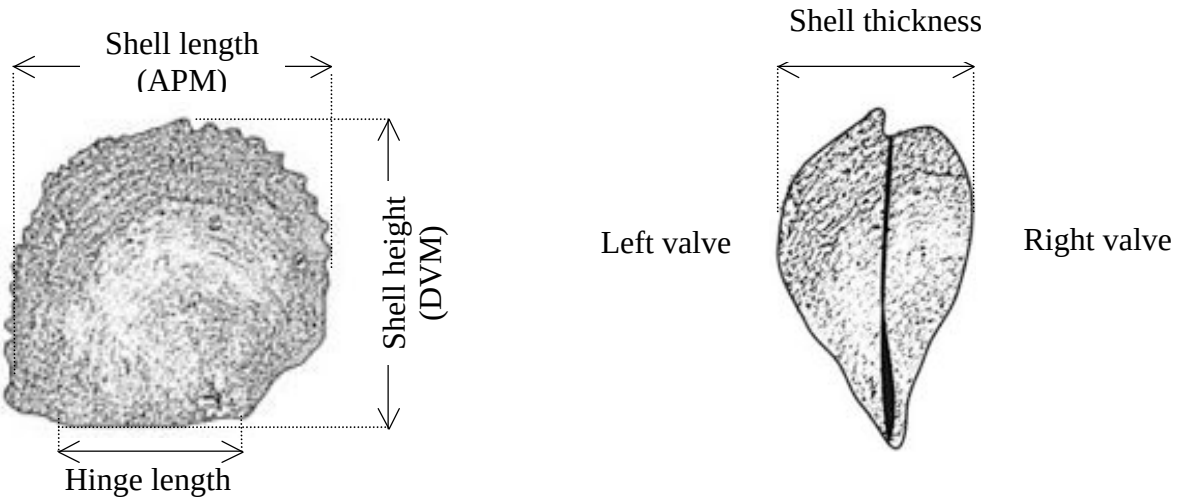

Fig. 2.1 Diagrammatic representation of the dimensions for measurement in a bivalve, based on the pearl oyster, *Pinctada maxima*.

2.3.1.2 Antero-posterior measurement (APM)

The APM is the greatest horizontal distance between the anterior and posterior margins of the shell usually taken parallel to the hinge line (Fig. 2.1). While the long axis of the oyster (APM) has been described as the height anatomically (Quayle and Newkirk, 1989), common usage indicates it as the length and it has been commonly referred to as such (Nicholls, 1931; Pilditch and Grant, 1999). Although, it has also been previously called the shell width (Al-Sayed et al., 1997), the term shell length will be used for the APM in this thesis.

2.3.1.3 Hinge Length (HL)

The hinge length is the distance between the ends of the anterior and posterior edge along the hinge line (Fig. 2.1). It is a dimension used in various growth and taxonomic studies for adult and spat bivalves (Narayanan and Michael, 1968; Wada, 1986a).

2.3.1.4 Shell thickness

Thickness is the greatest distance between the external surfaces of the two valves when they are fully closed (Beaumont and Khamdan, 1991; Nayar and Al-Rumaidh, 1993) (Fig. 2.1). This dimension is also known as the shell width (Franz, 1993, Gaytan-Mondragon *et al.*, 1993) but will be referred to as shell thickness in this thesis.

2.3.2 Weight measurements

While linear measurements are useful, they do not show tissue growth. Weight measurements are useful as an indicator of tissue or meat growth, especially since the meat (or shell, in some cases) is usually the product of concern to the grower. The commonly used measures of weights include wet weight, dry weight and ash-free dry weight.

2.3.2.1 Wet weight

Wet weights have been used effectively by previous authors to measure growth in bivalves (Parsons and Dadswell, 1992; Wada and Komaru, 1994; Numaguchi, 1995a). Wet weight may be measured in water or in air. Weighing bivalves in water eliminates inconsistencies in weight brought about by variable retention of seawater within the

internal cavity caused by gaping valves and is a sensitive method (Quayle and Newkirk, 1989). Weighing in air is easier although care must be taken that the animal is dried from the outside and not allowed to gape and leak liquid.

The difference between the weight in air and weight in water is the whole volume of the animal (Quayle and Newkirk, 1989). The meat and shells of the bivalve may be separated and measured individually, and shell volume and internal volume is calculated from the differences between the various air and water weights.

2.3.2.2 Dry weight

Dry weight is obtained by drying the shell and meat until a constant weight is reached. It is used as a measure of physiological condition and growth in bivalves (Bayne *et al.*, 1989; Macdonald and Bourne, 1989; Numaguchi, 1994). Dry weight is also measured to monitor changes in the reproductive cycles through seasonal oscillations (De Zwaan and Wijsman, 1976).

Measuring dry weights eliminates inaccuracies caused by retention of varying amounts of water within the bivalve body cavity and the water content of tissues which may vary with physiological status. However, the disadvantage of this parameter is the need to sacrifice the animal which does not allow for the monitoring an individual's growth by repeated measurement.

2.3.2.3 Ash-free dry weight (AFDW)

The AFDW is calculated as the difference between dry weight and ash weight of a sample and is the measure of organic content. Ash content is estimated by igniting the sample at a temperature that could range of around 450 – 500°C (Sprung and Borcherding, 1991; Martínez *et al.*, 1992; Emerson *et al.*, 1994).

This measurement is more precise than dry weight since ash content, like that of water, has been known to increase under unfavourable physiological conditions (Wilkins, 1967; Mayzeaud, 1976). It is a particularly useful measure for larvae and post-larvae where dissection of the animal is impractical. As with dry weight, the disadvantage of

this parameter is the need to sacrifice the sampled animal. AFDW was measured in this thesis to calculate condition index in later chapters.

2.3.3 Volume

Growth in molluscs can be measured by changes in volume (Lawrence and Scott, 1982; Rainer and Mann, 1992), since volume integrates length, width and thickness (Quayle and Newkirk, 1989). This is a particularly useful when there is considerable variation in the shape of the shell, as occurs in many bivalves such as oysters.

Shell cavity volume can be estimated from the difference between the volume of water displaced by whole live animal and volume displaced by the separate valves after removal of meat (Rainer and Mann, 1992). In general, volumetric measurements suffer from poor precision due to the displacements methods used to measure internal shell volume (Lucas and Beninger, 1985).

2.3.4 Condition index

In growth studies, it is important to be able to evaluate the quality and condition of the animal by using a simple index for comparison. The use of a physiological or condition index in bivalve aquaculture is widely utilised to characterise the quality of a marketed product. Additionally, condition indices are used to assess physiological activity such as growth, reproduction or secretion in the cultured animals (Lucas and Beninger, 1985) as well as an indicator of the nutritive state of the animal (Brown and Hartwick, 1988c; Littlewood and Gordon, 1988).

In bivalves, shell represents cumulative growth, while the amount of body tissue depends on the current sexual and metabolic activity of the organism. By comparing the amount of tissue to the amount of shell, it is possible to evaluate the current metabolic and reproductive status of the bivalve. A low condition index indicates a major biological effort has been expended. This could be energy used in response to poor environmental or disease condition, or for the production and release of gametes (Lucas and Beninger, 1985).

The most widely used condition index for adult bivalve is calculated as the dry tissue weight: dry shell weight ratio (Rainer and Mann, 1992; Numaguchi, 1994, Numaguchi, 1995a). In the case of post-larvae and spat, Walne and Millican (1978) used the ratio of dry ash weight: total dry weight which corresponds well to the more conventional dry tissue weight: dry shell weight (Lucas and Beninger, 1985). The use of dry tissue and shell measurements eliminates bias due to water content fluctuation.

Condition index can also be expressed as the dry weight: internal cavity volume ratio, and has been used primarily with oysters (Lawrence and Scott, 1982; Bressan and Marin, 1985; Rainer and Mann, 1992).

Due to the nature of the measurements of condition indices, they are easily standardised and give valuable information about the comparative physiological status of the animal.

2.3.5 Biochemical index

The RNA:DNA ratio, protein:DNA ratio and protein:RNA ratios are biochemical indices which have been shown to be directly related to on-going tissue growth in bivalves and other aquatic organisms (Haines, 1973; Pease, 1976; Paon and Kenchington, 1995). These biochemical indices are increasing being used in growth studies of bivalves as changes in these ratios serve as a sensitive and reliable indicator of condition (Kenchington, 1994).

The amount of somatic DNA remains relatively constant within the somatic cells of a species (Clemmesen, 1993) while the RNA content fluctuates with age, life stage, size and variation in environmental conditions (Pease, 1976; Bulow, 1987). Biochemical indices have been used successfully in growth studies on various bivalves including *C. virginica* (Pease, 1976; Wright and Hetzel, 1985), *Pecten maximus* (Robbins *et al.*, 1990), *Abra ovata* (Frantzis *et al.*, 1993) and *Mya arenaria* (Mayrand *et al.*, 1994). Although these indices provide early information about the ecological changes on an animal's growth (Mayrand *et al.*, 1994), RNA and DNA levels can only be measured using laboratory techniques seldom available to the aquaculturist, making it impractical for the grower to use as a measure of bivalve growth.

2.3.6 Mathematical model - von Bertalanffy growth function (VBGF)

Growth can be expressed mathematically and the method which has found widest application in fisheries science is the von Bertalanffy growth function (von Bertalanffy, 1938). The VBGF is a mathematical model which allows for comparative studies on growth. Although the conceptual analysis of the underlying theory is based on fish growth, it has been used successfully for computation of growth parameters in various species of bivalves including Pacific oyster *C. gigas* (Yoo *et. al.*, 1972), American oyster *C. virginica* (Shaw, 1962), the blue mussel *M. edulis* (Sukhotin and Maximovich, 1994), giant clam *Tridacna gigas* (Munro and Gwyther, 1981) and the quahog clam *Mercenaria mercenaria* (Menzel, 1963; Jones *et. al.*, 1989). VBGF has been used successfully for computation of growth parameters in pearl oysters including *P. margaritifera* (Nasr, 1984; Pouvreau *et al.*, 2000b; Pouvreau and Prasil, 2001), *P. imbricata* (Urban, 2000; Urban, 2002; Marcano *et al.*, 2005), *P. mazatlanica* (Saucedo and Monteforte, 1997B) and *P. maxima* (Hart and Joll, 2006; Yukihara *et al.*, 2006).

The VBGF reads for length:

$$L_t = L_\infty (1 - e^{-k(t- to)}), \qquad \text{(Equation 2.1)}$$

where L_∞ is the asymptotic length, K is the growth constant, L_t the length at age t and t_o the theoretic age of the animal at length equals zero.

While there are other mathematical models for describing growth mathematically such as the logistic growth curve or Gompertz curve (Moreau, 1987), the VBGF is generally considered to be superior than the others as it is biologically interpretable and can be used for comparative growth studies as well as its parameters are relatively easy to determine.

2.4 Allometry of growth

Changes in growth may be represented by a parameter of growth of the whole organism related to age, or the rate of growth of one part or dimension to that of another part, termed allometry of growth (Huxley and Teissier, 1936). The relationship between various measurements of growth is a useful source of information, with the relationship between the size of the animal and its meat content of particular interest to the grower (Quayle and Newkirk, 1989).

Significant differences in the allometric relationships between shell and body growth can be caused by local variations in the marine environment (Brown and Hartwick, 1988a; Franz, 1993). Site-related growth variations are apparent as an increase in absolute growth or change in the allometric relationship between shell and body tissue (Macdonald and Thompson, 1985; Aldrich and Crowley, 1986; Brown and Hartwick, 1988b).

Allometric relationships are an important consideration for growers interested in a particular shape of the cultured bivalve (for example, a pearl oyster with a greater shell thickness that would facilitate insertion of a larger nucleus) and may be utilised as a means of assessing the suitability of a potential site.

2.5 Bioenergetics of bivalve growth

Growth results when energy acquisition exceeds energy expenditure (Bayne and Newell, 1983). The difference between the energy of the food an animal consumes and all the other energy utilisations and loss is termed “scope for growth” (Warren and Davies, 1967).

The scope for growth has been widely used to examine the various components of growth in pearl oysters and their response to environmental changes (Pouvreau *et al.*, 2000a; Yukihira *et al.*, 1998a; Yukihira *et al.*, 1998b; Yukihira *et al.*, 2006; Lucas, 2008b). The different components of growth include ingestion, absorption, excretion and respiration (Bayne and Newell, 1983). Energy is acquired through ingestion and absorption while energy is expended through excretion and respiration. Any decrease in

energy expenditures will cause greater assimilation and increased capacity for growth and vice-versa (Bayne *et al.*, 1993).

Ingestion is determined by clearance rate of particulate organic matter (Bayne *et al.*, 1989; Bacon *et al.*, 1998, Yukihira *et al.*, 1998b) while absorption is estimated by using ash weight: organic weight ratio (Conover, 1966; Macdonald *et al.*, 1998). Respiration is measured through rates of oxygen consumption (Sobral and Widdows, 1997; Clausen and Riisgård, 1996, Yukihira *et al.*, 1998) and excretion is estimated by ammonia secretion rates (Widdows, 1985; Macdonald *et al.*, 1998).

Scope for growth calculations can be used to build a model of bioenergetics in pearl oysters (Pouvreau *et al.*, 2000b) to simulate growth, reproduction and spawning which may provide valuable information for management of aquaculture. Bivalves reported to have the highest scope for growth as well as highest clearance, respiration and excretion rates include the pearl oysters, *P. maxima* and *P. margaritifera* (Yukihira *et al.*, 1998a; Lucas, 2008a)

2.6 Factors that affect growth of bivalves

An outstanding feature of growth in bivalve molluscs is its variation in rate (Wilbur and Owen, 1964). Obviously many factors combine to exert an influence on the growth of bivalves. Jamieson *et al.* (1988) divided factors influencing growth of *M. edulis* into two categories: 1) biological factors and 2) physical factors. For bivalves under culture, a third factor which affects growth is culture method and husbandry. Quantification of these factors is useful for making predictions for the culture of economically important species.

2.6.1 Biological factors

Some of the biological factors are inherent which affect growth of bivalves includes genetics, age, size, health and physiological condition of the animal.

2.6.1.1 Genetic factors

Most commercially important bivalve molluscs show quantitative traits which can be influenced by genetic factors (Wada, 1987; Wada and Jerry, 2008). Quantitative traits,

also called polygenic traits, are those of most interest from a production viewpoint and are likely to be controlled by a large number of genes (Newkirk, 1980). These traits include growth rate, survival, meat yield and shell shape.

The expression of particular traits such as growth rate is due to a combination of both genetic and environmental factors. To determine the relative importance of the genetic component, an expression termed heritability (h^2) is used. Heritability is expressed as a ratio of genetic variance: total phenotypic variance (composed of genetic and environmental components). Some heritability estimates for bivalve growth and survival are given in Table 2.1.

Electrophoretic studies have shown that natural populations of bivalve species are genetically differentiated to some extent (Ahmed *et al.,* 1977; McDonald and Koehn, 1988; Blot *et al.,* 1988). If the populations experience different environments, one might expect to see adaptations to local environmental conditions (Newkirk, 1980). The interaction between genotype and environmental factors has been described for *C. virginica* where genetically dissimilar *C. virginica* spat from different estuaries displayed varying growth rates (Mallet and Haley, 1983). *C. virginica* larvae have also been shown to grow at different rates at different salinities (Newkirk, 1978a).

The origin of an bivalve affects its growth rate (Tedengren and Kautsky, 1986; Petersen and Beal, 1989; Rawson and Hilbish, 1991), filtration rate and net growth efficiency (Widdows *et al.,* 1984) and mortality (Dickie *et al.,* 1984, Kautsky *et al.,* 1990) and its influence is considered a manifestation of genetic differences (Sukhotin and Maximovich, 1994; Kvingedal *et al.*, 2007a, b; Kvingedal *et al.*, 2008).

The difference in quantitative traits can be exploited for selective breeding programs in bivalve aquaculture. The aim of selective breeding is to produce strains that are improved for certain economic traits. In oysters, this includes faster growth rates (Haley and Newkirk, 1977), greater resistance to disease (Haskin and Ford, 1978) and producing improved hybrid vigour or heterosis (Singh and Zourous, 1978). For some species of bivalves, a positive correlation has been established between heterosis and growth rate (Mitton and Grant, 1984; Zourous and Foltz; 1987; Britten, 1996). Pronounce difference has been reported in *O. edulis* of the same cohort reared under

uniform conditions (Walne, 1958) and this was correlated with the degree of heterozygosity (Singh and Zourous, 1978; Koehn and Shumway, 1982; Alvarez *et al.*, 1989).

Table 2.1 Heritabilities estimated for various species of marine bivalve molluscs including the pearl oyster, *P. fucata*. (Adapted from Wada, 1987)

Species	Traits	Heritabilities	Reference
C. virginica	Larval growth	0.07 – 0.85	Longwell (1976) Newkirk *et al.* (1977)
	Spat length	0.29 – 0.71	Losee (1978)
C. gigas	Larval survival	0.31	Lannan (1972)
	Size	0.15	Lannan (1972)
	Shape	0.13	Lannan (1972)
	Meat weight	0.37	Lannan (1972)
	Total weight	0.33	Lannan (1972)
M. edulis	Larval growth	0.12 – 0.78	Innes and Haley (1977) Newkirk (1980) Newkirk *et al.* (1980)
P. fucata martensii	Shell width	0.127 – 0.467	Wada (1984)
	Shell convexity	0.126 – 0.368	Wada (1986b)
	Larval shell length	0.078 – 0.335	Wada (1989)
	Shell height	0.304 – 0.921	He *et al.*(2008)

While selective breeding can produce favourable results, the effects of inbreeding must be taken into consideration. While inbreeding can be beneficial when used as a means of "purifying" lines such as in producing better colour nacre in pearl oysters, inbreeding can also produce deterioration in vigour and survival due to inbreeding depression. The deleterious effects of inbreeding have been reported in various cultured bivalves. For example, inbreeding of the Pacific oyster, *C. gigas*, produced offspring which were smaller in shell size, wet weight and dry weight (Beattie *et al.*, 1987) while abnormal die-off as with mortality as high as 72.5% was reported in inbred lines of Japanese pearl oyster, *P. fucata martensii* (Wada, 1984).

While growth of cultured bivalves may be improved through selective breeding in the hatchery, the advantages of improved growth performance must be weighed against the inadvertent effects of inbreeding depression caused by genetic drift (Newkirk, 1978b).

Chromosome manipulation by producing triploids through chemical or physical means has been studied as a means of retarding or stopping gonad development in favour of growth in aquaculture. Triploid animals can be produced by suppressing either meiosis I or II in bivalve molluscs (Gosling, 2003). Many authors reported that the size and weight of triploid adult bivalves were significantly greater than diploid animals (Stanley *et al.,* 1984; Tabarini, 1984; Allen and Downing, 1986; Mason *et al.*, 1988, Komaru and Wada, 1989). Triploidy has been reported to improve growth in the Japanese pearl oysters *Pinctada fucata martensii* (Jiang *et al.,* 1991, 1993; Komaru and Wada, 1994; Uchimura, 1999). It also has the advantage of making the pearl oysters more suitable for nuclei insertion as mature gonads in a pearl oyster is unfavourable for pearl operation. While triploidy may retard gonad development in most bivalves, Komaru and Wada (1990) reported some male and female triploids to be mature. More studies must be performed on the gonadal maturation in triploids before it can be safely be used as an inducement for growth in aquaculture.

2.6.1.2 Age

Previous studies on molluscan growth and age have relied on estimation of an animals' age using size-frequency study, growth interruption lines and release and recovery of tagged individuals (Haskin, 1954). With the advent of hatchery-production, the absolute age of bivalves is known and growth rate may be studied over the different stages in the life cycle.

The curve of absolute growth in bivalves as a function of age commonly describes a sigmoidal curve (Gibson, 1956; Wilbur and Owen, 1964; Seed, 1973). The general pattern is for rapid growth to occur in young individuals which declines with age. In older individuals, there may be an extended period of slow growth at a rate which does not change greatly with time (Wilbur and Owen, 1964). The decrease in growth rate with age has been reported in a number of species of molluscs including the European cockle *Cardium edule* (Kristensen, 1957), the American oyster *C. virginica* (Ingle and Dawson, 1952), the blue mussel *M. edulis* (Chalfant *et al.,* 1980), the fan mussel *Pinna nobilis* (Richardson *et al.,* 1999), the scallop *Pecten maximus* (Mason, 1957) and the pearl oysters, *Pinctada radiata* (Nayar and Al-Rumaidh, 1993), *P. margaritifera* (Pouvreau *et al.*, 2000b) and *P. mazatlanica* (Saucedo and Monteforte, 1995).

Age-related response may reflect variations in the allocation of assimilated energy into reproduction, storage, shell or somatic growth (Zandee *et al.*, 1980; Cigarría, 1999). The slower growth rate which occurs as the animal ages has been attributed to progressive reduction in metabolic activity (Wilbur and Owen, 1964; Chalfant *et al.*, 1980). For example, the activity of the enzyme carbonic anhydrase, which is associated with shell deposition in molluscs (Wilbur and Jodrey, 1955; Freeman, 1960), has been found to decrease with age in the pearl oyster, *P. fucata martensii* (Kawai, 1955) and American oyster *C. virginica* (Wilbur and Anderson, 1950). Studies on *P. fucata martensii* shell and pearl formation (Yamaguchi, 1958; Kobayashi and Watabe, 1959) have indicated a decrease in mantle efficiency with age in the deposition of calcium carbonate, $CaCO_3$. As pearl formation is a process closely related to shell deposition, the growth of pearls is affected by the age of both the host oyster and the inserted piece of mantle which forms it.

Ageing has an effect on changing the energy budget and scope of growth of the bivalve (Bayne and Newell, 1983). As discussed previously, scope of growth is equivalent to total production and is the sum of reproductive output and somatic growth. When an individual ages, a greater proportion of this is directed to the production of gametes while somatic production declines (Rodhouse, 1978; Branch, 1982). When somatic production reaches zero the animal achieves its maximum size.

The decreased growth rate in older bivalves has been attributed to size as well as age. Walne (1958) observed that individual oysters, *O. edulis,* of the same age exhibited decrease growth efficiency as their size increased, while Kristensen (1957) found a similar relationship in the cockle, *C. edule*. Since growth rates decrease as a bivalve becomes larger and older, one may expect to find metabolic correlations with size and age (Wilbur and Owen, 1964).

2.6.1.3 Size

As an animal increases in size, its growth rate becomes slower due to a reduction in metabolic activity and feeding efficiency. Smaller individuals have been found to have a faster growth rate than larger individuals in many bivalves (Petersen and Fegley, 1986; Hoffman *et al.*, 1994; Cigarría, 1999; Stiles *et al.*, 2000).

Studies of body size and metabolic rate of molluscs and their larvae have established that metabolism is proportional to a constant power of the body weight (Hemmingsen, 1960; Zeuthen, 1947, 1953; von Bertalanffy, 1957). The relationship between metabolic activities such as filtration, ingestion, oxygen consumption and maintenance ration, and body size, in molluscs, can be expressed as a function of dry-tissue weight (W) by the allometric equation

$$R = aW^b, \quad \text{(Equation 2.2)}$$

where R is the rate of the metabolic activity under consideration, and a and b are constants at specific experimental conditions (Winter, 1978). From studies on filtration and oxygen consumption in various bivalves, the b values were found to range from 0.46 to 0.87 (Table 2.2).) Generally, mature bivalves give priority of energy allocation to gonad growth and gamete development (Brown and Russell-Hunter, 1978). Rapid growth is observed when gonad development is blocked by inducing triploidy in *Pinctada martensii* (Jiang et al., 1993).

The surface that governs the rate of oxygen supply, i.e. the surface of the gills, is thought to physiologically limit growth (Pauly, 1979). As a relationship between body size and gill area was found to exist in *M. edulis* (Dral, 1967; Foster-Smith, 1975; Thiesen, 1982), it is justified to assume that as the animal becomes larger, it will approach a point where the oxygen-controlled rate of synthesis of body mass is lowered so that it is just sufficient to counteract catabolic processes (Vakily, 1992).

The surface of the gills affect filtration the same way it does oxygen supply; in larger animals, filtration is reduced, leading to less food being ingested which ultimately lessens the growth rate of the animal. For example, Jørgensen (1952) reported that the growth efficiency decreased from 84% in *M. edulis* and *M. californianus* of 0.35 – 6.0 mm in length to 11% in animals of approximately 90 mm in length while Winter (1973) calculated that small *M. edulis* filtered algae equivalent to 29% of its dry tissue weight daily, while larger *M. edulis* filtered just 5.5%.

2.6.1.4 Reproductive stage

Reproductive activity in molluscs has on effect on the growth of an organism by directing energy away from somatic growth and channelling it towards gametogenesis (Bayne and Newell, 1983). In some molluscs, reproduction is only initiated when somatic growth stops, while in others growth continues after maturity with increasing energy being allocated to gamete production.

Many bivalves have seasonal cycles of energy utilisation that are intimately associated with the reproductive cycle (Besnard, 1991; Paon and Kenchington, 1995; Roddick *et al.,* 1999). This is also true in pearl oysters (Saucedo and Southgate, 2008). The energy requirement for gametogenesis is supplied by food or utilisation of lipid, carbohydrate and protein reserves supplied to the gonads from various storage sites (Barber and Blake, 1991). Paon and Kenchington (1995) reported that gonad weight increase coincided with adductor muscle weight decrease in the sea scallop *Placopecten magellanicus* during gametogenesis, suggesting either a transfer of substrate from the adductor muscle or a preferential direction of resources towards gamete production at the expense of the adductor muscle. Another example of substrate transfer from muscle is glycogen peaks, originating mainly from the muscular tissues, and thought to trigger vitellogenesis in several bivalves such as *Argopecten irradians* (Barber and Blake, 1981), *M. edulis* (Lowe *et al.,* 1982; Bayne *et al.,* 1983), *O. edulis* (Ruiz *et al.,* 1992a), *O. puelchana* (Fernandez Castro and Vido de Mattio, 1987) and *Glycymeris glycymeris* (Galap *et al.,* 1997). Vitellogenesis in pearl oysters has been reported to be very energy demanding and sensitive to external factors and selective pressure (Saucedo and Southgate, 2008). Metabolic conversion from glycogen to lipid is thought to occur during gonad development (Gabbott, 1983; Deslous-Paoli and Héral, 1988; Ruiz *et al.,* 1992b). Many investigators have reported a relationship between reduced growth rate and spawning in bivalves (Quayle, 1951; Nasr, 1984).

Generally, mature bivalves give priority of energy allocation to gonad growth and gamete development (Brown and Russell-Hunter, 1978). Rapid growth is observed when gonad development is blocked by inducing triploidy in *Pinctada martensii* (Jiang *et al.,* 1993).

Table 2.2 Values of the constant b in various bivalves including pearl oysters *P. maxima* and *P. margaritifera*, based on the general allometric equation $R = aW^b$

Bivalve species	Metabolic activity	b	Reference
M. edulis	Filtration	0.60	Vahl (1973)
		0.73	Winter (1973)
	Oxygen consumption	0.70	Krüger (1960)
		0.64	Read (1962)
		0.75	Vahl (1973)
M. californianus	Filtration	0.46	Bayne *et al. (*1976)
Pecten irradians	Filtration	0.82	Chipman and Hopkins (1954)
	Oxygen consumption	0.87	Jørgensen (1976)
P. maxima	Clearance	0.61	Yukihara *et al.* (1998a)
	Respiration	0.56	Yukihara *et al.* (1998a)
	Ammonia excretion	0.78	Yukihara *et al.* (1998a)
P. margaritifera	Clearance	0.60	Yukihara *et al.* (1998a)
		0.61	Pouvreau *et al.* (2000a)
	Respiration	0.44	Yukihara *et al.* (1998a)
	Ammonia excretion	0.64	Yukihara *et al.* (1998a)
	Pseudofaecal production	0.77	Pouvreau *et al.* (2000a)
	Faecal production	0.49	Pouvreau *et al.* (2000a)

2.6.1.5 Disease and parasites

Bivalve culture throughout the world has been plagued by epizootics (Lester, 1989) that have affected the growth and survival of animal stocks. Disease and disease-related stress has been found to cause shortened life span, mass mortalities, reduction in total amino acid and carbohydrate content and invasion by other opportunistic organisms (Jeffries, 1982).

Disease causing agents and parasites of bivalves including pearl oysters encompass a wide range of organisms, ranging from protozoa to molluscs (Quayle and Newkirk, 1989; Humphrey, 2008). The range of deleterious effects on the host bivalve varies from food sharing to outright disease ending in mortality (Quayle and Newkirk, 1989). In the culture of pearl oysters, disease also impact on the industry by producing poor quality pearls (Humphrey, 2008)

Infections by pathogens can affect growth in bivalves by interfering with energy acquisition in the animal (Pérez Camacho *et al.*, 1997) as well as retard shell deposition at the shell-mantle interface (Perkins, 1996; Ruck and Cook, 1998; Humphrey, 2008). Some examples of reduced growth in pearl oysters caused by disease agents and parasites will be discussed.

Reduced shell growth and mass mortalities in farmed Akoya pearl oysters in Japan have been reported (Miyazaki *et al.*, 1999; Kobayashi *et al.*, 1999). While a causative agent is yet to be conclusively identified, there is evidence that an icosahedral virus is involved, with clinical symptoms of reduced growth, low condition indices, decreased body weigh and mortality caused by functional disturbance in various metabolic functions such as feeding, respiration, and excretion (Humphrey, 2008).

Studies have shown that polychaete worm *Polydora* infestation causes a decrease in *C. gigas* growth by burrowing in the shell surface at the growing margin (Zottoli and Carriker, 1974; Sato-Okoshi and Okoshi, 1993; Almeida *et al.*, 1997). Another spionid polychaete worm *Boccardia knoxi* was found to cause the static condition indices in *C. gigas* to decrease (Handley, 1998).

While most pathogens and parasites cause a decrease in growth in molluscs, there have been some reports for gigantism in gastropods caused by larval trematode infection (Rothschild, 1936; Boettger, 1952). Infected animals grew at a rate which was faster than normal (Rothschild and Rothschild, 1939) which may have brought about by the partial or complete destruction of the gonads thus favouring somatic growth. *Bucephalus sp.* trematodes have been reported to infect Akoya pearl oysters and invading the gonad and digestive glands (Ozaki and Ishibashi, 1934; Wada, 1991; Khamdan, 1998), making oysters unsuitable for pearl production but no observation of gigantism were reported. In the literature reviewed thus far, there has been no report of gigantism or accelerated growth in marine bivalves caused by a pathogenic agent.

2.6.2 Environmental factors

Variations in growth and survival of marine bivalves living under different environmental conditions have been widely studied as rapid expansion of aquaculture has placed increasing importance upon habitat requirements and selection. Environment-related variation in growth has been demonstrated in many commercially important bivalves, amongst them the pearl oysters *P. fucata martensii* (Numaguchi and Tanaka, 1986a, 1986b) and *P. maxima* (Mills, 2000). Growth in marine bivalves is affected by the interactions of several environmental variables.

2.6.2.1 Water temperature

The effects of water temperature on growth rate in bivalves have been examined in natural population and under controlled laboratory conditions. Field studies of temperature effects are very difficult because of the complex interaction of multiple factors. Temperature is one of the principal environmental factors affecting bivalve growth (Malouf and Breese, 1977; Incze *et al.*, 1980) and has a direct effect on the growth of the animal by influencing metabolic activity such as ingestion, absorption, excretion and respiration.

The range of temperatures over which growth is optimal differs considerably between species and between different populations of the same species. Below a given temperature, growth becomes very slow or ceases as the animal stops feeding and the digestive tract become empty (Bayne and Newell, 1983) due to insufficient energy for

tissue growth i.e. scope-for-growth nears zero or become negative (Lucas, 2008a). The temperature at which feeding stops occurs at different temperatures for different bivalves, depending on distribution of the species ie, temperate or tropical, ranging from 13°C for the pearl oyster *P. martensii* (Kobayashi and Watabe, 1959) and to below 5°C for *C. virginica* (Loosanoff, 1958) and *M. edulis* (Loosanoff, 1942) while the giant clam, *Tridacna gigas* was found to stop growing at 19.2°C (Lucas *et al.*, 1988) and growth becomes negligible for *M. mercenaria* at temperatures below 10°C (Pratt and Campbell, 1956).

From a physiological perspective, temperature affects growth in bivalves by regulating the division of net production into somatic and reproductive tissue, as well as regulating the relative rates of filtration and respiration (Fig. 2.2).

Many temperate species of bivalves follow a growth pattern with a low growth rate during winter and maximum growth in spring or summer. Some examples include the pearl oysters *P. margaritifera* (Nasr, 1984) and *P. fucata* (Kobayashi and Tobata, 1949; Kim, 1969). The slower growth rate at low temperatures, demonstrated experimentally in *C. virginica* (Loosanoff, 1950) has been attributed to the adverse affect of temperature on the opening of the valves and filtration rate.

Higher temperature favours growth by increasing soft tissue weight through gonadal development in some bivalves such as *Pecten magellanicus* (Pilditch and Grant, 1999), *C. gigas* (Almeida *et al.*, 1997) and pearl oysters *P. margaritifera* (Araya-Nunes *et al.*, 1991; Saucedo *et al.*, 2000), *P. fucata* (Behzadi *et al.*, 1997) and *Pteria sterna* (Vite-Garcia and Saucedo, 2008), as well as raises filtration and ingestion rate in *P. maxima* (Mills, 2000; Yukihira *et al.*, 2000), *P. margaritifera* (Yukihira *et al.*, 2000) and *Pteria sterna* (del Rio-Portilla, 1992).

Conversely, some authors have noted that some bivalves experience a cessation or decrease in growth during summer believed to result from higher temperature increasing the metabolic energy demand beyond what the feeding processes can cope with (Lammens, 1967; De Wilde, 1975). Unfed *P. fucata martensii* displayed a decrease in dry weight due to increased catabolic losses of carbon and nitrogen with increasing water temperature (Numaguchi, 1995b).

Slowing of growth in summer in some bivalves can be contributed to the channelling of energy to gametogenesis at the expense of somatic growth (Beaven, 1950). Browne and Russel-Hunter (1978) observed that gonad growth in bivalves is given priority over growth of other tissues during periods before spawning which is usually in warmer months. As water temperature increases, more net production is allocated to reproduction (Hofman *et al.*, 1994). This would account for no shell increment in some bivalves during summer.

While shell growth and tissue growth are obviously interrelated (Vakily, 1992), temperature may affect the growth of one more than the other. Calcium carbonate is crucial in the growth of bivalve shells as it is a major component of the shell, deposited in the form of an organic matrix (Crenshaw, 1980). Shell composition and secretion in pearl oyster was described in detail by Fougerouse *et al.* (2008). In seawater, the solubility of calcium carbonate increases with decreasing water temperature and high pressure (Dietrich *et al.*, 1980). The free energy of precipitation of calcium carbonate from seawater is affected by its solubility and increases linearly from 0°C to 40°C (Clarke, 1983), meaning that the metabolic cost of removing calcium carbonate for shell formation is greater at low than high temperature (Vakily, 1992).

Besides the direct effect of temperature on metabolic and active transport reactions which regulate bivalve growth, temperature may also act concomitantly with other multiple factors such as food quality and intake (Loosanoff *et al.*, 1953; Davis Guillard, 1958; Yukihira *et al.*, 2000), size and age (Hamai, 1935; Zandee *et al.*, 1980; Yukihira, 1998a) and latitude (Bullock, 1955, Hofmann *et al.*, 1994) to influence growth. This phenomenon has been shown for pearl oysters (Tomaru *et al.*, 2002(b); Mondal, 2006; Saucedo *et al.*, 2009).

2.6.2.2 Salinity

Salinity conditions beyond a species optimal zone can negatively affect growth and survival of bivalves (Quayle, 1969; Bernard, 1983) including pearl oysters (Lucas, 2008b). However, many bivalves are euryhaline and can survive over a wide range of salinities.

Bayne and Newell (1983) viewed salinity as a limiting factor (Fry, 1947) for marine molluscs growth; in a low salinity environment the potential for energy acquisition is restricted. Reduced growth rate of bivalves in low salinities have been reported in many studies (e.g. Sakuda, 1966; Thiesen, 1968; Remane and Schlieper, 1971; Forbes, 1973; Mok, 1974; Brown, 1988; Brown and Hartwick, 1988b, 1988c). A review of the effects and influences of varying salinities on pearl oysters is presented by Lucas (2008b). Jeyabaskarn *et al.* (1983) suggested that high salinities within the range 29 - 34 ‰ reduced growth of pearl oyster *P. fucata* in farms in India. Taylor *et al.* (2004) reported that *P. maxima* spat growth was optimal at 30 ‰ but a reduction to 25 ‰ or increment to 40 ‰ or 40 ‰ reduced shell growth.

Fluctuations in salinity levels may cause reduced growth of bivalves by acting directly on the physiological processes of the animal, or it can reduce the animal's tolerance to alteration in other environmental variables such as food availability and temperature (Medcof and Needler, 1941; Bernard, 1983). Low salinity causes shell closure in bivalves (Pierce, 1947; Brown, 1988) leading to reduction in food consumption and respiration as well as absorption efficiency, and may cause significant energy drain through excretory losses of amines (Bayne and Newell, 1983). It has been reported in the pearl oyster *P. fucata* that ciliary movements of the gills were abnormal at 13 ‰ salinity and ceased at 9.5 ‰ (Kobayashi and Matsui, 1953) and feeding is inhibited at approximately 14 ‰ (Wada, 1969). This, as well as the additional metabolic cost to the animal associated with maintenance of osmotic balance in a stressful salinity regime, can cause bivalves to stop somatic growth.

Rate of shell formation is partially dependent on the supply of calcium to the mantle by blood or the external medium (Wilbur and Saleuddin, 1983). Calcium concentration in water is related to salinity (Riley and Chester, 1971) and the limited availability of the shell-building substrate in a low-salinity environment may suppress growth in the bivalve.

While most bivalves cultured in coastal areas experience occasional low salinity stress due to monsoon rains and freshwater run off from the coast, some bivalve are cultured in environments where high salinities prevail. For example, the pearl oyster *P. radiata* cultured in the Arabian Gulf experience high and stressful salinity ranges of 50 to 60 ‰

due to excessive evaporation, shallow depths and little freshwater input. In these areas of high salinity, growth of *P. radiata* is stunted (Al-Sayed *et al.*, 1997). Hypersalinity changes the osmoregulatory capability of the membrane permeability system so that permeability to water decreases while permeability to ions increases. The uptake of nutrients and accumulation of catabolites is affected, leading to the possible slowing down of metabolism and growth (Al-Sayed *et al.*, 1997).

Salinity may act indirectly on bivalve growth by affecting the growth of phytoplankton as well as the species composition of the phytoplankton community, the source of food for marine bivalves (Angell, 1986). Alteration in phytoplankton amounts may lead to lower food level and subsequently inadequate nutrition for growth.

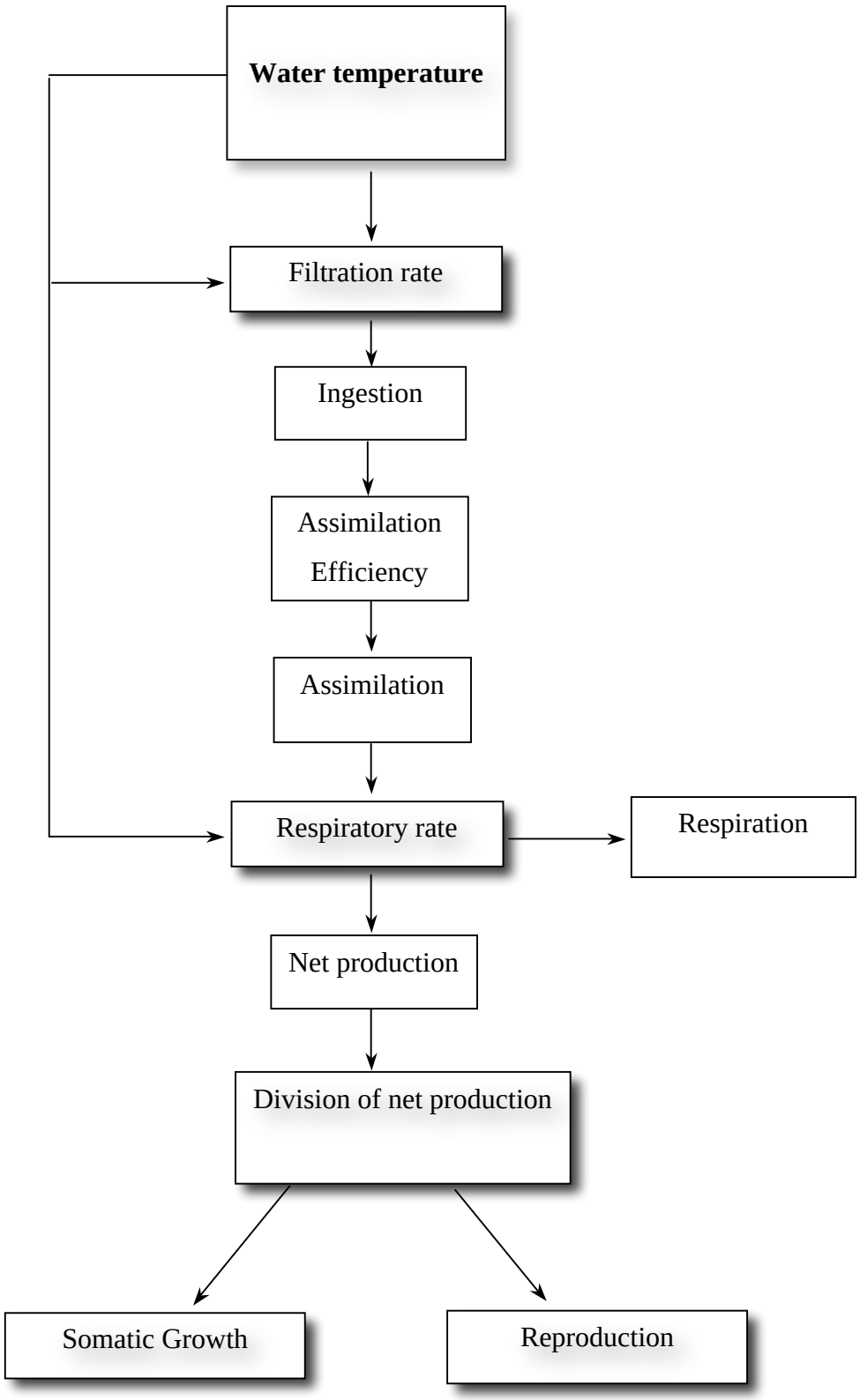

Fig. 2.2 Schematic of the influence of temperature on physiological activities and growth in bivalves (adapted from Hofmann *et al.*, 1994).

2.6.2.3 Food availability

An important consideration in aquaculture is food availability as this is a major factor affecting bivalve growth, having an equal if not greater influence upon growth rates compared with temperature (MacDonald and Thompson, 1985; Brown and Hartwick, 1988b; Lucas, 2008a).

The influence of food on growth is well documented in bivalves (Côté *et al.,* 1994; MacDonald *et al.,* 1998; Pilditch and Grant, 1999) including the pearl oysters *P. maxima* (Yukihara *et al.,* 1998b; Yukihara *et al.,* 1999; Mills, 2000), *P. fucata martensii* (Numaguchi, 1995a, 1995b; Yukihira *et al.*, 1999) and *P. margaritifera* (Yukihira *et al.*, 1999; Pouvreau *et al.*, 2000a; Yukihira *et al.,* 2006).

In a natural environment, bivalves experience a diet of suspended particulate matter (seston), which includes living plankton, organic detritus and inorganic particles or silt (Bayne and Newell, 1983). Factors such as the quality, quantity and size of the suspended particulate matter alter the physiological responses of the filter-feeding bivalves, having a direct effect on their growth rates and reproduction (Navarro and Ulloa, 1992). Therefore, changes in the composition of the seston can lead to changes in the nutritional quality of the food available, and growth of bivalves.

It has been reported that scope-for-growth in *M. edulis* (Bayne and Newell, 1983) and *Aulacomya ater* (Bayne and Newell, 1983; Navarro and Ulloa, 1992) increased with increased algal cell quantity. Conversely, it has been observed in *M. arenaria* (Gilfillan *et al.,* 1976), *M. edulis* (Bayne and Widdows, 1978) and *C. islandica* (Vahl, 1980) that low ration concentration resulted in lowered growth efficiency and subsequent weight loss. This has also been observed in the pearl oyster *P. maxima* (Yukihara *et al.,* 1998b; Lucas, 2008a). In environments of high seston concentration, ingestion rate increases until an asymptote is reached with no further increase at higher concentration (Bayne *et al.,* 1989; Bacon *et al.,* 1998). Bivalves regulate ingestion as food concentration increases by reducing clearance rates and producing pseudofaeces (Bacon *et al.,* 1998).

Correlation between seston concentration and growth may not necessarily be meaningful and may only indicate that conditions favourable for plankton growth also

favour bivalve growth (Coe, 1948). Food quality is perhaps the greatest constraint on bivalve energy budget and growth (Grant, 1996). For example, specific components of the seston were found to correlate bivalve growth of rather than the bulk of the seston (Grant, 1996). Many species of bivalves have the ability to select the quality of the material ingested through the production of pseudofaeces and preferential rejection of inorganic particles (MacDonald and Ward, 1994). The nutritional composition of ingested food particles have been reported to influence growth rates in pearl oysters. Martinez-Fernandez *et al.* (2006) reported that growth rate of *P. margaritifera* larvae fed a micro-algal diet was correlated to levels of nutrients in the food especially carbohydrates and highly unsaturated fatty acids.

In order to for growth to occur, bivalves need to ingest food particle of an appropriate size and favourable morphology (Saucedo and Southgate, 2008). For example, mortalities and loss of condition in the pearl oyster *P. fucata martensii* were reported in Japan when the marine diatom *Nitzschia* spp. was found to predominate in the water column (Tomaru *et al.*, 2001; Tomaru *et al.*, 2002b) due to the unsuitability of the algae as a food for akoya pearl oysters.

Van Stralen and Dijkema (1994) demonstrated that bottom mussel culture was related to chlorophyll levels, while Vahl (1980) reported the growth of *C. islandica* to be correlated to concentration of particulate organic matter (POM) as well as inorganic matter. It has been found that the presence of suspended inorganic material silt stimulated growth in the bivalve *M. edulis* (Kiørboe *et al.*, 1980, 1981; Møhlenberg and Kiørboe, 1981; Bayne and Newell, 1983). Kiørboe *et al.* (1980) suggested that the presence of silt may induce rapid feeding as well as absorb organic compounds for energy utilisation in the mussel.

The characterisation of food supply to bivalves and its effect on bivalve growth is a complex issue and involves interaction between variables such as food concentration, food quality, size and other diverse factors such as temperature, resuspension, water current and water flow. Feeding and metabolism in pearl oyster was recently reviewed by Lucas (2008a).

2.6.2.4 pH

As reduced pH is an infrequent natural occurrence in the sea, relatively little research has been done on its potential effects on bivalve growth (Knutzen, 1981), when compared to studies on the effects of temperature and salinity. However, with the ongoing decrease in the pH of the Earth's oceans caused by their uptake of anthropogenic carbon dioxide from the atmosphere, negative consequences is a threat to oceanic calcifying organisms such as pearl oysters. Ocean acidification may force bivalves to reallocate resources away from productive endpoints such as growth in order to maintain calcification.

Oxygen uptake in bivalves occurs when oxygen diffuses passively from the higher partial pressure of oxygen in mantle cavity water to the lower partial pressure of oxygen in the haemolymph. Rate of oxygen uptake depends on the partial pressure gradient and pH is one factor which influences partial pressure besides temperature and salinity (Lucas, 2008b). Reduced ventilation is correlated to reduction in gape with retraction of the mantle edges (Jorgensen, 1996) which in turn lowers feeding and consequently growth.

Some observed responses to pH in *O. edulis*, *C. gigas* and *M. edulis* include growth suppression, shell dissolution, tissue weight loss and feeding suppression at pH < 7.0, and abnormal and “narcotic” behaviour at pH < 6.5 such as gaping of the valves and the absence of byssal attachment (Bamber, 1990). Other responses to low pH in bivalves consist of reduced gamete respiration in *M. edulis* below pH 7.6 (Akberali *et al.*, 1985), increased adult mortality and shell dissolution in *P. fucata* at pH 7.4 - 7.6 (Kuwatani and Nishii, 1969), reduced pumping rate and abnormal shell movement in adult *C. virginica* below pH 7.0 (Loosanoff and Tommers, 1947), inhibition of feeding and shell growth, shell dissolution and increased mortality in *Venerupis decussata* below pH 7.0 (Bamber, 1987) and lowered shell deposition in *Cerastoderma edule* at below pH 7.0 (Richardson *et al.*, 1981). Recent research with the pearl oyster *P. fucata* showed that reduced pH (to pH 7.8 and 7.6) resulted in reduced activity, reduced byssal attachment and byssal strength as well as reduced shell strength (Welladsen, 2009).

The responses of bivalves towards a low pH are attributed to the direct affect of acidity (Bamber, 1990) and the disruption of the CO_2 carbonate buffering system in seawater (Whitfield and Turner, 1986).

2.6.2.5 Fouling, boring organisms and predators

Biofouling is the attachment of marine organisms to another organism or non-living object. Some fouling organisms of bivalves including pearl oyster include barnacles, bryozoans, molluscs, fouling sponges, ascidians, hydroids, algae and tunicates (Dharmaraj *et al.*, 1987; Quayle and Newkirk, 1989; de Nys and Ison, 2008). These organisms can affect bivalve growth by occupying the same ecological niche as the bivalve and contesting for available food and living space (Arakawa, 1990a; Fernández *et al.*, 1999). Additionally, they may be deleterious to bivalve growth through physical interruption to the opening and closing of the valves thereby affecting the efficiency of filtration (Alagarswami and Chellam, 1976; Paul and Davies, 1986), interfering with the shell growth margin (Taylor *et al.*, 1997a) and reducing oxygen supply to the animal (Wallace and Reinsnes, 1985).

Fouling communities have been reported to cause adverse effect on the growth and survival of cultured bivalves like the Pacific oyster *C. gigas* (Arakawa, 1990a), the northern quahog, *Mercenaria mercenaria* (Fernández *et al.*, 1999), the American oyster, *C. virginica* (Zajac *et al.*, 1989) and the pearl oysters *P. fucata* (Alagarswami and Chellam, 1976; Mohammad, 1976; Dharmaraj *et al.*, 1987), *P. margaritifera radiata* (Doroudi, 1996), *P. radiata* (Doroudi, 1996), *Pteria penguin* (Smitasiri *et al.*, 1994) and *P. maxima* (Taylor *et al.*, 1997a).

Another group of animals which adversely affect bivalve growth and survival are boring organisms comprising polychaetes, sponges, molluscs and isopods (Dharmaraj *et al.*, 1987). Polychaetes of *Polydora* spp., a common borer, cause damage by drilling into the bivalve's shell margin and penetrating the nacre (Quayle and Newkirk, 1989). This induces the bivalve to expend energy in secreting additional nacre to coat blisters created by *Polydora* spp. and leaving less energy for somatic growth. Boring sponges of the *Cliona* species bore into calcareous shells and create a honeycomb of tunnels, making the animal susceptible to further damage by polychaetes. In extreme cases, the

secretion of nacre to seal perforations may lead to death from physical exhaustion (Alagarswami and Chellam, 1976). The deleterious effects of *Cliona* such as destruction of shell have been reported in pearl oysters (Fromont *et al.*, 2005; Humphrey, 2008; de Nys and Ison, 2008) and can potentially lead to disruption in the pearling industry.

In addition to fouling and boring organisms, predators present a serious threat to the survival and growth of cultured bivalves. Predators can affect the dynamics of prey population not only by direct predation, but also by changing the rates of growth and reproduction of prey (Fraser and Gilliam, 1992). Growth would be affected if prey stops foraging to avoid predators (Milinski and Heller, 1978; Lima and Dill, 1990), or if greater energy is channelled to morphological or chemical defence against predators (Harvell, 1992; Stibor, 1992; Hernandez and Leon, 1995). Nakaoka (2000) reported that *M. mercenaria* showed lower growth rates in habitats where predation by whelks was higher and suggested that the lower growth rate was caused by feeding inhibition; a predator-avoidance behaviour. Pit and Southgate (2003a) reported that removing predators monthly had a significant effect on growth of blacklip pearl oyster *P. margaritifera.* Predators of pearl oyster include gastropods, starfish, crabs, turtles and fish and these attack their prey through drilling through shells, forcibly tearing open valves as well as crushing shells (Humphrey, 2008).

2.6.3 Culture methods

Aquaculture practices strive to provide optimal conditions to promote the rapid growth of a species. Long term viability of bivalve culture is dependent upon the selection of suitable sites and culture methods which provide the physical and biological conditions necessary to promote rapid growth and high survival (Brown and Hardwick, 1988a). Culture methods including depth, stocking density and cleaning regimes can affect and modify pearl oyster growth (Pouvreau *et al*., 2000b; Saucedo and Southgate, 2008; Southgate, 2008).

Growth in bivalves is influenced by depth. Lodeiros *et al.,* (2002) showed that growth and survival rate of the pearl oyster *P. imbricata* in Venezuela was higher in suspended culture than bottom culture. Depth has also been showed to affect growth of various other pearl oysters such as *Pteria penguin* (Smitasiri *et al.,* 1994) and *Pinctada fucata*

(Chellam, 1978). Research with scallops indicated a nutritional basis for this effect (Wallace and Reinsnes, 1985).

The spatial distribution of an organism can affect the growth of the animal through competition for food and space. Overstocking of animals can have the same deleterious affect on bivalve growth as fouling organisms. Cultured *Placopecten magellanicus* showed an inverse relationship between growth (measured by shell height, meat weight and whole weight) and stocking density (Parsons and Dadswell, 1992). A possible explanation put forward by Wildish and Kristmanson (1985) is the seston depletion effect, whereby food resources are depleted more rapidly by animals held in high densities. Another reason may be space limitation leading to retarded growth or shell deformation, as demonstrated by Taylor *et al.* (1998) for the pearl oysters *Pinctada maxima* stocked at high densities. Spatial variation in growth has also been demonstrated in other bivalves such as the bay scallop, *Argopecten irradians* (Duggan, 1973), the clam, *Mercenaria mercenaria* (Hadley and Manzi, 1983), Sydney rock oysters, *Saccostrea commercialis* (Holliday *et al.* 1991, 1993) and Pacific oysters, *Crassostrea gigas* (Roland and Albrecht, 1990) amongst others.

Methods of culture employed for commercial bivalve farming takes into consideration optimal conditions for growth. In the hatchery stage, larval growth and survival are improved through feed supplement, removal of predators and provision of an optimal growth environmental with controlled water parameters such as temperature, salinity and pH. During ocean grow-out, growth and survival may be significantly better in some structures over others. For example, the pearl oysters *Pinctada mazatlanica* and *Pteria penguin* grew larger when cultured in pocket nets compared to lantern nets and plastic cages (Gaytan-Mondragon *et al.*, 1993). Similarly, an evaluation into growth of *P. margaritifera* using 5 different culture techniques indentified optimum growth was attained when oysters were grown using pocket nets and by “ear” hanging (Southgate, 2000). Besides employing suitable culture structures, the judicious selection of sites with favourable conditions also affect growth of bivalves. Yukihira *et al.* (2006) showed that growth index (Φ) for *P. margaritifera* was significantly different between two culture sites in Queensland, Australia. Selection of a culture site which has appropriate physical and biological conditions to promote growth of pearl oyster is crucial to the success of pearl oyster culture.

2.7 Conclusion

Bivalve growth is affected by a complex combination of biological and environmental factors. Biological factors include the size, age, reproductive condition and genetic characteristic of the animals. These aspects of bivalve growth are often difficult if not impossible to manipulate in a culture situation to ensure optimum growth. On the other hand, limited control may be exerted over environmental factors that govern growth such as water temperature and salinity, which affect rates of biochemical reactions within an organism and food availability, which affects nutritional conditions, through careful selection of culture sites.

2.8 Aims of this study

The major objective of this study was to develop our understanding of what governs growth and mortality in *P. maxima* cultured at Aljui Bay in West Papua, Indonesia. Various temporal (month), spatial (site and depth) and biological factors (age and size) were investigated to determine how they affect somatic and reproductive growth in *P. maxima*. In addition, various environmental parameters were investigated to determine their influence on *P. maxima* growth.

The specific aims of this study were:

1. To examine the temporal and spatial variation in environmental parameters at three experimental sites within Aljui Bay (Chapter 4)

2. To evaluate the spatio-temporal and age-related variation in growth and mortality of *P. maxima* grown at three sites and two depths, with special emphasis on the environmental influence (Chapter 5)

3. To model the growth of *P. maxima* mathematically and use mathematical expressions to compare growth at three sites and two depths (Chapter 6)

4. To investigate the spatio-temporal variation in the recruitment of biofouling assemblages on *P. maxima* grown at three sites and two depths and examine the effect of biofouling on growth and mortality (Chapter 7)

5. To examine various factors affecting gender and gonad development in *P. maxima* cultured at two sites and depths (Chapter 8)

This research will provide valuable insight into factors which influence growth of *P. maxima* during ocean grow-out. The *in situ* nature of this study ensures that the research outcome is directly relevant to the pearl farmer.

CHAPTER 3

General Methods and Materials

3.1 Study area

Field study was conducted between May 2000 and February 2002 at a commercial pearl oyster farm, P. T. Cendana Indopearls. Cendana Indopearls is owned by an Australian parent company Atlas Pacific Limited. Cendana Indopearls is located in a remote area surrounded by pristine waters off Pulau Waigeo (Latitude 0° 11' S, Longitude 130° 14' E), West Papua (formerly Irian Jaya), Indonesia. Pulau Waigeo (Fig. 3.1) is the largest island in the Raja Ampat island group, lying approximately 148 km off the north-west of West Papua within the administrative regency of Sorong. Access to the farm is by boat from Sorong. Unless otherwise stated, all field experiments and environmental monitoring were conducted on site.

Fig. 3.1 Location of study area at Pulau Waigeo, Raja Ampat Island group, West Papua, Indonesia.

Cendana Indopearls has approximately 2,500 hectares of water leases in the area, and the farm consists of a land-based hatchery, ocean-based nursery and ocean grow-out stocked with approximately 800,000 oysters as well as pearl seeding and farming facilities. The farm's proximity to the equator and away from cyclonic activity makes it a favourable site for pearl oyster cultivation. Additionally, it presents an ideal site to conduct pearl oyster research as all stages of the life cycle are fully represented.

3.2 Physical features

The farm is located at the western end of Pulau Waigeo within Aljui Bay (Fig. 3.2). Aljui Bay consists of an inner bay and an outer bay. Oysters are cultivated in the outer bay by suspension on long-lines arranged in blocks close to shore and placed either parallel or perpendicular to the land mass. The sea within the Bay is relatively calm and protected by surrounding islands.

Aljui Bay encompasses numerous scattered islets and reefs within both bays. The inner bay is landlocked and fringed by extensive mangrove swamps. The outer bay is approximately 10 km long by 5 km wide (Knauer, Atlas Pacific, pers. comm., 2002) and access from the open sea is restricted to a few channels. The topography of the outer bay islets is characterised by lower montane forest on steep and rugged limestone karst, with ultra-basic peridotites and serpentine rock outcrops (Diamond, 1986). Most of the islets have no sandy shoreline; instead the perimeter slopes almost vertically into fringing reefs, with xerophytic vegetation over the basic parent rocks (Diamond, 1986).

3.3 Climate

West Papua lies within the humid tropical climatic zone with an approximate average annual air temperature and rainfall of 27.4 ℃ and 2767.7 mm, respectively[1] (Hoare, 1996). Historical climate data derived from the Global Historical Climatology Network indicated slight seasonal variation in average monthly temperature and rainfall within the region, with greater rainfall and lower temperatures observed in June, July and August (Hoare, 1996).

[1] Data from weather station at Jefman Airport (Latitude 0 ℃N, Longitude 131 ℃E) and covers an area approximately 111 km north to south and east to west (Hoare, 1996).

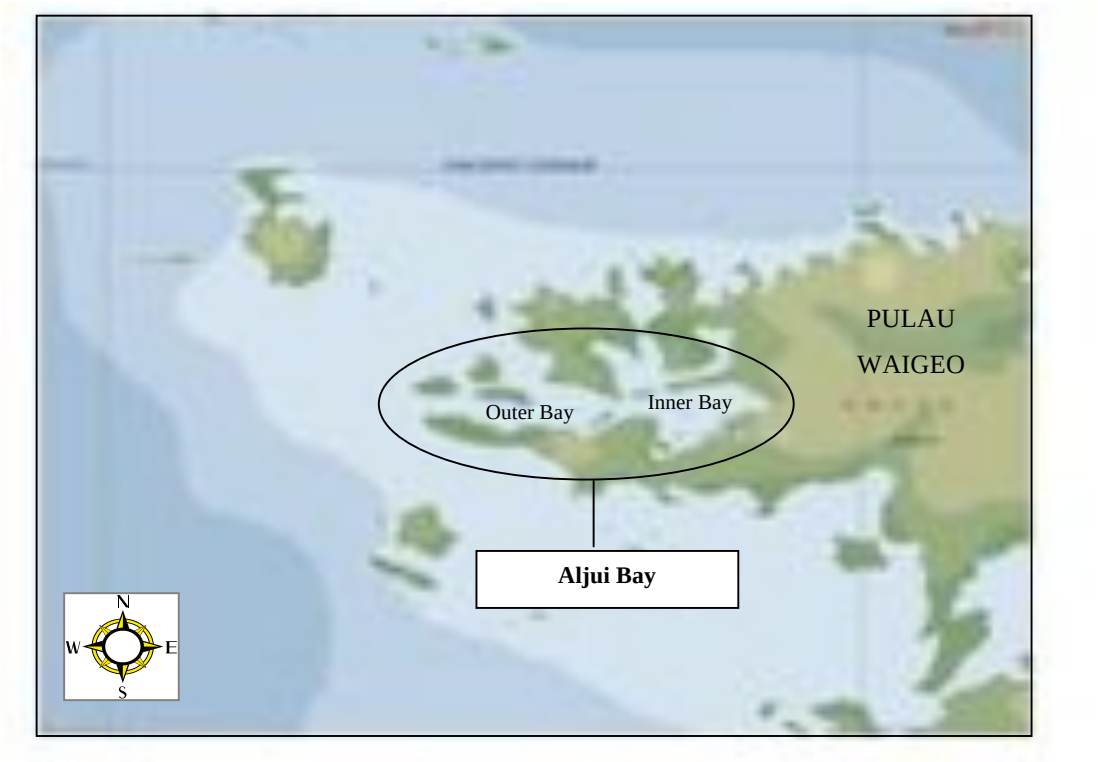

Fig. 3.2 Location of Aljui Bay, western Pulau Waigeo.

3.4 Experimental sites

Three locations within Aljui Bay were selected as experimental sites. The sites were used extensively for culturing farm oysters and differed in depth, surrounding topography, current and long-line positions. The micro-environment of the sites was monitored over a one and a half year period to determine if there were any differences in environmental parameters between sites and over season. The experimental sites are Ganan, Manselo and Batu Terio (Fig. 3.3)

3.4.1 Ganan

Ganan (Latitude 00° 11’ 59 S, Longitude 130° 15’ 52 E) is located at the entrance to the outer bay and faces strong upwelling currents sweeping in from the Pacific Ocean from two direction (Fig. 3.3) with high water exchange. The long-lines at Ganan are placed perpendicular to the current flow (cross-current) and secured to limestone karsts on the nearby land mass of Pulau Ganan. Depth soundings with a Simrad® EQ 30 echo sounder indicate a maximum depth at Ganan of approximately 23 m. The bottom topography consists mainly of coral reef and sand.

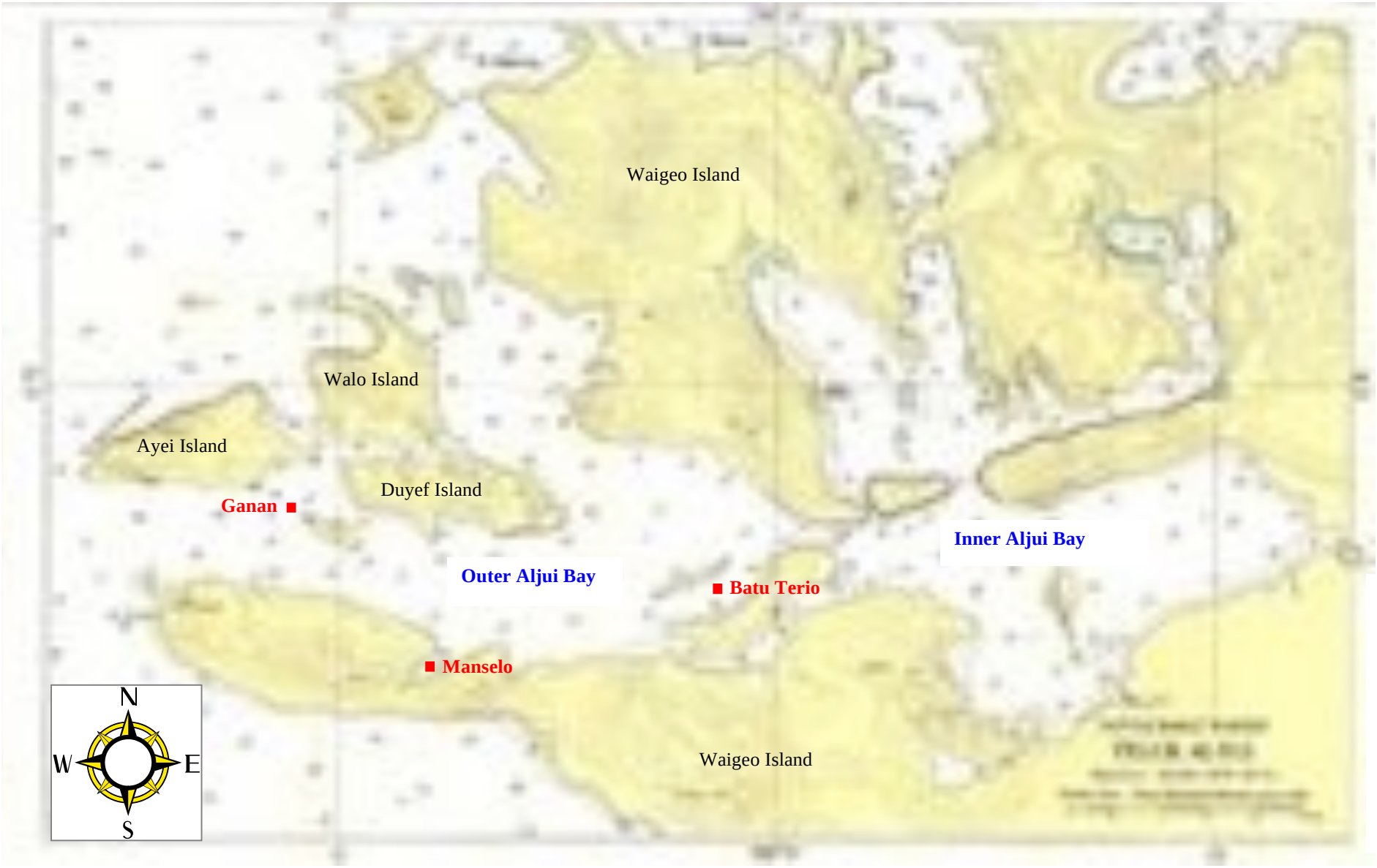

Fig. 3.3 Diagram of the experimental sites of Ganan, Manselo and Batu Terio within Aljui Bay, western Waigeo. Diagram is not to scale.

3.4.2 Manselo

Manselo (Latitude 00° 12’ 23 S, Longitude 130° 17’ 15 E) is located approximately 2.6 km south-east from Ganan and is separated from it by the island mass of Pulau Ganan (Fig. 3.3). The long-lines at Manselo are secured to the main island of Pulau Waigeo at the entrance of a small cove lined with fringing mangrove; hence water exchange at Manselo is lower than at Ganan. The long-lines are placed parallel to the main current flows and the maximum depth at Manselo is approximately 40 m. The sea bed consists mainly of mud and sand.

3.4.3 Batu Terio

Batu Terio (Latitude 00° 12’ 35 S, Longitude 130° 19’ 7 E) is located 6.1 km south-east of Ganan and 3.5 km north-east of Manselo (Fig. 3.3). Long-lines at Batu Terio are situated in a channel between groups of limestone islets with steep limestone cliffs that slant almost vertically into the sea. Long-lines at Batu Terio are set further away from shore than Ganan and Manselo and there is an intermediate rate of water exchange in the channel. Maximum depth at Batu Terio is approximately 32 m and the sea bed is mainly mud and sand.

3.5 Environmental monitoring

A range of environmental data were collected from June 2000 to February 2002 at the three sites; water temperature, salinity, pH, suspended particulate matter (SPM), particulate organic matter (POM) and chlorophyll *a*, *b* and *c*. Rainfall at Aljui Bay was also monitored.

Water temperature data was taken directly at all the sites using data loggers set at 5 m and 15 m depths. Salinity, pH, SPM, POM, chlorophyll *a*, *b* and *c* values were obtained by taking weekly water samples from both 5 and 15 m depths (measured from the surface) at the experimental sites using a 1 L discrete vertical water sampler (Viktoria Prima Perkasa, Indonesia). The water sampler was secured to a marked rope, lowered to the set depth and 1 L of seawater was taken from that depth. The procedure was repeated to obtain two 1 L replicates of seawater from each depth per sample.

3.5.1 Water Temperature

Water temperature was monitored at 6-hourly intervals using a submersible data logger (Onset Stowaway® Tidbit®). To determine water temperature at a discrete depth within the water column, a logger was secured at both 5 m and 15 m along a weighted line and suspended from a surface long-line (Fig. 3.4). Loggers were brought to the surface every week and data downloaded to a portable shuttle (Onset Stowaway® shuttle). After downloading, the submersible logger was immediately placed back into the sea. Data from the shuttle was then downloaded onto a computer using the BoxCar® Pro, Version 3.01 (Onset Computer Corporation) program.

3.5.2 Salinity

Salinity was measured with an Atago® S/Mill refractometer. The refractometer was calibrated with distilled water before each measurement. Unit of measurement for salinity was in parts per thousand (‰).

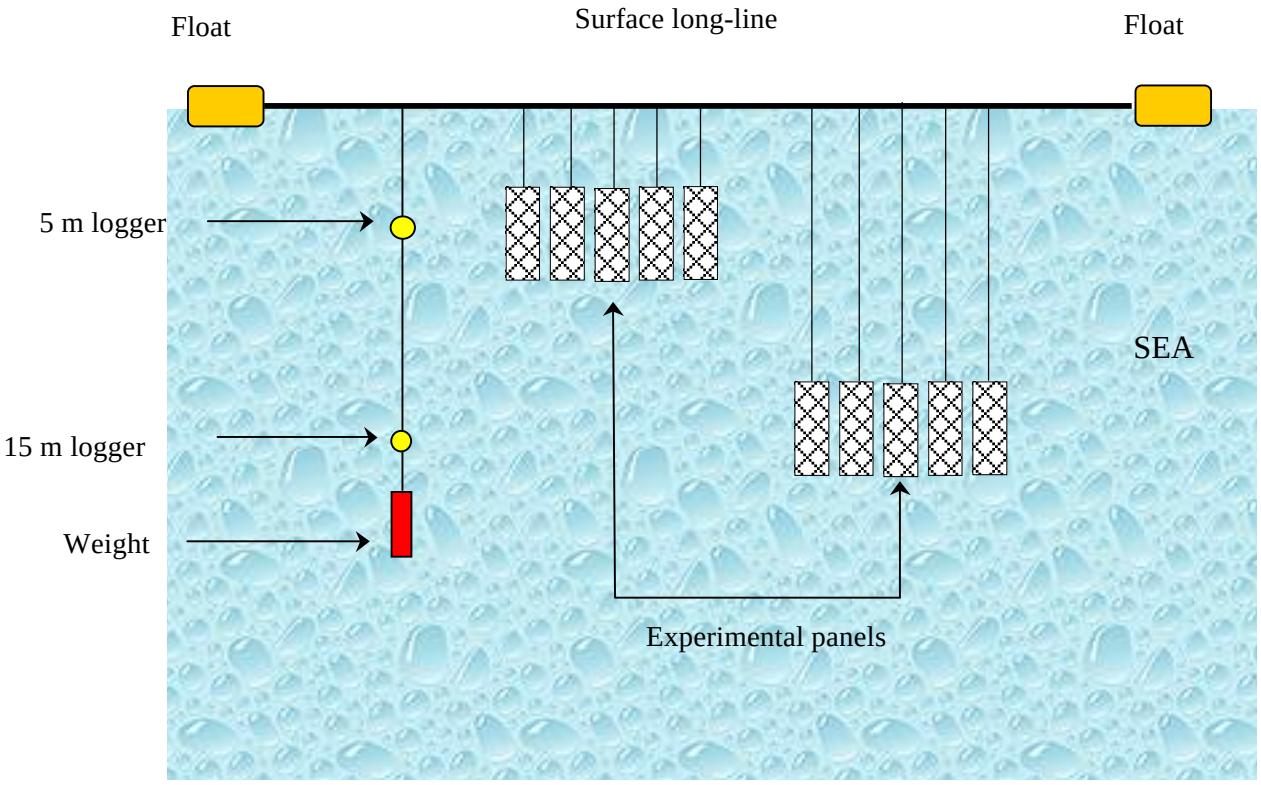

Fig. 3.4 Diagram showing position of temperature loggers used to monitor water temperature at 5 m and 15 m depths at the experimental sites.

3.5.3 pH

pH of seawater was determined using a Eutech® pH scan WP meter. The pH meter was calibrated before each use with a buffer solution of pH 7 standard and the electrode cleaned with distilled water between each sample measurement.

3.5.4 Determination of particulate matter

SPM and POM were determined according to the method described by Strickland and Parsons (1982) with slight modifications as described below.

3.5.4.1 Preparation of glass fibre filters

Whatman GF/F® glass fibre filter papers (47 mm in diameter) were placed on an aluminium foil sheet and pre-ignited for 5 h at 500 °C in a Thermolyne® muffle furnace. The filters were left to cool in the furnace for an hour, then removed with flat bladed forceps and weighed on a Sartorius® LP 6200S balance to two decimal places. The weight (pre-weight, PW) of individual filters was recorded in grams (g) and the filters were kept in an airtight glass container until use.

3.5.4.2 Measuring SPM and POM

To measure SPM, 1 L of seawater was transferred under suction by vacuum pump through a ceramic funnel lined with a pre-ignited Whatman® glass fibre filter paper. After filtration, the filtrate was discarded while the filter paper was removed with a pair of forceps and placed on a small slip of aluminium foil before being transferred to a desiccating oven. The filter paper was dried at 60 °C in the drying oven for 24 h, cooled and weighed to determine the dry weigh (DW). After weighing, the filter paper was placed into a muffle furnace and ignited for 6 h at 560 °C. The filter paper was allowed to cool for 30 min in the furnace before being weighed again to obtain ash weight (AW) and ash-free dry weight (AFDW) was then calculated as:

$$AFDW = DW - AW \qquad \text{(Equation 3.1)}$$

The unit of measurement for DW and AFDW is mg L^{-1}.

SPM and POM were determined as follows:

SPM = PW – DW (Equation 3.2)

POM = DW – AFDW (Equation 3.3)

Unit for SPM and POM is mg L^{-1}

3.5.5 Chlorophyll a, b and c

The analytical procedure for measuring the chlorophyll content of planktonic algae in seawater was based on the method described by Strickland and Parsons (1982).

One litre of seawater was filtered through a 300 μm mesh to remove zooplankton. The filtrate was passed under suction through a ceramic funnel lined with a 47 mm diameter Whatman® GF/C glass fibre filter. After the filter was thoroughly drained under suction, it was removed with a pair of forceps and placed in a 15 mL graduated centrifuge tube. Ten millilitres of 90% acetone (v/v) (Appendix B) was added to the tube, thoroughly ground in a tissue grinding tube and Teflon® pestle, stoppered and shaken vigorously to disintegrate the filter into a homogenate solution. Aluminium foil was wrapped around the centrifuge tube and then placed in the refrigerator in complete darkness for 15 – 20 h to allow for pigment extraction. After extraction, the tube was brought to room temperature and volume made up to 12 mL by the addition of 90% acetone. The tube was centrifuged for 5 - 10 min using a Hettich® 4-rotor swing out hand centrifuge with a maximum relative centrifugal force (rcf) of 1,300. After centrifugation, the clear supernatant was decanted and an aliquot transferred into a 1 cm light path glass cuvette. Optical density (extinction) of the solution was measured in a Hitachi® Model 101 spectrophotometer blanked with freshly prepared 90% acetone solution. The extinction coefficients (E) were measured at wavelengths of 6300, 6450, 6650 and 7500 Å. Concentrations of chlorophyll were calculated as follows:

Chlorophyll *a*: $11.6\ E_{665} - 1.31\ E_{645} - 0.14\ E_{630}$ (Equation 3.4)

Chlorophyll *b*: $20.7\ E_{665} - 4.34\ E_{645} – 4.42\ E_{630}$ (Equation 3.5)

Chlorophyll *c*: $55.0\ E_{665} – 4.64\ E_{645} – 16.3\ E_{630}$ (Equation 3.6)

Chlorophyll per unit was calculated as

$$\mu g\ L^{-1} = \frac{\text{Chlorophyll (value from equation)}}{\text{Volume of filtered seawater (L)}} \qquad \text{(Equation 3.7)}$$

3.5.6 Rainfall

Rainfall was recorded daily using a rain meter located on land near Ganan. As the experimental sites were less than 7 km apart, it was deemed unnecessary to measure rainfall at all sites. Temporal, not spatial comparison of rainfall was used in analyses later on in the thesis.

3.5.7 Environmental data

The mean of an environmental parameter at a site or depth over a particular monthly period (C_a) was determined by the equation:

$$C_a = (C_1 + C_2 + \ldots\ldots + C_n) / n \qquad \text{(Equation 3.8)}$$

where C_1, C_2, and C_n were individual measurements for a particular month, and n was the total number of measurements taken during the month. Means were computed using the pivot table function in Microsoft Excel 2003.

3.6 General oyster culture

Pinctada maxima used in the experiments were hatchery produced at Cendana Indopearl's hatcheries in Kupang (West Timor) and Aljui via mass spawning. Oysters were not produced *in vitro*, as the objective of this research was to investigate growth of *P. maxima* bred under farm protocol. Wild broodstock originated from Flores in the Nusa Tenggara region of Indonesia. Ages of experimental oysters were specified in the relevant chapters. First generation offspring of broodstock from the same locality were specifically selected for the experiments to minimise any potential genetic induced variability. Some aspects of oyster production and cultivation at the farm will be briefly discussed. The various stages of production include spawning, larval rearing, larval settlement, nursery rearing and ocean grow-out (Fig. 3.5).

SPAWNING

Broodstock oysters are sexed, then induced to spawn

LARVAL REARING

Larvae reared in tanks and fed cu;tured algae until settlement

SETTLEMENT

Spats settle on special collector frames hung in culture tanks

SPAT CULTURE

Spats transferred from collectors and place in frameless pocket nets, then suspended on longlines

JUVENILE AND ADULT GROWOUT

Oysters are grown in suspended culture on longlines and periodically graded and cleaned until used for seeding or as broodstock

Fig. 3.5 Summary of the various stages of *P. maxima* production at Cendana Indopearls.

3.6.1 Spawning

Broodstock oysters were taken from the sea and transferred to the laboratory. The oysters were placed upright in oyster baskets in 1000 L tanks constantly supplied with running seawater piped directly from the sea. Oysters were kept in this flow-through system at ambient salinity and water temperature. When oyster shells were agape, wooden pegs were inserted between shell valves at the antero-ventral areas of the shells to allow sagittal inspection of the internal part of the oysters and the sex and level of gonad maturation to be determined by visual inspection (Appendix C). Closed oysters were gently prised open with reverse pliers inserted into the postero-ventral area, and wedged with wooden pegs in the antero-ventral area to keep the valves apart. Only suitably conditioned males and females were selected for spawning.

The selected broodstock were cleaned and exposed to direct sun light for 45 min. Subsequently, the females were placed in a gently aerated, flow-through tank containing 1 µm and UV filtered seawater at ambient temperature. The males were placed in an air-conditioned room (22 to 24 °C) for 45 min. Following temperature shock, the males were removed to a separate, gently aerated spawning tank (250 to 500 L) filled with 1 µm and UV filtered seawater.

Male oysters were induced to spawn by repeatedly lowering and raising the water level. When spawning commenced, spawning males were placed into the spawning raceways containing the females. The introduction of spawning males into the tank triggered the release of eggs by female oysters after approximately 20 min. When spawning ceased, male and female oysters were removed to a new tank where further spawning was allowed to recommence. The tanks containing freshly spawned eggs and sperm were left undisturbed for 40 min to undergo fertilisation. The resulting zygotes were collected by siphoning, then counted and finally transferred to 250 or 500 L hatching tanks where they were stocked at a density of 10 to 30 zygotes mL^{-1}.

3.6.2 Larval rearing

Twenty hours after fertilisation, the hatching tanks were drained to collect D-stage larvae (Fig. 1.2). The larvae were counted and restocked into new tanks at a density of

between 5 to 7 D-stage larvae mL^{-1} and immediately fed on a mixture of the flagellates *Pavlova salina* and *Isochrysis* aff. *galbana* (strain T-ISO), and the diatom *Chaetoceros calcitrans*. For the first fortnight, seawater was changed regularly, depending on water quality and larval health. At the eyespot stage (Day 14 to 19) (Fig. 1.2), stocking density was reduced to two larvae mL^{-1}.

3.6.3 Settlement

Larvae were ready to settle once they reached the pediveliger stage, characterised by red eyespots and a functional foot (Rose and Baker, 1994). Once the eyespots were of a burgundy colour with a diameter of 10 μm, settlement tanks containing collectors were prepared. Collectors were made up of a black metal frame panels and old, black rope forming squares with holes 2 x 2 cm between them and provided a large substrate area to which spat could attach. Collectors were tied together horizontally with approximately 5 cm space between individual panels and placed in the settlement tanks suspended from wooden poles.

Pediveligers were transferred from the larval rearing tanks to the settlement tanks at a density of 1 to 2 pediveliger mL^{-1}. Settlement was completed within 3 to 7 days. After metamorphosis, spat were kept in the settlement tanks for approximately 20 more days. Once they had reached between 2 to 3 mm in shell length, all the collectors containing the spat were transferred to the ocean by tying two collectors together and covering them with 1 mm nylon mesh for protection from predators.

3.6.4 Nursery culture and grow-out

Collectors were suspended at 15 m depth from surface long-lines, and checked and cleaned every week. Initially, the collectors were gently raised and lowered to wash off diatomaceous films forming on the mesh. However, after 2 weeks the collectors were cleaned using a low pressure hose.

Spat were first graded according to size at 2.5 months of age. They were detached from the collector by carefully cutting the byssal threads using a razor blade and transferred to 64-pocket frameless mesh nets termed ‘flag nets’ made up of 3 to 5 mm nylon mesh. After the first selection, collectors containing spat too small to be put into flag nets (Fig.

3.6a) were covered with 3 mm black mesh and returned to the sea for selection at a later stage.

Flag nets were suspended at 5 m and 15 m depths from surface long-lines in various parts of the farm. Juveniles kept in flag nets were removed from the sea and cleaned every 4 to 5 weeks using a pressure hose to detach soft fouling, followed by the manual removal of hard fouling with a chisel. The juveniles were left in the flag nets until they reached a mean shell length of 5 cm at approximately 6 - 7 months old. The juveniles were again graded into size groups ranging from 3 - 8 cm shell length. Juveniles with a shell length from 3 - 7 cm were put into 28-pocket panel nets with frames (Fig. 3.6b) and juveniles with a shell length greater than 8 cm into 8-pocket panel nets (Fig. 3.6c). The panel nets were doubled up and hung from surface long-lines in the ocean. The same cleaning regime as before was resumed and grading was performed at regular intervals to sort oysters into similar sized groups. The final grading of juveniles took place approximately 6 months before seeding for pearl production to grade juveniles into size groups ranging from 12 - 16 cm.

3.6.5 Culture structures

Oysters were cultured using a surface long-line system (Fig. 3.7). This consisted of a length of line buoyed by floatation and anchored at both ends by mooring blocks on the seabed or when near shore, secured to land to limit movement from wave action. At Cendana Indopearls, long-lines were arranged in blocks; each block consisted of 10 parallel lines strung approximately 5 m equidistance apart. Drop lines (droppers) of 5 m or 15 m depth were hung at 1 m intervals along the long-line and oysters in panel nets were hung at the end of each dropper (Fig. 3.6).

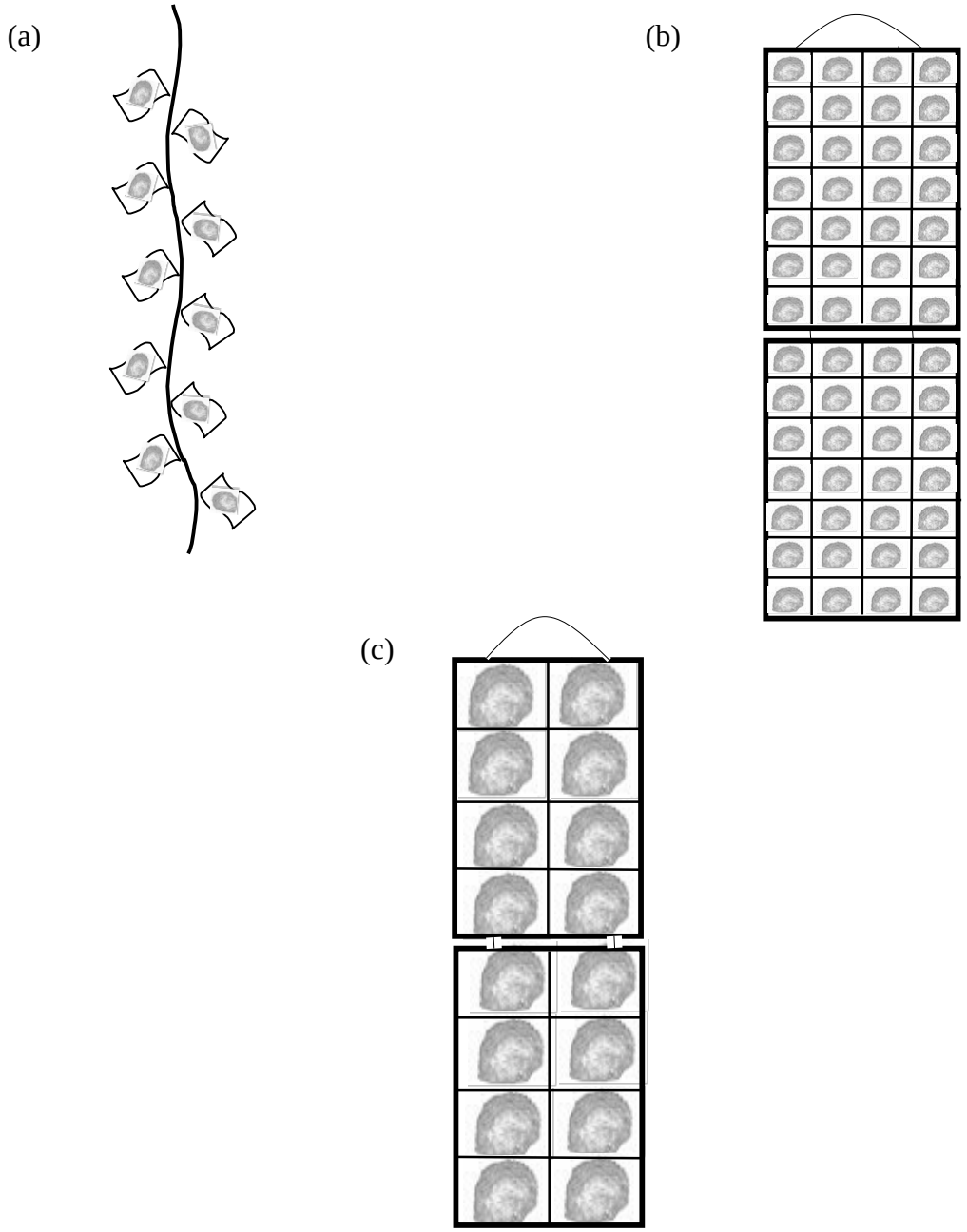

Fig. 3.6 Various nets used in the culture of *P. maxima* in Aljui Bay including flag nets for spat (a), 28-pocket panel nets for juveniles with 3 – 7 cm shell length (b) and 8-pocket panel nets for adult oysters with shell length > 8 cm (c).

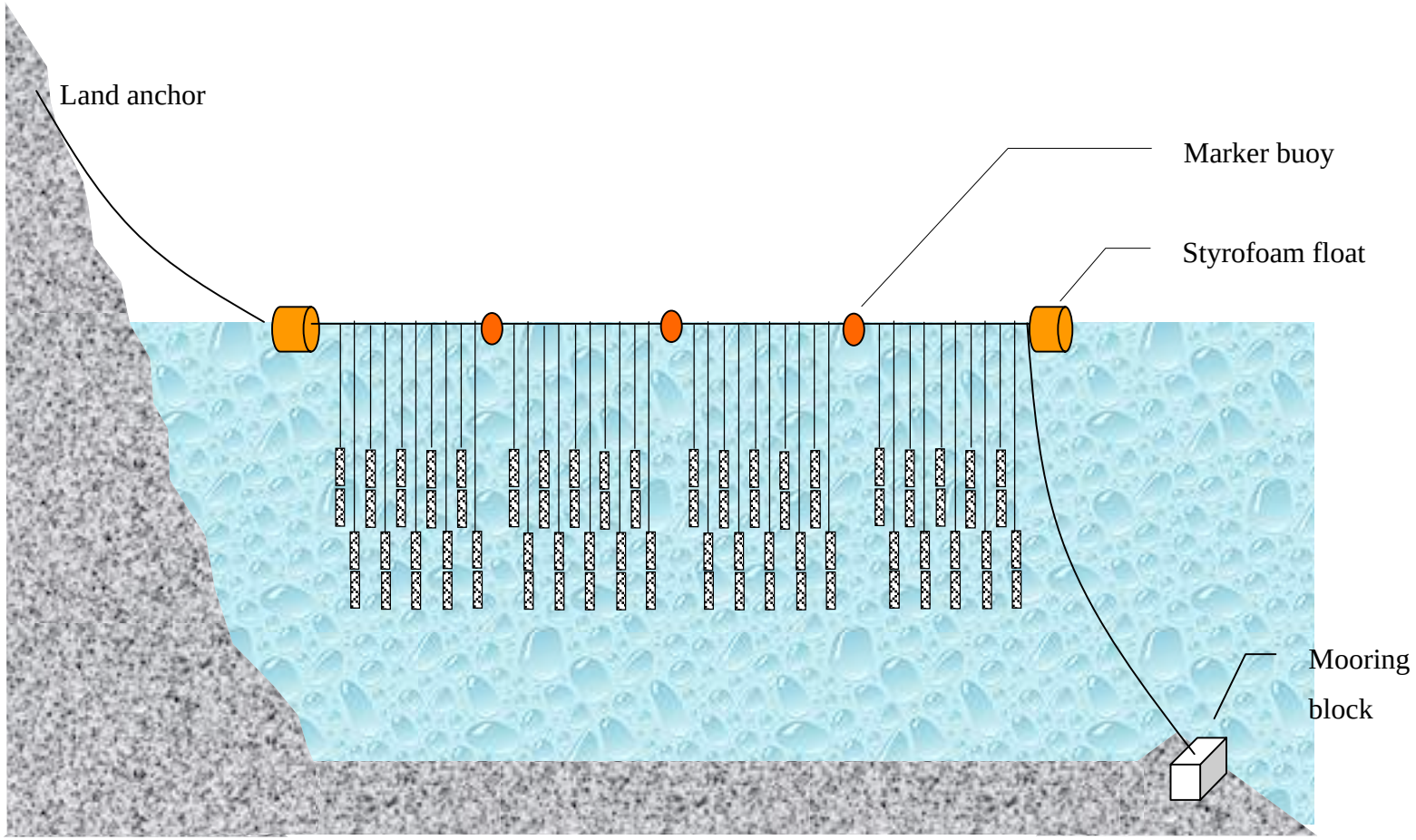

Fig. 3.7 Diagrammatic representation of a long-line system used for the suspended culture of *P. maxima* at Cendana Indopearls.

3.7 Culture of experimental oysters

Oysters used in experiments were randomly selected during grading and kept separate from farm oysters in different nets and long-lines at experimental sites. When oysters of a particular age or size were required to examine the effects of these parameters, stratified random sampling was employed where the populations of oysters were first divided into relevant groups; thereafter oysters were randomly chosen. Depending on the experiment, the oysters were subjected to the same regular cleaning regime as other oysters on the farm, with the difference that once an experiment commenced, oysters were not graded according to size as per normal husbandry practice for farmed oysters.

3.8 Sampling of experimental oysters

Oysters were transferred from the ocean grow-out to the laboratory and placed upright in oyster baskets in 1000 L raceways constantly supplied with running seawater from a flow-through system. The oysters were kept at ambient salinity and water temperature for no longer than 2 h before being sampled.

3.8.1 Linear growth measurements

Before linear growth sampling, oysters were cleaned of surface fouling with a knife and dried with tissue. Shell length, shell height and shell thickness (Section 2.3.1) were measured using a vernier calliper to the nearest 0.1 mm (Fig. 2.1 in Chapter 2). Before measuring shell thickness, oysters were checked to ensure both valves were firmly closed so measurement was not distorted by gaping.

3.8.2 Weight measurement

3.8.2.1 Wet weight

Before weight sampling, oysters were wiped dry with paper tissue then wet weight was measured using a digital balance (Sartorius® LP 6200 S) to two decimal places.

3.8.2.2 Dry weight

Dry weight of oyster components was determined by sacrificing and dissecting an oyster. The dissected oyster was separated into shell, adductor muscle, mantle, muscle and remaining soft tissue components. The muscle, mantle and soft tissue were dab-

dried with paper towel, placed on aluminium foil and dried in an oven at 55 °C until constant weight. Organic content was determined by weight difference after ashing the dried components at 400 °C for 4 h.

3.9 Statistical Analyses

All analyses were performed using Excel 2003 (Microsoft® Corp.) and SPSS Version 16.0 (SPSS© Inc.).

When an analysis of variance (ANOVA) was used, the two underlying assumptions of ANOVA, homocedascity and normality, were tested on the data using the Levene's and Shapiro-Wilk's test, respectively. In the event of a variable departing from the assumption of homocedascity, a suitable transformation was applied. In some instances where variance remain heteroscedastic after transformation due to outliers, analysis of variance was performed nonetheless, as ANOVA is robust, operating well even within considerable heterogeneity of variances as long as all n are equal or nearly equal (Zar, 1984). The test is also robust to violations of the normality assumption, provided that the samples are sufficiently large (Francis, 2004). The significance level (α) was set at 0.05 for all analysis of variance.

When two or more variables were compared, a multivariate analysis of variance (MANOVA) was utilised using the Wilks Lambda statistic. Assumptions of MANOVA i.e. multivariate normality and homogeneity of variance/covariance matrix was checked using outliers testing and Box M in SPSS. Fortunately, like ANOVA, provided that the sample sizes are equal, MANOVA is also very robust to violations of homogeneity of variance/covariance assumptions (Francis, 2004).

Principal component analysis (PCA) is a data reduction technique that reduces observed variables to a more meaningful and manageable number without excessive loss of information (Francis, 2004). PCA was performed in some analysis to gain a better understanding of the relationship among variables. Factors (components) with eigenvalues greater than one (known as Kaiser's criterion) were retained and rotated. The rotation used depended on whether the factors produced were expected to be uncorrelated (orthogonal rotation) or correlated (oblique rotation). Varimax rotation was

employed where an orthogonal rotation was required while a Direct Oblimin was selected for an oblique rotation (Francis, 2004). Each component is a composite variable made up of a linear combination of the original variables (Appeldoorn, 1983). In this thesis, when a new variable is created for each factor in the final solution, the method used for calculating the factor scores was the regression method.

Discriminant analysis was performed in some analyses to predict group membership and to identify variables that were useful in discriminating between groups (Francis, 2004). It is a multivariate technique that can be used to build rules that classify a subject into the appropriate population. It is similar to regression analysis except that the dependent variable is categorical rather than continuous and its objective is to be able to predict class membership on an individual observation based on a set of predictor variables (Steel *et al.,* 2004).

CHAPTER 4

Multivariate analyses of temporal and spatial variations in environmental parameters at three experimental sites within Aljui Bay

4.1 Introduction

Site selection is one of the most important considerations in aquaculture. Some of the factors that need to be taken into account when choosing a site include ease of access (e.g. proximity to land), protection from wind and wave action, security and most importantly, an optimal environment that satisfies the growth requirements of the cultured species. In land-based culture, the growing environment may be regulated in tanks and raceways. In pearl oyster culture, where oysters are grown out for long periods of time in the ocean, hydrological characteristics of the environment often exert a significant effect on growth.

Sukhotin and Maximovich (1994) divided the effects of the environment on bivalve growth into factors of general action and local action. The former include water temperature and salinity, which may affect the rate of biochemical reactions within the organism in temperate climates. Factors of local action include suspended particulate matter (SPM), particulate organic matter (POM), chlorophyll levels, water current and water depth that determine nutritional conditions to influence growth rate of the organism.

Besides affecting growth and mortality, the microenvironment also has other significant effects on bivalve culture. For example, the colour and quality of harvested pearls are of prime importance in pearl oyster culture, and factors such as depth of culture and light penetration, and the quality of phytoplankton have been reported to contribute to pearl colour and quality (Sonkar, 1998) by influencing the bio-mineralisation process of nacre (Wu *et al.*, 2003a).

While seasonal variation in factors of general action (e.g. water temperature) is not as distinct in the tropics as in temperate areas, there have been reports that experimental

production of pearls in the tropics shows differential nacre growth and pearl quality during different periods (Victor *et al.*, 1994).

The effects of the environment on various aspects of *Pinctada maxima* growth at the research sites of Ganan, Manselo and Batu Terio are important considerations in this study. This chapter is devoted to the multivariate analyses of temporal and spatial effects on environmental factors of both general and local action for the duration of the research. To avoid repeated descriptions of environmental data analyses in subsequent chapters, this chapter also introduces the various methods employed for statistical analyses of environmental data, provides an overall insight into the environmental conditions in which *P. maxima* were cultured over a 20-month period, and examines relationships between the various environmental parameters.

4.2 Methods and Materials

4.2.1 Environmental monitoring

Environmental parameters were measured weekly in Ganan and Batu Terio from June 2000 to February 2002. Manselo was sampled weekly from June 2000 to November 2001. Duplicate water samples (1 L) were taken at each sampling from 5 m and 15 m depths using a suspended water sampler for measurement of salinity, pH, SPM, POM and chlorophyll *a, b, c* according to the methods described in Chapter 3 (Section 3.5).

Environmental monitoring of each site corresponded to the timing of various experiments conducted at the particular site (detailed in subsequent chapters) and seawater was sampled close to experimental long-lines. While environmental sampling was continuous, the whole dataset was not used in the analyses of all experiments as the experiments were staggered and conducted within different time frames. The corresponding environmental dataset for the relevant period will be specified in each subsequent chapter. However, in this chapter, the complete dataset will be employed to provide a more comprehensive picture of the variation in environmental parameters between culture site and depth over the entire study period.

4.2.2 Statistical analysis

A mean value for each parameter for each site and at each depth over a particular monthly period (C_a) was determined using Equation 3.7 in Section 3.5.7.

Means were computed using the pivot table function in Microsoft Excel 2003 and used for graphical representation of environmental parameters from various sites, depths and sampling months.

A univariate analysis of covariance (ANCOVA) was used to analyse each environmental parameters to determine if they were significantly different between sites and depths over the sampling months, with the sampling month designated the covariate. Interaction between the covariate and factors was tested to determine homogeneity of regression (i.e. the relationship between the covariate and the dependent variables had the same slope in each treatment group). If the slopes were not significantly different, the use of ANCOVA was justified (i. e. the covariate did not have an effect on factors). Before analysis, assumptions of ANCOVA (i.e. homocedascity and normality), were tested on the data using the Levene's and Shapiro-Wilk's test, respectively, according to the methods outlined in Section 3.9.

To assess the simultaneous effects of spatial (site and depth) and temporal (month) factors on all environmental variables, the Wilks-Lambda multivariate analysis of covariance (MANCOVA) was performed on the complete dataset. SPM, POM, pH, salinity, water temperature, chlorophyll *a*, *b* and *c* were assigned the dependent variables, site and depth the fixed factors and month of sampling the covariate.

Pair-wise principal component analysis (PCA) was performed on the environmental data to determine relationships between environmental variables. Components with eigenvalues greater than one (known as Kaiser's criterion) were retained and rotated obliquely by Direct Oblimin method to produce components (Francis, 2004).

4.3 Results

Environmental data sampled from 5 m and 15 m at Ganan, Manselo and Batu Terio from June 2000 to February 2002 are summarised in Table 4.1, Table 4.2 and Table 4.3 , respectively. Each parameter will also be discussed individually. Descriptive statistics of each treatment are given in Appendix A.

4.3.1 Univariate analysis of environmental parameters

4.3.1.1 Water temperature

During the experimental period, seawater temperature ranged from 27.43°C to 29.87°C. When tested with univariate ANCOVA, mean water temperature between sites, depths and month of sampling was significantly different (site: $F_{(2,500)} = 91.349$, $p < 0.05$; depth: $F_{(1,500)} = 5.130$, $p < 0.05$; month: $F_{(1,500)} = 4.610$, $p < 0.05$). There were also differences in water temperature between site and depth combinations as the interaction between site and depth was significant ($F_{(2,500)} = 75.488$, $p < 0.05$).

There appeared to be a seasonal trend in seawater temperature with average water temperatures peaking in December 2000 and 2001 and dipping in August 2000 and 2001 at all sites and depths (Fig. 4.1). Mean water temperatures at Ganan were consistently lower than at Manselo and Batu Terio where water temperature decreased with depth. However, at Ganan, the water temperature was higher at 15 m than at 5 m. Overall, the highest water temperatures were consistently recorded at Manselo at a depth of 5 m while the lowest water temperatures were recorded at Ganan at a depth of 5 m.

Table 4.1 Means of various environmental parameters at Ganan at 5 m and 15 m depths. Missing data indicated by NA.

Month	Water temperature (°C)		Salinity (‰)		pH		SPM (mg L^{-1})		POM (mg L^{-1})		Chlorophyll *a* (µg L^{-1})		Chlorophyll *b* (µg L^{-1})		Chlorophyll *c* (µg L^{-1})	
	5 m	1 5 m	5 m	15 m	5 m	15 m	5 m	15 m	5 m	15 m	5 m	15 m	5 m	15 m	5 m	15 m
Jun 00	28.20	28.86	34.0	33.8	7.6	7.8	0.040	0.053	0.023	0.020	NA	0.616	NA	0.637	NA	1.867
Jul 00	27.96	28.75	35.2	34.8	7.9	7.9	0.042	0.034	0.020	0.010	0.58	0.514	0.726	0.569	2.047	1.528
Aug 00	27.89	28.74	35.0	35.0	8.1	8.1	0.045	0.043	0.010	0.018	0.35	0.249	0.471	0.299	1.310	0.900
Sep 00	27.92	28.74	34.8	35.0	7.8	7.8	0.033	0.038	0.015	0.018	0.30	0.267	0.390	0.381	1.052	1.048
Oct 00	28.13	28.85	34.8	34.8	7.8	7.8	0.047	0.043	0.020	0.023	0.63	0.419	0.691	0.413	1.925	1.301
Nov 00	28.89	29.16	34.5	34.3	8.0	8.0	0.078	0.083	0.058	0.060	0.69	0.454	1.019	0.296	2.637	1.020
Dec 00	29.21	29.08	35.0	34.8	7.9	7.9	0.075	0.068	0.050	0.050	0.42	0.340	0.534	0.435	1.555	1.271
Jan 01	28.71	28.72	35.0	35.0	7.7	7.7	0.070	0.068	0.048	0.048	0.60	0.413	0.717	0.499	2.102	1.386
Feb 01	28.50	28.71	35.0	35.0	7.9	7.9	0.050	0.055	0.028	0.030	0.25	0.520	0.335	0.670	0.987	1.878
Mar 01	28.36	28.74	35.0	35.0	7.8	7.8	0.053	0.050	0.025	0.033	0.45	0.439	0.717	0.681	2.040	2.001
Apr 01	28.37	28.63	36.0	35.0	NA	NA	0.040	0.030	0.020	0.010	0.54	0.544	0.706	0.816	1.819	2.227
May 01	28.42	28.99	35.8	35.8	7.0	7.2	0.053	0.050	0.028	0.025	0.60	0.382	0.919	0.503	2.614	1.481
Jun 01	28.30	28.89	33.5	34.3	6.7	7.0	0.043	0.038	0.020	0.018	0.75	0.619	1.178	1.028	3.302	2.876
Jul 01	27.87	28.52	35.0	35.3	6.7	6.9	0.036	0.036	0.014	0.028	0.58	0.700	0.810	1.106	2.269	3.145
Aug 01	27.60	28.22	34.4	34.8	7.4	7.6	0.034	0.036	0.014	0.006	0.45	0.489	0.632	0.769	1.825	2.043
Sep 01	27.72	28.34	34.0	34.0	7.5	7.7	0.032	0.035	0.012	0.005	0.33	0.167	0.456	0.170	1.297	0.654
Oct 01	28.10	28.72	35.0	35.2	7.7	7.6	0.034	0.034	0.010	0.010	0.47	0.421	0.669	0.587	1.905	1.653
Nov 01	28.18	28.82	35.0	35.0	7.6	7.7	0.035	0.030	0.008	0.010	0.40	0.300	0.557	0.291	1.539	0.905
Dec 01	29.12	29.02	35.0	35.0	7.7	7.8	0.034	0.030	0.010	0.004	0.63	0.517	0.915	0.707	2.661	2.052
Jan 02	28.70	28.71	34.5	35.0	8.0	8.0	0.023	0.028	0.007	0.005	0.36	0.511	0.394	0.708	1.292	2.111
Feb 02	28.50	28.71	35.0	35.0	8.0	8.0	0.030	0.035	0.005	0.010	0.47	0.697	0.714	1.102	1.985	2.943

Table 4.2 Means of various environmental parameters at Manselo at 5 m and 15 m depths. Missing data indicated by NA.

Month	Water Temperature (°C)		Salinity (‰)		pH		SPM (mg L^{-1})		POM (mg L^{-1})		Chlorophyll *a* (µg L^{-1})		Chlorophyll *b* (µg L^{-1})		Chlorophyll *c* (µg L^{-1})	
	5 m	1 5 m	5 m	15 m	5 m	15 m	5 m	15 m	5 m	15 m	5 m	15 m	5 m	15 m	5 m	15 m
Jun 00	29.25	28.81	33.0	33.0	7.7	7.7	0.033	0.033	0.020	0.015	0.626	0.715	0.719	0.903	2.100	2.502
Jul 00	28.99	28.79	34.0	34.0	8.0	8.0	0.036	0.038	0.010	0.014	0.734	0.449	1.040	0.564	2.865	1.607
Aug 00	29.07	28.75	35.0	34.8	8.1	8.1	0.030	0.030	0.010	0.013	0.303	0.399	0.403	0.576	1.149	1.607
Sep 00	29.19	28.76	34.8	35.0	7.8	7.8	0.038	0.040	0.015	0.018	0.630	0.669	0.873	0.910	2.415	2.449
Oct 00	29.24	29.10	34.3	34.5	7.8	7.8	0.045	0.050	0.015	0.023	0.489	0.470	0.460	0.323	1.339	0.976
Nov 00	29.59	29.32	33.8	34.3	8.0	8.0	0.090	0.088	0.065	0.063	0.470	0.510	0.323	0.431	0.976	1.019
Dec 00	29.58	29.25	34.8	35.0	8.0	7.9	0.068	0.070	0.045	0.050	0.593	0.326	0.733	0.325	2.123	0.961
Jan 01	29.11	28.88	35.0	35.0	7.8	7.8	0.066	0.064	0.044	0.046	0.255	0.565	0.343	0.678	1.015	1.897
Feb 01	29.13	28.72	35.0	35.0	7.9	7.9	0.050	0.043	0.025	0.015	0.434	0.437	0.534	0.644	1.458	1.865
Mar 01	29.26	28.94	35.0	35.0	7.8	7.8	0.053	0.048	0.033	0.023	0.459	0.639	0.717	0.952	1.943	2.549
Apr 01	29.34	28.88	35.0	35.0	NA	NA	0.040	0.050	0.020	0.030	0.837	0.547	1.444	0.927	4.009	2.634
May 01	29.40	28.88	34.3	35.0	7.4	7.4	0.055	0.053	0.038	0.028	0.610	0.954	0.974	1.511	2.745	4.202
Jun 01	29.31	28.87	33.0	33.0	7.4	7.4	0.045	0.043	0.028	0.018	0.953	0.883	1.483	1.409	4.100	3.960
Jul 01	28.94	28.50	35.5	35.3	7.2	7.3	0.042	0.040	0.018	0.014	0.311	0.211	0.380	0.305	1.045	0.867
Aug 01	28.61	28.18	34.2	34.0	7.6	7.6	0.036	0.032	0.014	0.014	0.824	0.870	1.251	1.244	3.369	3.422
Sep 01	28.81	28.34	33.5	33.3	7.6	7.5	0.033	0.033	0.008	0.008	0.399	0.255	0.557	0.326	1.539	0.953
Oct 01	29.30	28.78	35.0	35.0	7.9	7.9	0.034	0.028	0.008	0.006	0.676	0.747	0.908	1.055	2.586	2.908
Nov 01	29.34	28.70	34.5	34.5	7.8	7.8	0.030	0.033	0.005	0.010	0.787	0.600	1.005	0.891	2.743	2.512

Table 4.3 Means of various environmental parameters at Batu Terio at 5 m and 15 m depths. Missing data indicated by NA.

Month	Water Temperature (°C)		Salinity (‰)		pH		SPM (mg L^{-1})		POM (mg L^{-1})		Chlorophyll *a* (µg L^{-1})		Chlorophyll *b* (µg L^{-1})		Chlorophyll *c* (µg L^{-1})	
	5 m	1 5 m	5 m	15 m	5 m	15 m	5 m	15 m	5 m	15 m	5 m	15 m	5 m	15 m	5 m	15 m
Jun 00	29.06	28.91	33.3	33.5	7.8	7.7	0.040	0.040	0.020	0.023	0.618	0.716	0.692	0.930	2.071	2.603
Jul 00	28.88	28.67	35.0	35.0	7.9	8.0	0.038	0.040	0.010	0.016	0.770	0.658	0.950	0.800	2.685	2.114
Aug 00	28.89	28.70	35.3	35.3	8.1	8.1	0.043	0.053	0.010	0.025	0.298	0.309	0.422	0.458	1.265	1.304
Sep 00	28.99	28.85	35.0	35.0	7.8	7.8	0.045	0.035	0.020	0.010	0.327	0.543	0.442	0.802	1.246	2.176
Oct 00	29.08	28.86	34.3	34.8	7.8	7.8	0.047	0.050	0.023	0.020	0.508	0.634	0.597	0.746	1.703	2.129
Nov 00	29.47	29.30	34.3	34.8	8.0	8.0	0.085	0.080	0.065	0.055	0.725	0.486	0.677	0.377	2.031	1.034
Dec 00	29.37	29.22	35.0	35.0	7.9	7.9	0.073	0.063	0.050	0.045	0.518	0.324	0.633	0.251	1.742	0.689
Jan 01	28.94	28.80	35.2	35.2	7.8	7.8	0.068	0.070	0.048	0.052	0.516	0.298	0.685	0.337	1.971	0.999
Feb 01	29.22	28.76	35.0	35.0	7.9	7.9	0.055	0.055	0.038	0.035	0.326	0.340	0.325	0.435	0.961	1.271
Mar 01	29.12	28.93	35.0	35.0	7.8	7.8	0.055	0.055	0.030	0.038	0.267	0.436	0.408	0.607	1.162	1.730
Apr 01	29.17	28.90	35.0	35.0	NA	NA	0.040	0.040	0.020	0.020	0.798	0.511	1.115	0.708	3.078	2.111
May 01	29.28	28.93	35.8	34.8	7.2	7.3	0.055	0.050	0.033	0.028	0.727	0.600	1.040	0.891	2.938	2.512
Jun 01	29.26	28.90	34.8	34.0	7.1	7.3	0.043	0.035	0.025	0.023	0.891	0.874	1.436	1.354	3.989	3.829
Jul 01	28.90	28.54	34.5	34.3	7.1	7.1	0.042	0.040	0.022	0.016	0.742	0.481	1.077	0.735	3.048	2.091
Aug 01	28.53	28.16	34.2	34.2	7.6	7.6	0.042	0.034	0.012	0.010	0.518	0.727	0.707	1.040	2.038	2.938
Sep 01	28.77	28.41	34.3	33.8	7.7	7.4	0.030	0.028	0.008	0.008	0.326	0.373	0.428	0.448	1.195	1.350
Oct 01	28.84	28.81	35.2	35.2	7.8	7.9	0.028	0.030	0.004	0.006	0.452	0.538	0.560	0.816	1.673	2.285
Nov 01	28.95	28.85	34.8	34.8	7.8	7.8	0.033	0.030	0.010	0.005	0.526	0.369	0.707	0.310	1.965	0.841
Dec 01	29.51	29.17	35.0	35.0	7.7	7.7	0.030	0.032	0.006	0.004	0.532	0.568	0.817	0.767	2.343	2.223
Jan 02	29.07	28.88	34.8	34.8	8.0	8.0	0.033	0.030	0.008	0.003	1.299	1.244	2.048	2.028	5.659	5.577
Feb 02	29.13	28.72	35.0	35.0	8.0	8.0	0.028	0.028	0.008	0.005	0.399	0.546	0.557	0.871	1.539	2.430

4.3.1.2 Salinity

Salinity ranged from 30‰ to 38‰ over the 20 months of sampling. While salinity appeared to fluctuate between different months of sampling when plotted (Fig. 4.2), statistically salinity was not significantly different between depths ($F_{(1,438)} = 0.031$, $p > 0.05$) and over time ($F_{(1, 438)} = 4.610$, $p > 0.05$). ANCOVA indicated that site had an effect on salinity ($F_{(2,483)} = 7.522$, $p < 0.05$). Differences in mean salinities were observed between the three sites from April 2001 to August 2001. Salinity peaks were observed at 5 m in Ganan, Manselo and Batu Terio in April 2001, July 2001 and May 2001 respectively. The lowest salinities were recorded at Ganan and Manselo at 5 m on June 2001, while the lowest salinity at Batu Terio occurred in June 2000 at a depth of 5 m (Fig. 4.2). Post-hoc test showed that the salinity in Batu Terio was different from that of Ganan and Manselo.

4.3.1.3 pH

The pH of seawater at the three sites over the 20-month sampling period ranged from 6.4 to 8.3. There was a significant seasonal variation in pH ($F_{(1,447)} = 38.150$, $p < 0.05$) while site and depth did not affect pH (site: $F_{(2,447)} = 2.459$, $p > 0.05$; depth: $F_{(1,447)} = 0.934$, $p > 0.05$). The lowest pH levels were recorded from May to September 2001 at all three sites (Fig. 4.3). pH of seawater at Manselo and Batu Terio were almost identical at 5 m and 15 m. However, pH levels in Ganan differed slightly from May 2001 to July 2001 before becoming stable for the rest of the sampling period.

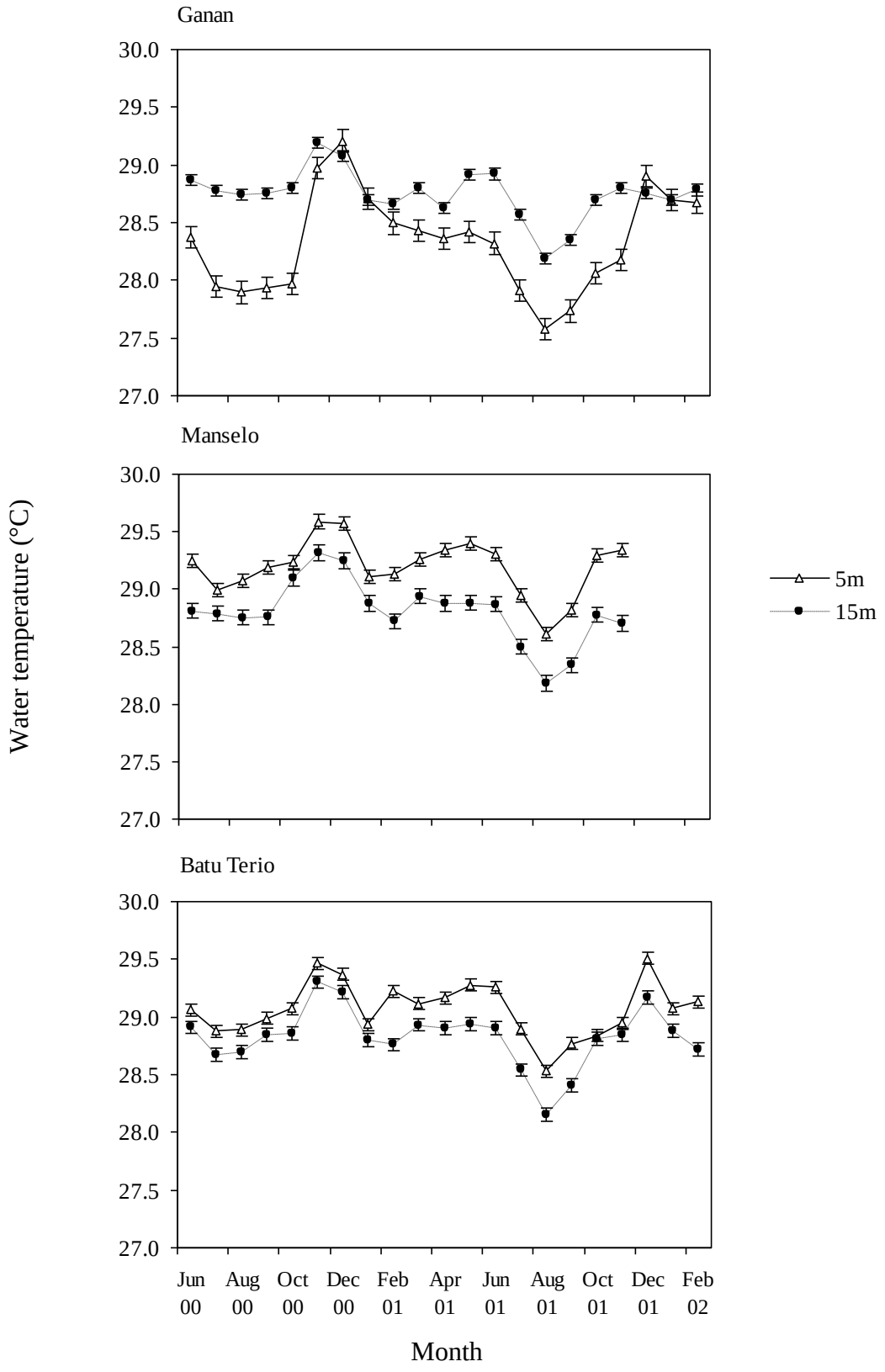

Fig. 4.1 Spatial and temporal variation in water temperature at depths of 5 m and 15 m in Ganan, Manselo and Batu Terio over the sampling period (June 2000 – February 2002 for Ganan and Batu Terio, June 2000 – October 2001 for Manselo). Vertical bars indicate Standard Error.

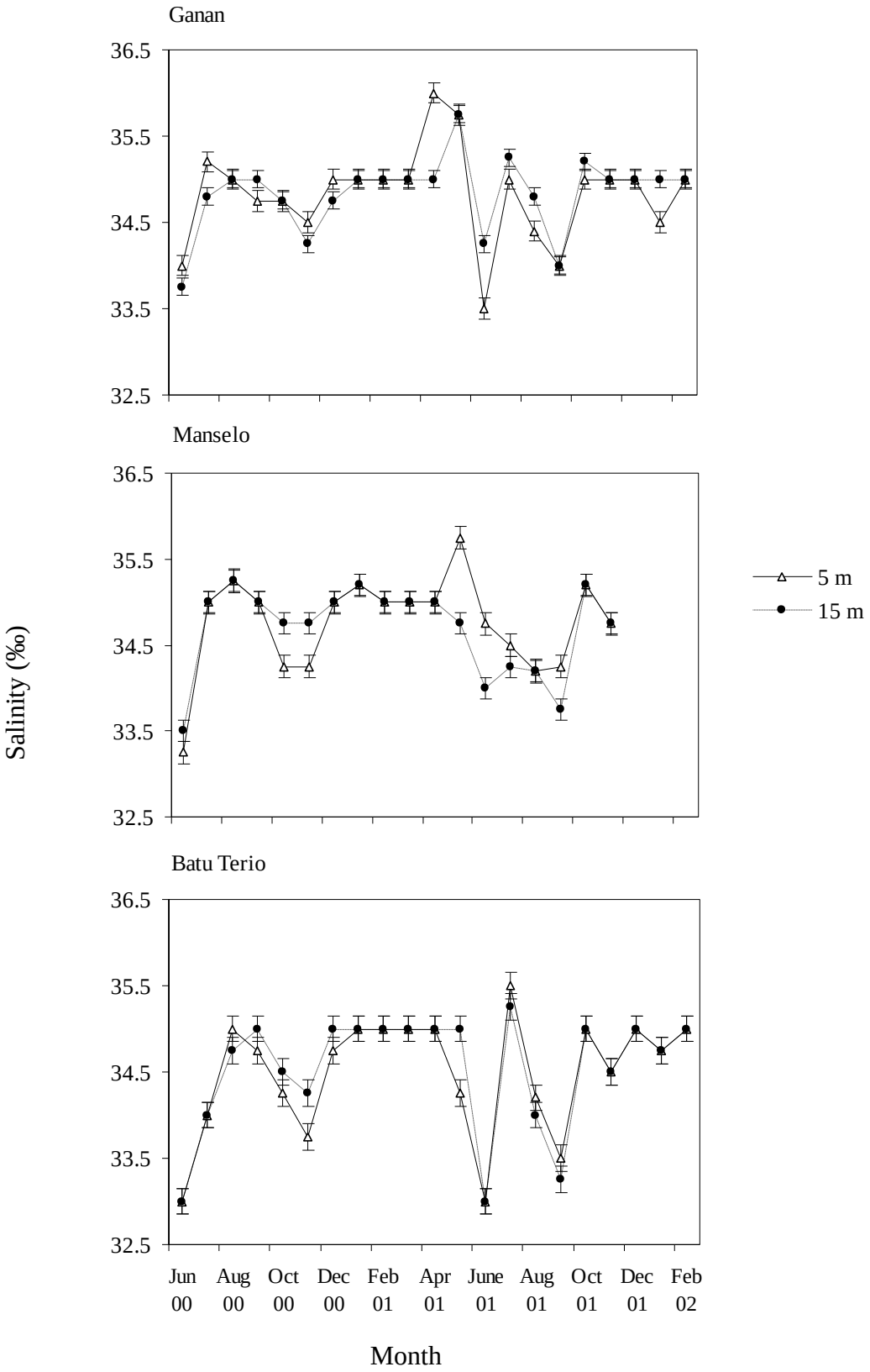

Fig. 4.2 Spatial and temporal variation in salinity at depths of 5 m and 15 m in Ganan, Manselo and Batu Terio over the sampling period (June 2000 – February 2002 for Ganan and Batu Terio, June 2000 – October 2001 for Manselo). Vertical bars indicate Standard Error.

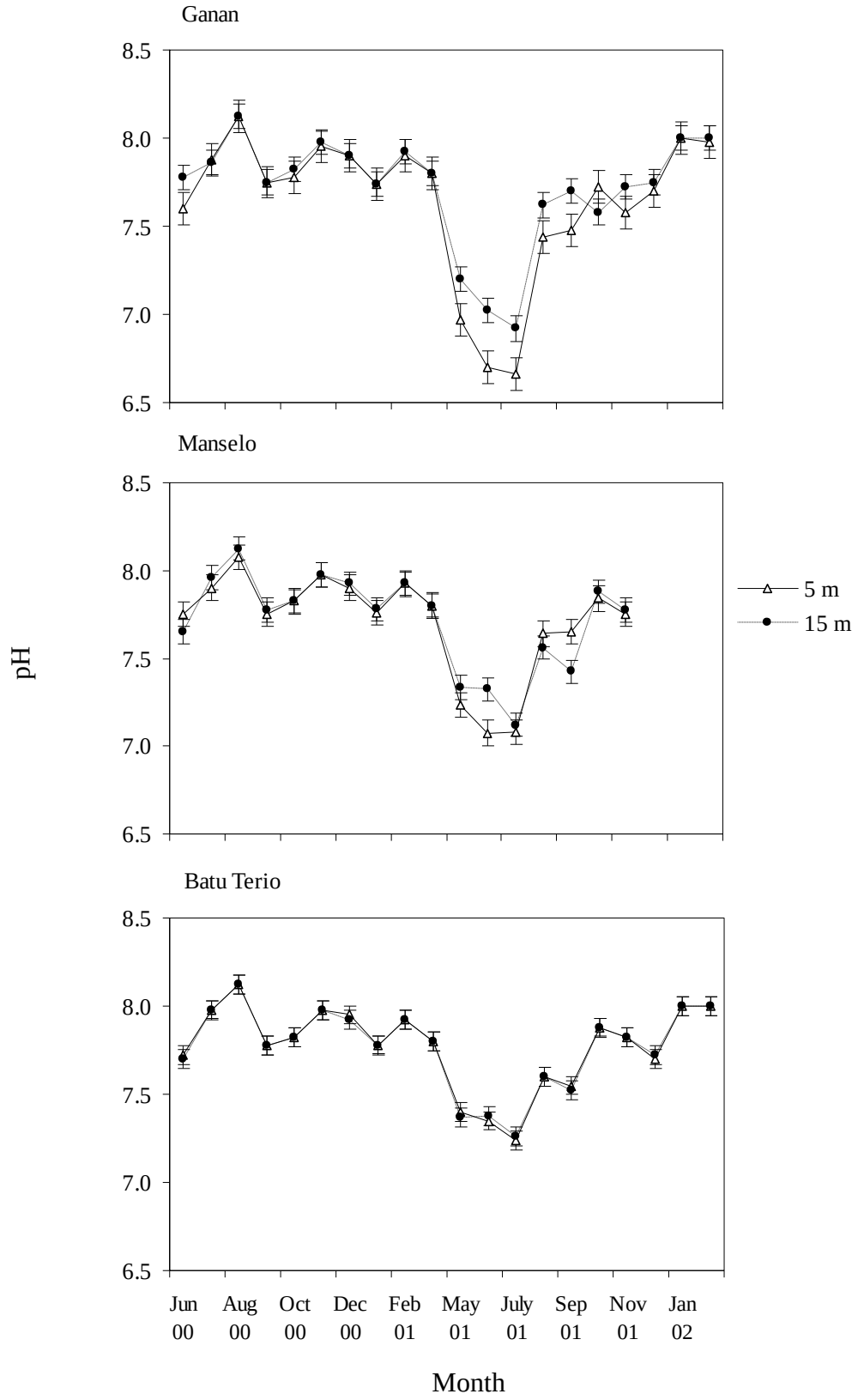

Fig. 4.3 Spatial and temporal variation in pH at depths of 5 m and 15 m in Ganan, Manselo and Batu Terio over the sampling period (June 2000 – February 2002 for Ganan and Batu Terio, June 2000 – October 2001 for Manselo). Vertical bars indicate Standard Error.

4.3.1.4 SPM

SPM levels ranged from 0 - 0.15 mg L^{-1} during the sampling period. Statistical analysis indicated that the levels of SPM were significantly affected by the months of sampling ($F_{(1, 485)} = 43.904$, $p < 0.05$) but were not significantly different between sites and depths (site: $F_{(2,485)} = 0.78$, $p > 0.05$; depth: $F_{(1,485)} = 0.23$, $p > 0.05$). At all three sites, SPM increased gradually from August 2000 and peaked in November 2000. Another smaller peak was observed in June 2001, but overall, SPM levels remained relatively constant throughout 2001 (Fig. 4.4).

4.3.1.5 POM

POM ranged from 0 – 0.12 mg L^{-1} during the experiment. As with SPM, which is the sum of POM and particulate inorganic matter (PIM), POM levels varied significantly over time ($F_{(1,485)} = 48.420$, $p < 0.05$) but were not significantly different between sites ($F_{(2,485)} = 4.258$, $p > 0.05$) and depths ($F_{(1,485)} = 0.333$, $p > 0.05$). As might be expected, POM levels followed an identical distribution as SPM over time at all sites and depths, with a large peak observed in November 2001 and a smaller peak observed in June 2001 (Fig. 4.5).

4.3.1.6 Chlorophyll a

Chlorophyll *a* levels ranged from 0.0 – 1.96 µg L^{-1}. Chlorophyll *a* levels were significantly different between sites ($F_{(2,448)} = 4.223$, $p < 0.05$) but were not different between depths ($F_{(1,448)} = 0.346$, $p > 0.05$). While sampling month did not appear to have an effect on chlorophyll *a* levels when tested with an ANCOVA ($F_{(1,448)} = 3.580$, $p > 0.05$), the probability ($p = 0.059$) was very close to α (0.05) indicating that sampling month very likely does exert an influence on chlorophyll *a* levels. This was supported by graphical data (Fig. 4.6), which showed chlorophyll *a* levels fluctuate over the sampling period. Chlorophyll *a* levels peaked between April and August 2001 at all the sites while the highest level of chlorophyll *a* was observed in Batu Terio from December 2001 to January 2002, with mean levels reaching 1.2 µg L^{-1}.

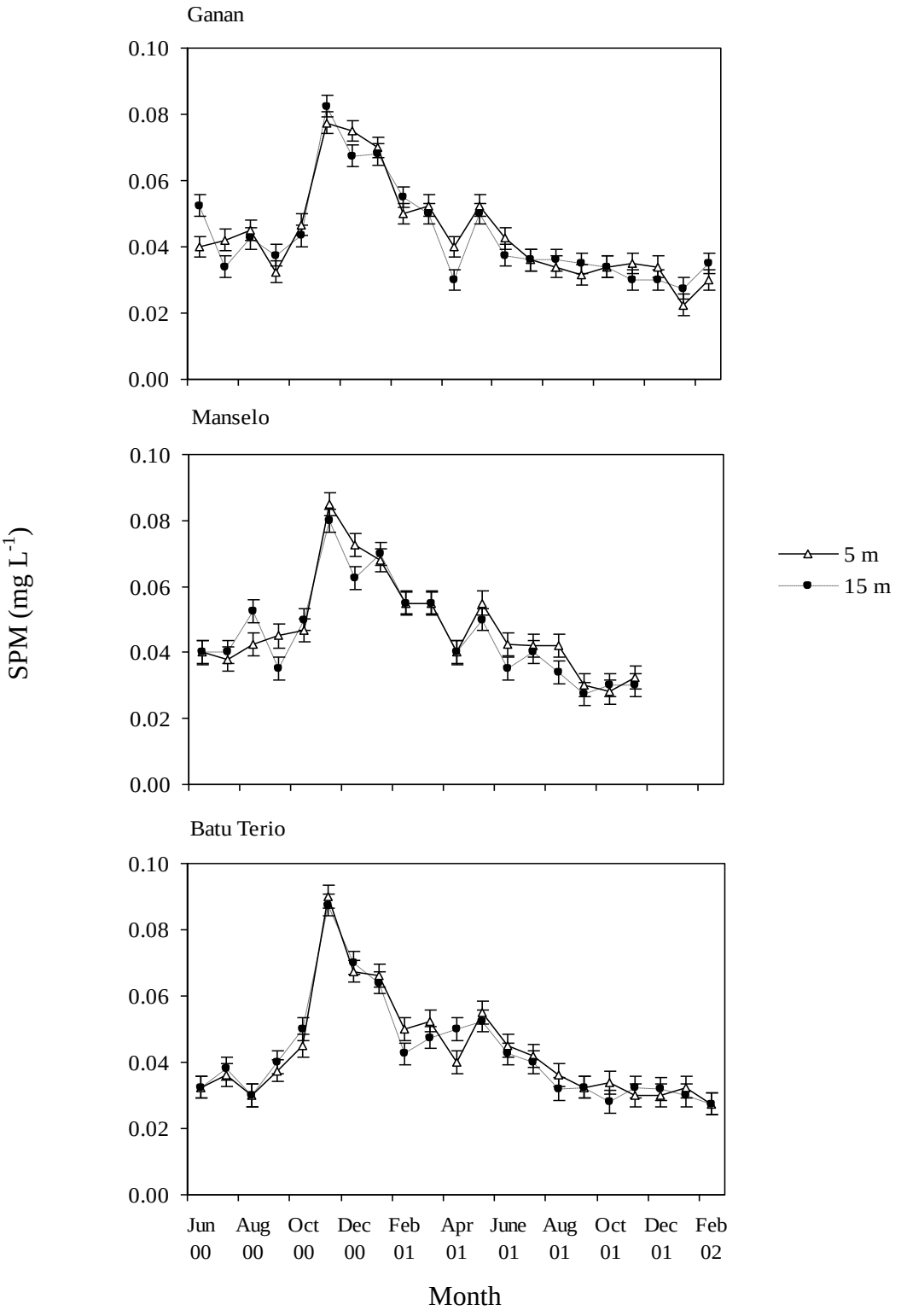

Fig. 4.4 Spatial and temporal variation in SPM at depths of 5 m and 15 m in Ganan, Manselo and Batu Terio over the sampling period (June 2000 – February 2002 for Ganan and Batu Terio, June 2000 – October 2001 for Manselo). Vertical bars indicate Standard Error.

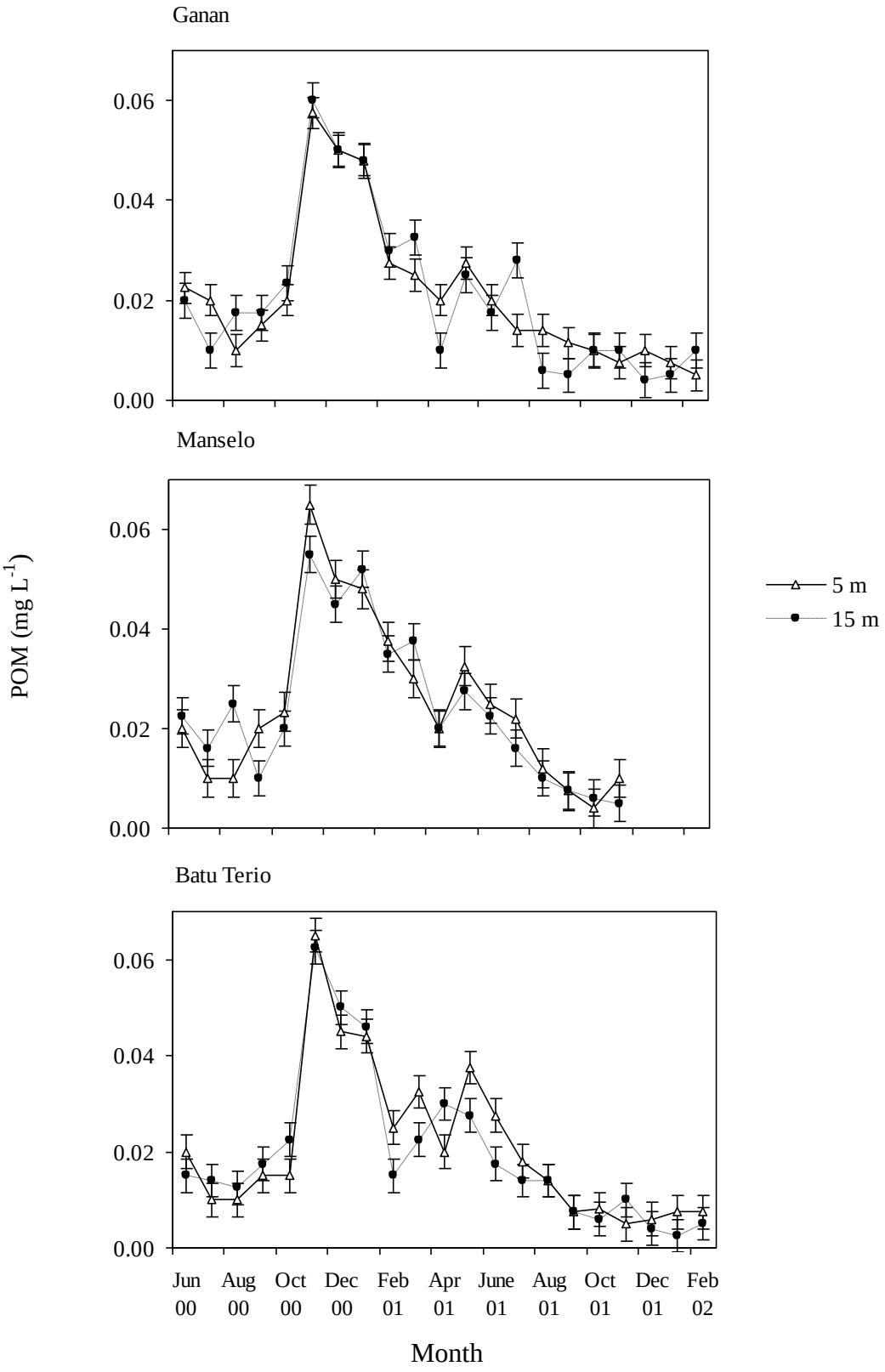

Fig. 4.5 Spatial and temporal variation in POM at depths of 5 m and 15 m in Ganan, Manselo and Batu Terio over the sampling period (June 2000 – February 2002 for Ganan and Batu Terio, June 2000 – October 2001 for Manselo). Vertical bars indicate Standard Error.

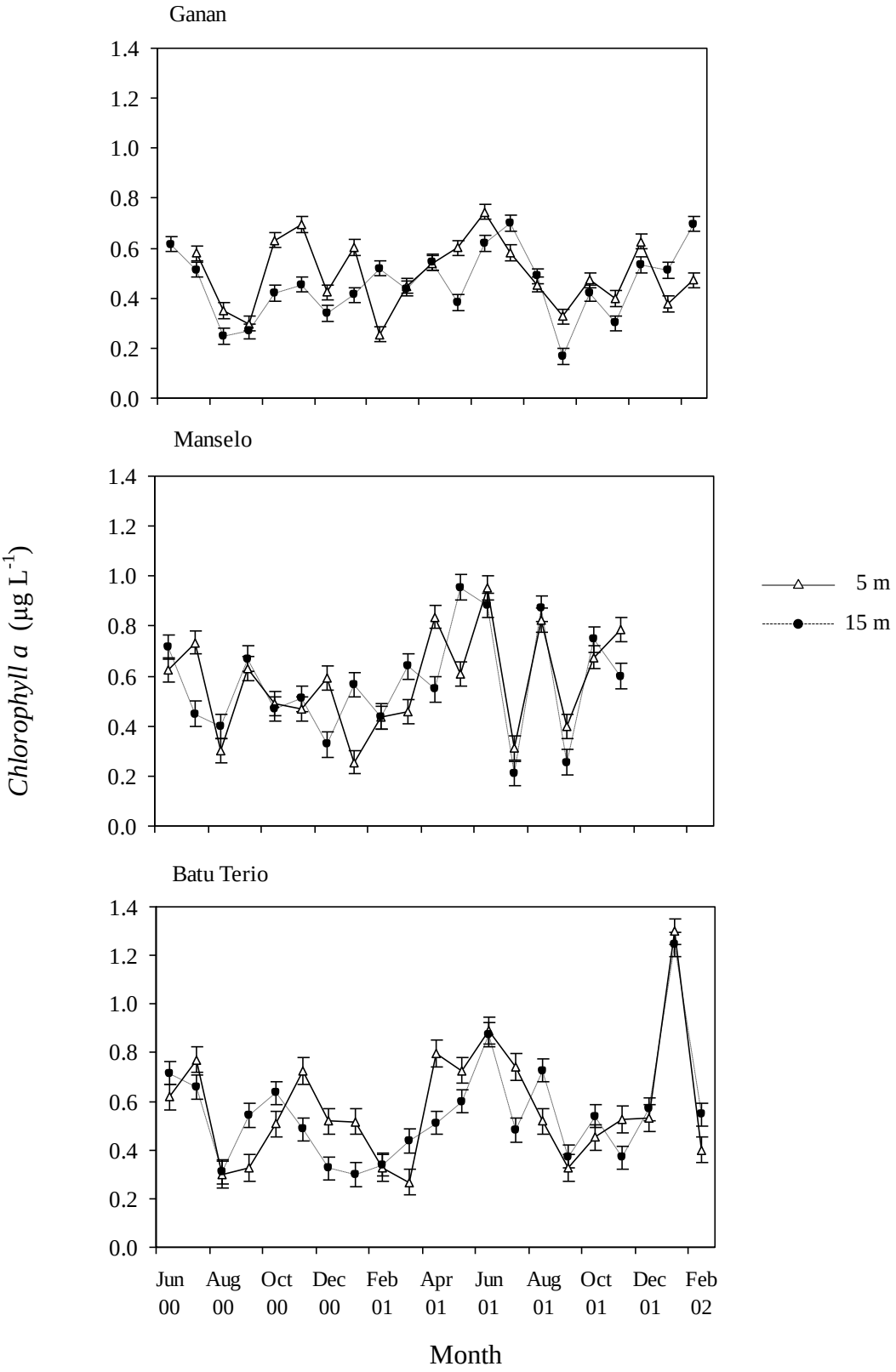

Fig. 4.6 Spatial and temporal variation in chlorophyll *a* at depths of 5 m and 15 m in Ganan, Manselo and Batu Terio over the sampling period (June 2000 – February 2002 for Ganan and Batu Terio, June 2000 – October 2001 for Manselo). Vertical bars indicate Standard Error.

4.3.1.7 Chlorophyll b

Chlorophyll *b* levels ranged from 0.0 – 3.18 μg L^{-1} during the sampling period. Statistical analysis indicated that chlorophyll *b* levels were significantly different between sampling months ($F_{(1,448)}$ = 15.812, $p < 0.05$) and sites ($F_{(2,448)}$ = 3.322, $p < 0.05$) but were not affected by depth ($F_{(1,448)}$ = 0.399, $p > 0.05$). Chlorophyll *b* levels were higher in Batu Terio and Manselo than at Ganan, and fluctuated with no discerning pattern throughout the sampling period at all three sites (Fig. 4.7). Between April 2001 and August 2001, levels of chlorophyll *b* showed several small peaks at the three sites. As with chlorophyll *a* levels, the highest chlorophyll *b* was found at Batu Terio in December 2001 – January 2002, with mean levels > 2 μg L^{-1} recorded.

4.3.1.8 Chlorophyll c

Chlorophyll *c* was found in greater concentration than chlorophyll *a* and chlorophyll *b* at all three sites, with concentration ranging from 0.0 – 8.5 μg L^{-1}. Chlorophyll *c* levels were significantly different in seawater sampled between different months ($F_{(1,448)}$ = 16.469, $p < 0.05$) and from different sites ($F_{(2,448)}$ = 3.036, $p < 0.05$), but was the same for the two depths ($F_{(1,448)}$ = 0.472, $p > 0.05$). As with chlorophyll *a* and chlorophyll *b*, chlorophyll *c* levels were highest in the period between December 2001 to January 2002 at Batu Terio reaching concentrations exceeding 5 μg L^{-1}, while smaller peaks between 3 – 4 μg L^{-1} were observed between April and August 2001 at all sites (Fig. 4.8).

4.3.1.9 Rainfall

There was significant temporal variation in rainfall during the sampling months ($F_{(17,428)}$ = 7.326, $p < 0.05$). There did not appear to be a distinct annual wet or dry season during the 20 months of sampling, with the highest rainfall recorded in June 2000 and the lowest rainfall recorded in August 2001 (Fig. 4.9).

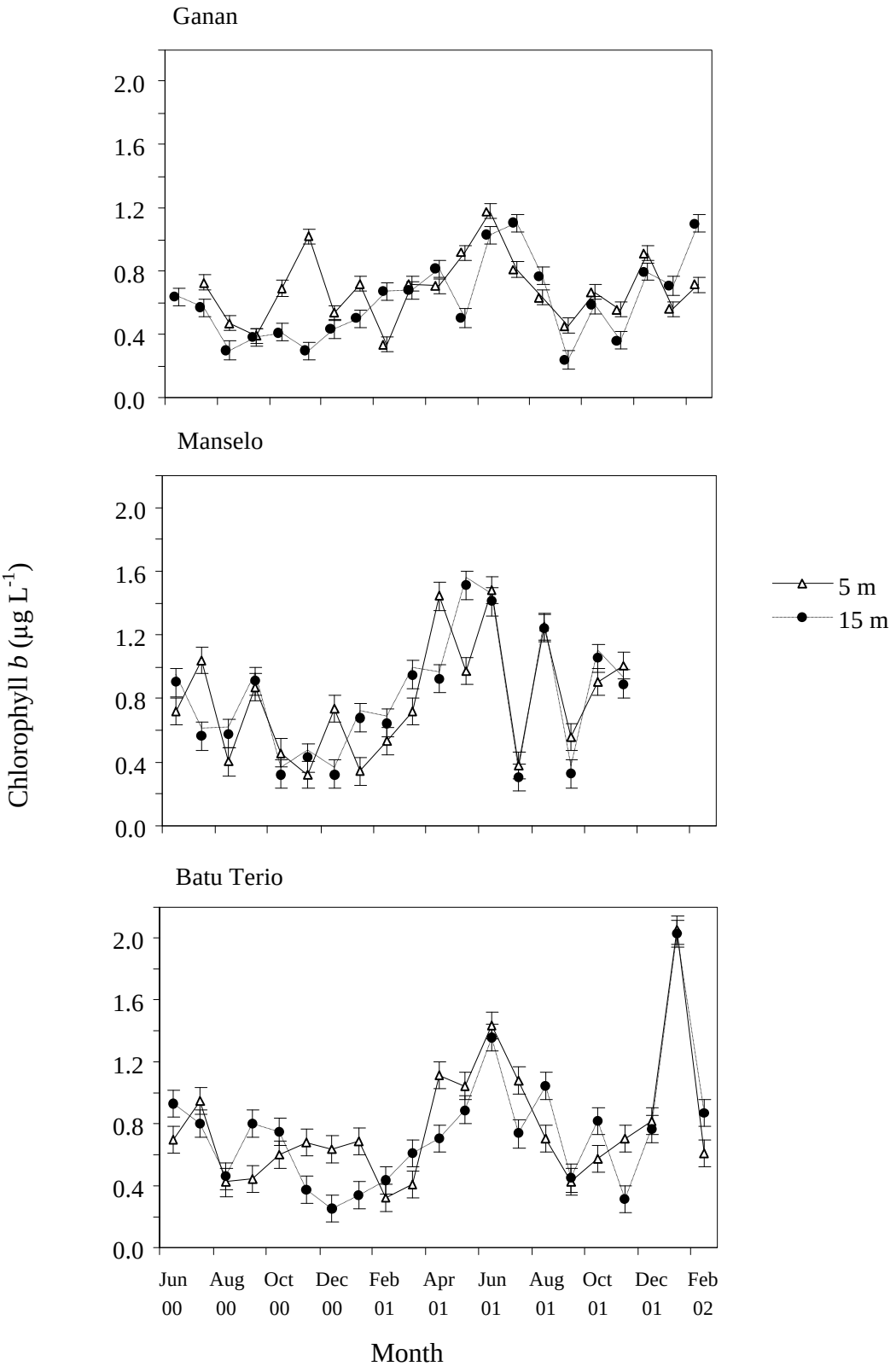

Fig. 4.7 Spatial and temporal variation in chlorophyll *b* at depths of 5 m and 15 m in Ganan, Manselo and Batu Terio over the sampling period (June 2000 – February 2002 for Ganan and Batu Terio, June 2000 – October 2001 for Manselo). Vertical bars indicate Standard Error.

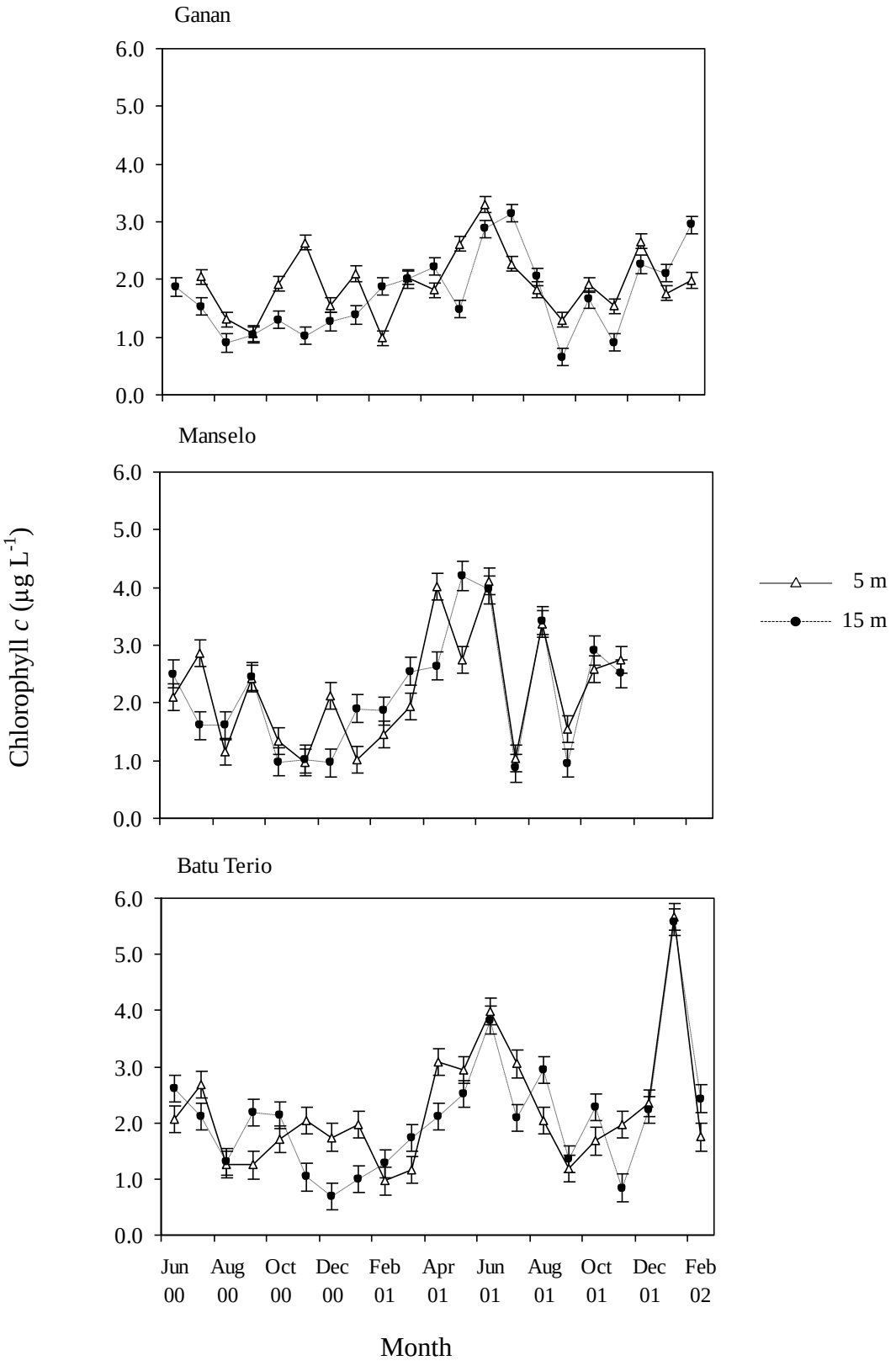

Fig. 4.8 Spatial and temporal variation in chlorophyll *c* at depths of 5 m and 15 m in Ganan, Manselo and Batu Terio over the sampling period (June 2000 – February 2002 for Ganan and Batu Terio, June 2000 – October 2001 for Manselo). Vertical bars indicate Standard Error.

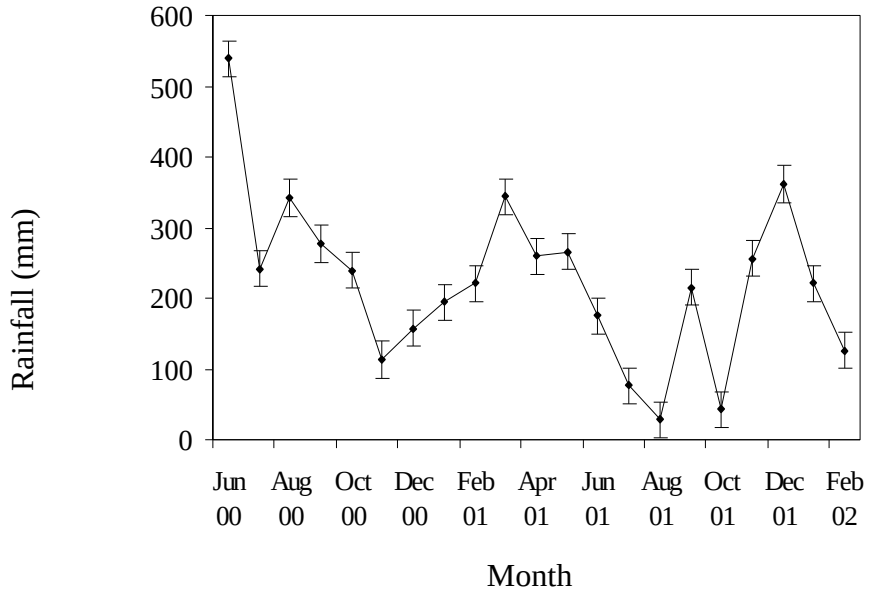

Fig. 4.9 Temporal variation in rainfall at all sites over sampling period (June 2000 – February 2002). Vertical bars indicate Standard Error.

4.3.2 Multivariate analysis of environmental parameters

Results of the MANCOVA showed that overall, site ($F_{(16,762)} = 3.614$, $p < 0.05$) and month of sampling ($F_{(8,380)} = 0.796$, $p < 0.05$) had an effect on environmental parameters while depth ($F_{(8,380)} = 0.552$, $p > 0.05$) did not. However, there was significant interaction between site and depth ($F_{(16,762)} = 0.909$, $p < 0.05$) indicating that there were differences in environmental parameters between depths at various sites.

Test of between subjects effect indicated that site had a significant effect on water temperature ($F_{(2,387)} = 21.743$, $p < 0.05$) and salinity ($F_{(2,387)} = 3.231$, $p < 0.05$) while sampling month affected water temperature ($F_{(1,387)} = 6.445$, $p < 0.05$), salinity ($F_{(1,387)} = 5.558$, $p < 0.05$), pH ($F_{(1,387)} = 27.384$, $p < 0.05$), SPM ($F_{(1,387)} = 30.877$, $p < 0.05$), POM ($F_{(1,387)} = 35.557$, $p < 0.05$), chlorophyll *b* ($F_{(1,387)} = 12.127$, $p < 0.05$) and chlorophyll *c* ($F_{(1,387)} = 12.561$, $p < 0.05$). Depth did not exert any significant effect on any of the environmental parameters.

4.3.3 Interrelationship between environmental parameters

The results of PCA are shown in Fig. 4.10 and Table 4.4. Four principal components were extracted and accounted for a total of 82.92% of the original variance. The loadings for each component given in Table 4.4 represent the partial correlation between the variable and the rotated component. Chlorophyll *a, b, c* were extracted with strong loadings with component 1. SPM and POM strongly loaded with component 2. Components 1 and 2 variables relate to the phytoplankton and other organic food sources in the seawater sampled. Component 3 had moderately strong loadings on pH and rainfall and a moderate loading on water temperature, while component 4 had very strong loading on salinity. Component 3 and 4 can be inferred as the physical components of the seawater sampled. There were significant correlations between the environmental parameters (Table 4.5), with the exception of salinity, which was not significantly correlated to any of the other parameters.

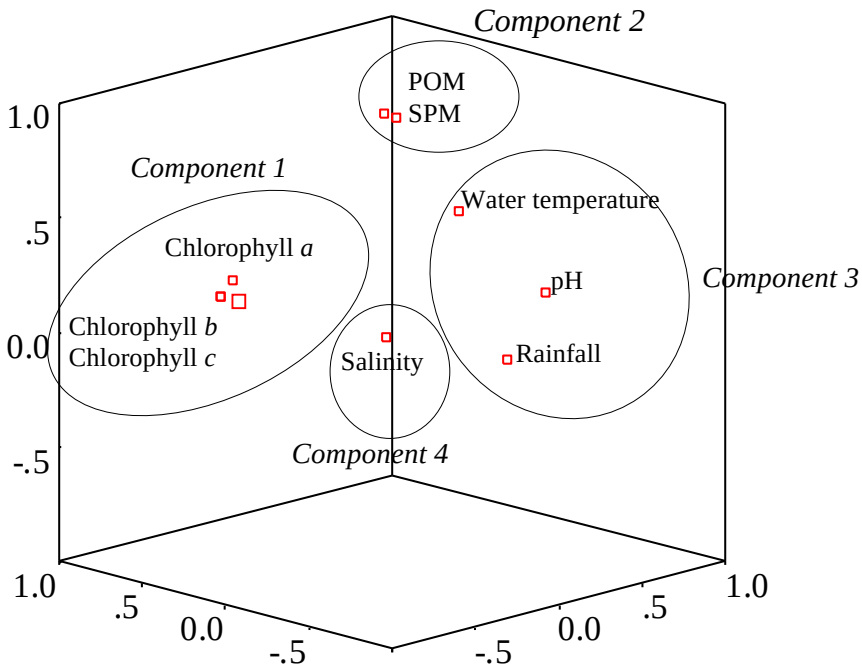

Fig. 4.10 Component plot of environmental parameters in rotated space.

Table 4.4 Rotated component matrix of PCA on environmental data. Rotation method: Direct Oblimin with Kaiser normalisation. Absolute partial correlation values less than 0.1 are suppressed.

Environmental parameter	*Principal components*				*Communality*
	1	*2*	*3*	*4*	
Chlorophyll *c*	0.993				0.988
Chlorophyll *b*	0.992				0.987
Chlorophyll *a*	0.978				0.959
POM		0.957			0.919
SPM		0.956			0.919
pH		0.150	0.748	0.158	0.628
Rainfall		0.214	0.720		0.615
Water temperature		0.439	0.554		0.475
Salinity				0.984	0.973
Initial eigenvalues	3.063	2.027	1.358	1.014	
% Variance	34.03	22.52	15.09	11.27	
% Cumulative variance	34.03	56.56	71.65	82.92	

Table 4.5 Correlation matrix from principal component analysis of environmental parameters. Asterisk (*) indicates significant correlation. Partial correlation < 0.1 have been suppressed.

		Water Temperature	Salinity	pH	SPM	POM	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll *c*	Rainfall
Correlation	Water temperature	1.000		0.229	0.256	0.264	0.066			0.107
	Salinity		1.000							
	pH	0.229		1.000	0.128		-0.124	-0.199	-0.202	0.248
	SPM	0.256		0.128	1.000	0.922		-0.155	-0.153	-0.140
	POM	0.264			0.922	1.000				-0.135
	Chlorophyll *a*			-0.124			1.000	0.957	0.956	
	Chlorophyll *b*			-0.199	-0.155		0.957	1.000	0.996	
	Chlorophyll *c*			-0.202	-0.153		0.956	0.996	1.000	
	Rainfall	0.107		0.248	-0.140	-0.135				1.000
Significance	Water temperature		0.428	0.000*	0.000*	0.000*	0.082	0.382	0.316	0.013*
(1-tailed)	Salinity	0.428		0.127	0.305	0.379	0.380	0.336	0.346	0.158
	pH	0.000*	0.127		0.003*	0.036*	0.006*	0.000*	0.000*	0.000*
	SPM	0.000*	0.305	0.003*		0.000*	0.019*	0.000*	0.001*	0.002*
	POM	0.000*	0.379	0.036*	0.000*		0.207	0.020*	0.019*	0.003*
	Chlorophyll *a*	0.082	0.380	0.006*	0.019*	0.207		0.000*	0.000*	0.103
	Chlorophyll *b*	0.382	0.336	0.000*	0.000*	0.020*	0.000*		0.000*	0.297
	Chlorophyll *c*	0.316	0.346	0.000*	0.001*	0.019*	0.000*	0.000*		0.185
	Rainfall	0.013*	0.158	0.000*	0.002*	0.003*	0.103	0.297	0.185	

4.4 Discussion

This study presents the usefulness of multivariate statistical techniques for evaluation and interpretation of large complex water quality data sets and apportionment of variance factors to sites and depths in order to deduce better information about the water quality at different sites within the pearl farm.

The choice of Ganan, Manselo and Batu Terio as experimental sites was based on a few considerations. They are used extensively for commercial pearl oyster culture and as such, are sites of interest. Another consideration was to determine if different topography and positions of the three sites within Aljui Bay (Fig. 4.11) resulted in a spatial difference in hydrological parameters.

Fig. 4.11 Diagram of the experimental sites of Ganan, Manselo and Batu Terio within Aljui Bay, western Waigeo. Diagram is not to scale.

Water quality monitoring typically involves the sampling of three main categories of hydrological descriptors: physico-chemical (water temperature, salinity, dissolved oxygen, pH and turbidity), particulate matter (total suspended matter, organic phosphate, nitrogen and carbon, chlorophyll pigments) and nutrients (nitrite, nitrate, ammonium, phosphate and silicate) (Aminot *et al*., 2004). For the scope of this study, only certain physico-chemical and particulate descriptors were monitored i.e. water temperature, salinity, pH, suspended particulate matter (SPM), particulate organic matter (POM) and chlorophyll *a, b* and *c,* as these have been reported to influence growth of pearl oysters (Lucas, 2008b) (see Table 5.1).

Univariate analysis allowed subtle differences in an environmental variable between different sites and depths and months of sampling to be compared without taking into consideration possible interaction with other environmental variables.

Results showed that water temperature at the three sampling sites and two depths was significantly different. The differences are likely accounted for by different levels of solar warming at various depths, different topography of the sites that impacted directly on how seawater is circulated and exchanged, as well as any upwelling of cold water at the sites. Manselo recorded the highest mean water temperature (29.59°C) and smallest water temperature range (1.83°C) of the three sampling sites. Its position of being closest to land and enclosed in a small bay (Section 3.4.2) produced poor water exchange and would account for its higher and less variable water temperature. Batu Terio's position in a channel (Section 3.4.3) ensured there was greater water movement at that site leading to greater variation in temperature range (1.99°C) over the sampling period. At both sites, water temperature at 5 m was consistently higher than water temperature at 15 m due to the effects of solar warming higher in the water column. This was not observed at Ganan where the average water temperature at 15 m was found to be higher than 5 m over the sampling period. This apparent anomaly at Ganan was associated with coastal current or cold water upwelling at the site, similar to that along the north coast of Papua New Guinea northern where upwelling of relatively cold water was reported (Hasegawa *et al.,* 2008). Ganan is positioned at the entrance of Aljui Bay and strong upwelling currents sweeping in from the Pacific Ocean from two direction (Section 3.4.1 and Fig. 4.11) resulted in very high water exchange at the site.

Salinity in surface seawater was significantly different between sites. Variation in ocean salinity is caused by a combination of factors including the relative amount of evaporation and precipitation, proximity to freshwater run-offs, wind action and topography of the seabed. Although sampling month did not have a significant effect on salinity, the lowest salinity was recorded at the start of the experiment in June 2000, which coincided with the highest rainfall recorded over the sampling period. The proximity of experimental sites to various islands would most likely expose the sites to freshwater run-offs and decrease overall salinity within the area during heavy rainfall. Post-hoc tests showed that Batu Terio had different salinity than Ganan and Manselo, which was likely caused by the variation in topography of the various sites.

The pH of seawater can be affected by several factors such as the benthic composition through which the seawater moves and the amount of marine plant growth and organic material in the microenvironment. In this study where the sites were located close to shore, pH values would additionally be related to the tidal flow and the acidity of the soils, which discharge into the sea during heavy rainfall (Sanderson and Taylor, 2003). There was no spatial effect on pH levels observed in this study but pH levels showed temporal variation over the sampling period. The variation in pH ranged from 6.4 to 8.3, indicating that the seawater pH varied from acidic to alkaline. Factor analysis indicated that pH levels in Aljui Bay were correlated to rainfall, which also differed significantly between months. pH level fell below 7.5 when rainfall fell below 100 mm month^{-1} (Fig. 4.3 and Fig. 4.9). It is likely that the soil on the islands surrounding the experimental sites is alkaline in nature, as freshwater run-off from land appeared to cause the seawater pH to increase when precipitation was high.

SPM levels were not affected spatially (site or depth) but showed significant variation over time. Various fluvial, oceanographic, biological and chemical factors including tidal flow, river discharge and interaction between fresh and salt water (Sanderson and Taylor, 2003) can affect the concentration of SPM in near-shore seawater. It has also been reported that SPM can be influenced by biogenous components that fluctuate seasonally and were concentrated in areas where upwelling occurs (Chester and Stoner, 1972). SPM in seawater is very heterogeneous in composition and varies in particle size and abundance over various habitats (Lucas, 2008b), and is made up of particulate inorganic matter (PIM) and particulate organic matter (POM). PIM is often composed

of resuspended benthic sediments, while POM may be derived from many sources including phytoplankton, terrestrial material, river run-offs, resuspension of sediments or aeolian inputs (Degens and Ittekkot, 1985) as well as non-living biogenous material such as faeces, decomposing cells and mucus (Lucas, 2008b). In this study, distribution of POM followed an identical distribution to SPM where there was a temporal but not spatial effect on concentration of POM. Varying levels of SPM and POM were likely related to both local factors, such as discharge from the land and tidal flow, as well as to more regional climatic factors – for example, variation of seasonal solar heating over Asia and Australia that drives the monsoon twice a year (Tomascik *et al.*, 1997; Webster *et al.*, 1998).

Chlorophyll levels were found to fluctuate between sites and between months of sampling, but not between depths. Chlorophyll concentration is an indication of the biomass of phytoplankton in seawater and can be affected by similar factors which influence SPM and POM concentrations, namely tidal flow, river discharge and interaction between fresh and salt water. In addition, light is an important factor affecting growth and multiplication of phytoplankton (Li *et al.*, 1999). The lack of a significant difference in the vertical distribution of phytoplankton may be due to both depths of 5 m and 15 m lying within the euphotic zone, or to the occurrence of upwelling causing mixing of POM in the water column.

In this study, four principal components were extracted which accounted for a total of 82.92% of the original variance. PCA showed that there were significant correlations between the various environmental parameters. As expected, there was a very strong correlation between SPM and POM as SPM is partly derived from POM. Similarly, chlorophyll levels were significantly correlated to POM, as phytoplankton represent part of the organic component measured in SPM. This is an example of how one environmental parameter directly influences the concentration of another.

While PCA is useful for examining relationships between environmental parameters, it does not give any indication of the way in which the environmental parameters might be related. However, certain inferences may be made e.g. while there was a significant correlation between POM and chlorophyll levels in this study, the partial correlation was relatively weak ($r < 0.01$), indicating that phytoplankton might not make a big

contribution towards POM in this study, and that the organic component of SPM might be derived from other sources, e.g. terrestrial run-offs. In a study by Fernandes *et al.*, (2008), a significant positive correlation of chlorophyll *a* with concentrations of particulate organic carbon (POC) was taken as an indication that phytoplankton abundance played an important role in controlling the concentration of POC.

In summary, this chapter describes the physico-chemical characteristics and particular matter at the two depths within the three experimental sites used in this study. These data will provide the basis for comparison of growth rates of *P. maxima* held at the three sites described in later chapters.

CHAPTER 5

Temporal, spatial and age-related growth variation and mortality in cultured *P. maxima*, with emphasis on environmental influence

5.1 Introduction

A prime aim of most aquaculture industries is to achieve maximum growth rate and to shorten grow-out time thus increasing the efficiency of production. While this aim is true for the nursery and grow-out phases of farming *P. maxima*, there are stages in the culture of pearl oysters in which slow growth is preferable; for example, in the pre-operative stage where pearl oysters are conditioned to be weak so as not to reject an inserted nucleus (Gervis and Sims, 1992; Taylor and Strack, 2008). While land-base culture using raceways, tanks or ponds can be utilised to manipulate and create a controlled growing environment, this is not an economically feasible option for pearl oysters, which are usually grown on long-lines, fence-lines, rafts or trestles in the sea (Gervis and Sims, 1992; Southgate, 2008). The practical alternative to a controlled land-based culture environment is to choose a grow-out site with favourable environmental conditions. The selection of a particular culture site or culture depth allows some control over the growth environment of cultured pearl oysters.

Growth variability in a population of molluscs living in the same locality has been reported to be related to differences in the microenvironments (Wilbur and Owen, 1964). This has been described for the Pacific oyster *C. gigas* (Brown and Hartwick, 1988b; Almeida *et al.*, 1997, Park *et al.*, 1999), the mussel *M. edulis* (Incze *et al.*, 1980) and the American oyster *C. virginica* (Mallet and Haley, 1983a; Bataller *et al.*, 1999). It has been also reported for the pearl oysters *P. fucata* (Alagarswami, 1970; Nalluchinnappan *et al.*, 1982), *P. margaritifera* (Friedman and Southgate, 1999), *P. maxima* (Saville-Kent, 1893; Gervis and Sims, 1992; Sims, 1993) and *Pteria penguin* (Smitasiri *et al.*, 1994). A further selection of literature on the effects of different environmental parameters on the growth of various species of pearl oysters is presented in Table 5.1.

Table 5.1 Environmental parameters investigated for their influence on physiology and growth of pearl oyster.

Environmental parameter	Species	Authors
Water temperature	*P. maxima*	Yukihira *et al.* (2000) Mills (2000) Pass *et al.* (1987)
	P. imbricata	O'Connor and Lawler (2004a) O'Connor and Lawler (2004b) Tomaru *et al.* (2002b)
	P. margaritifera	Pouvreau *et al.* (2000c) Yukihira *et al.* (2000) Doroudi *et al.* (1999a)
	Pteria sterna	Del Rio-Portilla *et al* (1992) Araya-Nuñes *et al.* (1991)
Salinity	*P. maxima*	Kvingedal *et al.* (2008)
	P. imbricata	O'Connor and Lawler (2004a) Urban (2000)
	P. margaritifera	Doroudi *et al.* (1999a)
pH	*P. maxima*	Taylor *et al.*, (1997b) Welladsen (2009)
	P. imbricata	Yu *et al.* (1998) Yu *et al.*, (1999)
Suspended particulate matter	*P. maxima*	Yukihira *et al.* (1999) Taylor *et al.* (1998) Taylor *et al.* (1997b) Rose and Baker (1994)
	P. imbricata	Hashimoto and Nakano (2003) Tomaru *et al*, (2002a) Urban (2000)
	P. margaritifera	Pit and Southgate (2000) Yukihira *et al.* (2000) Doroudi *et al.* (1999b) Southgate *et al.* (1998)
Chlorophyll	*P. imbricata*	Tomaru *et al.* (2002b)
	P. margaritifera	Vacelet *et al.* (1996)

Growth in marine bivalves is affected by the interaction of several environmental variables, particularly water temperature and food supply (Incze *et al.,* 1980; Bayne and Newell, 1983; Brown and Hartwick, 1988b). Growth studies have generally involved univariate empirical methods that manipulate environmental conditions in the laboratory or field experiments that monitor variations in these variables (Malouf and Breese, 1977; Bayne and Newell, 1983 and MacDonald and Thompson, 1985). These studies indicate that water temperature, food, salinity and pH all influence growth in bivalves (Bernard, 1983; Bamber, 1987, 1990; Al-Sayed *et al.,* 1997). It is clear that bivalve growth is a function of several environmental variables acting in concert.

In addition to environmental parameters, the age of a bivalve also influences growth. Growth rate has been reported to decrease with age in the pearl oysters, *Pinctada radiata* (Nayar and Al-Rumaidh, 1993), *P. margaritifera* (Pouvreau *et al.,* 2000a) and *P. mazatlanica* (Saucedo and Monteforte, 1995).

While univariate studies are useful in determining differences in growth caused by age or a particular environmental factor, it has limitations as interaction between parameters and age, apportionment of environmental factors or age towards growth, and the degree a particular variable affects growth are unknown. The method of choice for studies, which involve numerous variables whose effects on, or relationships with, other variables of interest is multivariate statistical techniques.

The research in this chapter was undertaken to establish if culture site, depth and oyster age had a significant impact on the growth of *P. maxima* cultured in commercial grow-out systems in West Papua, Indonesia.

5.2 Methods and materials

The experiment was conducted at Cendana Indopearls' farm at Aljui Bay in West Papua, Indonesia (refer Section 3.1) from May 2000 to November 2001.

5.2.1 Sites

Ganan, Manselo and Batu Terio (refer Section 3.4) were used as sites for the experiments. Within each site, a longline was established to culture the oysters used in the experiments according to the methods described in Section 3.6.5.

5.2.2 Oysters used in experiments

Hatchery produced oysters were reared according to the methods set out in Section 3.6. Oysters were randomly selected from three age classes of oysters spawned on January 1997, February 1999 and October 1999. At the start of the experiment in June 2000, the ages of the three groups were 41 months (3.4 years), 16 months (1.3 years) and 8 months (0.6 year); they had mean initial lengths of 153.03 ± 16.69 mm, 93.94 ± 5.04 mm and 65.66 ± 9.69 mm, respectively, when the study commenced. Size is assumed to be a factor of age in this study; therefore, oysters from the three age classes will be referred to as 'large', 'medium' and 'small' in this chapter. One hundred and fifty oysters were randomly selected from each age class and tagged by attaching labelled Dymo® tape to the left valve of the shells using marine epoxy. In total, 450 oysters were tagged.

5.2.3 Experimental design

Tagged oysters from each of the three age cohorts were divided into three groups consisting of 50 oysters and each group was allocated to Ganan, Manselo or Batu Terio for grow-out. At each site, tagged oysters from each age group were further divided into two groups consisting of 25 oysters per group, divided equally into five sub-groups of five oysters and placed in doubled-up 28-pocket panel nets. The remaining spaces in the 28-pocket panel nets were filled with untagged oysters from the same age group for fouling studies (Chapter 7). Five panel nets of oysters were hung from the long-line at a depth of 5 m below the surface, while the other five were hung 15 m below the surface (**Error! Reference source not found.**). Each treatment group was made of a different combination of variables (i.e. site, depth and age) as outlined in **Error! Reference source not found.**. In total, there were 18 treatment groups comprising of 5,040 oysters in which 450 oysters were tagged for the growth study. During the experiment, the oysters were not subject to the normal grading and cleaning husbandry practice undertaken at the farm and were instead cleaned during sampling.

At each experimental site:

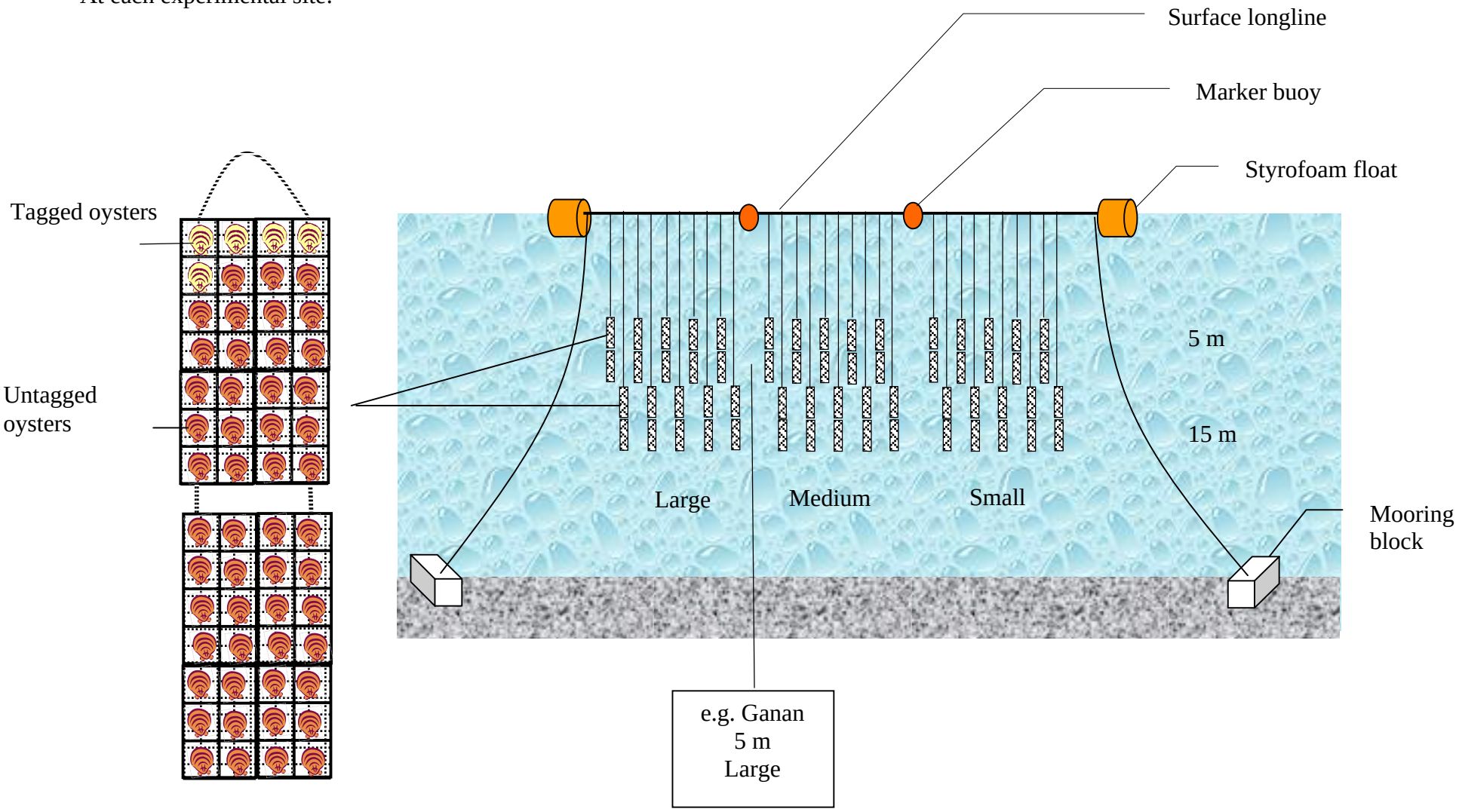

Fig. 5.1 Diagrammatic representation of the experiment. An example of a site/depth/site treatment group is shown in the square.

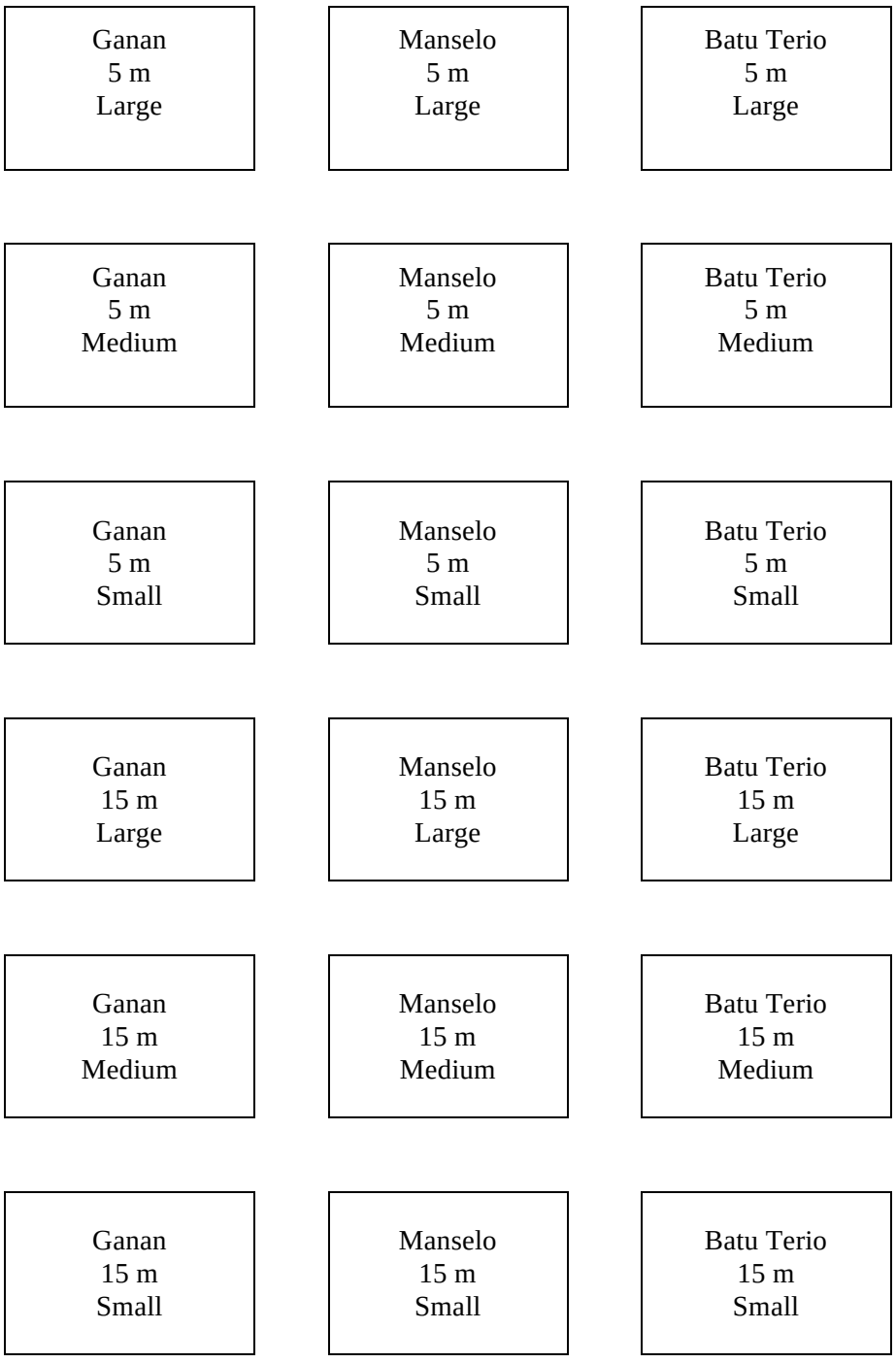

Fig. 5.2 Site, depth and size treatment groups for the experiment. There were 18 treatment groups, with each treatment consisting of 25 tagged oysters distributed equally between five double-hung 28-pocket panel nets.

5.2.4 Sampling of oysters

Sampling was performed monthly from May 2000 to November 2001, with the exception of December 2000 when bad weather prevented sampling. Oyster nets were removed from long-lines and placed in a 1000 L tank constantly supplied with running seawater piped directly from the sea, and maintained in this flow- through system at ambient salinity and water temperature until sampled.

Both tagged and untagged oysters were removed from the panel nets by cutting through the byssal threads attaching oysters to the net with a razor. Oysters were then cleaned of surface biofouling with high-pressure water spray and scraped with a knife.

5.2.5 Oyster growth

Tagged oysters were measured for shell length (antero-posterior measurement, refer Section 2.6.1.2) to the nearest 0.1 mm using vernier callipers according to the method described in Section 3.8.1. Wet weight of each tagged oyster was measured to the nearest 0.01 g following the method described in Section 3.8.2.

5.2.6 Oyster mortality

The number of mortalities of tagged oysters was recorded during sampling. Dead oysters were removed from the panel nets, cleaned and placed back into the net for fouling studies (Chapter 7) and were not replaced with live oysters. Mortality was recorded as the number of dead oysters per month as well as calculated as overall percentage survival.

5.2.7 Condition index (CI)

Two randomly selected untagged oysters from each treatment group were sacrificed every month during the experiment. The oysters were cleaned of surface fouling and total dry tissue and shell weight were determined according to the methods described in Section 3.8.2.2. CI was calculated using the formula of Walne and Mann (1975):

$$\text{Condition index} = \left(\frac{\text{Dry total tissue weight}}{\text{Dry shell weight}} \right) \text{x } 1000 \quad \text{(Equation 5.1)}$$

Dry tissue weight was measured as the sum of soft tissue, mantle and muscle dry weight.

5.2.8 Environmental monitoring

Environmental parameters were measured weekly from June 2000 to November 2001 according to the methods described in Section 3.5. As there was no growth sampling in December 2000, the corresponding environmental data for that month were excluded from the analysis. A mean value for each environmental parameter at each of the three sites and two depths over a particular sampling month (C_a) was determined using Equation 3.7 in Section 3.5.7 and used for graphical representation of environmental data in this chapter.

5.2.9 Statistical analyses

To measure overall growth, total growth (G_T) over the sampling period was calculated as:

$$(G_T) = G_n - G_1, \qquad \text{(Equation 5.2)}$$

where G_n = measurement at last sampling and G_1 = measurement at first sampling.

The units for G_T length and G_T weight are mm and g, respectively. To determine if oyster age, culture site and culture depth significantly affected G_T, G_T length and G_T weight were entered into a multivariate analysis of variance (MANOVA) using the Wilks Lambda test of significance. Means were tested using the Bonferroni post-hoc range test to determine which means differ.

When comparing monthly growth of tagged oysters, the absolute measurement of the oysters was unsuitable as there were obvious size differences between the various age classes. Instead, a more standard measure, monthly instantaneous growth rate, (G_{30}) was used to compare growth of oysters over a particular month and was defined by Brown (1988) as:

$$G_{30} = \left[\frac{\log_e \left(\frac{G_{t+1}}{G_t} \right)}{\log_e D} \right] \times 30 \qquad \text{(Equation 5.3)}$$

Where G_{t+1} = mean growth measurement (length or weight) of the current month; G_t = mean growth measurement of the previous month and D = number of days between observations.

Log_e transformation of D served to further detrend residuals in multiple regression analysis (Brown, 1988). The unit of measurement for G_{30} length is mm $month^{-1}$ and for G_{30} weight g $month^{-1}$. The terms 'total growth' and $G_{T,}$ as well as 'growth rate' and G_{30} are used interchangeably.

To examine the effects of sampling month, oyster age, culture site and culture depth on G_{30} length and G_{30} weight, pair-wise repeated measure ANOVA using the Wilks Lambda multivariate test of significance was employed using month as a repeated measure. Besides the usual assumptions for ANOVA, the assumption for sphericity was also tested using Mauchly's test. If the assumption was violated, the Greenhouse-Geissner (ε) adjustment test was used (Francis, 2004).

Analysis of mortality using full factorial ANOVA with 4-way interaction could not be employed due to insufficient replication of data as only one count of mortality (number of dead oysters per month) could be recorded at each sampling. Instead, a main effect and 3-way factorial ANOVA model was employed. Similarly, CI was examined using ANOVA to test for significant differences between the different sites, depths, size and month. The site and depth combinations, which produced the best growth for each size class of oysters, were identified by calculating the average G_{30} for each site/depth combination.

To determine the relationship between growth and environmental parameters, average G_{30} of length and weight were entered into a multivariate stepwise regression with environmental parameters assigned the independent variables. The criteria set for F to enter and F to remove were ≤ 0.05 and ≥ 0.10, respectively. Regression against environmental parameters was tested for all oysters as well as different sized oysters grown at the various sites and depths. Potential predictor environmental variables included in the analysis were water temperature, salinity, pH, levels of chlorophyll *a*, *b*, *c*, SPM and POM. Rainfall was not included in the analyses, as only temporal data for

rainfall was available with spatial data not sampled. Variables not included in the equations were rejected in favour of remaining variables at $\alpha = 0.05$.

5.3 Results

Growth is discussed in terms of the total growth G_T as well as monthly instantaneous growth rate, G_{30}.

5.3.1 Total Growth (G_T)

Mean shell length and mean wet weight increased in oysters of all sizes and grown at every site and depth over the sampling period (Table 5.2). Final mean length at the end of the experiment in November 2001 ranged from 96.01 ± 18.18 mm (mean ± SD) for medium oysters grown at Ganan at a depth of 15 m to 205.93 ± 32.43 mm for large oysters grown at Ganan at a depth of 5 m. Mean length () and weight () of oysters from different treatments were graphed over time on a standard Y axis scale to allow for visual comparison of growth between different sized oysters.

Length growth in small oysters showed a steeper growth trajectory than that of medium and large oysters regardless of culture site or depth. The magnitude of growth, seen by the height difference between the initial and final length measurement, was also largest in small oysters, indicating shell growth was greater in small oysters than in medium and large oysters over the experimental period. Similarly, medium oysters consistently had a steeper slope curve than large oysters. While site and depth differences were also observed in shell growth curves, they were not as obvious as that seen for oysters of different sizes (). Total growth was not perceivably greater at depths of 5 m or 15 m or at any particular site when observed in the graph. When mean G_T was calculated, oysters at Manselo grew more at 5 m than at 15 m for all size groups (Table 5.3)

This was also observed in G_T of small oysters at Ganan and Batu Terio. However, with large and medium oysters, some exceptions were observed. For example, average G_T length of large oysters grown at Batu Terio at a depth of 15 m was 105.40 ± 12.70 mm compared to 100.00 ± 15.48 mm for oysters cultured at 5 m.

Table 5.2 Mean (± SD) initial, final and G_T length and weight of three size classes of *P. maxima* grown at Ganan, Manselo and Batu Terio.

Site		Size					
		Large		Medium		Small	
		Length (mm)	Weight (g)	Length (mm)	Weight (g)	Length (mm)	Weight (g)
Ganan	Initial	149.86 (19.48)	439.49 (129.99)	94.39 (6.02)	114.22 (14.50)	53.03 (9.85)	20.79 (7.54)
	Final	170.23 (16.51)	645.96 (172.48)	147.72 (14.77)	376.00 (84.68)	149.89 (9.28)	313.42 (43.10)
	G_T	20.36 (12.29)	206.47 (99.46)	53.32 (14.47)	261.79 (69.41)	96.86 (15.50)	292.63 (71.92)
Manselo	Initial	156.08 (16.48)	571.99 (159.37)	93.74 (5.18)	115.77 (13.55)	57.16 (7.35)	25.45 (8.18)
	Final	166.53 (13.97)	742.81 (126.59)	155.35 (14.54)	473.05 (83.04)	156.56 (13.05)	416.87 (68.77)
	G_T	10.45 (8.06)	170.82 (101.14)	61.61 (14.13)	357.28 (80.68)	99.40 (15.33)	391.43 (65.88)
Batu Terio	Initial	153.16 (13.28)	495.87 (81.68)	93.68 (3.72)	116.59 (12.54)	51.61 (7.14)	19.49 (7.54)
	Final	169.97 (16.63)	677.79 (178.45)	151.03 (11.09)	452.28 (57.76)	152.57 (14.95)	337.17 (91.14)
	G_T	16.81 (11.67)	181.92 (140.92)	57.35 (12.89)	335.69 (60.72)	100.96 (14.65)	317.68 (90.94)

MANOVA (Appendix 5.1) indicated that oyster size, culture site and culture depth had an effect on both G_T length and weight (size: $F_{(4,576)} = 265.975$, $p < 0.05$; site: $F_{(4,576)} = 6.292$, $p < 0.05$; depth: ($F_{(2,288)} = 5.922$, $p < 0.05$). Between subject results showed that G_T length was significantly affected by culture depth ($F_{(1,289)} = 11.153$, $p < 0.05$) and oyster size ($F_{(2,289)} = 839.420$, $p < 0.05$) but not culture site. However, there was significant interaction between site and size ($F_{(4,289)} = 4.551$, $p < 0.05$). G_T weight was significantly affected by oyster size ($F_{(2,289)} = 60.796$, $p < 0.05$) and culture site ($F_{(2,289)} = 11.005$, $p < 0.05$) but there was no difference in G_T weight between culture depth. Interaction between site and size was significant ($F_{(4,289)} = 7.542$, $p < 0.05$).

Bonferroni post-hoc tests (Appendix 5.1) on sites indicated that G_T weight was different between oysters from Ganan and Manselo. Similar multiple comparisons on size showed that G_T length differed between large, medium and small oysters while G_T weight was only different between small and large oysters. Graphical representations of G_T length (**Error! Reference source not found.**) and G_T weight (Fig. 5.6) show the differences between sizes, sites and depths, while statistical results are summarised in Table 5.4.

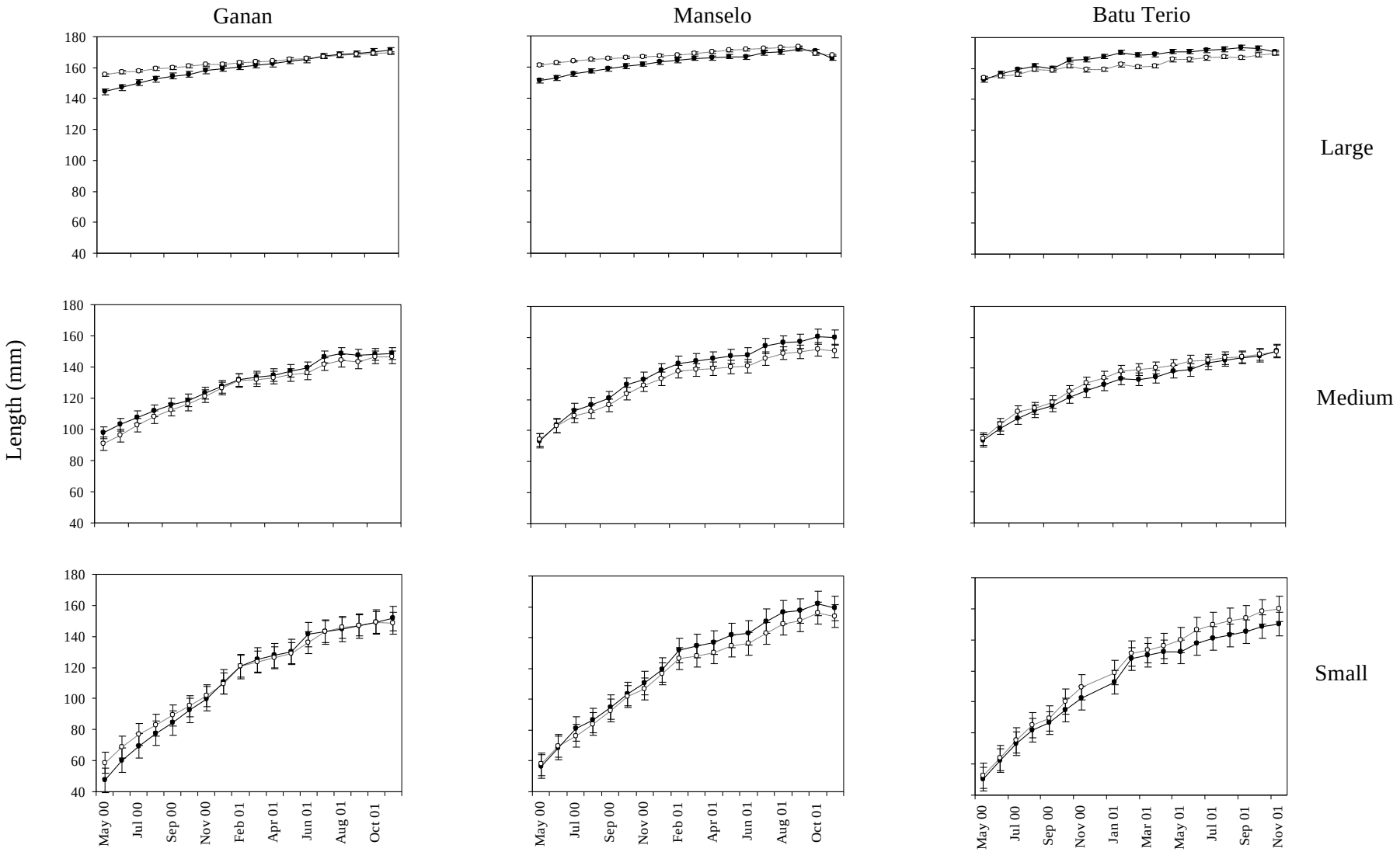

Fig. 5.3 Mean (± SE) monthly length of three size (age) classes of *P. maxima* grown at Ganan, Manselo and Batu Terio at 5 m (●) and 15 m (○) from May 2000 to November 2001.

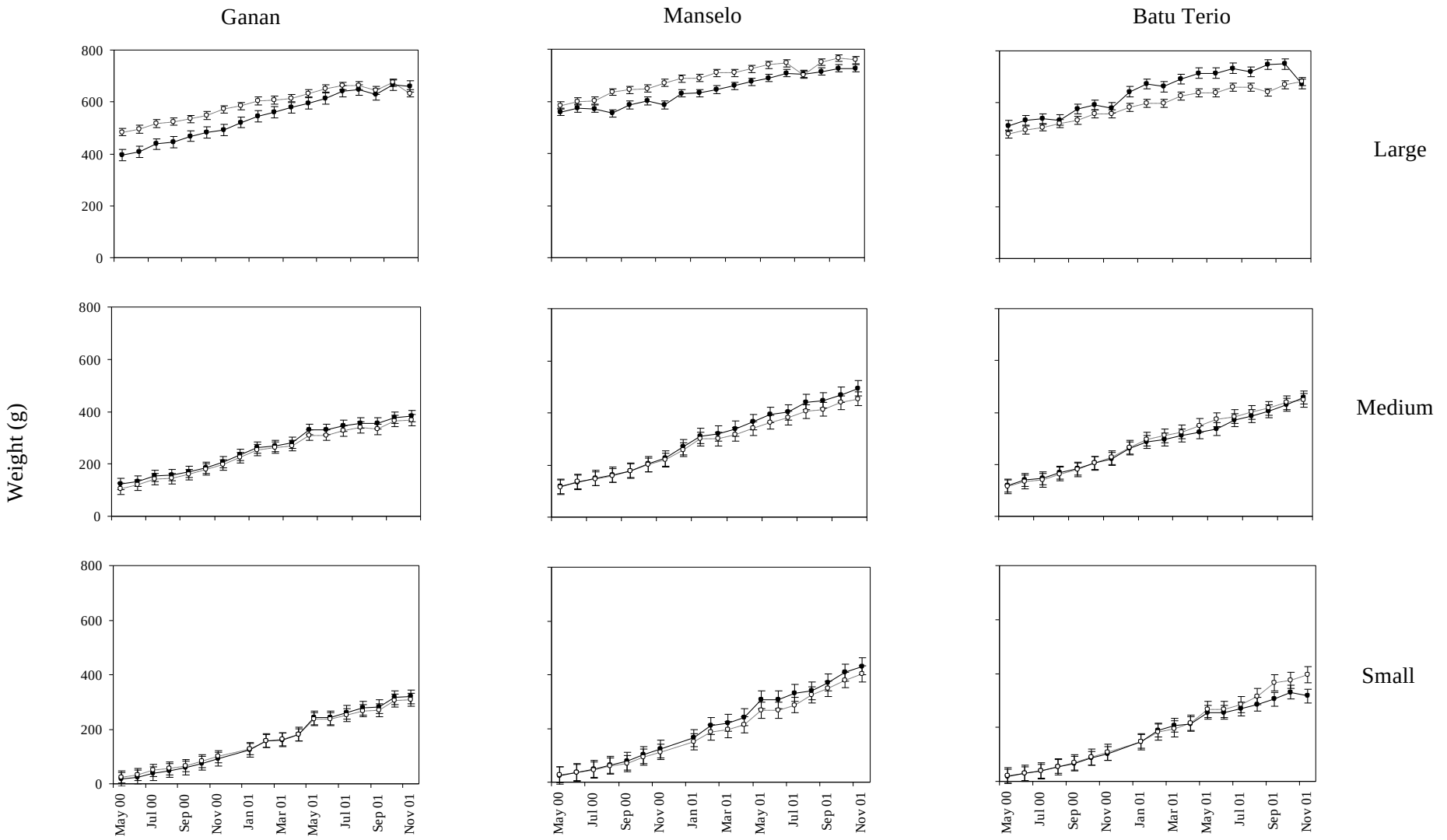

Fig. 5.4 Mean (± SE) monthly weight of three size (age) classes of *P. maxima* grown at Ganan, Manselo and Batu Terio at 5 m (●) and 15 m (○) from May 2000 to November 2001.

Table 5.3 Mean (± SD) G_T length and weight of three size classes of *P. maxima* grown at Ganan, Manselo and Batu Terio at 5 m and 15 m. Asterisk * indicates the mean G_T of a growth parameter when it is greater at 15 m than 5 m.

Growth Parameter	Size	Site					
		Ganan		Manselo		Batu Terio	
		5 m	15 m	5 m	15 m	5 m	15 m
G_T Length (mm)	Small	26.99 (13.69)	14.50 (6.86)	19.00 (15.90)	8.49 (5.23)	18.98 (10.76)	17.03 (12.53)
	Medium	50.77 (12.79)	*55.23 (16.52)	65.81 (15.28)	57.17 (11.74)	57.45 (14.37)	56.99 (11.69)
	Large	103.37 (12.98)	87.34 (13.86)	103.54 (14.98)	96.57 (15.35)	100.00 (15.48)	*105.40 (12.70)
G_T Weight (g)	Small	268.13 (101.23)	189.23 (82.34)	197.80 (112.88)	194.84 (91.53)	322.17 (51.97)	205.79 (96.45)
	Medium	271.68 (61.97)	258.87 (79.40)	377.33 (87.48)	339.84 (71.05)	333.95 (59.64)	325.81 (65.35)
	Large	277.25 (93.01)	*282.57 (51.11)	406.97 (57.09)	376.07 (72.13)	297.28 (90.73)	*374.32 (70.71)

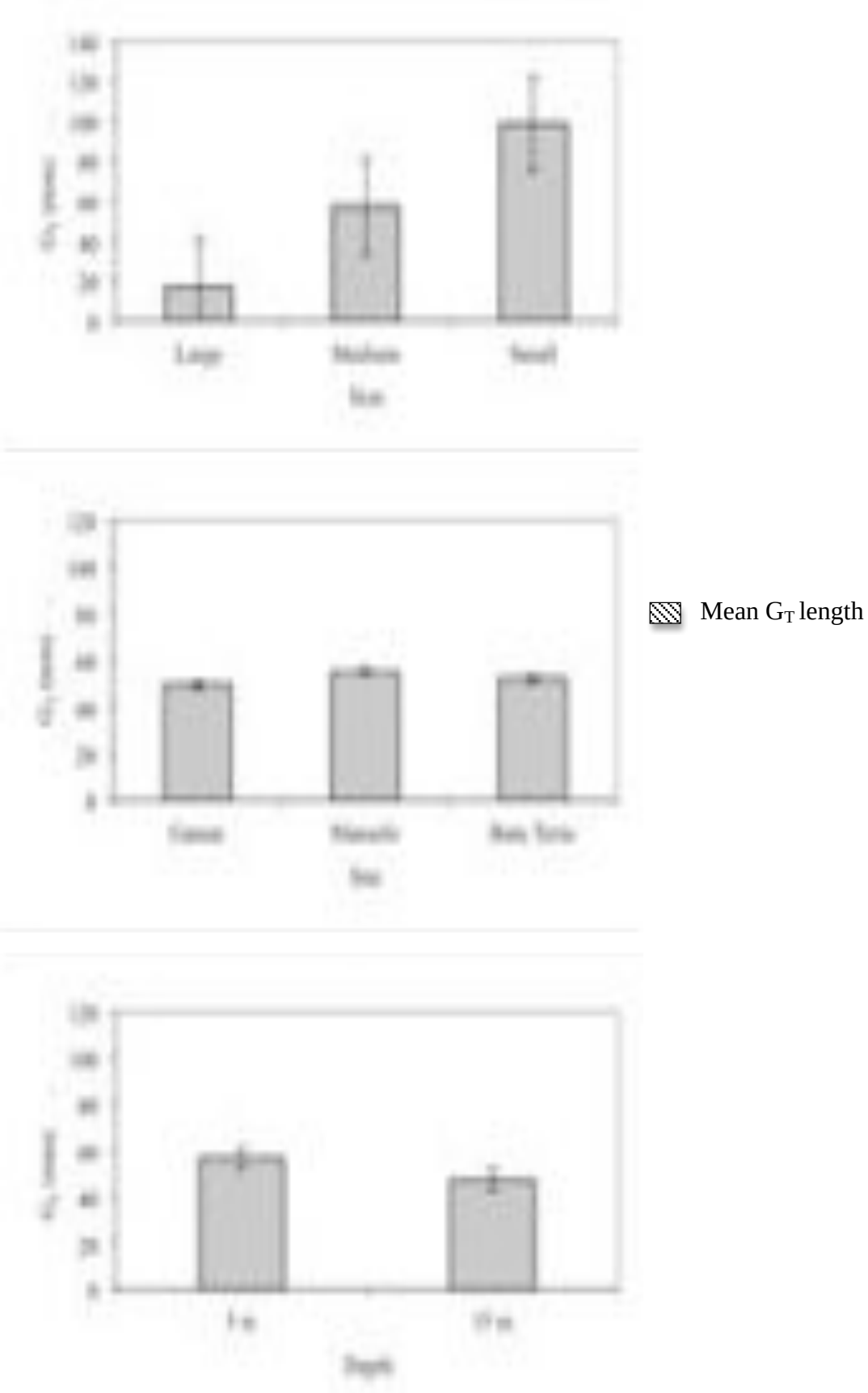

Fig. 5.5 Mean (± SE) total growth (G_T) of length of *P. maxima* from three age classes and grown at Ganan, Manselo and Batu Terio at 5m and 15 m from May 2000 to November 2001.

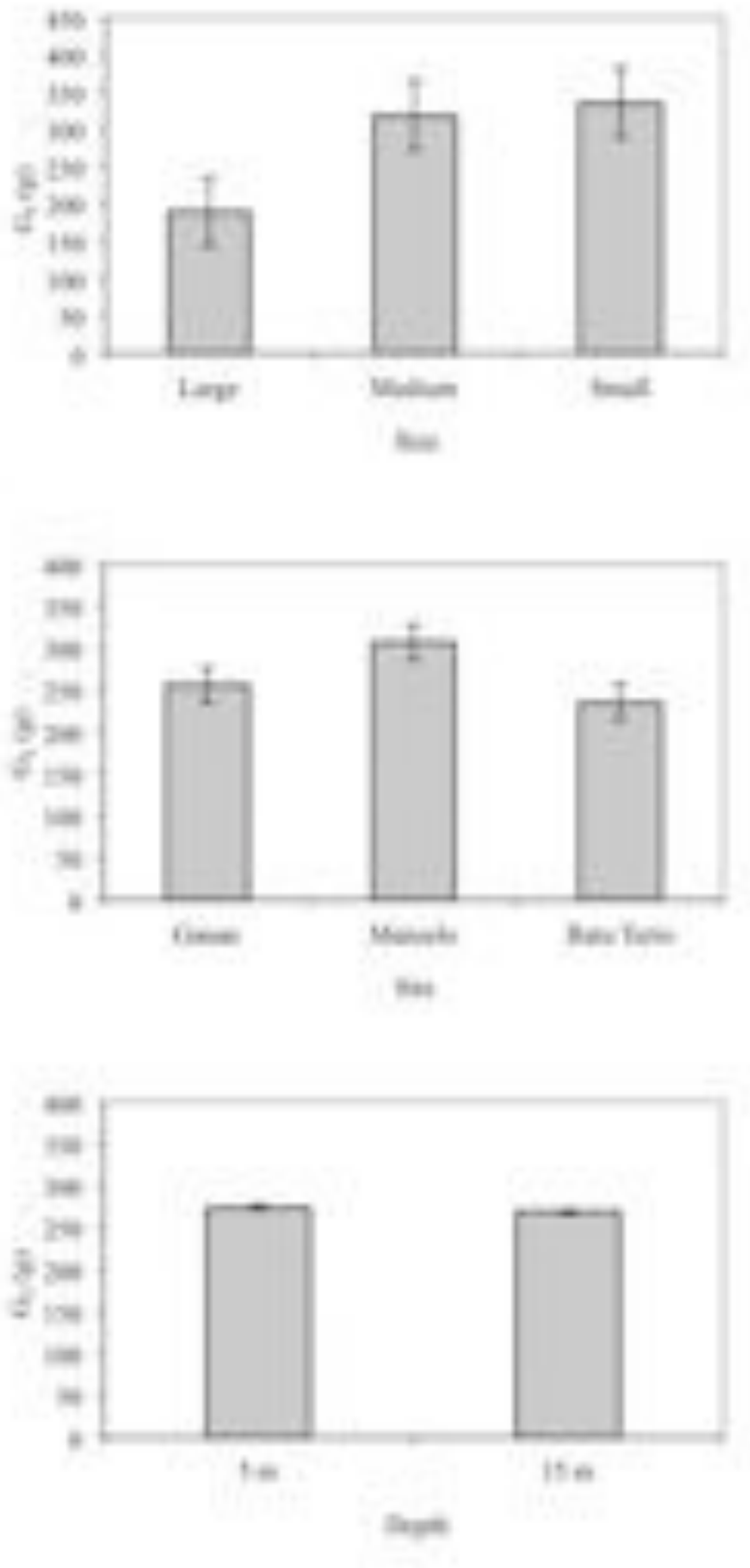

Fig. 5.6 Mean (± SE) total growth (G_T) of weight of *P. maxima* from three age classes and grown at Ganan, Manselo and Batu Terio at 5m and 15 m from May 2000 to November 2001.

Table 5.4 A summary of results from statistical analyses of G_T length and weight.

Measurement	Test	Effect	Significant ($\alpha = 0.05$)
G_T length and weight	Wilks Lambda multivariate	Site	Yes
		Depth	Yes
		Size	Yes
		Site * Depth	No
		Site * Size	Yes
		Depth * Size	No
		Site * Depth * Size	Yes
G_T length	Between subject test	Site	No
		Depth	Yes
		Size	Yes
		Site * Depth	Yes
		Site * Size	Yes
		Depth * Size	No
		Site * Depth * Size	No
G_T weight	Between subject test	Site	Yes
		Depth	No
		Size	Yes
		Site * Depth	No
		Site * Size	Yes
		Depth * Size	No
		Site * Depth * Size	No

5.3.2 Monthly Instantaneous Growth Rate (G_{30})

G_{30} of length and weight for all size, site and depth varied over time. G_{30} length was positive for all treatments while G_{30} weight was negative during some sampling months, e.g. large oysters sampled at Manselo in July and August 2001 indicate loss of weight in oysters during those months.

5.3.2.1 Temporal effect

When represented graphically on standard axes over time, differences in average G_{30} length () and G_{30} weight () were observed. Small oysters were the most affected temporally and displayed the greatest variation in G_{30}, followed by medium, then large oysters. G_{30} lengths of small and medium oysters were higher than that of large oysters, although all three sizes were similar in having the highest G_{30} recorded at the start of sampling in June 2000. Medium oysters displayed a similar G_{30} pattern to small oysters, with lesser magnitude in fluctuation, over time while changes in the G_{30} of large oysters were very slight over the sampling period. In all the site, depth and size treatments for G_{30} length, there appeared to be a trend for growth rate to be lowest from March - April 2001 before increasing again from June – July 2001, although to a lesser magnitude compared to the start of the experiment ().

G_{30} weight over time was similar to G_{30} length in that there was greatest fluctuation in growth in small and medium oysters, with the greatest growth observed at the start of the experiment for all sizes of oysters. The main difference between G_{30} length and weight was that negative values were recorded in G_{30} weight for small and large oysters grown at all sites from July 2001 onwards, while values of G_{30} length were all positive.

Multivariate within-subject test showed that the month of sampling had an overall effect on G_{30} length and weight ($F_{(32,252)} = 280.759$, $p < 0.05$). Interaction at all levels was also significant (month and site: $F_{(64,504)} = 11.05$, $p < 0.05$; month and depth: $F_{(32,252)} = 1.943$, $p < 0.05$; month and size: $F_{(64,504)} = 35.633$, $p < 0.05$; month, site and depth: $F_{(64,504)} = 1.692$, $p < 0.05$; month, site and size: $F_{(128,1005.2)} = 3.674$, $p < 0.05$; month, depth and size: $F_{(64,504)} = 2.444$, $p < 0.05$; month, site, depth and size: $F_{(128,1005.2)} = 1.756$, $p < 0.05$). Within subject ANOVA with repeated measures using the Greenhouse-Geisser adjustment (ε) showed a significant difference in G_{30} length

($F_{(8.5,2429.9)}$ = 253.56, $p < 0.05$, η^2= 0.537) and G_{30} weight ($F_{(2.5,695.1)}$ = 51.15, $p < 0.05$, η^2= 0.154) over time (Appendix 5.3).

5.3.2.2 Spatial and size effect

Differences in G_{30} length and weight between the three size groups could clearly be seen in the plots of G_{30} length () and G_{30} weight () over time. In contrast, the effect of site and depth on G_{30} was not possible to distinguish in the graphs.

Multivariate between-subject tests on both G_{30} length and weight (Appendix 5.2) showed that oyster size and culture depth had an overall effect on both measurements (size: $F_{(4,564)}$ = 335.168, $p < 0.05$; site: $F_{(2,282)}$ = 8.846, $p < 0.05$). Culture site did not exert a significant effect on G_{30} length and weight. Interactions between site and size ($F_{(8,564)}$ = 2.198, $p < 0.05$), depth and size ($F_{(4,564)}$ = 2.758, $p < 0.05$) and site, depth and size ($F_{(8,564)}$ = 2.067, $p < 0.05$) were significant.

In the within subject test, G_{30} length was found to be significantly affected by oyster size ($F_{(2,283)}$ = 1373.727, $p < 0.05$), culture site ($F_{(2,283)}$ = 3.764, $p < 0.05$) and culture depth ($F_{(1,283)}$ = 16.898, $p < 0.05$) over the months of sampling. There was also significant interaction between depth and size ($F_{(2,283)}$ = 5.012, $p < 0.05$) and site, depth and size ($F_{(4,283)}$ = 3.912, $p < 0.05$). While interaction between site and size was also significant ($F_{(4,283)}$ = 2.495 , $p < 0.05$), the p value was 0.043 indicating a relatively weak interaction. No interaction was found between site and depth.

As age has been reported to affect growth rate of bivalves, an ANOVA was performed which partitioned the analysis according to size to examine the effects of culture depth and site on G_{30} length of oysters from different size classes. Results (Appendix 5.3) showed G_{30} length of large oysters were significantly affected by depth ($F_{(1,2362)}$ = 42.601, $p < 0.05$) but not site. Medium oysters growth rate were significantly affected by site ($F_{(2,2172)}$ = 5.692, $p < 0.05$) but not depth. Similarly, small oyster G_{30} length were affected by site ($F_{(2,1905)}$ = 6.829, $p < 0.05$) but not depth.

Within subject analysis showed G_{30} weight was significantly affected by oyster size ($F_{(2,289)}$ = 929.433, $p < 0.05$) but not culture site or depth. The only significant interaction for G_{30} weight was between site and size ($F_{(4,289)}$ = 3.861 , $p < 0.05$). Partitioning the analysis (Appendix 5.3) by size showed that site significantly affected

the G_{30} weight of medium ($F_{(2,2162)}$ = 9.839, $p < 0.05$) and small oysters ($F_{(2,1899)}$ = 5.655, $p < 0.05$) but not of large oysters.

The magnitude of G_{30} length and weight appeared to be inversely proportional to the size of the oysters, with small oysters displaying largest G_{30} values and large oysters smallest G_{30} values (Table 5.5). Small oysters were also observed to have higher fluctuations in G_{30} than medium oysters and large oysters.

A summary of results from the statistical analyses of G_{30} is presented in Table 5.6

5.3.2.3 Average G_{30} from different treatments

Average G_{30} length from the 18 combinations of size, site and depth treatments were highly variable, ranging from 0.0485 mm month^{-1} for large oysters grown at Ganan at a depth of 15 m to 0.7231 mm month^{-1}, the average G_{30} length for small oysters grown at Batu Terio at 15 m (Table 5.5). Similarly, average G_{30} weight ranged from 0.1306 g month^{-1} for large oysters from Manselo at a depth of 15 m to 1.9199 g month^{-1}, which represented the average G_{30} weight for small oysters from Batu Terio at a depth of 15 m (Table 5.5).

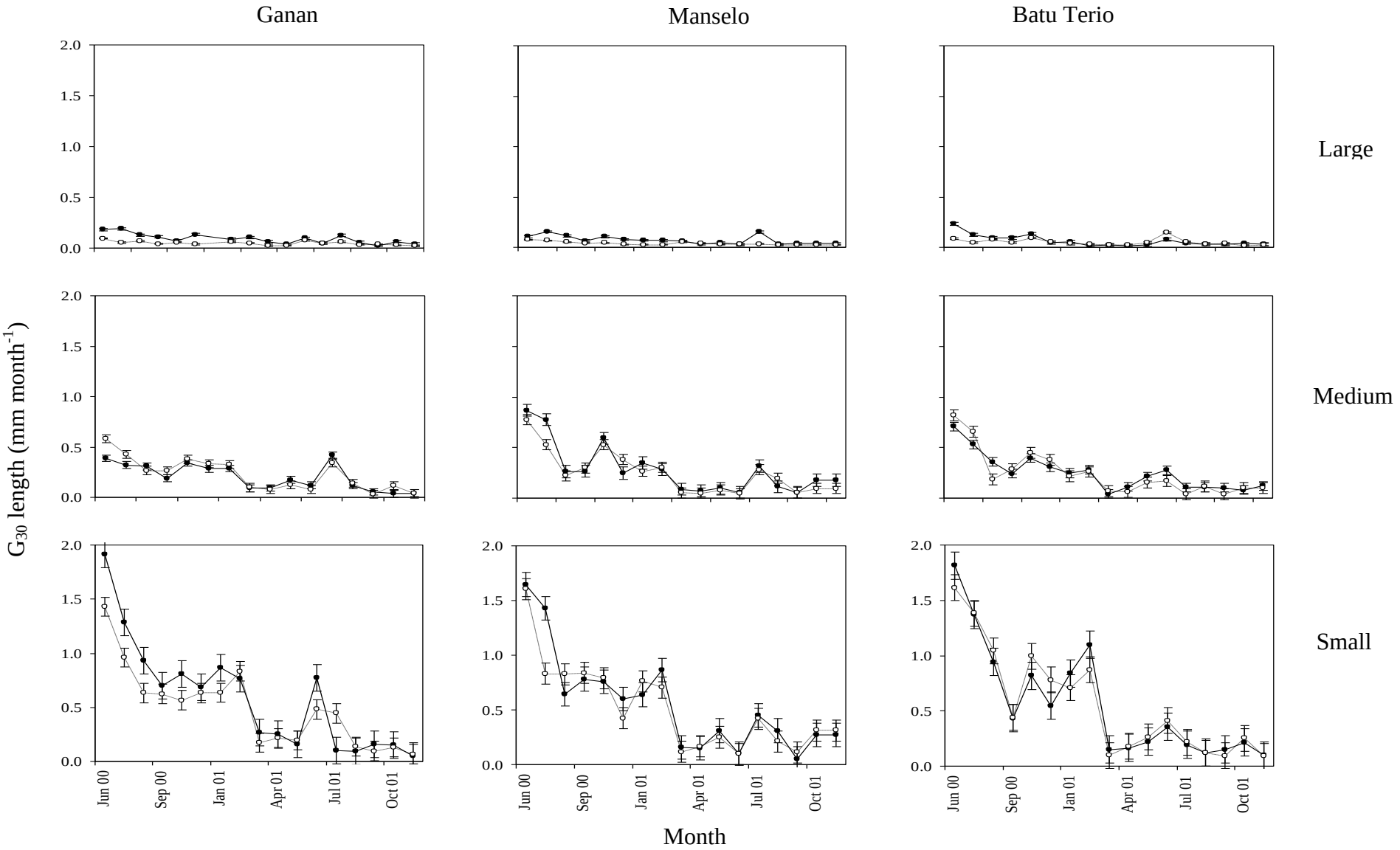

Fig. 5.7 Mean (± SE) monthly instantaneous growth (G_{30}) of length of three size (age) classes of *P. maxima* grown at Ganan, Manselo and Batu Terio at 5m ( ) and 15 m ( ) from May 2000 to November 2001.

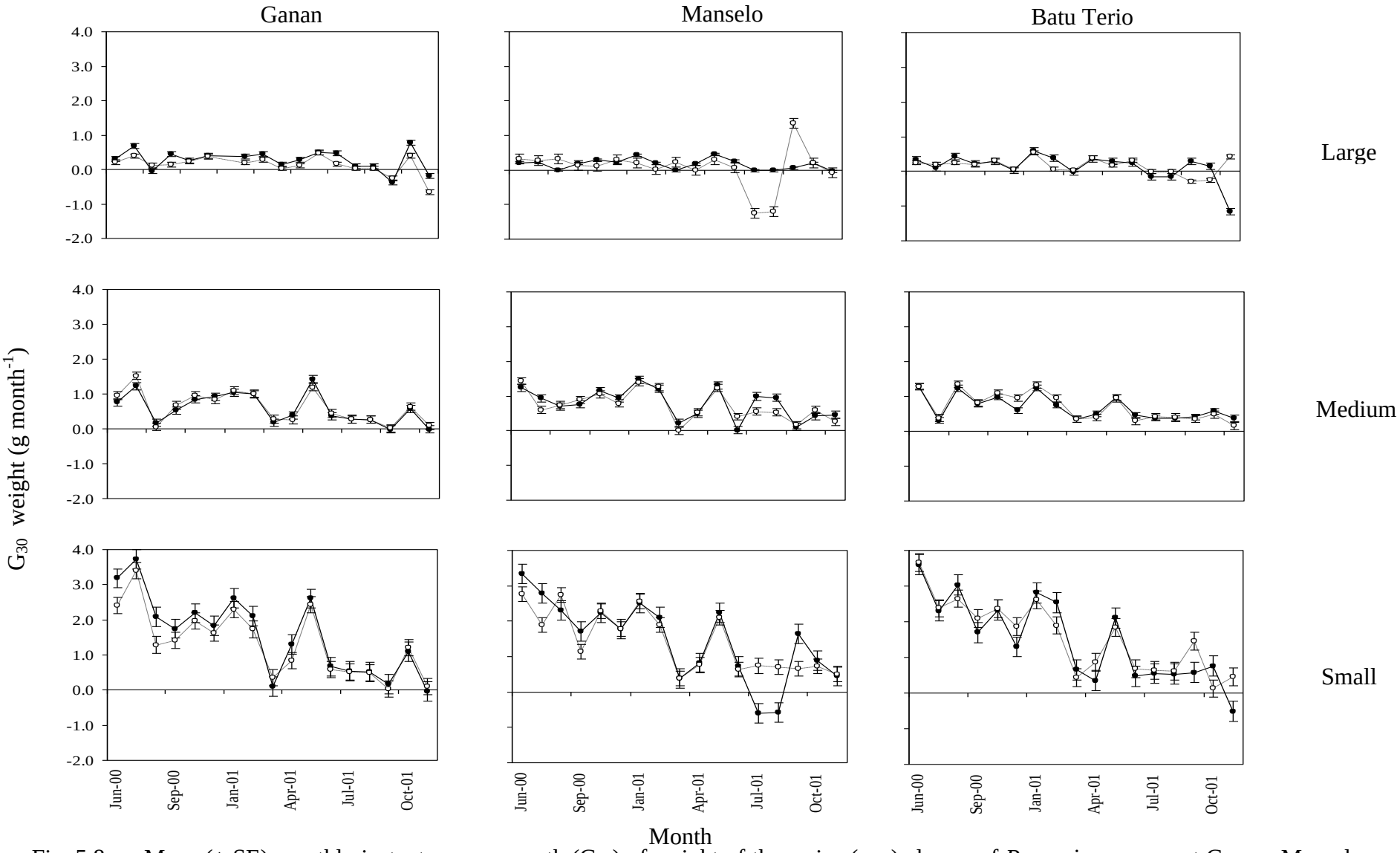

Fig. 5.8 Mean (± SE) monthly instantaneous growth (G_{30}) of weight of three size (age) classes of *P. maxima* grown at Ganan, Manselo and Batu Terio at 5m (●) and 15 m (○) from May 2000 to November 2001.

Table 5.5 Mean (± SD) G_{30} length and weight of three size classes of *P. maxima* grown at Ganan, Manselo and Batu Terio at 5 m and 15 m for the whole sampling period.

Growth Parameter	Size	Site					
		Ganan		Manselo		Batu Terio	
		5 m	15 m	5 m	15 m	5 m	15 m
G_{30} Length (mm)	Small	0.62 (0.59)	0.51 (0.49)	0.59 (0.57)	0.54 (0.53)	0.61 (0.63)	0.72 (0.59)
	Medium	0.22 (0.26)	0.25 (0.27)	0.29 (0.33)	0.26 (0.29)	0.28 (0.29)	0.29 (0.32)
	Large	0.09 (0.15)	0.05 (0.07)	0.09 (0.19)	0.05 (0.08)	0.07 (0.12)	0.05 (0.11)
G_{30} Weight (g)	Small	1.63 (1.24)	1.39 (1.19)	1.64 (1.16)	1.50 (1.14)	1.63 (1.47)	1.92 (1.23)
	Medium	0.59 (0.69)	0.63 (0.73)	0.79 (1.11)	0.73 (0.60)	0.73 (0.63)	0.79 (0.50)
	Large	0.28 (0.72)	0.13 (0.71)	0.16 (0.40)	0.13 (0.79)	0.16 (0.73)	0.16 (0.79)

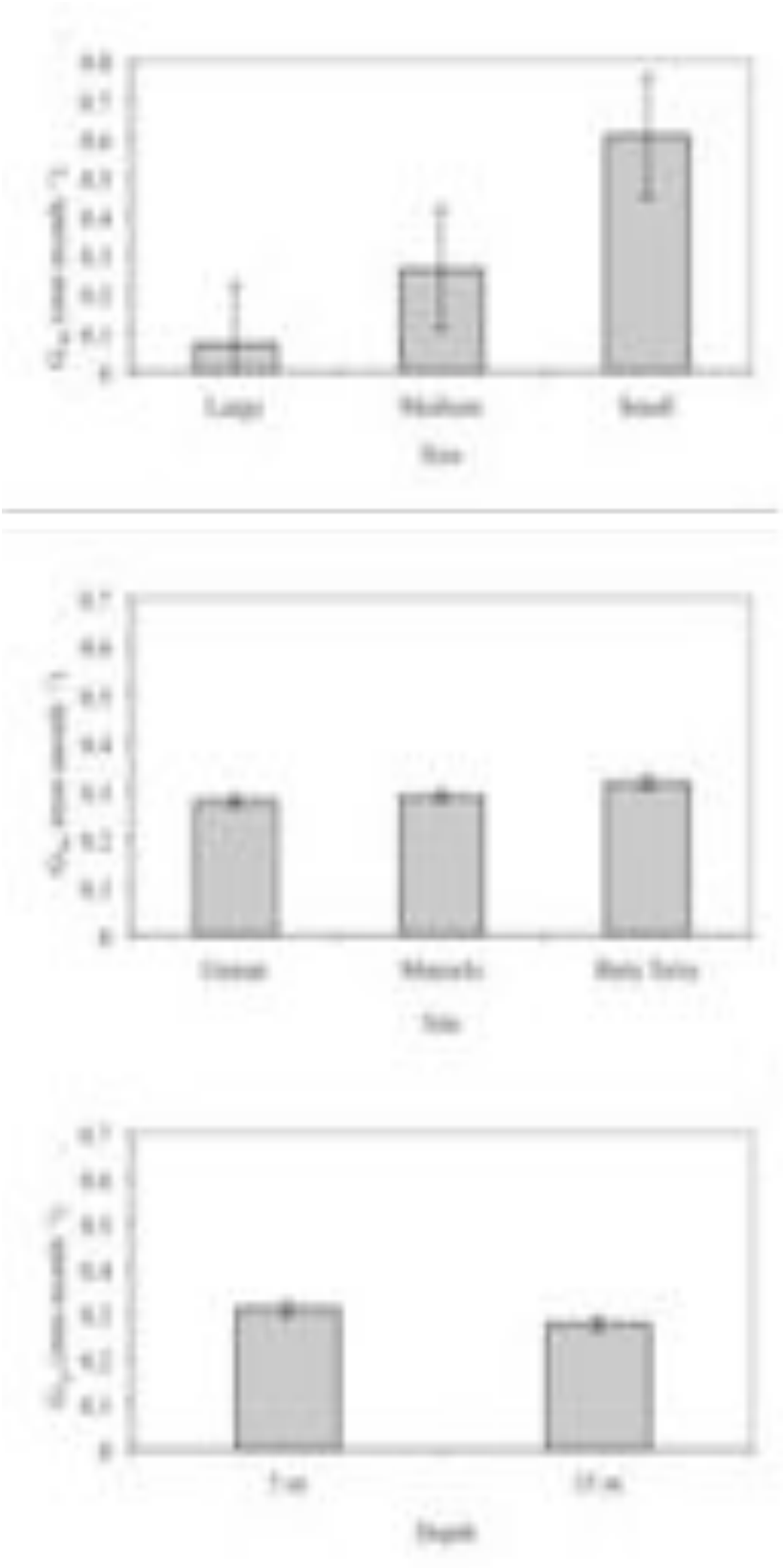

Fig. 5.9 Mean (± SE) monthly instantaneous growth (G_{30}) of length of *P. maxima* from three age classes and grown at Ganan, Manselo and Batu Terio at 5m and 15 m from May 2000 to November 2001.

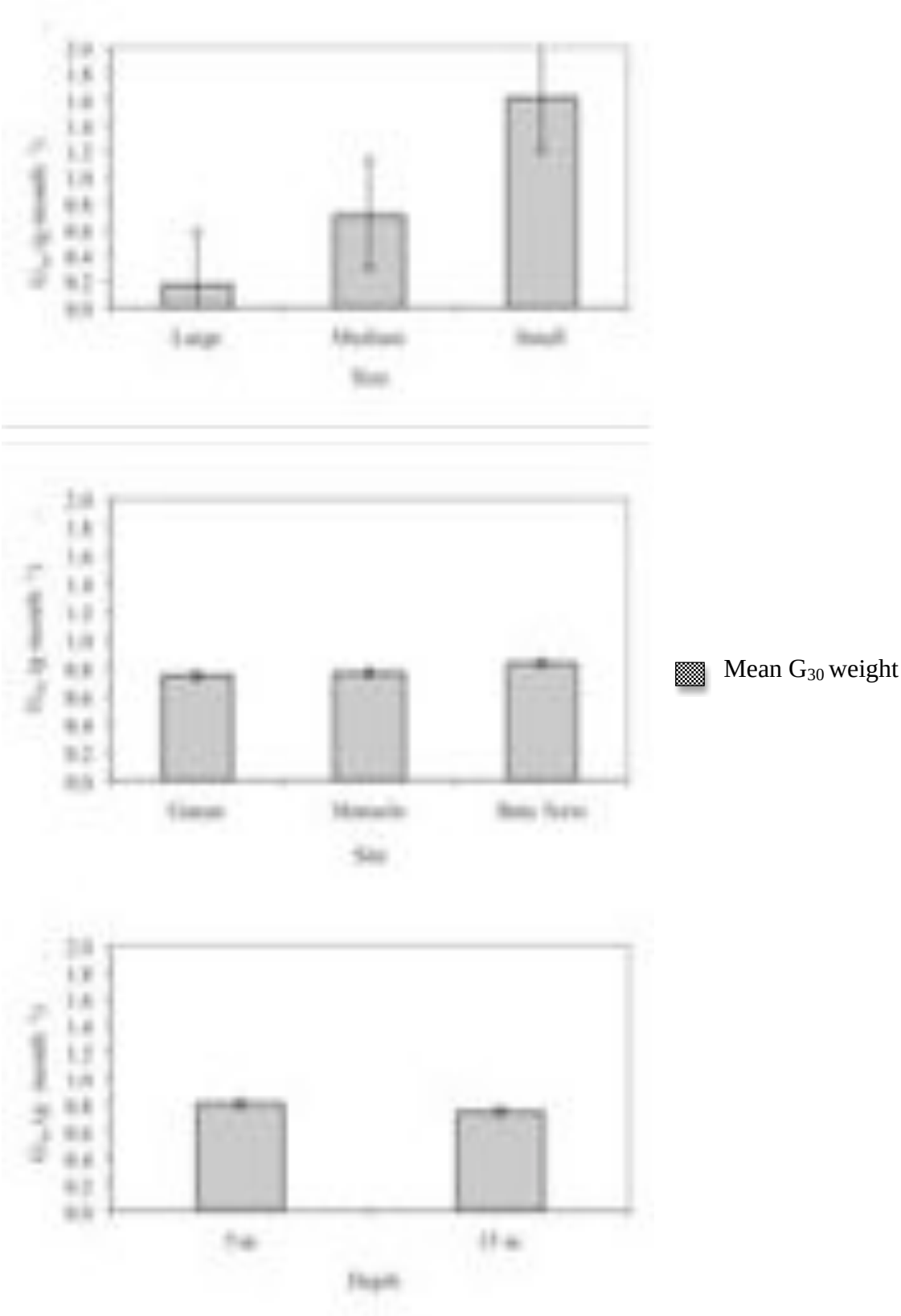

Fig. 5.10 Mean (± SE) total growth (G_{30}) of weight of *P. maxima* from three age classes and grown at Ganan, Manselo and Batu Terio at 5m and 15 m from May 2000 to November 2001.

Table 5.6 A summary of results from statistical analyses of G_{30} length and G_{30} weight

Measurement	Test	Effect	Significant ($\alpha = 0.05$)
G_{30} length and weight	Wilks Lambda Multivariate	Site	No
	with repeated measure	Depth	Yes
	Between subjects	Size	Yes
		Site * Depth	No
		Site * Size	Yes
		Depth * Size	Yes
		Site * Depth * Size	Yes
	Within subjects test	Month	Yes
		Month * Site	Yes
		Month * Depth	Yes
		Month * Size	Yes
		Month * Site * Depth	Yes
		Month * Depth * Size	Yes
		Month * Site * Depth * Size	Yes
G_{30} length	Between subject effect	Site	Yes
		Depth	Yes
		Size	Yes
		Site * Depth	$p = 0.51$
		Site * Size	Yes
		Depth * Size	Yes
		Site * Depth * Size	Yes
G_{30} weight	Between subject effect	Site	No
		Depth	No
		Size	Yes
		Site * Depth	No
		Site * Size	Yes
		Depth * Size	No
		Site * Depth * Size	No

5.3.3 Oyster mortality

Over the course of this experiment, 143 out of 450 oysters died which represented approximately 32% of tagged specimens. When graphed against time, mortality was observed to peak in May 2001 at Batu Terio (). Results from an ANOVA (Appendix 5.5) showed that size ($F_{(2,68)} = 6.91$, $p < 0.05$), site ($F_{(2,68)} = 10.19$, $p < 0.05$) and month ($F_{(17,68)} = 3.34$, $p < 0.05$) exerted a significant effect on mortality. While depth did not have a main effect on mortality, interaction between depth and month was significant ($F_{(17,68)} = 2.28$, $p < 0.05$). Interaction between month and size ($F_{(34,68)} = 1.63$, $p < 0.05$) and month and site ($F_{(34,68)} = 3.12$, $p < 0.05$) were also significant. There was also a significant three-way interaction effect between month, site and size ($F_{(68,68)} = 2.39$, $p < 0.05$)

Post-hoc Bonferroni tests showed the difference in mortality over the months was driven by high mortality in May 2001 of oysters in Batu Terio. Multiple comparisons of sites and sizes showed that mortality was significantly higher at Batu Terio than at Manselo and Ganan, and in small oysters when compared to medium or large ones (Appendix 5.5). When oysters were pooled into various age groups to detect if sampling month affected death of oysters of various ages, the highest mortalities were observed in young oysters sampled in August 2000, May 2001 and November 2001 ( ).

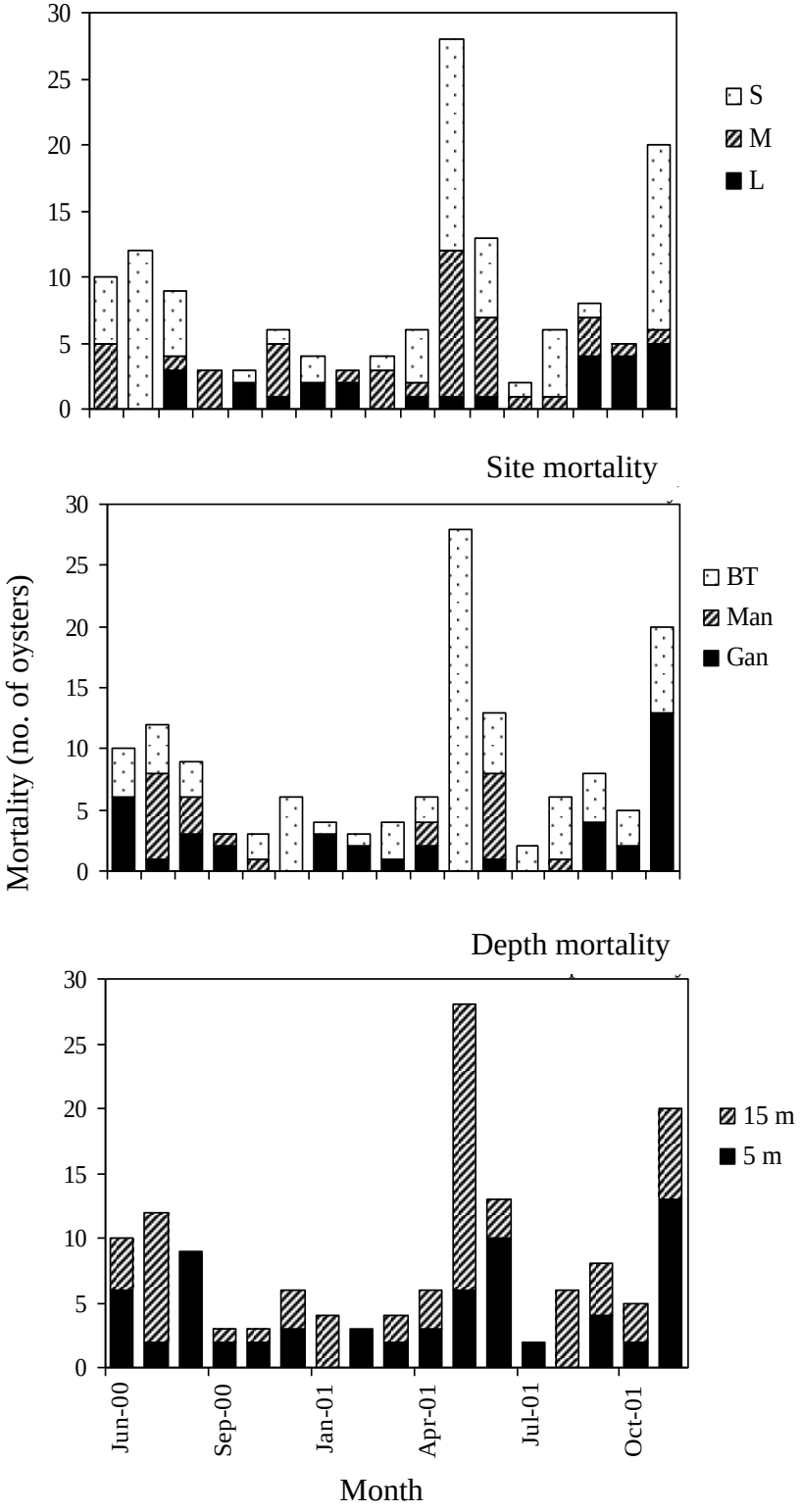

Fig. 5.11 Comparison of size, site and depth-related mortalities of oysters sampled from June 2000 – November 2001.

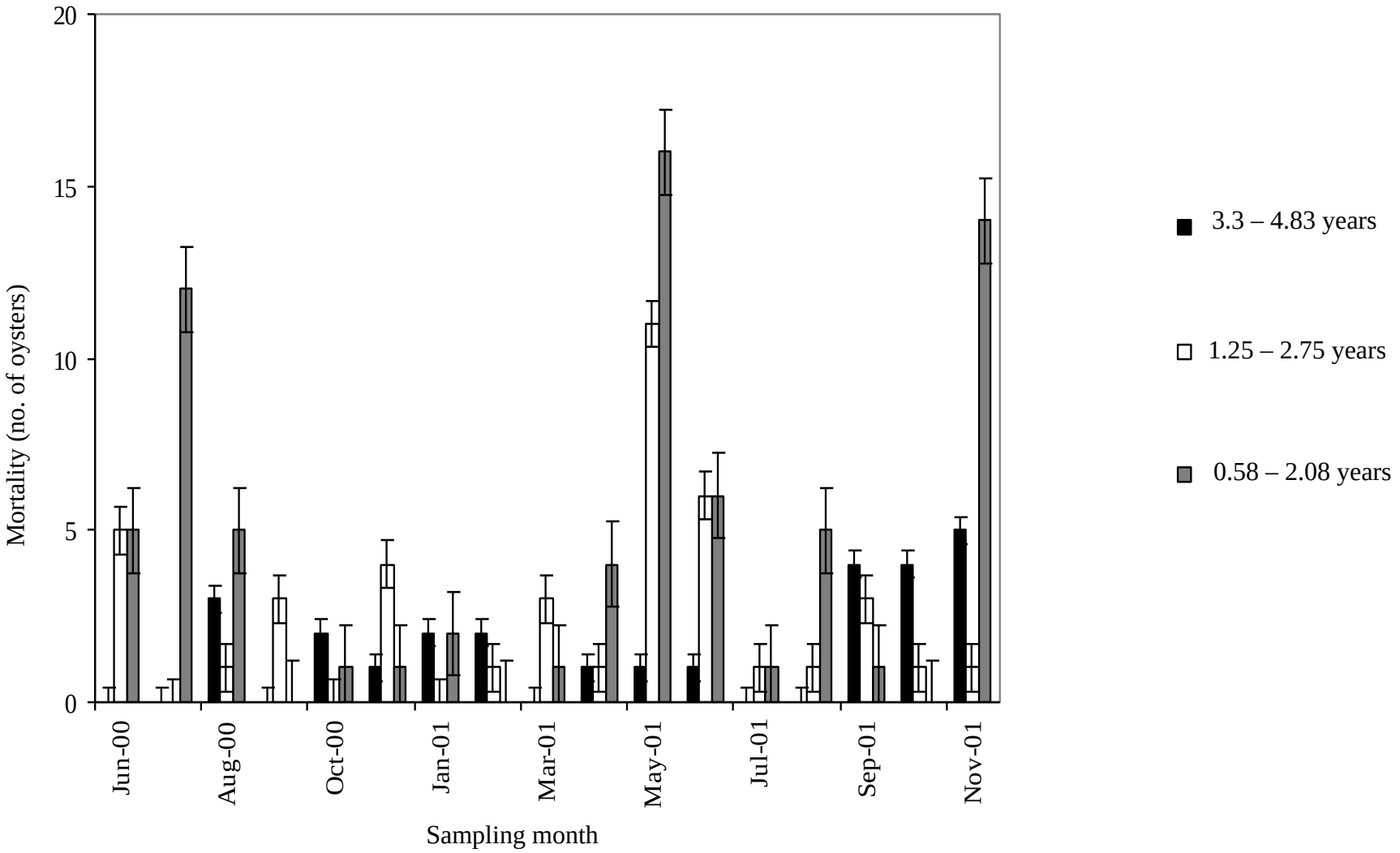

Fig. 5.12 Mortality of oysters from different age groups during sampling months.

5.3.4 Condition Index (CI)

Temporal, spatial and size-related differences could be observed in CI when the data were graphed in . CI of large oysters grown at the three sites showed very little change during the experimental period with the exception of oysters grown at Ganan at 5 m and Batu Terio at 15 m during June 2000 and August 2001, respectively (). During these two months, the index peaked sharply. There was a sharp peak in the CI of medium and small oysters during October – November 2000 at all sites. In August – October 2001, a smaller peak was observed in medium oysters grown at 15 m at Batu Terio as well as small oysters grown at 5 m in Ganan.

ANOVA of CI (Appendix 5.5) confirmed that CI was significantly affected by month of sampling ($F_{(17,392)} = 35.49$, $p < 0.05$) , site ($F_{(2,392)} = 17.72$, $p < 0.05$), depth ($F_{(1,392)} = 16.25$, $p < 0.05$) and size ($F_{(2,392)} = 237.79$, $p < 0.05$). All two-way and three-way interactions were also significant with the exception of depth and size and month, depth and size (Appendix 5.6)

5.3.5 Environmental parameters

Environmental data were presented in detail in Chapter 4 and will not be repeated in depth in this chapter. In summary, MANCOVA of environmental parameters showed that site ($F_{(16,762)} = 3.614$, $p < 0.05$) and month ($F_{(8,380)} = 0.796$, $p < 0.05$) had an overall effect on environmental parameters while depth ($F_{(8,380)} = 0.552$, $p > 0.05$) did not. However, there was significant interaction between site and depth ($F_{(16,762)} = 0.909$, $p < 0.05$) indicating there were differences in environmental parameters between depths at various sites.

5.3.6 Relationship between G_{30} length and environmental parameters

Multiple regression analyses were used to explore the relationship between G_{30} length and environmental predictors (Appendix 5.5). Regression was calculated from data collected from all oysters (overall), as well as from selected data from the different site, depth and size treatments.

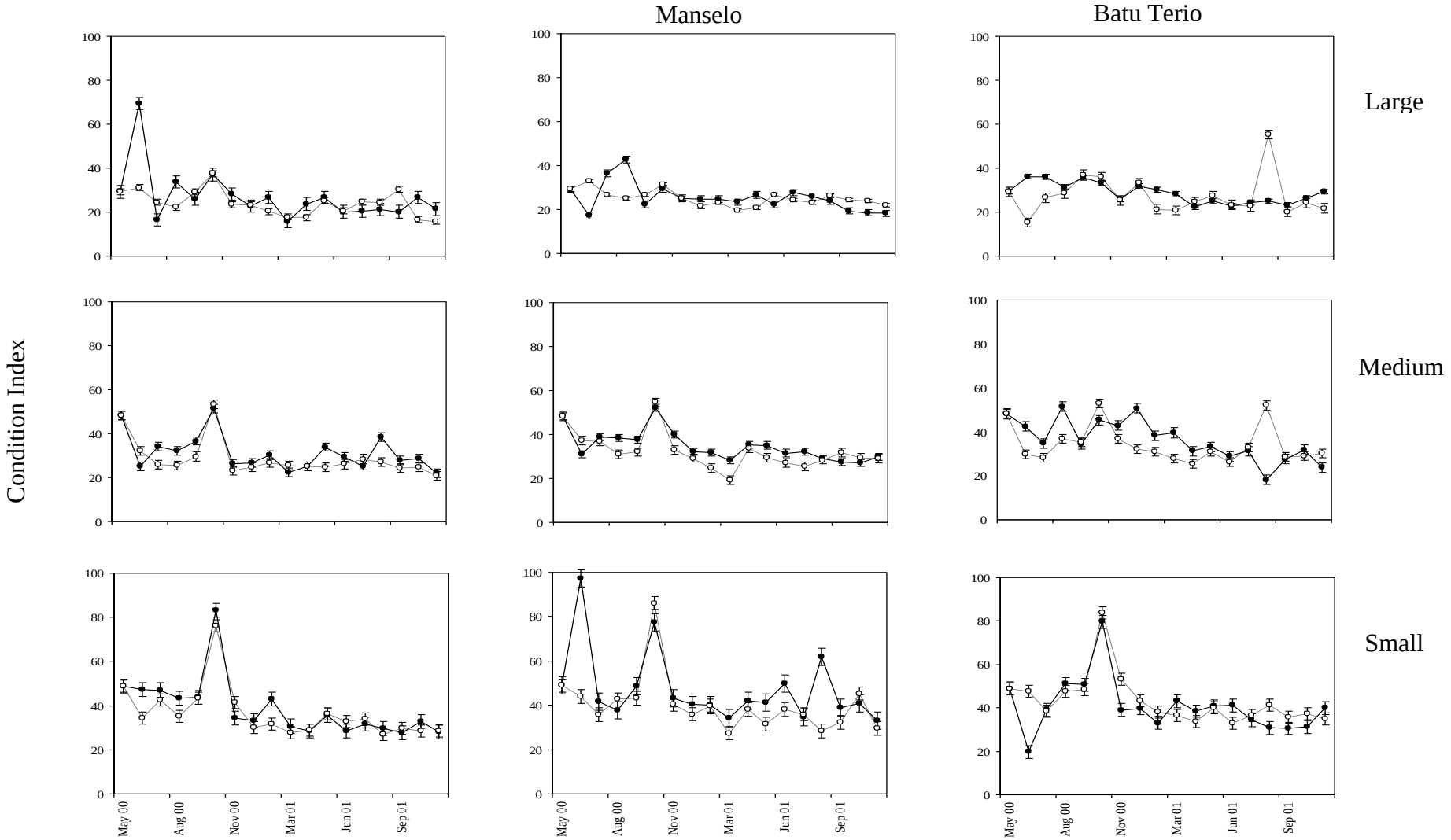

Fig. 5.13 Mean (± SE) CI of three size (age) classes of *P. maxima* grown at Ganan, Manselo and Batu Terio at 5m (♀) and 15 m (♀) from May 2000 to November 2001.

5.3.6.1 Overall G_{30} length

Stepwise multiple regression of G_{30} length of all oysters against environmental indicators (Appendix 5.5) showed that pH and salinity accounted for approximately 7% ($p < 0.001$) of variation in G_{30} length (Table 5.6). By comparing the standardised regression coefficients, which indicate relative influence of individual independent variables upon the dependent variable (Zar, 1984; Brown, 1988), results showed that pH with a regression coefficient of 0.232 had a greater effect on G_{30} length than salinity which had a regression coefficient of 0.166 (Table 5.7). pH was positively correlated to G_{30} length while salinity had a lesser inverse effect on G_{30} length as indicated by the negative value of the salinity coefficient.

5.3.6.2 Site-related difference in G_{30} length

There was no relationship between G_{30} length of oysters grown in Ganan and any of the predictor environmental parameters when stepwise regression was performed on G_{30} length of oysters from the three sites ($p > 0.05$). However, at Manselo, approximately 6% ($p < 0.05$) of the variation in G_{30} length could be explained by pH, while approximately 16% ($p < 0.05$) of the variation in G_{30} length at Batu Terio was influenced by a combination of pH, chlorophyll *a* and salinity (Table 5.7). At Batu Terio, pH with a standard regression coefficient of 0.430 had a greater influence on G_{30} length than salinity, which had a standard regression coefficient of -0.207.

5.3.6.3 Depth-related difference in G_{30} length

Multiple regression of G30 length from oysters grown at different depths showed pH and salinity influenced growth rate of oysters grown at 5 m while only pH affected the G30 of oysters grown at 15 m (Table 5.8). pH and salinity explained approximately 8% ($p < 0.05$) of variation in G_{30} length at a depth of 5 m. pH with a standardised regression coefficient of 0.223 had the greater influence over G_{30} length at a depth of 5 m than salinity with a coefficient of 0.180.

5.3.6.4 Size-related difference in G_{30} length

G_{30} lengths of large oysters were not significantly affected by the environment, but G_{30} lengths of medium and small oysters were. In addition, regression models for medium

and small oysters were improved when partitioned by size (Table 5.9). pH and salinity accounted for approximately 15% ($p < 0.001$) of variation in G_{30} length of medium oysters and approximately 20% ($p < 0.001$) of variation in small oysters, with pH exerting a greater influence on G_{30} length in both sizes. This was indicated by the higher standardised regression coefficients of pH (0.315 for medium oysters and 0.394 for small oysters) when compared to salinity (-0.268 for medium oysters and -0.260 for small oysters).

5.3.7 Relationship between G_{30} weight and environmental parameters

In addition to performing stepwise multiple regression analysis of G_{30} weight of all oysters against various environmental parameters, testing was also carried out on G_{30} weight of oysters partitioned by site, depth and weight to determine if partitioning the data improved the empirical models relating G_{30} weight to environmental variables (Appendix 5.7).

5.3.7.1 Overall G_{30} weight

Stepwise multiple regression of G30 weight of all oysters against environmental parameters indicated that SPM and pH accounted for approximately 6% ($p < 0.001$) of variation in G30 weight (Table 5.10). With a standardised regression coefficient of 0.180, SPM appear to have a marginally greater influence over variations in G30 weight of all the oysters than pH, which had a standardised regression coefficient of 0.135.

5.3.7.2 Site-related difference in G_{30} weight

G30 weight of oysters from all three sites was affected by the environment. SPM accounted for approximately 7% ($p < 0.05$) and 5% ($p < 0.05$) of the variation in G30 weight in Ganan and Batu Terio oysters, respectively (Table 5.10). At Manselo, the G30 weight of approximately 5% ($p < 0.05$) of oyster was influenced by pH. While the regression models showed G30 weight of oysters grown at the three sites had a relationship to either pH or SPM, partitioning the oysters by site did not improve the r2 value of the regression model for any of the sites.

Table 5.7 Stepwise multiple regression of G_{30} length of oysters from different sites against environmental variables. Standardised regression coefficients are in italics within parenthesis. All regressions were significant ($p < 0.05$) with the exception of Ganan.

Site	Model	β^0							r^2	Significance of regression	n
All sites	1	-1.540	+	0.238 (pH) (*0.215*)					0.046	$p < 0.001$	102
	2	1.426	+	0.257 (pH) (*0.232*)	-	0.090 (Salinity) (*-0.166*)			0.073	$p < 0.001$	102
Manselo	1	-2.378	+	0.345 (pH) (*0.249*)					0.062	$p < 0.05$	34
Batu Terio	1	-2.202	+	0.324 (pH) *(0.250*)					0.062	$p < 0.05$	34
	2	-3.902	+	0.504 (pH) (*0.389*)	+	0.586 (Chlorophyll *a*) (*0.285*)			0.124	$p < 0.05$	34
	3	0.574	+	0.556 (pH) (*0.430*)	+	0.519 (Chlorophyll *a*) *(0.252*)	-	0.140 (Salinity) *(-0.207*)	0.163	$P < 0.05$	34

Table 5.8 Stepwise multiple regression of G_{30} length of oysters from different depths against environmental variables. Standardised regression coefficients are in italics within parenthesis. All regressions were significant ($p < 0.05$).

Depth	Model	β^0					r^2	Significance of regression	n
All depths	1	-1.540	+	0.238 (pH) (*0.215*)			0.046	$p < 0.001$	102
	2	1.426	+	0.257 (pH) (*0.232*)	-	0.090 (Salinity) (*-0.166*)	0.073	$p < 0.001$	102
5 m	1	-1.434	+	0.226 (pH) (*0.211*)			0.045	$p < 0.05$	51
	2	1.862	+	0.239 (pH) (*0.223*)	-	0.098 (Salinity) (*0.180*)	0.077	$p < 0.05$	51
15 m	1	-1.744	+	0.262 (pH) (*0.277*)			0.052	$p < 0.05$	51

Table 5.9 Stepwise multiple regression of G_{30} length of oysters of different sizes against environmental variables. Standardised regression coefficients are in italics within parenthesis. All regressions were significant ($p < 0.05$) with the exception of large oysters.

Size	Model	β^0					r^2	Significance of regression	n
All sizes	1	-1.540	+	0.238 (pH) (*0.215*)			0.046	$p < 0.001$	102
	2	1.426	+	0.257 (pH) (*0.232*)	-	0.090 (Salinity) (*-0.166*)	0.073	$p < 0.001$	102
Medium	1	-1.103	+	0.176 (pH) (*0.287*)			0.083	$p < 0.05$	34
	2	1.554	+	0.193 (pH) (*0.315*)	-	0.080 (Salinity) (*-0.268*)	0.153	$p < 0.001$	34
Small	1	-3.497	+	0.526 (pH) (*0.365*)			0.135	$p < 0.001$	34
	2	2.536	+	0.564 (pH) (*0.394*)	-	0.183 (Salinity) (*-0.260*)	0.201	$p < 0.001$	34

5.3.7.3 Depth-related difference in G_{30} weight

Approximately 4% ($p < 0.05$) of oysters grown at a depth of 5 m had G_{30} weights significantly affected by POM (Table 5.11).At 15 m depth, water temperature had an influence on approximately 6% ($p < 0.05$) of oysters' G_{30} weight.

5.3.7.4 Size-related difference in G_{30} weight

G_{30} weights of large oysters were less affected by environmental parameters than G_{30} weights of medium and small oysters. Regression results showed 5% ($p < 0.05$) of large oyster G_{30} weight was affected by POM, while approximately 16% ($p < 0.001$) of medium oysters were affected by SPM (Table 5.12). Approximately 17% ($p < 0.05$) of small oysters G_{30} weight was significantly correlated to pH and POM. pH with a standardised regression coefficient of 0.310 had a greater effect on G_{30} weigh than POM, which had a standardised regression coefficient of 0.241.

Table 5.10 Stepwise multiple regression of G_{30} weight of oysters from different sites against environmental variables. Standardised regression coefficients are in italics within parenthesis. All regressions were significant ($p < 0.05$)

Site	Model	β^0					r^2	Significance of regression	n
All sites	1	0.196	+	12.820 (SPM) *(0.208)*			0.043	$p < 0.001$	102
	2	-2.601	+	11.083 (SPM) *(0.180)*	+	0.374 (pH) *(0.135)*	0.061	$p < 0.001$	102
Ganan	1	-0.012	+	17.088 (SPM) *(6.458)*			0.071	$p < 0.05$	34
Manselo	1	-5.118	+	0.762 (pH) *(0.214)*			0.046	$p < 0.05$	34
Batu Terio	1	0.135	+	14.227 (SPM) *(0.225)*			0.051	$p < 0.05$	34

Table 5.11 Stepwise multiple regression of G_{30} weight of oysters from different depths against environmental variables. Standardised regression coefficients are in italics within parenthesis. Both regressions were significant ($p < 0.05$).

Depth	Model	β^0					r^2	Significance of regression	n
All depths	1	0.196	+	12.820 (SPM) *(0.208)*			0.043	$p < 0.001$	102
	2	-2.601	+	11.083 (SPM) *(0.180)*	+	0.374 (pH) *(0.135)*	0.061	$p < 0.001$	102
5 m	1	0.524	+	11.838 (POM) *(0.194)*			0.038	$p < 0.05$	51
15 m	1	-22.758	+	0.817 (Temperature) *(0.236)*			0.056	$p < 0.05$	51

Table 5.12 Stepwise multiple regression of G_{30} weight of oysters of different sizes against environmental variables. Standardised regression coefficients are in italics within parenthesis. All regressions were significant ($p < 0.05$).

Size	Model	β^0					r^2	Significance of regression	n
All sizes	1	0.196	+	12.820 (SPM) *(0.208)*			0.043	$p < 0.001$	102
	2	-2.601	+	11.083 (SPM) *(0.180)*	+	0.374 (pH) *(0.135)*	0.061	$p < 0.001$	102
Large	1	0.038	+	5.289 (POM) *(0.223)*			0.050	$p < 0.05$	34
Medium	1	0.158	+	11.737 (SPM) *(0.397)*			0.158	$p < 0.001$	34
Small	1	-7.106	+	1.116 (pH) *(0.339)*			0.115	$p < 0.05$	34
	2	-6.744	+	1.020 (pH) *(0.310)*	+	17.008 (POM) *(0.241)*	0.172	$p < 0.05$	34

5.4 Discussion

Growth in molluscs consists of increases in both shell and soft body mass. In this study, *P. maxima* growth was examined using two dimensions, shell length and wet weight, to represent these changes. Variations in these dimensions were computed using total growth, G_T and monthly instantaneous growth rate, G_{30}. G_T measures the magnitude of growth attained by oysters over a specific period (e.g. over 18 months of sampling) and is an absolute measure or "velocity of growth" (Malouf and Bricelj, 1989 in Saucedo and Southgate, 2008) while G_{30} measures relative growth rate and plots growth increment per unit time. G_T is represented by the height difference between the lowest and highest point on the Y-axis when average lengths or weights of oysters are plotted over time. Comparing graphical growth trajectories of oysters grown under different regimes allows the magnitude of oyster growth to be evaluated and ordered. For example, graphical representation of growth (Fig 5.3) showed mean shell length of small oysters increased more than mean shell lengths of medium and large oysters over 18 months of sampling. From this observation, it could be concluded that younger oysters displayed faster growth rate than older oysters. However, limitations for G_T are that it does not allow for quantitative comparison of growth at a particular point in time. In this respect, G_{30} is a better indicator of growth as it is a standardised measure and permits comparison to be made between various age-classes of oysters with different shell sizes as well as at a specific point in time. As G_{30} is a better index of growth, subsequent discussion will focus on G_{30}.

G_{30} length was significantly affected by oyster size with smaller oysters displaying a higher G_{30} than larger oysters. Mean G_{30} length of small oysters was approximately 1.5 mm month^{-1} at the start of the experiment before slowing down to less than 0.1 mm month^{-1} by the end of the experiment, while medium oysters had an average shell growth rate of 0.7 mm month^{-1} at the start of the experiment and approximately 0.1 mm month^{-1} during final sampling. Large oysters did not show much difference in G_{30} length, averaging 0.06 mm month^{-1} for the duration of the experiment. These results are consistent with the general presumption that bivalve growth rates are directly related to age (Pouvreau and Prasil, 2001). The relationship between size and age in bivalves is characterised by a sinusoidal curve with an exponential phase in the early stages, followed by an inflection and gentler slope towards asymptotic length (Gibson, 1956;

Wilbur and Owen, 1964; Seed, 1973; Southgate and Lucas, 2008). Pearl oysters such *P. margaritifera* (Nalluchinnappan *et al.*, 1982, Nasr, 1984, Gervis and Sims, 1992; Pouvreau, 1999), *P. imbricata* (Chellam, 1978; Prajneshu and Venugopalan, 1999; Urban, 2002), *P. mazatlanica* (Saucedo and Monteforte, 1997b) and *P. maxima* (Yukihara *et al.*, 2006) show a rapid initial increase in shell size to near maximum size before slowing down significantly as the oyster ages. The decrease in shell size with age in molluscs has been strongly linked to the progressive investment of energy from somatic growth to reproductive growth as the animal ages and reaches sexual maturity. Yukihira *et al.* (1998) proposed that higher relative growth rates in small *P. maxima* and *P. margaritifera* compared with larger oysters in comparable environmental conditions were caused by relative scope for growth (SFG) being inversely related to size. SFG is expressed as:

$$SFG = AE - (RE + EE) \qquad \text{(Equation 5.4)}$$

where SFG is the surplus energy available for growth and reproduction, AE is absorbed energy which is the difference between ingested and faecal energy, RE is respired energy or energy cost of metabolism and EE is excreted energy (Lucas, 2008). The unit for SFG is J h^{-1}. As an animal ages, the part of the SFG allocated to reproduction, termed "reproductive effort" (ReE) (Thompson, 1984) is lost for shell or tissue growth.

Besides the effect of oyster size, overall G_{30} length was significantly affected by culture depth; most oysters grown at a depth of 5 m had a higher G_{30} length than oysters grown at 15 m in this study. When the effect of depth on different ages of oysters was investigated by partitioning the analysis by size, results showed that depth had a significant effect on only large oysters. This implies other physiological factors are likely to exert a greater influence on growth rate in G_{30} length in younger oysters, either in conjunction with or independent of depth-related environmental factors. Although *P. maxima* occur naturally over a wide range of depths ranging from the intertidal zone to 80 m (Gervis and Sims, 1992), previous studies have established that depth has a significant effect on growth in *P. maxima* (Chinh *et al.*, 2000) and also many other bivalves (Yamaguchi and Hasuo, 1978; Zhang *et al.*, 1991; Lodeiros *et al.*, 2002; Tomaru *et al.*, 2002b; Freeman and Denny, 2003; Rupp *et al.*, 2004; Larrivee *et al.*, 2004), with a few exceptions (Smitasiri *et al.*, 1994; Kurihara *et al.*, 2005). The

influence of depth on growth is not attributed to hydrostatic pressure *per se* but to differences in environmental factors associated with depth, such as water temperature, salinity or available food, which could be present as clines or gradients in the water column (Lucas, 2008). Besides the physicochemical properties of seawater, depth can also affect other environmental aspects like the distribution of fouling organisms, predators and disease-causing organisms that could consequently lead to changes in growth and mortality in bivalves (Itoh and Muzamoto, 1978; Nasr, 1982; Kitamura *et al.*, 2002; Tomaru *et al.*, 2002b). For example, studies by Kitamura *et al.*, (2002) showed a marine birnavirus associated with cultured *P. fucata* in Japan was present throughout the year at a depth of 15 m but was not detected at 2 m in summer, which suggested the birnavirus might be destroyed by ultraviolet rays or other factors at 2 m but was stable in deeper waters. In another study by Itoh and Muzamoto (1978), infestation of Akoya pearl oysters by the shell boring sponge *Polydora ciliate* tended to increase with depth, thus affecting growth and mortality.

Light intensity is a depth-related feature but was not included in this study. While light intensity reportedly influences the settlement of pearl oyster pediveligers (Nayar *et al.*, 1987; Tomaru *et al.*, 1999; Lucas, 2008), there has not been any report of light intensity affecting growth in adult pearl oysters and is unlikely to be the cause of growth difference in this study.

Results show spatial differences in G_{30} length of oysters grown at different sites on the farm with the highest overall mean G_{30} length recorded for oysters grown at Batu Terio (0.317 mm month^{-1}), followed by Manselo (0.287 mm month^{-1}) and Ganan (0.277 mm month^{-1}). When the analysis was partitioned by size, results indicated that only medium and small oysters were affected by culture site, with Batu Terio providing the best environment for G_{30} length in medium (0.286 mm month^{-1}) and small (0.671 mm month^{-1}) oysters. It has been postulated that growth variability in a population of molluscs living in the same locality (see Section 5.1) is related to differences in microenvironments (Wilbur and Owen, 1964). Regression analysis in this study showed that the environmental parameters which distinguished the difference in G_{30} length of oysters from all treatments were pH and salinity, while site-specific difference in G_{30} length of oysters at Manselo and Batu Terio were related to variations in pH and a combination of pH, chlorophyll *a* and salinity, respectively.

Similarly, depth-specific disparity in growth rates was affected by variation in pH and salinity. These two environmental factors were also significant in separating the size-specific differences in small and medium sized oysters grown at all sites, but did not appear to influence the growth rate of large oysters. In a similar study on the effects of environmental parameters on growth of *P. maxima* and *P. margaritifera*, Yukihira *et al.*, (2006) suggested that the absence of any significant effects of water temperature on growth rates in large oysters was likely due to their persistently low growth rates which concealed seasonal effects. This was possibly the case in this study as well, where similarly, G_{30} length of large oysters was consistently low and did not appear have any relationship to sampled environmental factors.

While there is no literature to date on the effects of pH on growth of *P. maxima* specifically, the effect of pH on *P. maxima* spat detachment has been shown (Taylor *et al.*, 1997b). Recent research with the pearl oyster *P. fucata* showed that reduced pH (to pH 7.8 and 7.6) resulted in reduced activity, reduced byssal attachment and byssal strength as well as reduced shell strength (Welladsen, 2009). In the same study, reduced pH was also shown to lower CI and shell thickness of the pearl oyster.

pH is reported to exert an effect on growth in other bivalves, such as growth and feeding rates of the oyster *Ostrea edulis* (Enright *et al.*, 1986) and the specific growth rates and survival of abalones *Haliotis laevigata* and *H. rubra* (Harris *et al.*, 1999). The study by Harris *et al.* (1999) indicated pH could reduce respiratory activity, alter kidney definition, tubule and lumen size and increase hyperplasia and abnormalities in abalones. pH levels influence seawater chemistry such as solubility and thus the bioavailability of various substances in seawater, and carbonic acid equilibrium (Knutzen, 1981) and can significantly alter the physiology of marine invertebrates either directly through the calcification process, or by having a synergistic effects with other environmental factors such as elevated temperatures (O'Donnell *et al.*, 2009). The levels of pH in this study ranged from 6.4 – 8.3, representing a significant variation, which not surprisingly exerted an effect on the physiology of *P. maxima*. In light of ocean acidification, a worldwide phenomenon related to the accumulation of excess carbon dioxide in the atmosphere, research into the effects of pH on bivalve growth merits greater attention in the future.

Salinity was another significant factor in distinguishing G_{30} length of oysters between treatments in this study. The effect of salinity on growth has been reported in *P. maxima* (Kvingedal *et al.*, 2008; Taylor *et al.*, 2004) and other pearl oyster species in numerous publications (e.g. Alagarswami and Victor, 1976; Numaguchi and Tanaka, 1986a; Dholakia *et al.*, 1997; Al-Sayed *et al.*, 1997; Doroudi *et al.*, 1999a; O'Connor and Lawler, 2004a; Yu *et al.*, 2005, amongst others). While salinity has been shown to affect the physiological function and hence, growth rates in a wide range of osmo-conforming marine invertebrates (Lucas, 2008), the precise mechanism by which salinity exerts an influence on *P. maxima* metabolism is unknown. Salinity has been reported to influence clearance rate in *P. imbricata* (Ota and Fukushima, 1961; Alagarswami and Victor, 1976; Abo and Toda, 2001), pumping rate in *P. imbricata* (Kobayashi and Matsui, 1953; Numaguchi and Tanaka, 1986a; Yamamoto, 2000) and is likely to affect the growth by having a direct affect on SFG in pearl oysters. In adverse salinity conditions, some bivalves may close their shells (Pierce, 1971). Reduction in assimilation food ration due to shell closure, as well as the metabolic cost of maintaining osmotic balance within the shell cavity in response to salinity changes could further lead to discrepancy in growth between oysters growing in dissimilar salinity environment.

The positive correlation between chlorophyll *a* and G_{30} length in *P. maxima* may be indicative of the role of specific phytoplankton, which constitute food for pearl oysters. Chlorophyll *a* is the most common of the photosynthetic pigments and ubiquitous to all species of phytoplankton (Brown, 1988). A high biomass of phytoplankton rich in chlorophyll *a* represents an available source of energy for *P. maxima* and would ultimately lead to an increase in SFG and growth. There have been conflicting results about the effect of chlorophyll *a* on pearl oyster growth in previous research. Pages and Prasil (2002) reported that growth in *P. margaritifera* was inversely correlated to chlorophyll *a* levels, while Lodeiros *et al*. (2002) showed that growth of *Pinctada imbricata* tended to increase with increases in chlorophyll *a* during periods of upwelling. Pages and Prasil (2002) suggested that the seemingly paradoxical high growth rate observed in *P. margaritifera* grown in oligotrophic water was caused by high water renewal rates and a trophic web that offered a qualitatively better diet despite the low particulate matter. It is probable that the fluctuating levels of chlorophyll *a* (Section 4.3.1.6) caused by dissimilar spatial and temporal water exchange over the

sampling period affected food composition and ultimately physiological function and growth in *P. maxima* in this study. A number of mechanisms, including tidal mixing, wind-driven upwelling, and coastal run-off likely influence the magnitude and distribution of chlorophyll at the different sites.

Shell size alone is not always the best index of overall growth due to shell tissue not responding rapidly to undesirable environmental conditions and often maintaining positive linear growth even when soft tissue is lost (Kautsky, 1982, Hilbish, 1986, Emerson *et al.*, 1994). In most bivalves, shell and flesh weight increases are separate as shell increase is related to somatic growth while fluctuation in weight is associated with reproductive cycle growth (Gosling, 2003). This was demonstrated here by the differences observed between G_{30} length and G_{30} weight. G_{30} length was always positive indicating the ongoing development of growth processes in the distal margins of oyster valves over time, while G_{30} weight was either positive or negative depending on the reproductive state of the oyster with periods of negative growth observed before gonad development. In addition, there was less fluctuation in mean G_{30} length compared to G_{30} weight, as somatic growth occur at a relatively slower rate than the build-up of gonad. Decreases in shell length have been reported in *P. margaritifera* due to sloughing or grazing by fishes (Sims, 1994) but was not observed in this study.

Condition indices (CI) are used to assess physiological activity such as growth, reproduction or secretion in cultured bivalves (Lucas and Beninger, 1985) and as an indicator of the nutritive state of the animal (Brown and Hartwick, 1988c; Littlewood and Gordon, 1988). By comparing the amount of tissue to the amount of shell, it is possible to evaluate the metabolic and reproductive status of a bivalve. A low CI indicates a major biological effort has been expended. In this study, CI of *P. maxima* showed temporal, spatial and age-related variation. This is not surprising as the two variables used to calculate CI (i.e. shell and soft tissue) showed similar variation. As the index changes proportionally with soft tissue (gonad) weight in the oyster, CI is highly correlated to G_{30} weight. The effects of various environmental parameters on gonad growth is explored in detail in chapter 8, so will not be discussed in detail here.

In summary, environmental factors that were found to contribute to variability in G_{30} weight were pH, SPM, POM and water temperature. Published data on the effect of pH

on reproduction in pearl oysters showed pH affected the spermatozoa activation rate and vitality of the pearl oyster, *P. fucata* (Yu *et al.*, 1998: Yu *et al.*, 1999; Ohta *et al.*, 2007) as well as induced spawning in *P. fucata* (Alargarswami *et al.*, 1983c). In addition, there have been studies detailing the effect of water temperature and food on reproduction in pearl oysters(e.g. Wada, 1953a, 1953b; Rose *et al.*, 1990; Wada *et al.*, 1995; Behzadi *et al.*, 1997; Pouvreau, 1999; Pouvreau *et al.*, 2000b; Saucedo *et al.*, 2002a; Garcia-Cuellar *et al.*, 2004; Vite-Garcia and Saucedo, 2008).

Mortalities of *P. maxima*, which occurred in this study, were significantly affected by oyster age, culture site, culture depth and month of sampling. Relatively high mortalities were observed in Batu Terio but it is not known what contributed to this event. High mortalities of *P. fucata martensii* observed in Japan were hypothesised to be caused by a combination of poor water temperature, chlorophyll *a* and phytoplankton conditions which weakened oysters and exposed them to infectious disease agents (Tomaru *et al.*, 2001). A combination of environmental and physiological factors may be the cause of the mortalities observed in this study. Results of this study showed that *P. maxima* aged 1.5 to 3.5 years old grown at Batu Terio had the lowest survival rate. In light of these data, alternative sites should be selected for the culture of younger *P. maxima* in Aljui Bay, and Batu Terio be utilised for the culture of older and larger oysters.

Although this study showed various relationships between oyster growth and environmental descriptors, the results presented here should be interpreted with caution. By itself, this evidence does not indicate that other environmental factors, for example, water temperature and food did not influence G_{30} length in *P. maxima*. It may simply be an indication that during the relatively short sampling period in this study, the ranges of environmental factors were insufficient to produce a measurable effect on somatic growth of *P. maxima*. In experiments under tightly controlled laboratory conditions and over a short time period, the effects of individual environmental variables such as water temperature, salinity and food on growth rate may be quantified readily, but in *in situ* experiments where such parameters cannot be regulated, careful interpretation of results is required as some modulators of growth such as water temperature and salinity interact, often synergistically and it is very difficult to quantify the precise effect of one environmental parameter on growth in a population of bivalves (Gosling, 2003).

The disparity in G_{30} length and G_{30} weight caused by variations in environmental parameters highlights the differences in the local physico-chemical profile of the various culture sites and depths that were discussed in chapter 4. A consequence of these differences is that they could be utilised as a method of exerting some control over *P. maxima* growth in a dynamic and largely random environment. In summary, differences in somatic growth of *P. maxima* from various treatments were governed by variations in pH, salinity and chlorophyll, while gonad growth was influenced by variations in pH, SPM, POM and water temperature.

The implication of the results in this study is that judicious selection of culture depths and sites could be utilised as a method of regulating growth in *P. maxima*. *P. maxima* oysters are seeded when they attain a shell length greater than 120 mm (Gervis and Sims, 1992). Culturing young *P. maxima* at the more favourable depth of 5 m would favour faster somatic growth and shorten the time it takes for pearl oysters to reach operable size. In addition, the use of less rope for suspending oysters at 5 m reduces entanglement and saves on the cost of culture material. Conversely, as the quality of the resulting pearls has been reported to depend on slower growth and nacreous deposition (Wada, 1973; Pouvreau and Prasil, 2001), this can be achieved by suspending *P. maxima* at a depth of 15 m or greater during the pearl culture phase. In the same way, sites could be selected based on how their physico-chemical profile affects growth in *P. maxima*.

CHAPTER 6

Mathematical expression and comparison of growth in the silver-lip pearl oyster cultured at three sites and two depths[1]

6.1 Introduction

Unlike most other aquaculture industries where rapid growth of a cultured species is desirable to shorten time to harvest, pearl oysters at different stages of culture have variable growth requirements. As discussed in Chapter 1, the first two years of culture requires a rapid increase in size and high survival rate is essential to ensure that large numbers of oysters are produced for pearl grafting. In addition, larger asymptotic size allows larger nuclei to be used to produce bigger and more valuable pearls. Conversely, slow growth is preferable at other stages such as prior to the insertion of a nucleus or grafting (see Chapter 5). It has also been reported that the quality of the resulting pearls depends on growth rate and nacreous deposition (Wada, 1973; Pouvreau and Prasil, 2001) and oysters which have been implanted with nuclei are generally grown in deep waters to reduce the metabolic rate and growth rates slow (Gervis and Sims, 1992). Therefore, the selection of suitable culture sites which satisfy the requirements for the different stages of pearl oyster culture are important for improved pearl farm management.

The previous chapter discussed variation in growth in *P. maxima* and the influence of fixed factors such as culture site, depth, age, as well as the effects of variable environment parameters. Site-related variation in growth of *P. maxima* has previously been reported by Saville-Kent (1893) and Gervis and Sims (1992) as well as in other species of pearl oysters such as *P. fucata* (Alagarswami, 1970; Nalluchinnappan *et al.*, 1982), *P. margaritifera* (Friedman and Southgate, 1999) and *Pteria penguin* (Smitasiri *et al.*, 1994). While it is to be expected that growth differences might exist between

[1] Data presented in this chapter have been published as: Lee, A. M, Ashley J. Williams and Paul C. Southgate (2008) Modelling and comparison of growth of the silver-lip pearl oyster *Pinctada maxima* (Jameson)(Mollusca:Pteriidae) cultured in West Papua, Indonesia. Marine and Freshwater Research 59: 22- 31 (Appendix D)

oysters grown at different geographical locations, latitudes or climate, it has also been reported that considerable difference in growth can be found in molluscs living in close proximity at the same locality due to local variations in the microenvironment (Wilbur and Owens, 1964). Comparative studies of *P. maxima* growth at various sites around a pearl farm would ensure the best sites are chosen for the various stages of *P. maxima* culture.

Ultimately, the study of growth should encompass the rates of chemical reactions that govern body composition and dimensions. However, most studies on bivalve growth examine less detailed aspects such as change in body dimension, increase in mass and changes in chemical constituents of tissues (Wilbur and Owen, 1964) as they are easier to measure and more relevant to the aquaculturist. Growth of pearl oysters, like that of other bivalves, consists of both increases in the shell size and soft body mass, but changes in linear dimensions are easier to measure.

Measurement of size by itself is useful in comparing growth in *P. maxima* as shown in the previous chapter. However, it has its limitations because it does not provide sufficient information to describe or compare growth performance in great detail, for example, the maximum size an animal could theoretically attain at a particular culture site or depth. A combination of factors such as the shell size of an animal at a certain age, the time it takes to reach a particular size (i.e. growth rate) and the maximum size an animal can hypothetically attain would much better describe growth performance of a species under given culture conditions (Vakily, 1999). One general approach which combines all this information is to quantify growth by fitting mathematical models to length or weight-at-age data (Vakily, 1999). Some of the more common models used to describe growth in fisheries science include the von Bertalanffy Growth Function (VBGF) (von Bertalanffy, 1938), the Gompertz (Gompertz, 1825), the Richards (Richards, 1959) and the Logistic models (Urban, 2002).

The most important model and that with the widest application in fisheries science is the VBGF (Prajneshu and Venugopalan, 1999). The VBGF is generally preferred over other models as it is biologically interpretable, can be used for comparative growth studies and its parameters are relatively easy to determine (Vakily, 1999).

Although the conceptual analysis of the underlying theory is based on fish growth, the VBGF has been used successfully for computation of growth parameters in various species of pearl oysters including *P. margaritifera* (Nasr, 1984; Pouvreau *et al.,* 2000b; Pouvreau and Prasil, 2001), *P. imbricata* (Prajneshu and Venugopalan, 1999; Urban, 2000; Urban, 2002; Marcano *et al.,* 2005) and *P. mazatlanica* (Saucedo and Monteforte, 1997a). To date the only study on growth modelling of adult *P. maxima* related to wild stocks in Western Australia (Hart and Joll, 2006) and experimented cultured oysters in the Great Barrier Reef (Yukihara *et al*., 2006); while modelling in these studies was based on the VBGF, it is unknown which model best describes the growth of *P. maxima* under commercial culture in Indonesia.

Previously in bivalve mollusc fisheries, age and length-at-age have often been estimated on the basis of measurement of annual growth rings (Stevenson and Dickie, 1954; Chalfant *et al.,* 1980; Quayle and Newkirk, 1989), successive measurement of marked individuals (Loosanoff, 1954; Lutz and Hess, 1979; Estacion and Braley, 1988), comparison of successive length frequencies of a large random sample (Quayle, 1951; Haskin, 1954) and acetate peels of cut shells (Lutz, 1976; Quayle and Newkirk, 1989). However, with the advent of hatchery production, the absolute age of an oyster is known and the parameters of growth models may be estimated with greater accuracy.

The research described in this chapter aims to evaluate the mathematical model which best describes growth of juvenile and adult *P. maxima* by fitting length-at-age data to the various historical models. A similar approach was used to describe growth of *P. imbricata* in the Caribbean (Urban, 2002). Once the best-fitting model was identified, this chapter takes the research a step further by comparing oyster growth between various sites and depths within the farm to determine if they had an effect on growth parameters. The data generated would ultimately lead to the better management and husbandry of *P. maxima* at this farm.

6.2 Materials and Methods

6.2.1 Study site

The research was conducted at P. T. Cendana Indopearls (refer Section 3.1). Details of the three sites within Aljui Bay that were selected for comparative growth studies are given in Section 3.4.1 to Section 3.4.3. The sites i.e. Ganan, Manselo and Batu Terio are used extensively for culturing farm oysters and are located approximately 8 to 10 km apart and have varied depth, surrounding topography and current (Refer Fig. 4.11).

6.2.2 Experimental and sampling designs

The experimental setup is described in detail in Chapter 5 (Section 5.2.3). Briefly, a total of 150 oysters were randomly selected from three cohorts of hatchery-bred oysters aged 0.58, 1.25 and 3.33 years old at the start of the experiment. The selection of three staggered age groups allowed for a wider age-span to be sampled over a shorter period. The oysters were pooled, tagged then evenly divided into three groups consisting of equal number of oysters of the same age and randomly allocated to the three experimental sites and grown on panel nets suspended at 5 m and 15 m as described in Section 5.2.3. Tagged oysters were sampled every month over 18 months from May 2000 and November 2001, with the exception of December 2000. Length (antero-posterior measurement) of oysters was measured using a vernier calliper to the nearest 0.1 mm as described in Section 3.8.1. Mortality was recorded for each site and depth, and dead oysters were discarded and not replaced.

6.2.3 Analysis of growth rate

Growth of oyster shell lengths over a particular month was measured using monthly instantaneous growth rate (G_{30}) as defined by Brown (1988) in Equation 5.3 (Section 5.2.9)

Growth data for December 2000 was estimated as the average of November 2000 and January 2001. Analysis of variance (ANOVA) with repeated measure was performed to determine if there was any difference in growth rate of oysters of the same age range cultured at different sites and depths: the level of significance was set at 0.05. As with

all data prior to ANOVA analyses, data were tested for homocedascity and normality using Levene's and Shapiro-Wilk's test, respectively.

6.2.4 Fitting growth models to length-at-age data

Length-at-age *(L_t)* data for sites and depths were collectively analysed for growth analysis modelling. When there was an overlap of ages from the three age groups sampled over time, the oysters were pooled and analysed as one age group. Length-at-age data were fitted into five different mathematical growth models using the Levenberg-Marquardt iterative non-linear regression algorithm in SPSS Version 14 in order to estimate growth parameters using. The models were:

Special VBGF:	$L_t =$	$L_\infty[1-e^{-K(t-to)}]$	(Equation 6.1)
General VBGF:	$L_t =$	$L_\infty[1-e^{-K(t-to)}]^b$	(Equation 6.2)
Gompertz:	$L_t =$	$L_\infty e[-e^{(-K(t-to))}]$	(Equation 6.3)
Richards:	$L_t =$	$L_\infty[1-be^{(-K(t-to))}]^{1/b}$	(Equation 6.4)
Logistic:	$L_t =$	$L_\infty[1+e^{-K(t-to)}]^{-1}$	(Equation 6.5)

Where L_∞ is the asymptotic length (mm), K is the growth constant (y^{-1}), L_t (mm) the length at age t (years), t_o the theoretic age of the animal at length equals zero and b an exponent which is an additional growth parameter to be estimated.

The criteria used for estimating best fit were adapted from Urban (2002) with several differences. A low mean residual sum of squares (MRSS) instead of mean square error (MSE) was used and an additional criterion, a high coefficient of determination (r^2) was employed. The third criterion, a low deviation of the asymptotic length (L_∞) from the maximum length (L_{max}) as calculated by

$$\sqrt{(L\infty - L\max)^2} \qquad \text{(Equation 6.6)}$$

was similar to that used by Urban (2002).

6.2.5 Comparison of growth curves

When the model of best-fit was determined, variation in growth parameters of oysters cultured at the different sites and depths were compared using likelihood ratio tests (Kimura, 1980) which is considered the most reliable procedure for such comparisons (Cerrato, 1990).

6.3 Results

6.3.1 Growth rate and mortality

Results for growth rate and mortality were presented in detail in Chapter 5. In summary, monthly growth rate based on shell length was positive throughout the sampling period but there was clearly a difference in growth rate in oysters of different ages as shown in the site and depth plots of G_{30} against age (Fig. 6.1). It was clearly seen that there was a sharp decline in G_{30} with age and younger oysters sampled at the start of the experiment had a higher G_{30} than older oysters (Fig. 6.1). Oysters were pooled into various age groups to detect if sampling month effected mortality of oysters of various ages (Section 5.3.3). The highest mortalities were observed in the young oysters sampled in August 2000, May 2001 and November 2001 (Fig. 6.2).

6.3.2 Growth modelling

When different growth models were fitted to pooled length-at-age data, estimates for L_∞, K, t_0 and b were obtained as shown in Table 6.1. Growth rate for all models are plotted in Fig. 6.4.

Based on the criteria for best fit [i.e. low mean residual sum of squares (MRSS), high coefficient of determination (r^2) and low deviation of the asymptotic length (L_∞) from the maximum length (L_{max}), the Special VBGF and the General VBGF equally provided the best fit to length-at-age data for all the pearl oysters grown at the area (Table 6.1). However, when the data were plotted and compared (Fig. 6.4), the Special VBGF appeared a better fit than the General VBGF as the predicted asymptotic length L_∞ in the General model appeared to be substantially lower than the observed L_{max}. Based on this observation, and the criteria for best fit, the Special VBGF was deemed the model

which best described growth in *P. maxima* cultured at this farm in West Papua, Indonesia.

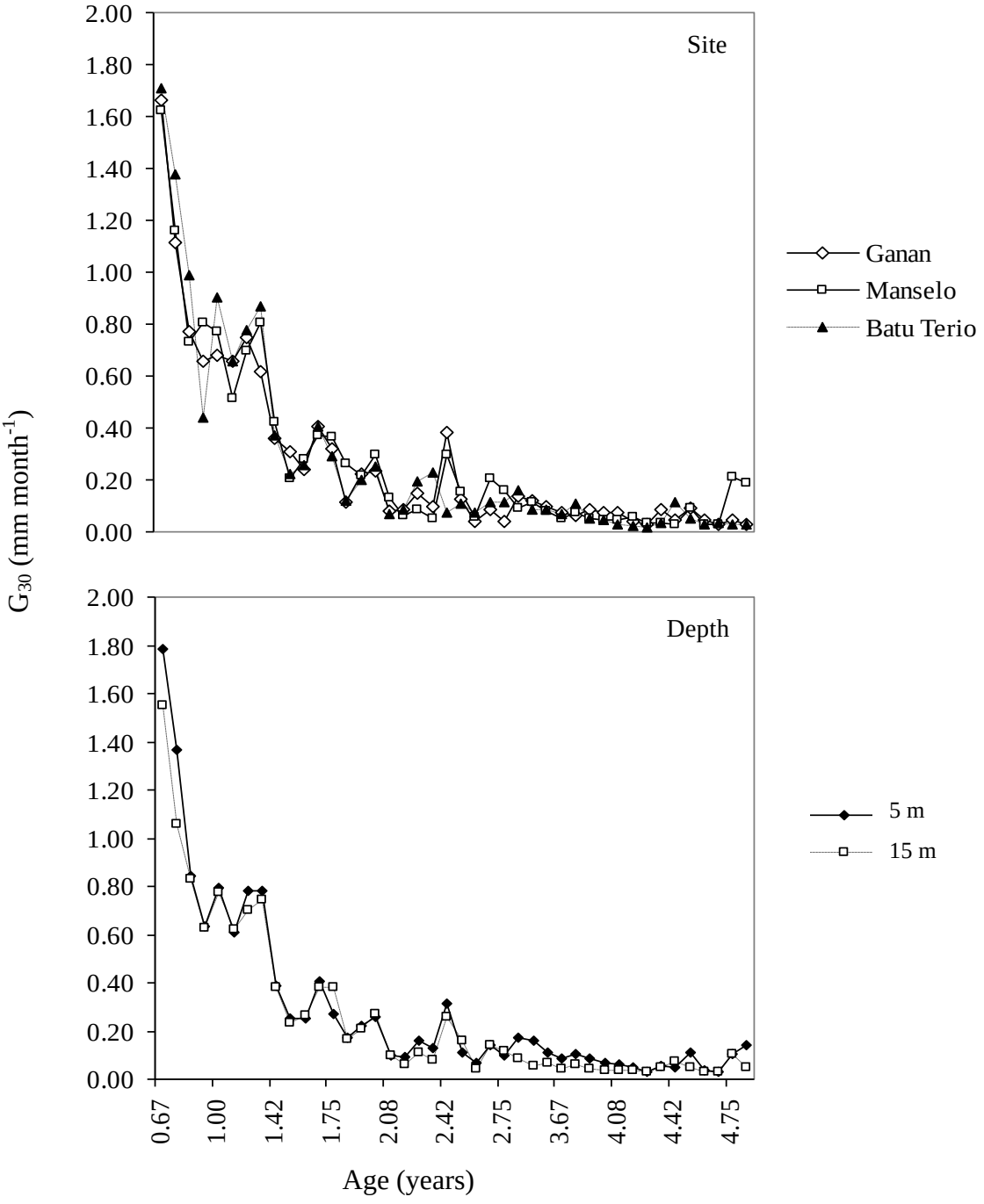

Fig. 6.1 Monthly instantaneous growth ($G_{30,}$ defined by Brown, 1988) of different aged *P. maxima* cultured at various sites and depths.

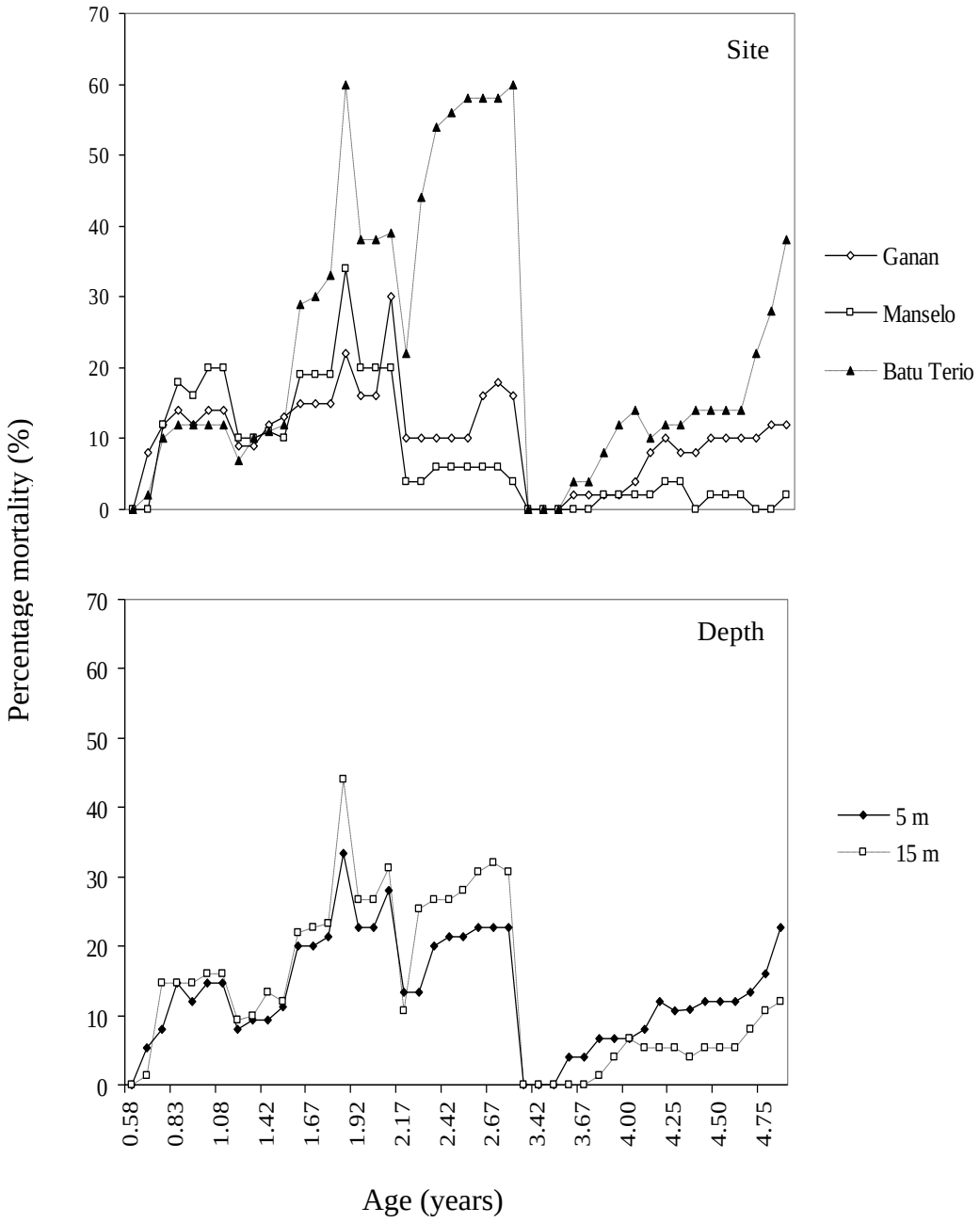

Fig. 6.2 Mortality of different ages of *P. maxima* at various sites and depths.

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Table 6.1 Growth parameters of models fitted with growth data of oysters aged 0.58 – 4.83 years. (n = 8010). L_∞ : asymptotic length, K growth constant, t_0 : theoretical age when length = 0, MRSS : mean residual sum of squares, b_{Richards} : growth parameter*, b_{GenVBGF} : surface factor*, Dev : deviation of L_∞ from L_{max}*. * As defined in Urban, 2002.

Model	Formula	L_∞ (mm)	K (y^{-1})	t_0	b (y^{-1})	MRSS	r^2	Dev
Special VBGF	$L_\infty[1-e^{-K(t-to)}]$	168.38	0.930	0.126		208.64	0.802	37.52
General VBGF	$L_\infty[1-e^{-K(t-to)}]^b$	172.86	0.651	0.414	0.514	207.23	0.803	33.04
Gompertz	$L_\infty e[-e^{(-K(t-to))}]$	165.85	1.232	0.565		212.20	0.798	40.07
Richards	$L_\infty[1-be^{(-K(t-to))}]^{1/b}$	165.85	0.0001	1.232	0.00006	212.23	0.798	40.05
Logistic	$L_\infty[1+e^{-K(t-to)}]^{-1}$	172.43	0.200	0.748		240.79	0.771	33.47

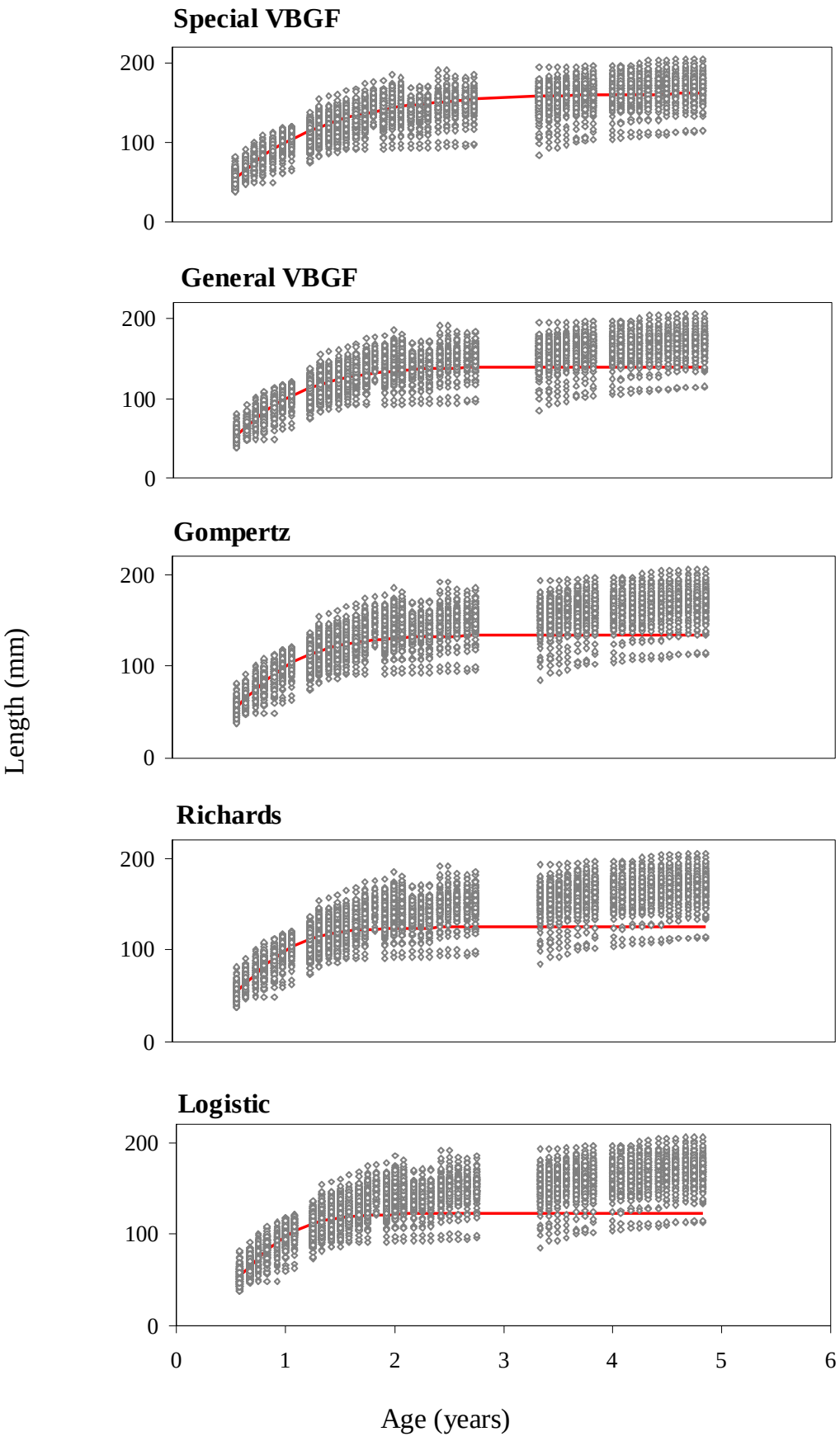

Fig. 6.3 Shell length (mm) of oysters plotted against age (years) and fitted with various growth models (Special VBGF, General VBGF, Gompertz, Richards and Logistic).

Accordingly, the Special VBGF was used to compare spatial variation in growth of *P. maxima* from various sites and depths using likelihood ratio tests. Results indicated that growth models of oysters cultured at different sites and depths when expressed by the Special VBGF varied significantly, with the difference driven by the growth rate, K (Table 6.2). When paired comparisons were made, the results confirmed that oysters displayed different growth patterns between each of the culture sites. However, the variation was due to the combined variation in $L_{\infty,}$ K and t_0 rather than just K.

When length-at-age data from oysters grown at Ganan, Manselo, Batu Terio as well as 5 m and 15 m at all sites were fitted into Special VBGF equation, estimates for $L_{\infty,}$ K and t_0 were obtained and are shown in Table 6.3

Table 6.2 Results of likelihood ratio tests comparing estimates of Special VBGF parameters from oysters aged 0.58 – 4.83 years cultured at 3 sites and 2 depths in West Papua, Indonesia. χ^2 = likelihood ratio Chi-squared statistic for length based on comparison of growth. Each comparison tests the hypothesis that the overall Special VBGF as well as the parameters L_∞ , K and t_0 were similar for oysters grown at different microenvironments. Significant differences are underlined.

Source of variation	Comparison	Growth curve				L_∞			K			t_0		
		df	χ^2	p	n	df	χ^2	p	df	χ^2	p	df	χ^2	p
Site	All sites	6	178.3	< 0.05	6910	2	5.6	> 0.05	2	8.7	< 0.05	2	1.8	> 0.05
	Ganan vs Batu Terio	3	1407.1	< 0.05	3272	1	84.6	< 0.05	1	43.9	< 0.05	1	41.6	< 0.05
	Ganan vs Manselo	3	58.7	< 0.05	2427	1	3.7	< 0.05	1	0.8	> 0.05	1	1.0	> 0.05
	Manselo vs Batu Terio	3	127.3	< 0.05	4518	1	46.9	< 0.05	1	43.0	< 0.05	1	51.9	< 0.05
Depth	5 m vs 15 m	3	47.4	< 0.05	6910	1	2.2	> 0.05	1	0.3	> 0.05	1	2.5	> 0.05

Table 6.3 Growth parameters of the Special VBGF fitted with growth data of oysters aged 0.58 – 4.83 years grown at different depths and sites (n = 8010). L_∞ : asymptotic length, K : growth constant, t_0 : theoretical age when length = 0, MRSS : mean residual sum of squares, Dev : deviation of L_∞ from L_{max} (Urban, 2002).

Site	L_∞ (mm)	K (y^{-1})	t_0	MRSS	r^2	Dev
Ganan	167.17	0.879	0.103	215.785	0.79	38.73
Manselo	169.63	0.975	0.131	193.835	0.80	30.37
Batu Terio	168.36	0.939	0.141	200.723	0.82	27.64
5 m	167.15	0.990	0.173	245.894	0.77	38.75
15 m	170.33	0.892	0.097	166.382	0.83	25.67

6.4 Discussion

Because of the different requirements for the various stages of *P. maxima* culture, the selection of suitable culture sites which satisfy the growth needs for a particular stage is essential for optimal management of *P. maxima* culture. It is important to measure growth from various sites as the results assist in assessing the suitability of a given site for culture.

Measuring G_{30} gave a good indication of the overall growth pattern of shell length in *P. maxima* (Chapter 5). For instance it could be concluded by G_{30} analysis that shell length of juvenile oysters of less than 0.6 years old grew more than 1.5 mm month^{-1} at the start of the experiment and growth progressively slowed to approximately 0.8 mm month^{-1} at 1 year of age. By two years of age, growth had virtually ceased to less than 0.1 mm month^{-1}. This was consistent with the general rule that growth rates of pearl oysters are directly related to age (Pouvreau and Prasil, 2001). Decreasing shell growth as oyster ages is due to energy being invested into reproduction. Site and depth did not affect the G_{30}, implying that physiological concepts of anabolism and catabolism (von Bertalanffy, 1957) were affecting growth rates rather than environmental parameters.

While G_{30} and survival rate data from a particular site or depth might be a good indication of the suitability of the site for growth of *P. maxima*, it failed to establish how fast or at what size an oyster might attain asymptotic length. Modelling growth using mathematical expressions provided greater insight into oyster growth than just G_{30} analysis alone.

It was established that the Special VBGF was the model that best described the growth of *P. maxima* cultured in West Papua. According to the criteria of lowest MRSS, high r^2 and least deviation of L_∞ from L_{max} it appeared that the General VBGF would be a better fitting model than the Special VBGF. However, when the curves were plotted, it was shown that the General VBGF and the other models tended to underestimate $L_{\infty,}$ while the Special VBGF did not. Taking both criteria of best fit and growth curves into consideration, the best fit models which explained growth of *P. maxima* cultured in West Papua could be ranked as follows: Special VBGF > General VBGF > Gompertz > Richards > Logistic. This was consistent with the results of studies on other pearl oyster species which indicated the VBGF (without the additional exponent *b* to be estimated) described growth in adult oysters. The only other two

studies that have modelled growth of *P. maxima* using VBGF were based on the dorso-ventral measurement of the oyster rather than the antero-posterior measurement used in this study. Hart and Joll (2006) used a maximum likelihood reformation of the Von Bertalanffy curve to study growth of *P. maxima* wild stocks in Western Australia and estimated L_∞ and K to be between 194 – 210 mm and 0.72 – 0.79 L_∞ and K respectively. Similar Von Bertalanffy curve analyses on field specimens of *P. maxima* on the Great Barrier Reef by Yukihara *et al*. (2006) reported an asymptotic size of between 205 – 229 mm and a K value of between 0.39 to 0.41 y^{-1}. The L_∞ (167.15 – 170.33 mm) estimated in this study was lower than the other results for *P. maxima* while K (0.879 – 0,990 yr^{-1}) was higher. This might be due to the age of the oysters. Relatively young oysters with smaller sizes and faster growth were sampled in this study, with the absolute known age of the oldest oysters < 5 years old. In other studies where oysters were sampled from the wild, the ages of the oysters were unknown and likely to be older than those in this study. Another reason for differences in L_∞ and K might be due to the latitude of the study site. Modelling has also been performed on *P. margaritifera* (Yukihira *et al*. 2006; Pouvreau and Prasil, 2001), *P. fucata* (Kandala and Prajneshu, 2004), *P. imbricata (*Urban, 2002) and *P. margaritifera* (Saucedo and Monteforte, 1998). Some values of L_∞ and K for pearl oysters are given in Table 6.4.

Requirements to fit a VBGF are known age and a wide range of age data (Pouvreau and Prasil, 2001). The data set for this study satisfied the former requirement in that the exact ages of hatchery bred oysters were known, and the latter requirement in that a large number of oysters encompassing a relatively wide age range was sampled. Due to limitations in time, modelling larval and early spat development was not possible in this study. Future effort to obtain length-at-age data for the early stages would allow for the modelling of all developmental stages of *P. maxima* comparable to *P. imbricata* by Urban (2002).

The curve of absolute growth in bivalves as a function of age is commonly a sigmoidal curve (Gibson, 1956; Wilbur and Owen, 1964; Seed, 1973). The general pattern is for rapid growth in young individuals which declines with age, as seen in this study. In older individuals, there may be an extended period of slow growth at a rate which does not change greatly with time (Wilbur and Owen, 1964). Comparison of spatial patterns of growth using the Special VBGF parameters was possible because a common range of age classes was used in each likelihood

ratio analysis (Kimura, 1980). Results showed that there was a spatial difference in oysters grown at various sites on the farm. When growth curves of oysters grown at Ganan, Manselo and Batu Terio were compared, the results showed they were significantly different (χ^2= 178.3, $p < 0.05$, df=6).

Table 6.4 von Bertalanffy growth parameters reported for pearl oysters at various locations (adapted from Saucedo and Southgate, 2008)

		Growth parameters		
Species	Location	L_∞ (mm)	K (y^{-1})	Author(s)
Pinctada margaritifera	Cook Islands (wild)	183	0.260	Sims (1994)
	Cook Islands (cultured)	310 - 157	0.254 - 0.528	Sims (1994)
	French Polynesia	161	0.46	Pouvreau *et al.* (2000a)
	French Polynesia	147 - 186.5	0.42 - 0..58	Pouvreau and Prasil (2001)
	Queensland, Australia	136 - 157	0.54 - 0.58	Yukihira *et al.* (2006)
	Kenya	127.2	0.3	Mavuti *et al.* (2005)
	Red Sea	153	1.52	Nasr (1984)
Pinctada maxima	Vietnam	260	0.4816	Tuyen and Tuan (2000)
	Queensland, Australia	205 - 229	0.39 - 0.41	Yukihira *et al.* (2006)
	Western Australia	194 - 210	0.72 - 0.79	Hart and Joll (2006)
	West Papua, Indonesia	167.15 – 170.33	0.879 – 0.990	This study
Pinctada imbricata	Venezuela	85.15	1.42	Marcano *et al.* (2005)
	Colombia (Caribbean)	84	0.939	Urban (2000)
	Colombia (Caribbean)	65.7	1.767	Urban (2002)
Pinctada fucata	India	79.31	0.075 ($month^{-1}$)	Chellam (1988)
Pinctada mazatlanica	Mexico	110	0.45	Saucedo *et al.* (1998)
Pteria sterna	Mexico	100	0.69	Saucedo *et al.* (1998)

The estimated Special VBGF parameters indicated that growth rate, as estimated by *K*, could be ranked from highest to lowest as follows for sites: Manselo (K =0.975 y^{-1}), Batu Terio (K =0.939 y^{-1}) and Ganan (K =0.879 y^{-1}). The depth for best growth was 5 m (K =0.990 y^{-1}) as compared to lower growth rate recorded at 15 m (K=0.892 y^{-1}). Sites which attained larger asymptotic length were also ranked similarly: Manselo (L_∞ =169.63 mm), Batu Terio (L_∞ =168.36 mm) followed by Ganan (L_∞ = 167.17 mm). However, although oysters grown at a depth of 15 m produced slower growth, they tended to reach a larger size (L_∞ = 170.33 mm) compared to oysters grown at 5 m (L_∞ = 167.15 mm). This is probably due to the difference in water temperature at the two depths (Chapter 4). Water temperature is one of the principal environmental factors affecting growth in bivalves (Malouf and Breese, 1977; Incze *et al.*, 1980) including pearl oysters (Lucas, 2008) and has a direct effect on the growth of the animal by influencing metabolic activity such as ingestion, absorption, excretion and respiration. Other species of pearl oysters which show low growth rates at lower water temperatures and maximum growth at higher water temperature include *P. margaritifera* (Nasr, 1984) and *P. radiata* (Nayar and Al-Rumaidh, 1993). Oyster size is also influenced by water temperature. In the Gulf of Mexico, oyster size declined at lower latitudes because increased water temperature decreases the allocation of net production to somatic growth (Klinck *et al.*, 1992).

In summary, this study determined that the best mathematical model for quantifying growth of *P. maxima* cultured in West Papua was the Special VBGF. It also demonstrated that there was significant difference in growth performance at various sites and depths within the pearl oyster farm in West Papua.

Based on survival, estimated growth rate and asymptotic size, it is recommended that in the first two years of culture when fast growth, high survival and larger asymptotic size are essential to produce large numbers of oysters for grafting, the best site for grow-out of grafted oysters would be Manselo at a depth of 5 m. On the other hand, oysters grown at Ganan and at a depth of 15 m had lower *K* values and would be suitable to condition oysters prior to grafting and also for pearl grow-out. Bivalve growth is affected by a complex combination of biological and environmental factors. Biological factors include the size, age, reproductive condition and genetic characteristic of the animals.

These aspects of bivalve growth are often difficult if not impossible to manipulate in a culture situation to ensure optimum growth. On the other hand, limited control may be exerted over environmental factors that govern growth such as water temperature and salinity, which affect rates of biochemical reactions within an organism and food availability through careful selection of culture sites. This study allowed for the recommendation of optimal culture sites through mathematical modelling of *P. maxima* growth.

CHAPTER 7

Temporal and spatial variation in recruitment and composition of biofouling found on three age classes of *Pinctada maxima* and their effect on growth and mortality

7.1 Introduction

In an ocean-based aquaculture environment, the establishment of an assemblage of unwanted organisms upon the surfaces of cultured animals and culture equipment impacts greatly upon functionality and cost of production. In the culture of sessile marine species which utilises structures such as ropes, cages and suspended net systems in the water column, the colonisation of unwanted species is particularly significant (Adams *et al*., 2006). Pearl production which employs underwater panels or nets suspended from long-lines, is a prime example.

The cost allocated to combat or prevent biofouling in the pearling industry has been variously estimated to be between 25 - 30% (Crossland, 1957; Lewis, 1994) and up to 80% (Fulton-Howard and Fort, 2004) of the total cost of pearl production. These costs are associated with the regular cleaning of pearl oysters and culture equipment which is labour intensive. Furthermore, biofouling assemblages add excess weight to the suspended culture systems which impairs floatation and requires regular maintenance (Lodeiros *et al*., 2007; De Nys and Ison, 2008). While the exact monetary cost of biofouling for the pearl industry is unknown, the detrimental impact upon the industry is well documented. Heavy fouling can directly cause oyster mortality (de Nys and Ison, 2008) with major impacts on pearl production (Thomas, 1979). There have been cases reported where fouling did not affect oyster growth (Lodeiros *et al*., 1999) or may exert a favourable influence on oyster growth by increasing food abundance (Lodeiros *et al*., 2002; Lodeiros and Garcia, 2004; de Nys and Ison, 2008) but this is the exception – fouling is a major problem in most pearl oyster culture systems.

In most studies, biofouling has been reported to deleteriously affect the pearling industry by decreasing growth and survival in pearl oysters (Taylor *et al*., 1997a, 1999;

Pit and Southgate, 2003a; Fromont *et al*., 2005) by reducing water flow which in turn decreases food (Claereboudt *et al*., 1994) and oxygen availability (Wallace and Reinsnes, 1985). Filter-feeding fouling organisms may also compete with cultured pearl oysters for available food (Taylor, 1999). A review by de Nys *et al.* (2008) categorised the impact of biofouling on pearl oysters into five major groups including: 1) physical damage to the shell; 2) mechanical interference with the opening of oyster valves; 3) biological competition for food; 4) environmental modification, and 5) increased friction on culture structures.

Biofouling on cultivated bivalves can be divided into microfouling and macrofouling. Microfouling or "soft" fouling includes biofilm formation and microorganism adhesion, with bacteria and diatoms representing the primary colonisers, followed by spores of microalgae and protozoa as secondary colonisers (Abarzua and Jakubowski, 1995). While microfouling in great quantities can add significantly to weight in suspended culture, the effects of live microfouling, such as algae, on oyster culture is not of particular concern (Quayle and Newkirk, 1989). Microfouling is relatively easy to remove by hosing oysters in culture panel nets with a high pressure seawater pump (Taylor *et al*., 1997a). The attachment of larger organisms such as barnacles, polychaete worms and other bivalves, which constitutes macrofouling, poses a greater problem due to the greater impact of macrofouling on bivalve growth, survival and culture systems (Mohammad, 1976; Chellam, 1978; Taylor *et al*., 1997a; Pit and Southgate, 2003). The removal of macrofouling is also more labour intensive as it involves scraping or chiselling the unwanted organism from shells of individual oysters and culture equipment (Knauer, Hatchery Manager, P. T Cendana Indopearls, *pers. comm*., 2001).

Previous studies on biofouling of pearl oysters have concentrated mainly on quantifying and identifying the species of macrofouling organisms which infest pearl oysters over time (e.g. Scardino *et al.*, 2003; Guenther and de Nys, 2006; Guenther *et al.*, 2006) and investigating the effects of fouling organisms on pearl oyster growth and survival (eg. Taylor *et al.*, 1997a; Lodeiros *et al.*, 2002; Pit and Southgate, 2003a). No investigation has been conducted to examine the influence of culture site, culture depth and age of oysters, upon the settlement and composition of biofouling assemblages on pearl oysters. In particular, the effects of various environmental parameters on pearl oyster biofouling are not known.

Based on the results of a previous study on *P. maxima* (Taylor *et al.*, 1997a), an optimal cleaning regime for cultured 1-year old pearl oysters was to remove fouling every four weeks from oyster shells. This regime maximised growth of 1-year old *P. maxima* and reduced operational costs by avoiding unnecessary cleaning. However, it has been shown previously that there is a relationship between age of pearl oysters and accumulation of biofouling organisms (Takemura and Okutani, 1955; Mohammad, 1972; Guenther *et al.*, 2006). A cleaning regime based on the susceptibility of different ages of oysters to fouling could improve husbandry through the scheduling of cleaning to when it is required. For example, older oysters might have to be cleaned more frequently than younger oysters to offset greater accumulation of biofouling (Guenther *et al.*, 2006).

Besides regulating the frequency of cleaning, another method in the management of biofouling would be to judiciously select sites which are unfavourable for the settlement of various fouling communities. It has been observed that fouling communities and biomass weight of fouling varied between localities (about 40 km apart) and depths used for scallop culture (Claereboudt *et al.*, 1994). Monitoring the ecological succession of biofouling on shells of cultured pearl oysters from various sites within a farm would provide qualitative and quantitative data on fouling which could assist in identifying and selecting sites where biofouling infestation is reduced.

The impact of biofouling is therefore of great research importance. This chapter analyses the recruitment of initial colonisers to cleaned cultured *P. maxima* to determine if temporal, spatial and biological cues for settlement could be identified which could ultimately be used to minimise fouling and assist in the effective management of *P. maxima* culture in Aljui Bay.

Specifically, this experiment aims to quantify hard macro-biofouling, which settles on the shells of three age groups of *P. maxima* oysters cultured at various sites and depths, over a 14-month sampling period. Furthermore, this research examines the succession variability of biofouling species settling on the shells of oysters and to determine if there are any differences between fouling species found on oysters of different ages and grown at different sites and depths. Finally, the effects of environmental parameters on

the distribution and magnitude of biofouling assemblages, and the impact of biofouling on oyster growth and mortality, are examined.

7.2 Methods and Materials

7.2.1 Site

Ganan, Manselo and Batu Terio (Section 3.4) were used as sites for the experiments. At each site, a longline was established to rear the experimental oysters according to the methods described in Section 3.6.5.

7.2.2 Pearl oysters

Specimens of *P. maxima* in this experiment were hatchery produced animals used in the growth experiment described in Chapter 5. At the start of sampling, the ages of the three oyster groups were 0.58, 1.25 and 3.33 years old and the mean initial shell lengths (antero-posterior measurement) were 65.66 ± 9.69 mm (mean ± SD), 93.94 ± 5.04 mm and 153.03 ± 16.69 mm respectively. Oysters from these three age classes will be referred to as ‘large’, ‘medium’ and ‘small’ in this chapter. The term ‘size’ and ‘age’ will also be used interchangeably in subsequent discussion.

7.2.3 Experimental design

The experiment was set-up as described in Section 5.3.2. Tagged and untagged oysters were sampled for growth and biofouling every month from August 2000 to November 2001, with the exception of December 2000 and March 2001 when inclement weather forced field sampling to be cancelled. The timing of sampling was based on the results of Taylor *et al.*, (1999) who reported the optimal cleaning regime for *P. maxima* cultured in North Maluku, Indonesia to be monthly (4 weeks).

7.2.4 Sampling for growth and biofouling

During sampling, panel nets containing oysters were removed from long-lines, brought to the surface and soft fouling was removed by high-pressure spraying. All oysters were removed from the nets by severing the byssus with a scalpel. Hard fouling on the shells of tagged oysters was visually identified to Class level. The number (proportion) of tagged oysters from each treatment fouled with a particular organism was recorded.

Biofouling from both tagged and untagged oysters was removed by scraping the shell with a knife and collected. The fouling assemblage from each treatment group were combined and drained of excess seawater. Biofouling from the different treatments were placed separately on aluminium plates and dried in an oven at 55°C to constant weight. The total dry weight of the fouling was then determined for each treatment.

Tagged oysters were measured for antero-posterior shell length using vernier callipers. Mortalities of tagged oysters were recorded. Dead oysters were not replaced with live ones, but were sampled for fouling, cleaned and placed back into the panel nets for subsequent samplings. Mortality was recorded on a monthly basis.

7.2.5 Environmental monitoring

A range of environmental data was collected from August 2000 to November 2001 at the three sites according to the methods described in Section 3.5. Environmental parameters monitored for this experiment were water temperature, salinity, pH, suspended particulate matter (SPM), particulate organic matter (POM) and chlorophyll *a*, *b* and *c*.

7.2.6 Statistical Analyses

The proportion of animals fouled with the various taxa of biofouling was compared with multivariate analysis of variance (MANOVA) using Wilks-Lambda statistic to determine if there were any differences in frequencies of fouling between treatments. Analysis using full factorial MANOVA with 4-way interaction could not be employed due to insufficient replication of data as only one count (number of oysters fouled with a particular species of fouling organism per month) could be recorded at each sampling. Instead, a main effect and 3-way factorial MANOVA model was employed. Prior to employing MANOVA, the two underlying assumptions, homocedascity and normality, were tested on the data using the Levene's and Shapiro-Wilk's, test respectively.

Dry weight of fouling on oysters from different months of sampling, sites and depths were compared using a univariate analysis of variance (ANOVA) with the significance level $\alpha = 0.05$. Prior to ANOVA, the unequal shell surface area from oysters of different ages was offset by standardising fouling weight – this was done by dividing the mean

dry weight of fouling by mean oyster shell length to calculate dry weight per unit of length for each oyster group.

The relationship between dry weight and fouling taxa was investigated using stepwise linear regression analysis. Comparison between the standardised regression coefficients (β), which indicate the relative influence of individual independent variables has upon the dependent variable (Zar, 1984; Brown, 1988) ascertained which fouling taxa contributed most to the total dry weight of fouling.

The standard measure, monthly instantaneous growth rate, (G_{30}) described in Section 5.2.9 was used to compare growth of oysters over a particular month. The unit for G_{30} was mm month^{-1}. G_{30} of oysters from different treatment groups were compared using univariate ANOVA to determine if month, site, depth and age of oyster had an effect on growth. Mortality between treatments was similarly compared using ANOVA.

A two-tailed Pearson Correlation using standardised weight per unit length and $\log_e$ transformed G_{30} as variables was performed to examine if there was a correlation between dry weight of biofouling and growth rate of oysters. The analysis was partitioned by size to compare the correlation between fouling weight and G_{30} in oysters from different ages, as age has been reported to have an effect on growth rate of bivalves (Gervis and Sims, 1992; Pouvreau *et al.*, 2000; Gosling, 2003). Pearson correlation was also used to investigate the association between dry weight and mortality, but without the partitioning by size, as ANOVA showed that age did not significantly affect mortality in this study. Pearson Correlation analysis was further employed to determine if there were any relationship between various taxa of fouling with G_{30} and mortality.

To determine the relationship between different taxa of biofouling and environmental parameters, a multivariate stepwise regression of each taxon was performed with environmental parameters assigned the independent variables. The criteria set for F to enter and F to remove were ≤ 0.05 and ≥ 0.10, respectively. Potential predictor environmental variables included in the analysis were water temperature, salinity, pH, concentrations of chlorophyll *a, b, c,* POM and SPM. Environmental data were those

obtained from Chapter 4. Variables not included in the equations were rejected in favour of remaining variables at $\alpha = 0.05$.

A similar regression analysis was conducted to investigate the association between fouling weight and environmental parameters.

While multivariate regression analysis constitutes a useful method to investigate the relationship between a fouling taxa and environmental factors, it does not allow for a coordinated analysis of all taxa and environmental parameters, nor does it provide a clear representation of the associations between the different taxa and the environment. To this end, a pair-wise principal component analysis (PCA) was performed. Components with eigenvalues greater than one were retained and rotated by Varimax method to produce components.

7.3 Results

7.3.1 Biofouling composition

Macrofouling organisms (Fig. 7.1) belonging to six taxonomic classes settled on *P. maxima* cultured at different sites in varying proportions over the sampling period. The fouling species belonged to classes Maxillopoda (barnacles), Polychaeta (polychaete tubeworms), Bivalvia (bivalves), Demospongiae (sponges), Foraminifera (forams) and Ascidicea (ascidians).

The most prevalent species observed during successive samplings were polychaetes and forams, while sponges fouled the least number of oysters over the sampling period (Fig. 7.2).

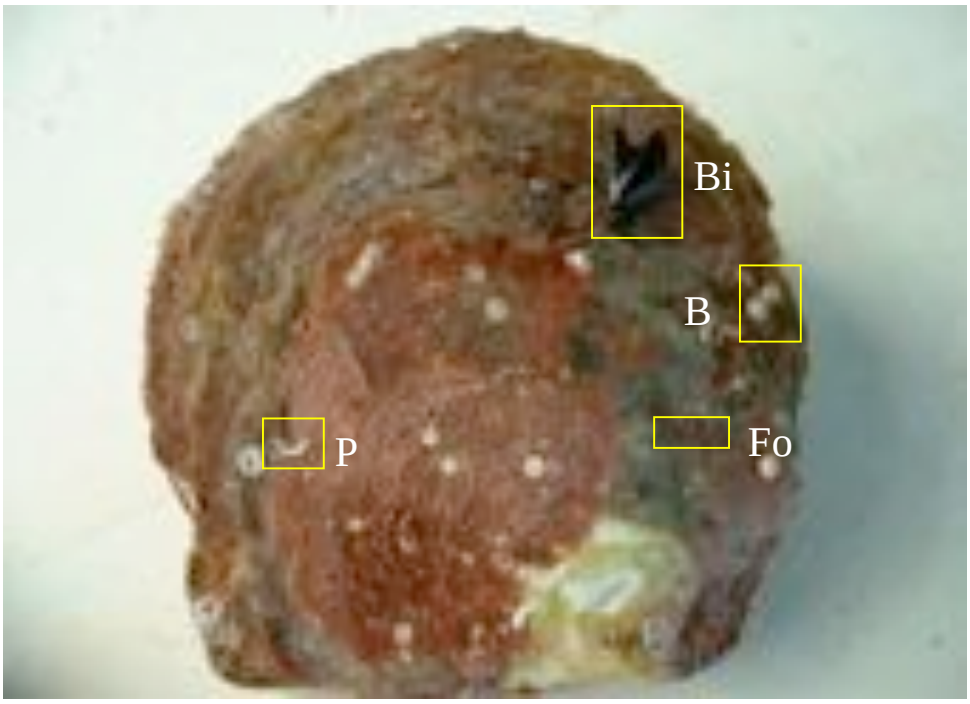

Fig. 7.1 Some species of hard macro-biofouling on the shell of *P. maxima* cleaned of soft fouling. Ba: barnacles, Bi: bivalves, Fo: forams and Po: polychaetes.

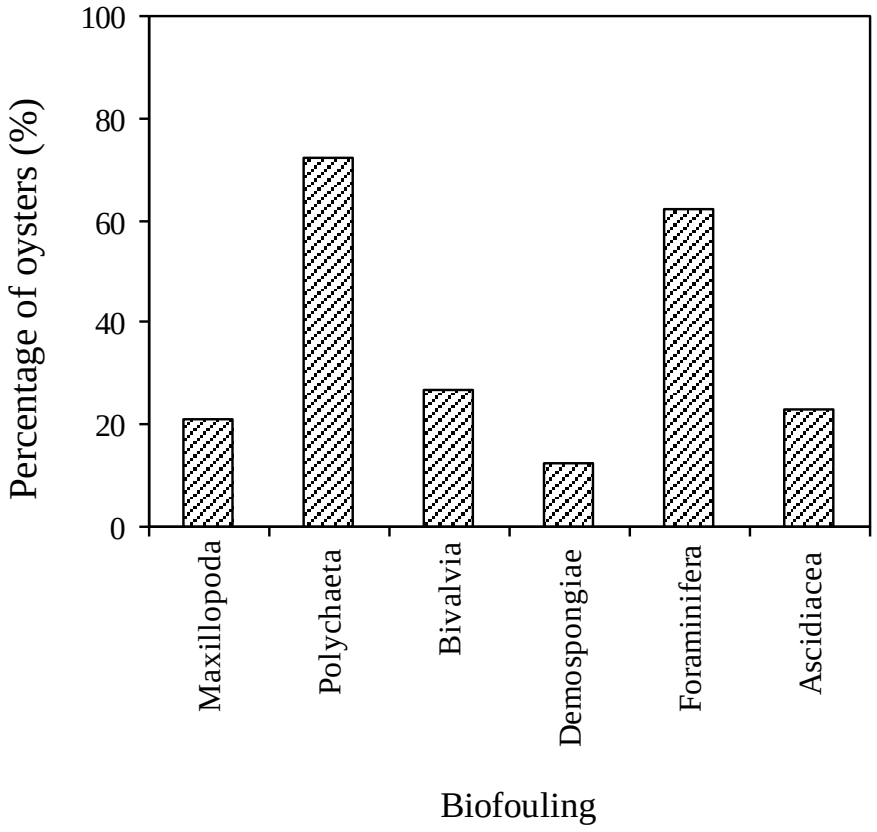

Fig. 7.2 Mean percentage of oysters fouled with various taxa of biofouling during the study period.

7.3.2 Temporal effect

Multivariate analysis showed that the prevalence of the various species of biofouling was significantly different over time ($F_{(78,243)} = 23.14$, $p < 0.05$). Differential settlement of biofouling communities wase detected every month, with marked increase in barnacle and ascidian colonisation observed in June, August and October 2001 (Fig. 7.3a, Fig. 7.3b) and bivalve settlement in January and February 2001 (Fig. 7.3e). Fouling by polychaetes and forams was relatively high throughout the experimental period except during May 2001 when polychaete settlement was low (Fig. 7.3c) and from January - May 2001 where less oysters were found to be fouled by forams (Fig. 7.3d). Settlement by sponges remained low during sampling although the number of oysters fouled by sponges was marginally higher from August – November 2000 (Fig. 7.3b). There was significant interaction between month of sampling and site ($F_{(156,260)} = 5.63$, $p < 0.05$), between month and size ($F_{(156,260)} = 2.253$, $p < 0.05$) and between month and depth ($F_{(78,243)} = 3.034$, $p < 0.05$).

7.3.3 Spatial effect

7.3.3.1 Culture site

Significantly different proportions of oysters at Ganan, Manselo and Batu Terio were colonised with the various species of biofouling ($F_{(12,86)} = 34.13$, $p < 0.05$). Between-subject effect showed that all classes of biofouling differed between sites, except bivalves and sponges. Forams appeared most abundantly at Ganan while fouling by polychaetes and ascidians were found mostly on oysters grown at Manselo (Fig. 7.4a). Although the number of oysters fouled with bivalves and sponges were not significantly different between sites, bivalves and sponges were found to be marginally higher at Ganan and Batu Terio, respectively (Fig. 7.4a). There was a significant interaction between site and size ($F_{(24,151)} = 3.53$, $p < 0.05$), and site and depth ($F_{(12,86)} = 2.87$, $p < 0.05$) .

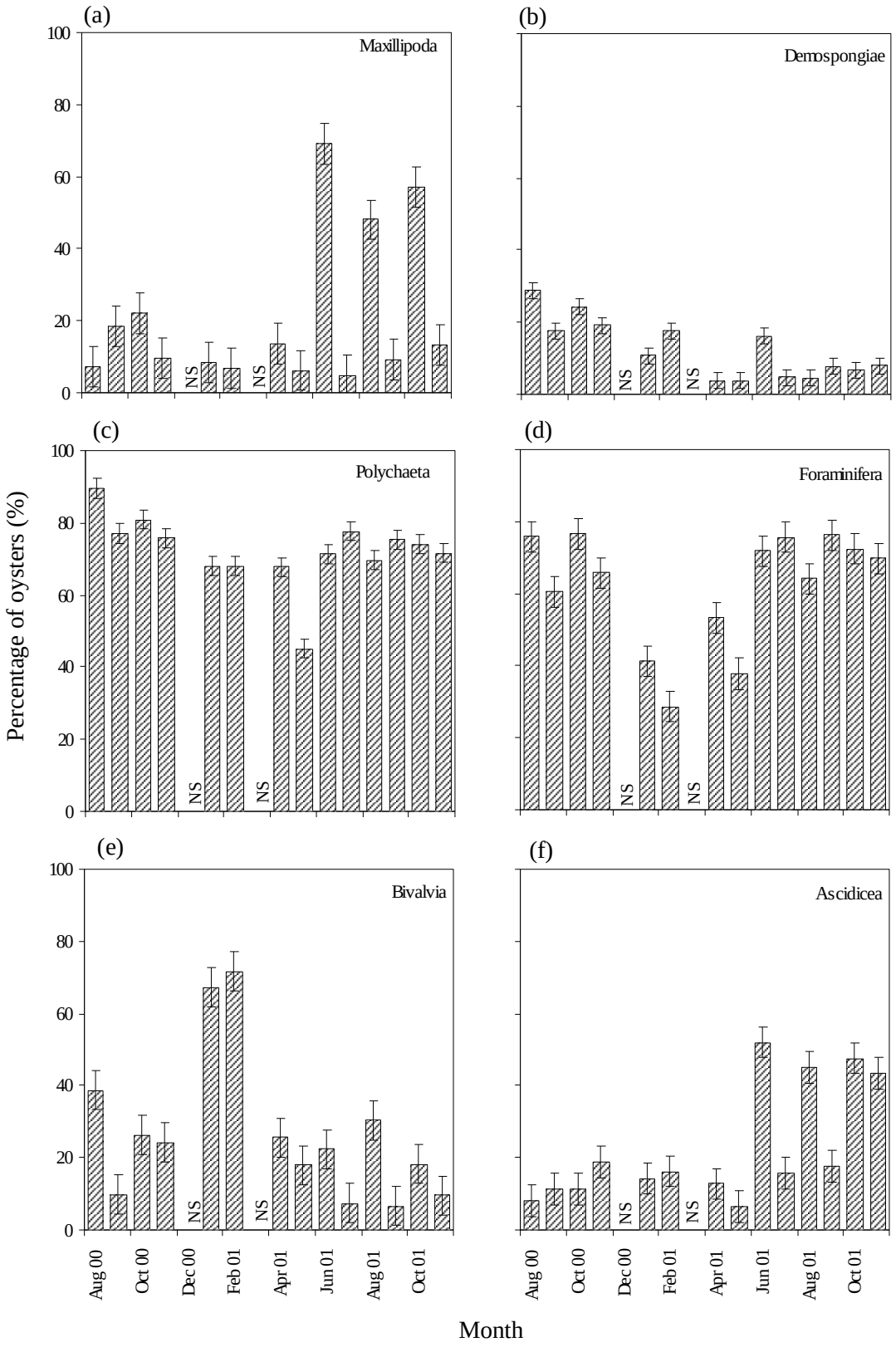

Fig. 7.3 Temporal variations in proportion of oysters fouled by Maxillopoda (a) Demospongiae (b), Polychaeta (c), Foraminifera (d), Bivalvia (e) and Ascidicea (f) during successive months of sampling. Vertical bars indicate Standard Error. NS signify no sampling was performed during the month.

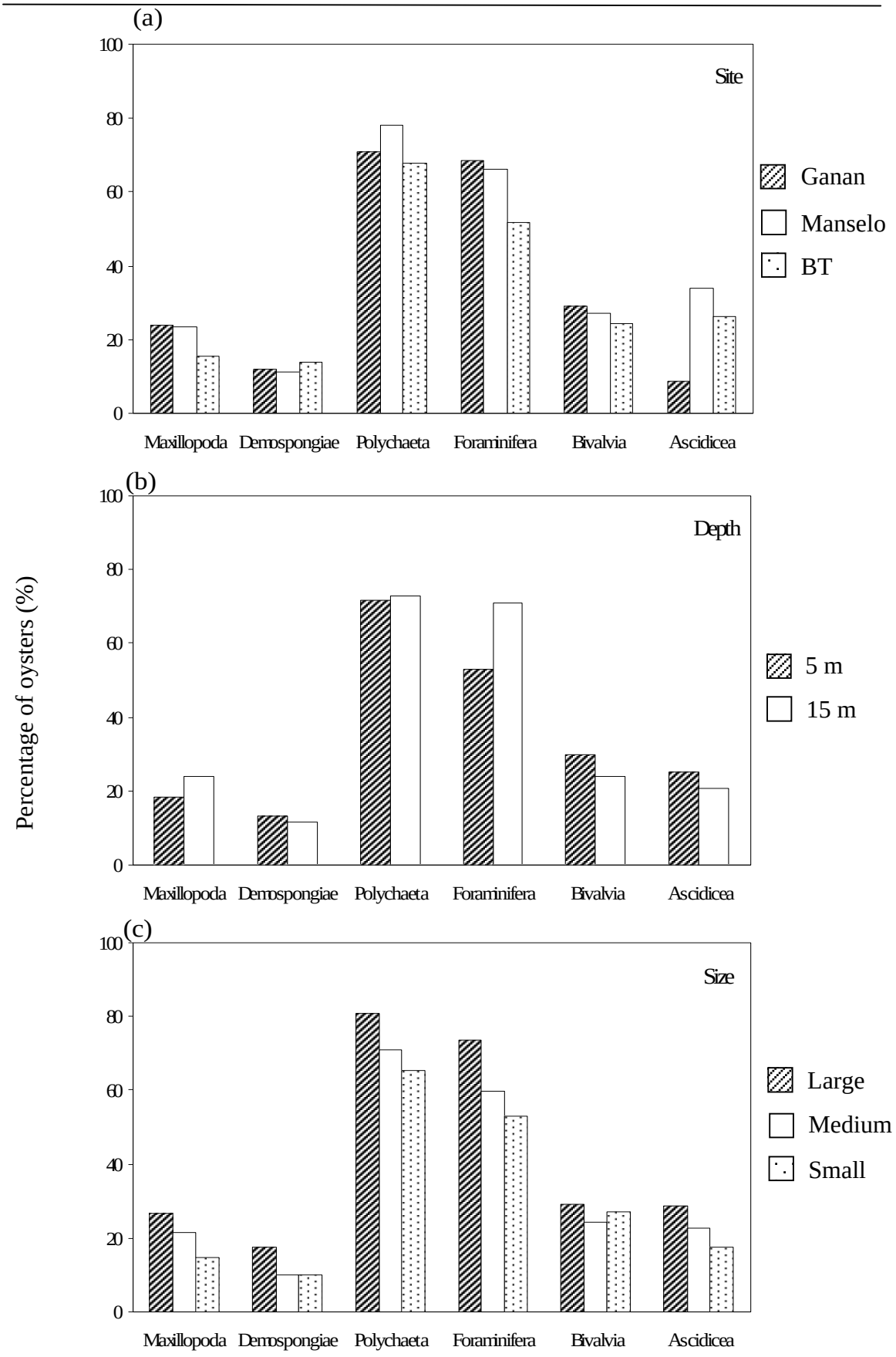

Fig. 7.4 Proportion of oysters from different sites (a), depths (b) and size of oysters (c) fouled with organisms from classes Maxillopoda, Polychaeta, Bivalvia, Demospongiae, Foraminifera and Ascidicea from Aug 2000 to Nov 2001.

7.3.3.2 Culture depth

Overall, depth significantly affected the distribution of biofouling classes ($F_{(6,43)}$ = 31.91, $p < 0.05$). Between-subject tests showed that the only exceptions were polychaetes and sponges infestation, which were not significantly different between oysters grown at 5 and 15 m (Fig. 7.4b). Bivalves and ascidians fouled more oysters at 5 m, while more barnacles and forams settled on oysters grown at 15 m (Fig. 7.4b).

7.3.4 The effect of oyster size

The size of *P. maxima* shells had a significant effect on the degree of fouling settlement on oysters ($F_{(12,86)}$ = 17.73, $p < 0.05$). Between-subject analysis showed that all taxa settled differentially on oysters of different sizes with the exception of bivalves. More of the larger oysters were successively infested with biofouling than medium oysters while small oysters were the least fouled (Fig. 7.4c).

7.3.5 Dry weight of fouling

The dry weight of biofouling from various treatments ranged from 1.36 g collected in August 2001 from small oysters grown at Batu Terio at a depth of 15 m, to 65.34 g sampled in the same month from shells of large oysters at Ganan grown at a depth of 5 m.

Analysis of variance showed that dry weight of fouling was significantly affected by month of sampling ($F_{(12,152)}$ = 6.54, $p < 0.05$), culture site ($F_{(2,152)}$ = 3.15, $p < 0.05$) and culture depth ($F_{(1,152)}$ = 9.83, $p < 0.05$). The highest total dry weight of fouling was recorded in January 2001 (Fig. 7.5a). Overall fouling levels were found to be elevated at the start of the experiment in August 2000 and again from July – October 2001 (Fig. 7.5a). Spatially, levels of fouling at Batu Terio were less than at Ganan and Manselo (Fig. 7.5b). This was confirmed by post-hoc tests on site variation. Fouling mass was consistently higher on oysters cultured at 5 m during all months of sampling except November 2001, where biofouling on oysters grown at a depth of 15 m was fractionally higher (Fig. 7.6b).

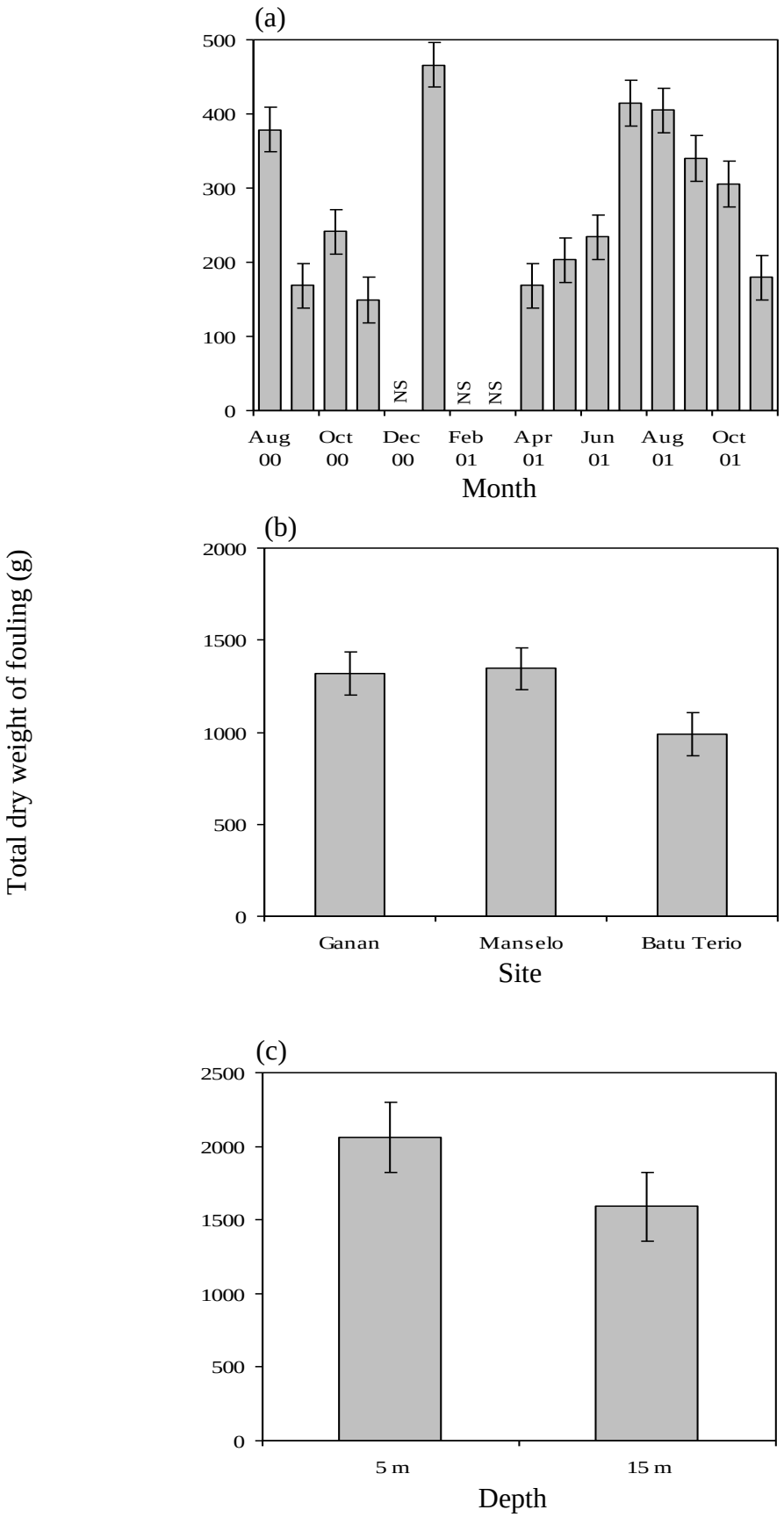

Fig. 7.5 Total dry weight of biofouling sampled from tagged *P. maxima* shells during various months (a) and cultured at different sites (b) and depths (c). Vertical bars indicate Standard Error. NS signifies no sampling was performed during the month.

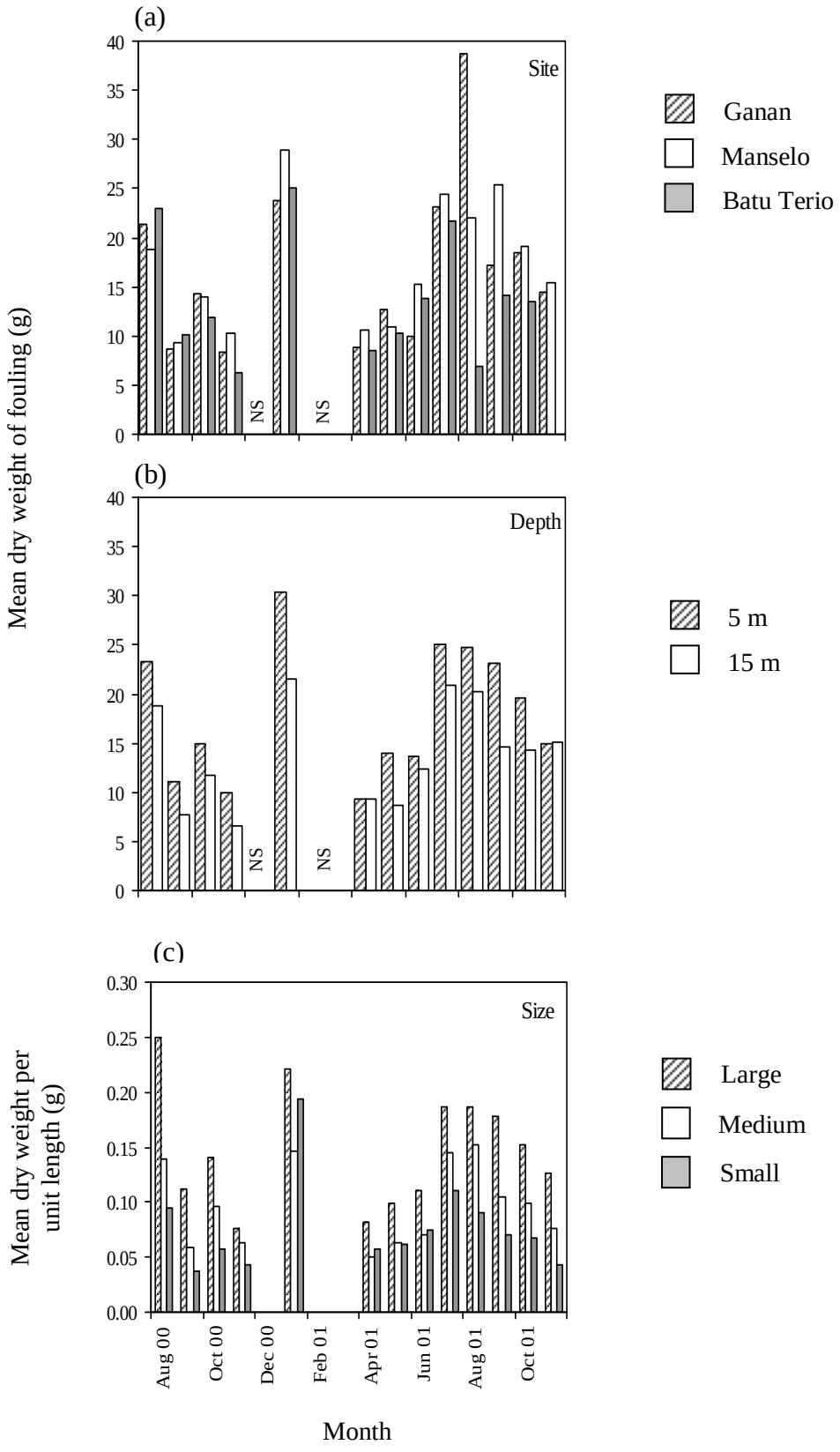

Fig. 7.6 Temporal variation in mean dry weight of fouling collected from shells of grown at different sites (a) and depths (b) and from three size groups (c). Mean dry weight of fouling from oysters of different sizes have been standardised per unit length. NS signify no sampling was performed during the month.

In the analysis of weight per unit length of biofouling using ANOVA, dry weight of fouling was significantly affected by the size of the oyster ($F_{(2,231)} = 27.79$, $p < 0.05$). Post-hoc tests indicated that the difference in dry weight was driven by the variation between fouling on large oysters and small oysters as well as between large oysters and medium oysters. There was no significant difference in fouling amount between small and medium oysters.

Linear regression of dry weight against fouling taxa (Table 7.1) showed that 20% ($p < 0.05$) of the variance in dry weight could be explained by polychaetes, bivalves and foram fouling. The relationship between dry weight of fouling and fouling taxa could be explained by the regression equation:

Dry weight = -1.397 + 0.329 (Polychaetes) + 0.590 (Bivalves) + 0.443 (Forams)

Comparison between the standardised coefficients ($\beta_{polychaete} = 0.15$, $\beta_{bivalve} = 0.33$, $\beta_{foram} = 0.27$) showed that bivalves contributed most to the total dry weight of fouling, followed by forams and polychaetes. The other fouling taxa did not contribute significantly to the variation in dry weight of fouling.

7.3.6 Environmental parameters

When multivariate analysis using the Wilks-Lambda statistic was applied to determine if there was an overall spatio-temporal difference in environmental parameters during the sampling period, results showed that environmental parameters were overall affected by month of sampling ($F_{(8, 220)} = 19.689$, $p < 0.05$) and culture site ($F_{(16, 440)} = 13.684$, $p < 0.05$) but not by culture depth. All interactions were also significant with the exception of the 2-way interaction between size and depth, and the 3-way interaction between month, size and depth.

The time scale for this experiment largely overlapped that for Chapter 4 and seawater quality results will not be repeated here. Refer to Chapter 4 for a detailed discussion on variation in environmental parameters between sites, depths and over time.

Table 7.1 Stepwise multiple regression models of dry weight of fouling against fouling taxa. β^0 is the unstandardised regression coefficient. Standardised regression coefficients (β) are in italics within parenthesis. All regressions were significant ($p < 0.05$).

	Model	β^0							r^2
Dry weight	1	3.145	+	0.684 (Polychaetes) *(0.311)*					0.097
	2	0.674	+	0.653 (Polychaetes) *(0.297)*	+	0.447 (Bivalves) *(0.253)*			0.161
	3	-1.397	+	0.329 (Polychaetes) *(0.150)*	+	0.590 (Bivalves) *(0.334)*	+	0.443 (Forams) *(0.270)*	0.207

7.3.7 Oyster growth

Over 18 months of sampling, mean (± S.E) shell length of large, medium and small oysters increased 9.60 ± 3.76 mm, 39.16 ± 12.56 mm and 70.45 ± 23.44 mm, respectively.

Stepwise multiple regression of G_{30} of oysters against environmental indicators (Table 7.2) showed that SPM and pH accounted for approximately 10% ($p < 0.05$) of variation in G_{30}.

Analysis of variance showed that size had a significant effect on oyster G_{30} ($F_{(2,234)} = 80.74$, $p < 0.05$) while culture site and depth did not affect G_{30} during the experimental period. G_{30} of small oysters was the highest of the size groups, followed by medium and large oysters (Fig. 7.7a). Irrespective of size, G_{30} of oysters were at a maximum from August 2000 to March 2001 before decreasing in rate until the end of the experiment (Fig. 7.7a).

As the size of oysters had an effect on G_{30}, a decision was made to partition the correlation analysis by size to investigate the relationship between oyster growth and standardised dry weight of fouling. Pearson Correlation analysis showed that there was a significant positive correlation between dry weight of fouling and G_{30} in medium (Pearson correlation = 0.304, $p < 0.05$) and small oysters (Pearson correlation = 0.370. $p < 0.05$) but no significant correlation was recorded for large oysters (Fig. 7.8). This result implies that the amount of fouling had a slight relationship to growth in medium and small oysters but not large oysters.

The analysis to determine association between taxa of fouling and growth was similarly partitioned by size. There was no significant correlation between growth and fouling taxa in large oysters. G_{30} was found to be significantly correlated to polychaetes (Pearson correlation = 0.278, $p < 0.05$) and sponges (Pearson correlation = 0.550, $p < 0.05$) in medium oysters while growth in small oysters was significantly affected by the most classes of biofouling including barnacles (Pearson correlation = -0.242, $p < 0.05$), polychaetes (Pearson correlation = 0.349, $p < 0.05$), bivalves (Pearson correlation = 0.514, $p < 0.05$), sponges (Pearson correlation = 0.414, $p < 0.05$) and ascidians (Pearson correlation = -0.241, $p < 0.05$).

Table 7.2 Stepwise multiple regression models of G_{30} against environment parameters. β^0 is the unstandardised regression coefficient. Standardised regression coefficients (β) are in italics within parenthesis. All regressions were significant ($p < 0.05$).

	Model	β^0					r^2
G_{30}	1	0.047	+	4.216 (SPM) *(0.255)*			0.065
	2	-1.091	+	3.390 (SPM) *(0.205)*	+	0.153 (pH) *(0.204)*	0.104

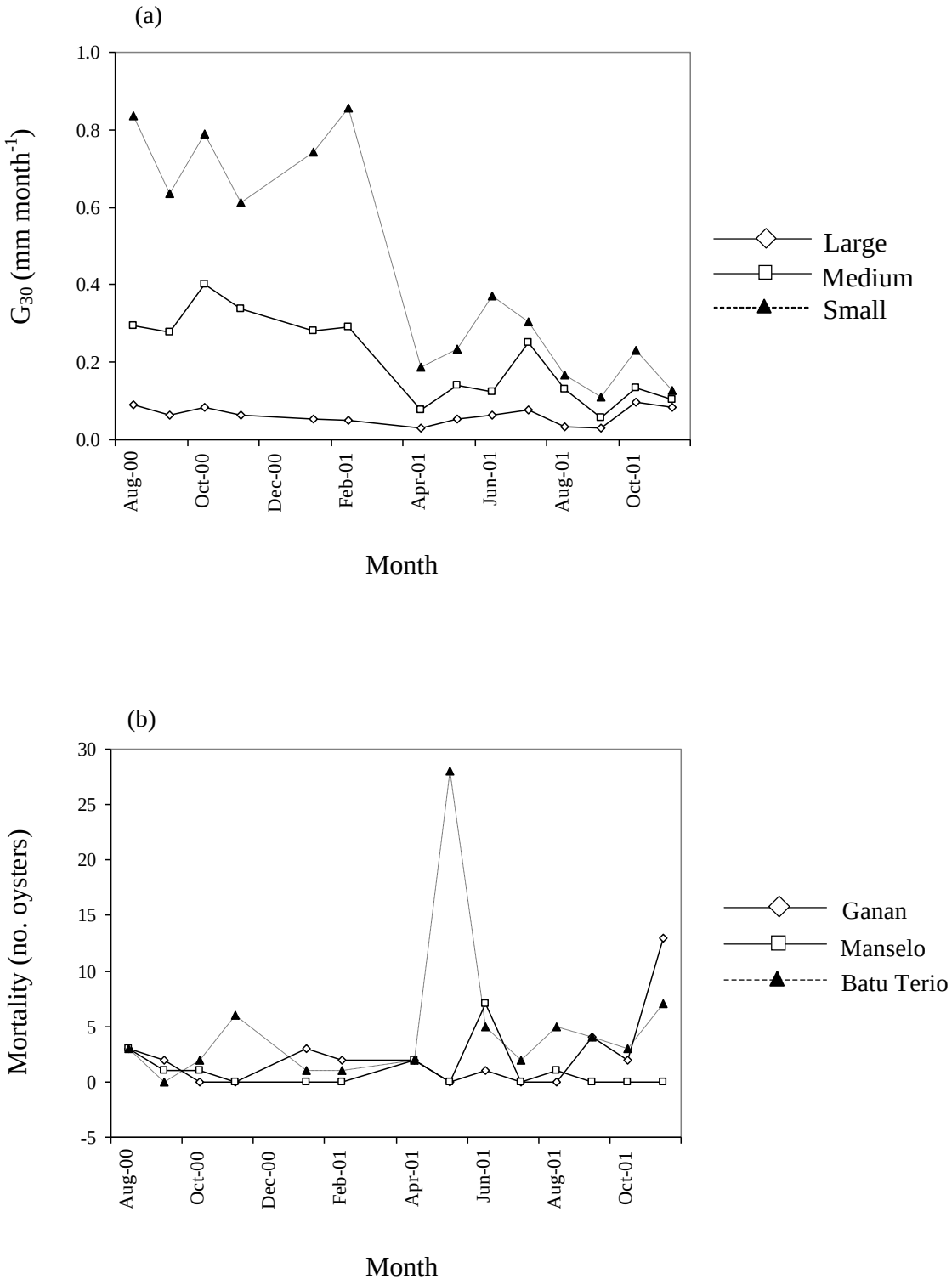

Fig. 7.7 Mean monthly instantaneous growth rate (G_{30}) (a) of oysters of different ages and number of mortalities of oysters (b) from various sites.

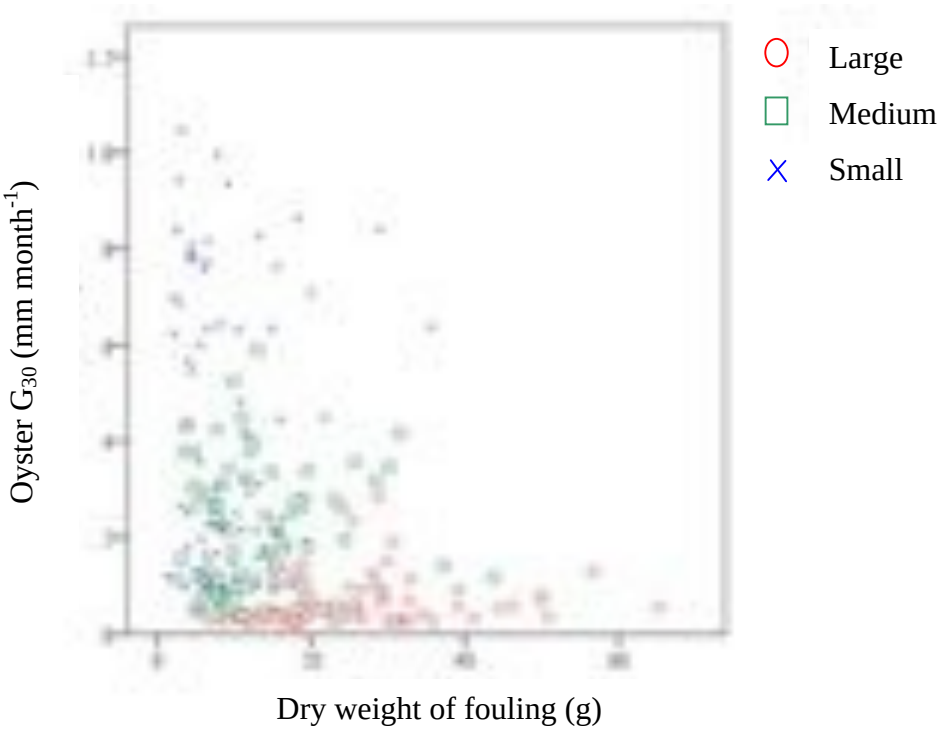

Fig. 7.8 Scatter-plot showing interaction between oyster G_{30} and biomass of fouling for large, medium and large oysters. Medium and small oyster G_{30} and biomass of fouling were significantly correlated ($p < 0.05$), but large oyster G_{30} was not correlated to biomass.

7.3.8 Oyster mortality

Mortality was significantly affected by culture site ($F_{(2,234)} = 4.95$, $p < 0.05$) but not by size and culture depth. This significant effect appeared to be driven by high mortalities of oysters in Batu Terio in May 2001 (Fig. 7.7b). As mortality was not affected by the size of oysters, there was no partitioning by size in the following correlation analyses. No significant correlation between oyster mortality and dry weight of fouling was found in all three sizes of oysters. In the analysis to determine the relationship between oyster death and biofouling taxa, there was a significant correlation between oyster mortality and polychaetes fouling (Pearson correlation = -0.209, $p < 0.05$) but no relationship for the other fouling classes.

7.3.9 Relationship between biofouling and the environment

7.3.9.1 Fouling dry weight

Stepwise multiple regression of dry weight of fouling against environmental indicators showed that water temperature accounted for approximately 6% ($p < 0.05$) of variation in dry weight of fouling on shells of *P. maxima*. The relationship between dry weight of fouling and water temperature could be explained by the regression equation:

$$\text{Dry weight of fouling} = 206.618 - 6.631\ (\text{Water temperature})$$

There were no significant relationships between fouling dry weight and other environmental parameters.

7.3.9.2 Fouling composition

Stepwise regression of each biofouling taxon against environmental parameters showed different taxa were affected differently by the various environmental parameters (Table 7.3). Bivalves and ascidians were affected by most parameters. Approximately 3% ($p < 0.05$) of variation in the number of shells fouled by bivalves could be accounted for by SPM, pH, water temperature, salinity, and chlorophyll *a*, *b* and *c*. Comparison between the standardised regression coefficients results showed that chlorophyll *a* had the greatest inverse effect on the occurrence of bivalves (as indicated by the negative value of the chlorophyll *a* coefficient), followed by chlorophyll *c*, and SPM, while pH, water temperature, salinity, and chlorophyll *b* exerted a lesser influence.

Ascidians were similarly affected by chlorophyll *b*, pH, salinity, POM and water temperature, which together accounted for approximately 40% ($p < 0.05$) of the variation in oysters fouled with ascidians. Chlorophyll *b* had the most influence in the occurrence of ascidians, followed by water temperature and POM.

Approximately 28% ($p < 0.05$) of maxillopod numbers were affected by chlorophyll *b*, POM and salinity, with chlorophyll *b* having the most influence, while only 9% ($p < 0.05$) of difference in the presence of forams could be explained by POM and salinity, with POM exerting the greater influence. Sponges and polychaetes numbers appeared to be affected by only a single environmental parameter, pH, which accounted for 9% ($p < 0.05$) and 3% ($p < 0.05$) variation in sponge and polychaete numbers, respectively.

Table 7.3 Stepwise multiple regression models of fouling taxa occurrence against environmental variables [Temp: temperature; Sal: salinity; pH; SPM; POM; Ch *a*: chlorophyll *a*; Ch *b*: chlorophyll *b*; Ch *c*: chlorophyll *c*]. β^0 is the unstandardised regression coefficient. Standardised regression coefficients (β) are in italics within parenthesis. All regressions were significant ($p < 0.05$).

Fouling organisms	Model	β^0													r^2
Maxillopods	1	-1.03	+	9.39(Ch *b*) *(0.46)*											0.215
	2	1.17	+	9.08(Ch *b*) *(0.45)*	-	86.31(POM) *(-0.21)*									0.259
	3	64.28	+	8.29(Ch *b*) *(0.41)*	-	83.83(POM) *(-0.20)*	-	1.80(Sal) *(-0.16)*							0.284
Polychaetes	1	-3.00	+	2.82(pH) *(0.18)*											0.029
Bivalves	1	-0.08	+	154.52(SPM) *(0.37)*											0.135
	2	-28.64	+	133.80(SPM) *(0.32)*	+	3.85(pH) *(0.20)*									0.173
	3	58.90	+	167.99(SPM) *(0.39)*	+	4.49(pH) *(0.24)*	-	3.27(Temp) *(-0.21)*							0.210
	4	-0.34	+	167.09(SPM) *(0.39)*	+	4.11(pH) *(0.22)*	-	3.21(Temp) *(-0.21)*	+	1.74(Sal) *(0.16)*					0.235
	5	-8.401	+	178.81(SPM) *(0.43)*	+	6.11(pH) *(0.32)*	-	4.08(Temp) *(-0.27)*	+	2.16(Sal) *(0.20)*	+	4.20(Ch *b*) *(0.21)*			0.265
	6	-16.38	+	187.21(SPM) *(0.45)*	+	6.09(pH) *(0.32)*	-	3.65(Temp) *(-0.24)*	+	2.06(Sal) *(0.19)*	+	8.08(Ch *b*) *(0.41)*	-	7.73(Ch *a*) *(-0.23)*	0.279

	7	-13.70	+	216.28(SPM) *(0.51)*	+	7.32(pH) *(0.39)*	-	3.73(Temp) *(-0.25)*	+	1.77(Sal) *(0.16)*	+	4.99(Ch *b*) *(0.25)*	-	21.95(Ch *a*) *(-0.66)*	+	4.28(Ch *c*) *(0.62)*			0.301
Sponges	1	-22.85	+	3.10(pH) *(0.30)*															0.089
Forams	1	18.40	-	104.71(POM) *(-0.24)*															0.058
	2	86.24	-	100.79(POM) *(-0.23)*	+	1.96(Sal) *(-0.17)*													.087
Ascidians	1	-0.591	+	9.47(Ch *b*) *(0.48)*															0.226
	2	-36.06	+	11.69(Ch *b*) *(0.58)*	+	4.43(pH) *(0.23)*													0.267
	3	-41.42	+	10.71(Ch *b*) *(0.54)*	+	4.45(pH) *(0.23)*	-	2.22(Sal) *(-0.20)*											0.307
	4	35-78	+	10.77(Ch *b*) *(0.54)*	+	5.05(pH) *(0.26)*	-	2.14(Sal) *(-0.20)*	-	77.9(POM) *(-0.19)*									0.343
	5	-57.71	+	9.41(Ch *b*) *(0.47)*	+	3.56(pH) *(0.19)*	-	2.19(Sal) *(-0.20)*	-	118.9(POM) *(-0.29)*	+	3.78(Temp) *(0.25)*							0.387

When the relationship between the various classes of biofouling organisms and environmental parameters were analysed using PCA, four different components were extracted which together accounted for 68% of the original variance in the factors (Table 7.4). The loading for each component given in Table 7.4 represents the partial correlation between the variable and the rotated component.

Ascidians, maxillopods and chlorophylls *a*, *b*, *c* were extracted with strong to medium loadings with component 1. POM, SPM and water temperature strongly loaded with component 2. Component 3 had moderately strong loadings on polychaetes and forams and lesser loadings on sponges and pH, while component 4 had moderate loadings on bivalves and salinity. A component plot of the various components in rotated space is summarised in Fig 7.9.

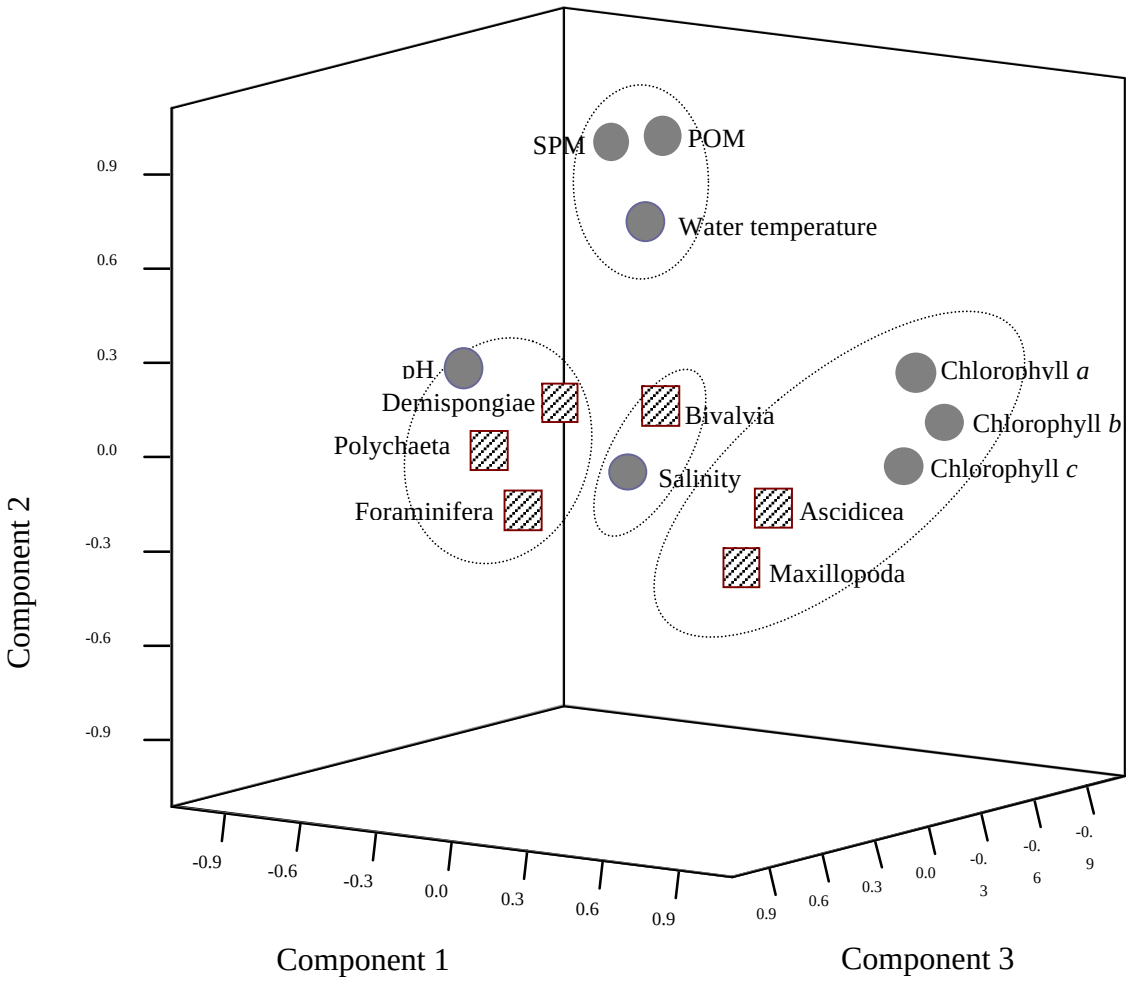

Fig. 7.9 Component plot of biofouling species and environmental parameters in rotated space. Rotation was by Varimax, with Kaiser normalisation.

Table 7.4 Rotated component matrix of PCA on biofouling classes and environmental data. Rotation method: Oblimin with Kaiser normalisation. Absolute partial correlation values less than 0.1 are suppressed.

Fouling classes and environmental parameters	Principal component				Communality
	1	2	3	4	
Maxillopoda	.598	-.245	.381		.594
Polychaeta			.803	-.111	.659
Bivalvia	.147	.102	.155	.836	.729
Demospongiae		.163	.499	.239	.355
Foraminifera		-.154	.604	-.520	.689
Ascidicea	.660		.366		.577
Water temperature	.161	.711	.170		.544
Salinity	-.190	-.172	-.129	.546	.390
pH	-.361	.190	.468	.320	.574
SPM	-.124	.905			.862
POM		.919	-.117		.872
Chlorophyll *a*	.910	.214	-.167		.894
Chlorophyll *b*	.928		-.164		.879
Chlorophyll *c*	.936		-.218		.915
Initial eigenvalues	3.942	2.586	1.934	1.071	
% Variance	28.159	18.474	13.812	7.652	
% Cumulative variance	28.159	46.634	60.445	68.097	

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Table 7.5 Correlation matrix from principal component analysis of biofouling species and environmental parameters. Asterisk (*) indicate significant correlation. Partial correlation less than 0.1 have been suppressed. [Temp: water temperature; Sal: salinity; pH; SPM; POM; Ch *a*: chlorophyll *a*; Ch *b*: chlorophyll *b*; Ch *c:* chlorophyll *c*].

		Maxillopoda	Polychaeta	Bivalvia	Demospongiae	Foraminifera	Ascidicea	Temp	Sal	ph	SPM	POM	Ch *a*	Ch *b*	Ch *c*
Correlation	Maxillopoda	1.000	.232			.340	.455		-.274	-.206	-.241	-.242	.396	.464	.427
	Polychaeta	.232	1.000		.204	.530	.205			.184			-.116	-.123	-.124
	Bivalvia			1.000	.173	-.270			.197	.279	.367	.350	-.100		-.115
	Demospongiae		.204	.173	1.000	.084		.185		.299	.160	.104		-.108	-.123
	Foraminifera	.340	.530	-.270		1.000	.168	-.118	-.180		-.216	-.242			
	Ascidicea	.455	.205			.168	1.000	.204	-.307		-.203	-.200	.465	.476	.438
	Temp				.185	-.118	.204	1.000		.249	.418	.441	.233		.121
	Sal	-.274		.197		-.180	-.307		1.000	.121			-.238	-.243	-.200
	pH	-.206	.184	.279	.299			.249	.121	1.000	.244	.159	-.359	-.482	-.496
	SPM	-.241		.367	.160	-.216	-.203	.418		.244	1.000	.924		-.146	-.161
	POM	-.242		.350	.104	-.242	-.200	.441		.159	.924	1.000	.113		
	Ch *a*	.396	-.116	-.100			.465	.233	-.238	-.359		.113	1.000	.846	.938
	Ch *b*	.464	-.123		-.108	.033	.476		-.243	-.482	-.146		.846	1.000	.898
	Ch *c*	.427	-.124	-.115	-.123		.438	.121	-.200	-.496	-.161		.938	.898	1.000
Sig. (1-tailed)	Maxillopoda		.000*	.144	.454	.000*	.000*	.120	.000*	.001*	.000*	.000*	.000*	.000*	.000*
	Polychaeta	.000*		.189	.001*	.000*	.001*	.064	.416	.003*	.353	.176	.035*	.027*	.027*
	Bivalvia	.144	.189		.003*	.000*	.312	.432	.001*	.000*	.000*	.000*	.060	.113	.036*
	Demospongiae	.454	.001*	.003*		.096	.255	.002*	.419	.000*	.006*	.052	.086	.046*	.027*
	Foraminifera	.000*	.000*	.000*	.096		.004*	.033*	.002*	.209	.000*	.000*	.354	.301	.356
	Ascidicea	.000*	.001*	.312	.255	.004*		.001*	.000*	.222	.001*	.001*	.000*	.000*	.000*
	Temp	.120	.064	.432	.002*	.033*	.001*		.422	.000*	.000*	.000*	.000*	.075	.027*
	Sal	.000*	.416	.001*	.419	.002*	.000*	.422		.032*	.297	.196	.000*	.000*	.001*
	pH	.001*	.003*	.000*	.000*	.209	.222	.000*	.032*		.000*	.007*	.000*	.000*	.000*
	SPM	.000*	.353	.000*	.006	.000*	.001*	.000*	.297	.000*		.000*	.420	.010*	.005*
	POM	.000*	.176	.000*	.052	.000*	.001*	.000*	.196	.007*	.000*		.037*	.131	.265
	Ch *a*	.000*	.035*	.060	.086	.354	.000*	.000*	.000*	.000*	.420	.037*		.000*	.000*
	Ch *b*	.000*	.027*	.113	.046*	.301	.000*	.075	.000*	.000*	.010*	.131	.000*		.000*
	Ch *c*	.000*	.027*	.036*	.027*	.356	.000*	.027*	.001*	.000*	.005*	.265	.000*	.000*	

7.4 Discussion

Previous literature on biofouling of pearl oysters has focused primarily on quantifying or identifying the progressive accumulation of various taxonomic groups of macrofouling organisms over time (e.g. Mohammad, 1976; Alargarswami and Chellam, 1976; Taylor *et al.*, 1997a; Pit and Southgate, 2003a; Scardino *et al.*, 2003; Guenther and de Nys, 2006). In those studies, oyster shells or culture units submerged for different periods of time were examined to identify colonisation patterns and successive settlement of various fouling taxa – this included initial colonisation as well as secondary recruitment of subsequent fouling organisms. This study examined only colonisation of macrofouling organism onto free substrata i.e. cleaned oyster shells, over time. Taylor *et al.* (1997a) established that the optimal cleaning frequency for *P. maxima* cultured in Indonesia was every four weeks. This cleaning frequency is now a routine in commercial culture operations for this species and prevents secondary infestation by subsequent hard-foulers (Taylor *et al.*, 1997a).

Biofouling can be quantified by scraping fouling organisms from the shells of oysters and measuring the wet weight (Dubost *et al.*, 1996) or dry weight (Smitasiri *et al.*, 1994; Taylor *et al.*, 1997a; Lodeiros *et al.*, 2002). Other measures include volume by displacement (Alagarswami and Chellam, 1976), determining the proportion of fouled oysters from a known total (Scardino *et al.*, 2003), counting the number of fouling organisms per oyster (Mohammad, 1976; Dharmaraj *et al.*, 1987) and measuring the percentage of fouling cover on shell surface using grids and point intersect (Mohammad, 1976; Meese and Tomich, 1992; Scardino *et al.*, 2003; Guenther and de Nys, 2006; Guenther *et al.*, 2006). The method used in this study was to measure the dry weight of fouling and count the proportion of oysters fouled with a particular fouling taxon. The decision to employ these methods was two-fold. Firstly, the short immersion time between sampling meant that minimal fouling emerged on shells and made measuring percentage cover impracticable. Secondly, measuring dry weigh and proportion of shells fouled enabled an approximation of the quantity of biofouling and the relative frequency of a particular class of biofouling over time and space.

Hard fouling was ubiquitous on shells of *P. maxima* during sampling as an accelerated rate of fouling is characteristic of many tropical environments (Lodeiros and

Himmelman, 1996). However, there were marked differences in the quantity and quality of novel biofouling communities emerging on *P. maxima* from the different treatments.

There is a vast amount of literature documenting seasonal variation in biofouling recruitment (e.g. Nair and Meenakumari, 1998; Lodeiros *et al.*, 2002; Rodriguez and Ibarra-Obando, 2008). Seasonal variation in biofouling is thought to be related to the seasonal reproductive periodicities of adult biofouling organisms and larval availability (Watson and Barnes, 2004) in the nearby vicinity and to varying patterns of settlement in different species. These, in turn, are governed by a complex interaction of parameters such as water temperature (Dubost *et al.*, 1996; Mazouni *et al.*, 2001; Rodriguez and Ibarra-Obando, 2008), salinity (Verween *et al.*, 2007), pH (Forrest *et al.*, 2007), nutrient availability (Hughes *et al.*, 2005), available substrate (Swain *et al.*, 1998), light (Dobretsov *et al.*, 2005), size and age of adult fouling species (Fong, 1994; Lardicci *et al.*, 1997; Watson and Barnes, 2004) and spatial distribution of planktonic larvae (Keough, 1984). As these parameters constantly change throughout the season and between sites, so do temporal and spatial patterns of biofouling recruitment, as was demonstrated in this study. For instance, dry weight of fouling on oysters in Aljui Bay varied significantly over time with the highest dry weight of 466 g recorded in January 2001 and the lowest of 149 g recorded in November 2000. In addition, oysters cultured at Ganan and Manselo showed higher levels of biofouling than oysters grown in Batu Terio. There was also a decrease in biofouling with depth. This observation has been reported in previous studies with other bivalves (Wallace and Reisnes, 1958; Claereboudt *et al.*, 1994). The three sites and two culture depths showed significantly differences in seawater temperature, salinity, pH and nutrient content (POM, SPM and chlorophyll) and it is these environmental parameters that are likely responsible for spatial variability in biofouling through affecting the biology (Keough, 1983) and nutritional requirement of biofouling.

Many invertebrates which constitute biofouling in the marine environment are sessile as adults and have short-lived larvae which disperse within relatively short distances (Watson and Barnes, 2004). The relative brevity of this study makes it difficult to evaluate patterns in reproductive periodicity of biofouling in Aljui Bay beyond a monthly scale, and inter-seasonal, sub-annual, or annual patterns would require a longer

term study. The seemingly stochastic nature of settlement seen in this study might reveal patterns of periodicity in a long term study.

Another factor which has been cited as influencing larval settlement of fouling organisms (but was not investigated in this study) is water current and velocity which might possibly modify larval settlement or dislodge settled organisms (Dubost *et al.*, 1996; Archambault and Bourget, 1999; Mazouni *et al.*, 2001). Future monitoring of water current at the three sites would assist in identifying if this factor has an effect on biofouling assemblage on *P. maxima*.

The six species of macro-fouling which settled on the shells of *P. maxima* during the experiment were invertebrates from the classes Maxillopoda, Polychaeta, Bivalvia, Demospongiae, Foraminifera and Ascidicea. While species heterogeneity was relatively low, the proportion of *P. maxima* fouled was high, with up to 80% of oysters exhibiting shell fouling during some sampling months. Archambault and Bourget (1999) reported that diversity and density of benthic community recruitment is influenced by the configuration of the shoreline, and with increasing bay size, diversity of recruited species increases while density decreases. Keough (1984) similarly proposed that the size of an enclosed body relates positively to the number of species present. In this study, all the experimental sites were located within the boundaries of outer Aljui Bay, an inlet which, due to its confined nature, is likely to have a hydrographic environment different from typical coastal conditions. The semi-enclosed nature of the bay and restricted water exchange may be a factor limiting species diversity and may also facilitate greater retention of planktonic propagules of fouling organisms. The presence of large surface area of suitable substratum in the form of culture structures and oyster shells further present greater opportunities for the settlement of planktonic larvae.

A high proportion of *P. maxima* were fouled with polychaetes and forams in this study. This was possibly due to adult sources of forams and polychaetes in close proximity to the culture systems as the recruitment of a species is often a reflection of the predominant species composition of the surrounding habitat (Holmes, *et al.*, 1997). The prevalence of polychaetes and forams over the sampling period suggests a contiguous reproductive cycle for the two biofouling organisms. Seasonal studies on forams showed some species reproduce continuously while others have reproductive peaks

during the year (Alve, 1999; Debenay *et al.*, 2006). Similar results have also been reported for various species of polychaetes (Fong, 1994; Lardicci *et al.*, 1997). Settlement of biofouling has been described as taking place uninterrupted, recurring in seasonal fluctuations or in irregular fluctuations over time (Keough, 1983; Watson and Barnes, 2004), and it would appear that the first scenario was the case with forams and polychaetes in this study.

Barnacle and ascidians were recorded in varying numbers throughout the sampling period. However, in contrast to forams and polychaetes which were found in high numbers throughout the experiment, these were only found in high numbers between June and November 2001, where up to 70% of oysters were found to be infested. A peak seasonal reproductive period for barnacles and ascidians encompassing those months of sampling would account for the fluctuation in proportions of *P. maxima* fouled, but it is not known if the reproductive period was sustained by continuous production of gametes or by a series of smaller separate spawnings by the biofoulers. Reproductive seasonality has been reported in barnacles (Patel and Crisp, 1960; Brown and Swearingen, 1998; Desai *et al.*, 2006; Yan *et al.*, 2006) and ascidians (Bates, 2005) and has been linked to water temperature, food availability, photoperiod, ocean hydrodynamics and proximity to co-specifics (Bates, 2005; Lambert, 2005). Therefore, reproductive periodicity for barnacles is likely to be site-specific depending on local environmental conditions and may vary according to geographic location and local climatic conditions. For example, Taylor *et al.* (1997a) reported high levels of barnacles settled on *P. maxima* farmed in northern Australia following the onset of the monsoonal rains around December to April, while the peak period for barnacle infestation in this study was June to November.

Bivalve fouling peaked in January and February 2001 and consisted of other pearl oysters of *Pteria* and *Pinctada* species. Heavy settlement of these biofouling bivalves fell shortly after the optimum spawning period identified for *P. maxima* cultured in Aljui Bay, which is between November to January (Knauer, Hatchery Manager, P. T Cendana Indopearls, *pers. comm.*, 2001). Most consisted of non-commercial *Pteria* and other *Pinctada* species although some *P. maxima* spat also recruited to the shells of cultured *P. maxima*. While forming part of the fouling complex which was sampled every month, it is noteworthy that they cannot be strictly considered an unwanted

fouling species as the settlement of *P. maxima* spat in the farm is an integral part of pearl production (Alagarswami, 1970). Infestation of cultured pearl oysters by other species of pearl oysters has been documented in prior studies (Alargarswami and Chellam, 1976; Doroudi, 1996; Taylor *et al.*, 1997a; Pit and Southgate, 2003a) and its negative impacts include competition for food and possible deformities to the shells of cultured oysters (Taylor *et al.*, 1997a).

Sponges fouled the least number of oysters in this study, with the peak period for sponge infestation the first four months of sampling from August to November 2000, when proportion of oysters fouled reached 30%. Sponges that fouled *P. maxima* during the study did not include the genus *Cliona*, which reportedly contributes the most damage to pearl oysters (Alagarswami and Chellam, 1976; Mao Che *et al.*, 1996) by boring and excavating cavities within the shells of the oyster (de Nys and Ison, 2008). Although clionid sponges were observed within the farm on other oysters, they did not constitute an initial colonist of free space on oysters used in this study.

Regression analysis showed that bivalves, followed by forams and polychaetes were the taxa which contributed most to biofouling dry weight on *P. maxima*. Although bivalves did not foul the greatest number of *P. maxima*, they have been identified as the species responsible for contributing most to fouling weight as shown by the highest standardised coefficient for bivalves ($\beta_{bivalve}$ = 0.33) in the regression analysis. The sharp increase in overall dry weight of fouling was restricted to January - February 2001 when bivalve settlement was most prolific. This observation could be used to help mitigate excess weight on culture lines. For example, more regular cleaning of *P. maxima* during and immediately after periods of peak bivalve settlement could be employed to reduce excess weight on culture structures.

Larger oysters in this study accumulated a greater biomass of fouling per unit length than medium or small oysters. Size is assumed to be a function of age in this study, so the conclusion that was drawn was older shells accumulated more biofouling than younger oysters. As the oysters were not further partitioned according to size, no further conclusion could be drawn as to whether it was age or size which exerted a greater effect on biofouling deposition. Guenther *et al.* (2006) reported that age, not size, was a factor in the accumulation of biofouling in the shells of *P. fucata*, and linked this to the

reduction or absence of periostracum, the hard chitinous outer sheath covering bivalve shells in older shells (Scardino *et al.*, 2003; Guenther *et al.*, 2006). Fouling is generally greater on older oysters where the periostracum layer has been abraded (Wahl *et al.*, 1998; Scardino *et al.*, 2003; Guenther *et al.*, 2006; de Nys and Ison, 2008). The implication of this for farm management is more frequent scheduled cleaning of older oysters to offset this.

Before considering the impact of fouling on oyster growth, it is necessary to discuss the impacts of environmental parameters on growth because bivalve growth is a function of several environmental variables acting in concert, as was indicated in Chapter 5. The only parameters to significantly effect growth in this study were SPM and pH, which together accounted for 10% of oyster G_{30}. This in itself is not evidence that other environmental factors did not influence growth; it may simply be an indication that during the relatively short sampling period, the ranges of environmental factors were insufficient to produce a measurable effect on growth. Overall, investigation into oyster growth rates in this study showed that growth was affected by time and age, but not site or depth of culture and is similar to the findings in Chapter 5. The shell size of small oysters increased more than that of medium and large oysters. This is consistent with previous bivalve literature which states that bivalve growth is rapid in the first years of life but progressively slows down with increasing age (Gosling, 2003) and has previously been shown in pearl oysters (Nasr, 1984; Gervis and Sims, 1992; Pouvreau *et al.*, 2000).

Bivariate correlation analysis showed G_{30} of medium sized and small oysters correlated to both quantity and diversity of biofouling. G_{30} of large oyster was not influenced by biofouling, indicating that fouling did not impair large oyster growth, possibly due to the optimal cleaning protocol for *P. maxima* recommended by Taylor *et al.* (1997) where minimal biofouling accumulated. Low biofouling may be insufficient to cause physical impairment of oyster shells or limit the opening of shell valves to reduce food intake in large oysters. Furthermore, as the sampling period overlapped the peak reproductive period for cultured *P. maxima* in Aljui Bay ((Knauer, Hatchery Manager, P. T Cendana Indopearls, *pers. comm*., 2001)), this might favour energy allocation toward reproduction over somatic growth in larger sexually mature oysters, where

intrinsic physiological processes of anabolism and catabolism (von Bertalanffy, 1957) influence oyster growth processes more than external factors such as biofouling.

While correlation provides a useful description of the relationships between oyster growth and fouling and allows prediction about how different types of fouling might affect oyster growth, it does not offer any conclusions regarding cause and effect. For example, it is not known if quantity of biofouling had a direct effect on oyster growth, or if a third variable might have exerted an influence on both oyster growth and fouling. Some authors report that biofouling had a deleterious effect on growth of oysters (e.g. Lodeiros and Himmelman, 1996; Lodeiros and Himmelman, 2000; Pit and Southgate, 2003a) while others reported a lack of a negative effect (e.g. Wallace and Reinsnes, 1985; Lodeiros *et al.*, 2002). Furthermore, it has been reported that biofouling may even have a favourable effect on oyster growth by increasing the planktonic food sources (Mohammad, 1976; Lodeiros *et al.*, 2002; de Nys and Ison, 2008). The correlation between biofouling and growth rate of *P. maxima* in this study was confined to small and medium sized oysters with relatively weak positive correlation (Pearson correlation strengths of < 0.50). However, there was no indication that biofouling exerted a detrimental effect on growth. This suggests that the cleaning regime recommended by Taylor *et al.*, (1997a) was adequate in preventing excess accumulation of biofouling from affecting growth of medium and small sized cultured *P. maxima*.

The cause of the mass mortality of *P. maxima* at Batu Terio in July 2001 is unknown, but statistical analysis showed it was not significantly correlated to biomass of fouling. However, correlation analysis did indicate that polychaete infestation was very weakly associated with oyster mortality. While polychaetes are known to prey on bivalves (Humphrey, 2008), carnivorous polychaetes *Palola* sp. and *Eunice* sp. did not cause mortality of juvenile black-lip pearl oysters, *P. margaritifera* in aquarium studies (Pit and Southgate, 2003a) and is unlikely to be the cause of oyster mortalities in this study.

While regression analysis provided information on environmental parameters acting in concert to affect biofouling, PCA gave an overall picture of the interaction between different biofouling taxa and environmental parameter. Together, they allowed examination of the interaction between various parameters, apportionment of environmental factors towards taxa of fouling and the degree a particular environmental

variable affects fouling. For example, in the rotated component plot from the PCA analysis, ascidians and barnacles were observed to coexist during the study period, while polychaetes, sponges and forams appeared together. Coexistence between suspension feeders has been reported before (Dalby and Young, 1993; Petersen *et al.*, 1997; Wahl, 2001) and has been suggested to occur through food resource partitioning i.e. each population of filter feeder utilises different sizes of food particles in the water column, or one may feed on the bio-deposits of another (Mazouni *et al.*, 2001). Coexistence might also be a reflection of the difference taxa responding to similar environmental requirements for settlement. From the structure of the component plot, it would appear that overall the six classes of biofouling were less influenced by SPM, POM and seawater temperature than by chlorophyll levels, pH and salinity for settlement.

The information gained from this study may be transferred to the field to assist in management of biofouling control in *P. maxima* culture in Indonesia. For example, the scheduling of a cleaning regime should consider oyster size and the interval between cleaning adjusted accordingly. Besides minimising biofouling, this would also ensure that valuable resources were not wasted in superfluous cleaning. This study also found that bivalves contributed the greatest proportion of biofouling by weight. When selecting sites to place new culture systems for pearl culture, it would be judicious to avoid sites with high bivalve infestations that could potentially add excess weight to the suspended culture systems and impair floatation. A preliminary survey of the benthic composition at prospective sites would provide information on potential biofouling at those sites, as the settlement of biofouling organism is often a reflection of the predominant species composition of the surrounding habitat (Holmes, *et al.*, 1997). Regression analysis showed the relationship between various environmental factors and taxa of biofouling. By monitoring the environmental parameters of potential sites and extrapolating the information obtained in this study, a prediction might be made on which site has favourable traits to reduce biofouling. Finally, the low mortality and high G_{30} of oysters in this study showed that the cleaning regime recommended by Taylor *et al.* (1997a) for *P. maxima* cultured in Bacan, North Maluku, Indonesia (Latitude 0.5 ° S, Longitude 127° E) was effective at keeping biofouling minimal and growth and survival optimal in *P. maxima* cultured at a different latitude in Indonesia.

CHAPTER 8

Factors affecting gender and gonad development in *Pinctada maxima* cultured at two sites and depths

8.1 Introduction

Artificial propagation of *P. maxima* has become more widespread in recent years due to advances in technology and hatchery techniques for this species. It has great significance in the pearl industry in Indonesia, as a regulated quota for hatchery production (Southgate *et al.*, 2008), such as that which exists in Australia, does not apply.

An essential aspect of hatchery production is selection of suitable broodstock for breeding. This is a management tool that can be utilise commercially to favour desirable traits such as fast growth, nacre colour or shape in the next generation of oysters (Le Moullac *et al.*, 2003). An understanding of the reproductive biology of the pearl oyster and the factors that govern gonad development is therefore important for the broodstock management of *P. maxima* in commercial farms.

Previous studies showed that the reproductive biology of different species of *Pinctada* pearl oysters are very similar, all being protandrous hermaphrodites (Wada, 1942; Tranter, 1958a, 1958b, 1958c, 1958d; Rose *et al*., 1990; Behzadi *et. al.*, 1997; Saucedo and Monteforte, 1997a). Protandrous hermaphrodites exhibit asynchronous hermaphroditism, with young oysters reaching sexual maturity as males first, after which gonads may become female and regular alternation may continue throughout life. For example, *P. maxima* from northwestern Australia were reported to reach male maturity in the first year of their lives when shell height is 110 – 120 mm (Rose *et. al.*, 1990) and female sexuality increases with age or size.

An optimal breeding program in mariculture requires an equal ratio of both sexes. In the wild, a sex ratio approaching 1:1 is found in *P. maxima* with shell height greater than 200 mm (Rose *et. al.,* 1990). However, previous research on *P. maxima, P. mazatlanica* and *Pt. sterna* under farm situations indicates bias towards male development (Saucedo and Monteforte, 1997a; Taylor, 1999).

Information on the various parameters that could affect gonad development and sex ratio is important to the commercial culturist. Favourable conditions for optimal growth may then be attained by manipulating the environment in which the oysters are grown. While culturing large numbers of oysters in a strictly controlled environment (i.e. in tanks) may not always be practical for the industry in terms of cost and effort, choosing a grow-out site with favourable conditions often is.

Given the importance of Indonesia as an emerging leader in pearl production, there has been a surprising paucity of information on the reproductive biology of *P. maxima* in Indonesian waters. The specific aim of this study was to investigate the quantitative aspects of sexual development in mature *P. maxima* cultured at two sites and depths in West Papua, with particular emphasis on the influence of environmental and biological conditions. Multivariate statistical methods which relate environmental and culture parameters to oyster growth and gonad development were used to assess variation in growth and development, and their relationships with environmental parameters. Comparison of sex ratio in two age and size classes of oysters cultured at two sites and depths was made to determine the effects of age and size as well as culture condition. From the results, recommendations are made towards developing management guidelines that would ensure *P. maxima* broodstock cultured in an environment most favourable for reproductive development and growth.

8.2 Methods and materials

All field experiments were conducted Cendana Indopearls' farm in Aljui Bay, West Papua, Indonesia (Section 3.1). Additional histological work was conducted at site at the School of Marine and Tropical Biology, James Cook University, Townville, Australia.

8.2.1 Timing of experiment

Effective broodstock management includes identifying an "optimum window" for gametogenesis (Lannan, 1980) and this was observed to be from October to February for *P. maxima* at Aljui Bay (Knauer, Hatchery Manager, P. T Cendana Indopearls, *pers. comm*., 2001). Previous unpublished data from the farm indicate that during the months of March through September, oysters held in Aljui Bay had little or no visible male or female gamete development. The gonadal area was flaccid and oysters were sexually indeterminate. As it was impractical to study sexual development during those months, the experiment was timed to start at the beginning of August 2001, with four weeks of prior conditioning beginning July 2001.

8.2.2 Sites

Ganan (00° 12' 35 S, 130° 19' 7 E) and Batu Terio (00° 12' 35 S, 130°19'7 E) were chosen as sites for the experiments. The selection of these two sites was made based on their geomorphologically and environmentally diverse characteristics (Chapter 4).

8.2.3 Oysters used in the experiment

Two age groups and two size groups of *P. maxima* were used in this experiment to determine whether age and size had an effect on sex ratio or reproductive development. The oysters were produced in P. T. Cendana Indopearls' hatchery in February 1999 and December 1999. Each cohort was produced from a single spawning of common parental stocks to reduce the influence of genetic variability on the results. They were grown-out in the ocean following standard farm husbandry regimes of grading and cleaning (Chapter 3). At the start of the experiment in July 2001, the ages of the oysters were 18 months (1.5 years) and 28 months (2.5 years). Oysters from the two cohorts are subsequently referred to as Year 1 and Year 2 oysters, respectively, in this Chapter.

This experiment was established to study the gender development of sexually mature *P. maxima*, not the onset of first maturity. Therefore, only animals older than 1 year were selected for this experiment as they were considered to have already reached sexual maturity (Rose *et al.*, 1990).

Six hundred and forty Year 1 oysters were selected and divided into two groups of 320; large oysters had an initial mean (± SD) antero-posterior shell length of 126.84 ± 6.22 mm and small oysters had an initial mean shell length of 92.81 ± 10.78 mm. Similarly, two sizes of Year 2 oysters were collected; 320 oysters with an initial mean shell length of 148.77 ± 7.16 mm and another 320 oysters with a mean shell length of 108.83 ± 11.59 mm. The oysters were all placed into 16-pocket panel nets, consisting of two 8-pocket panel nets tied one beneath the other (Chapter 3, Section 3.6.4).

8.2.4 Pre-experimental conditioning of oysters

Before the start of the experiment, all panel nets were covered by an open-ended shade cloth sleeve with a mesh pore size of 1 mm and placed on the seabed at a depth of 40 m for 4 weeks. This procedure reduced water flow, available oxygen and food; it is a weakening process used as a preoperative conditioning phase for pearl oysters to degenerate the musculature and gonad epithelium (Gervis and Sims, 1992).

The conditioning procedure was performed to induce all oysters into an equally inactive initial state and to reduce variability in condition. Periodic examination of the oysters was carried out during the 4-week conditioning period to determine gonad condition. The experiment commenced when all oysters showed no visible gonad development. No mortalities occurred during the conditioning period.

8.2.5 Experimental design

After the conditioning period, an equal number of large and small Year 1 and Year 2 oysters were removed to the experimental sites. The oysters were suspended at depths of 5 m and 15 m in 16-pocket panel nets from a surface longline in an identical manner at both sites (Fig. 8.1).

Sixteen groups of 80 oysters were formed. Each group was subject to different combination of variables, (i.e. site, age, size and depth) as outlined in Fig. 8.2. Each treatment group used five 16-pocket panel nets. In total, 1,280 oysters were used in the experiment. During the experiment, the oysters were not subject to the normal grading and cleaning husbandry practice undertaken at the farm. Instead, they were cleaned during sampling.

8.2.6 Sampling of oysters

Sampling was performed monthly from August 2001 to February 2002, with the exception of October 2001, where bad weather forced sampling to be cancelled. Oysters were transferred from the two sites to the laboratory and cleaned of surface fouling with a knife. Thirty oysters from each treatment group (Fig. 8.1) were randomly removed from the panel nets. Sampling was stratified so that an equal number of oysters were removed from each of the five panel nets holding a treatment group. The oysters were placed upright inside oyster baskets in a 1000 L tank constantly supplied with running seawater piped directly from the sea. Oysters were kept in this flow- through system at ambient salinity and water temperature for no longer than 2 h until sampled.

Wooden pegs were inserted into the antero-ventral corners of gaping oysters to allow for a sagittal inspection of oyster soft tissue. Closed oysters were gently prised open with reverse pliers inserted into the postero-ventral corner, and wedged with wooden pegs in the antero-ventral corner to keep the valves apart. Oysters were examined macroscopically for sex and gonad condition and measured for shell length, height, thickness and wet weight. Oysters were randomly placed back into the panel nets and returned to the longline immediately after sampling. Any dead oysters were removed from the nets and were not replaced.

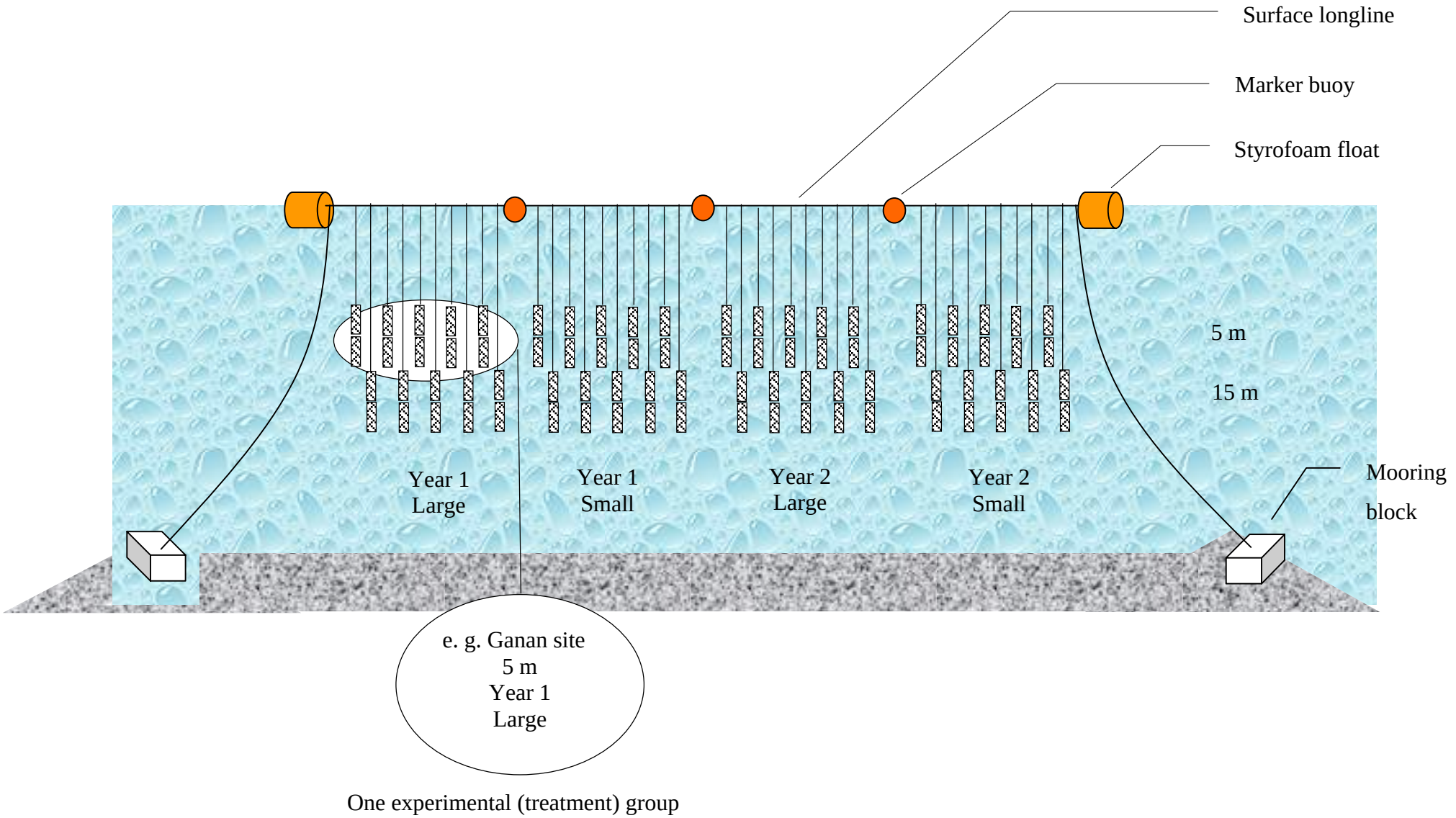


Fig. 8.1 Diagrammatic representation of the experimental longline set up at Ganan and Batu Terio.

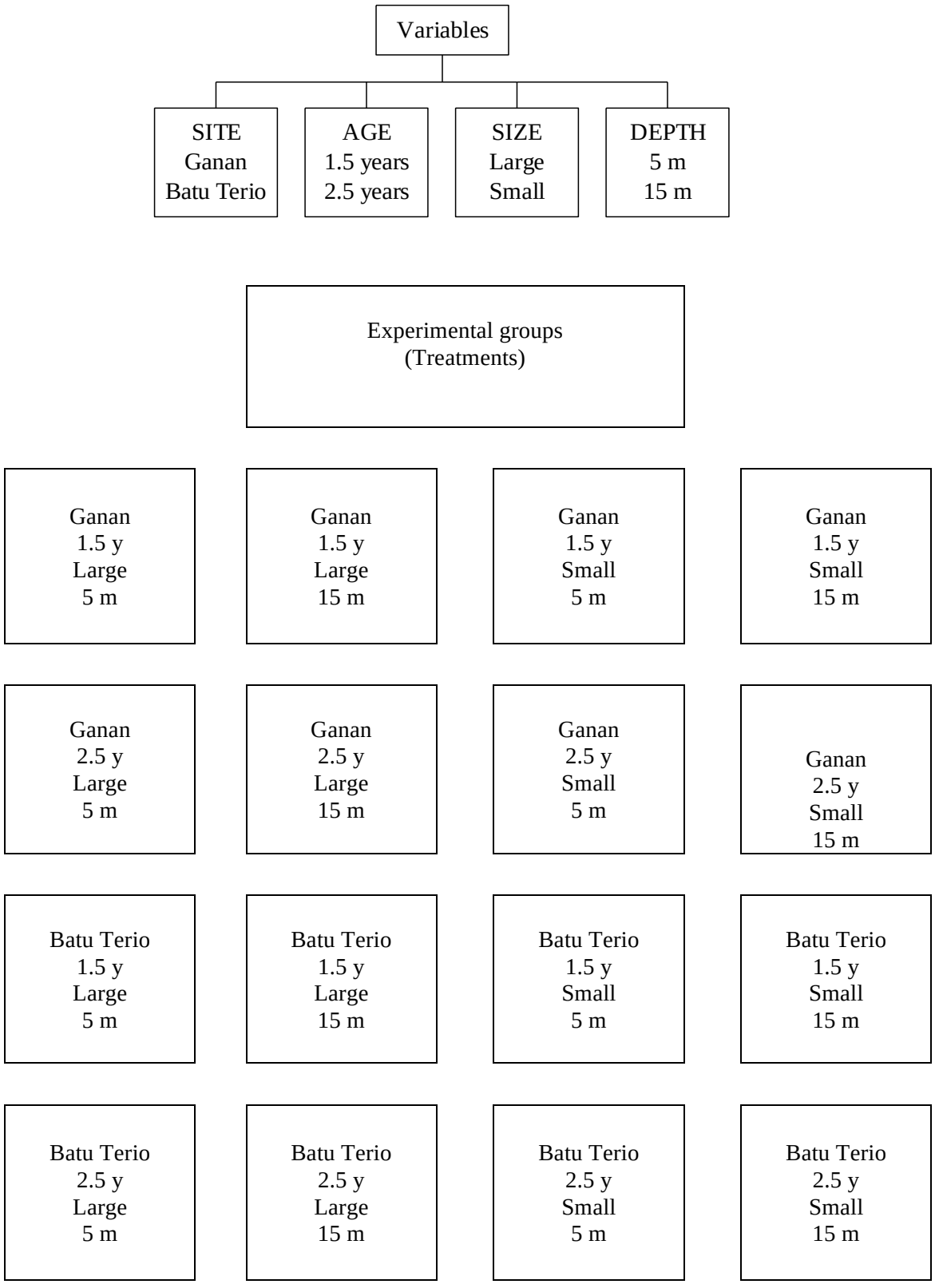


Fig. 8.2 Summary of variables and treatments of oysters. (y = years, m = meters)

8.2.6.1 Determination of sex and gonad index (GI)

Using a spatula to push aside the mantle and gills, each oyster was gently probed in the region of the digestive diverticulum (O'Connor and Lawler, 2004) to examine gonad development (Fig. 8.3). The digestive diverticulum is also referred to as the pearl-sac (Fougerouse-Tsing and Herbaut, 1994) and the gut loop (Tranter, 1958a).

A series of criteria for sexing and scoring different stages of gonad development based on the superficial appearance of gonads when examined macroscopically was developed for *P. maxima* by Taylor (unpublished data, 2000). These criteria were used to sex and score gonad development of oysters in this experiment from a scale of 0 to 6 (Table 8.1). Male and female oysters were distinguishable by the colour of the gonads; creamy-white for male and orange yellow for females. The colours are derived from the gametes that show through the transparent external epithelium (Tranter, 1958a).

The scale provided an indication of the ripeness of the oyster, similar to the scale described by Tranter (1958a), where the staging was based on the area occupied by gonad follicles relative to that of a ripe oyster. The term "ripeness" is used to indicate fullness of the gonad, and is distinguished from "maturity" which refers to adulthood (Tranter, 1958a).

Based on the scale developed by Taylor (2000), GI was calculated according to Gosling (2003) as follows:

$$GI = (S_n \times N_s)/N \qquad \text{(Equation 8.1)}$$

Where S_n is the numerical ranking of a particular stage (n = 0 to 6), N_s is the number of oysters from stage S_n, and N is the total number of oysters in the sample. Mean GI was the calculated for each sampling month.

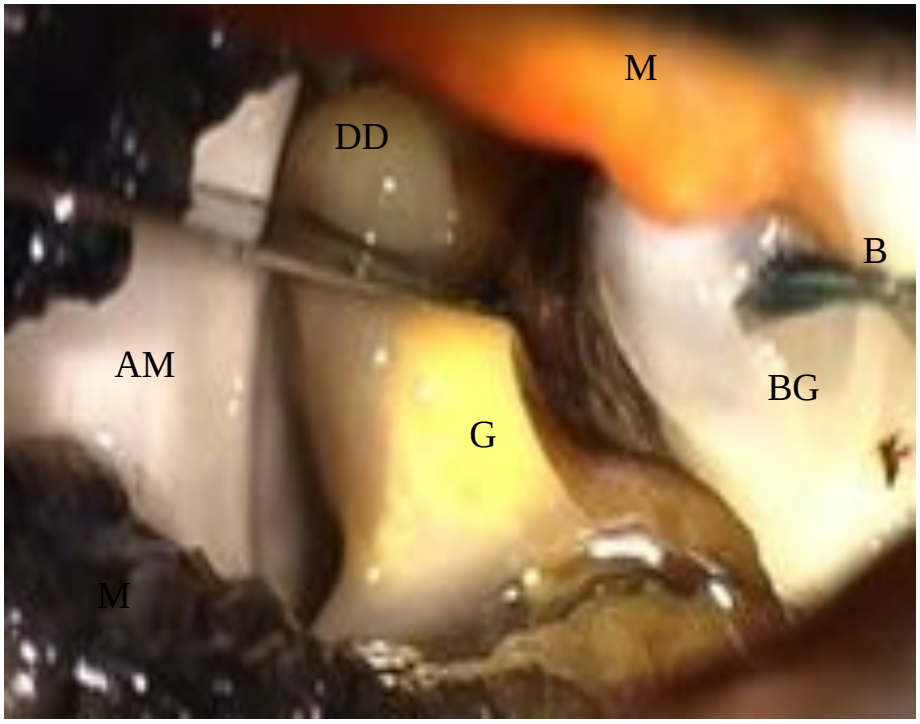


Fig. 8.3 Examination of gonad development around the digestive diverticular region of *P. maxima* using a spatula. AM: adductor muscle; DD: digestive diverticulum; G: gonad; M: mantle; B: byssus; BG: byssal gland.

Table 8.1 Criteria for macroscopic scoring of gonad condition in *P. maxima*. Scoring and description of gonad development are derived from J. J. Taylor (unpublished data, 2000) and adapted from Tranter (1958a). Gonad developmental stages are adapted from Garcia-Dominguez *et al.* (1996) and Saucedo and Monteforte (1997).

Score	Gonad Appearance	Developmental Stages
0	Gonad flaccid and sex indeterminate. Gonad area translucent and digestive diverticula visible.	Indeterminate and inactive or spent
1	Sex can be determined based on colour (cream white for males, yellow-orange for females) but development is slight and patchy. The gonad is still flaccid and the colour somewhat dull.	Developing
2	Development has progressed, with the gamete patches becoming larger and more closely associated. The sexes can be easily distinguished.	Developing
3	Patches of gametes more confluent and have taken on a cloudy appearance.	Developing and near ripe
4	Gonad is turgid; however, only one side of the gonad is well developed.	Ripe
5	Gonads now appear as dark bands of colour with both sides of the gonad well developed.	Ripe
6	Gonad is enlarged with development on either side of the gonad now confluent. The dark colouration has almost reached the distal end of the gonad; the gonad is turgid with a glossy appearance.	Ripe

8.2.6.2 Histological examination

Oysters were sacrificed, dissected and the gonad appearance recorded with a digital camera. The visceral mass encompassing gonad tissues was removed, fixed in a gonad fixative (FAACC, Appendix B) and processed for histology. A standard section of the visceral mass was taken and dehydrated through an increasing ethanol concentration series, embedded in paraffin and cut into 5 µm sections. Sections were mounted on glass slides, and stained with a solution of Hematoxylin and Eosin and examined microscopically. The criteria used to score gonad development stages through visual examination were confirmed through comparison with histological examination (Appendix C).

8.2.6.3 Oyster growth

Oysters were measured for length, height, and thickness to the nearest 0.1 mm using vernier callipers, according to the methods outlined in Chapter 2 (Section 2.3.1). Wet weight of each oyster was measured to the nearest 0.01 g using a digital balance. Prior to weighing, the outer shell surface of the oyster was blotted dry with paper towel. Growth of oysters over a particular month was measured with monthly instantaneous growth rate (G_{30}) defined by Brown (1988) as well as total growth (G_T) as outlined in Section 5.2.9.

Growth data for October was estimated by adding the average growth for September and November and dividing the result by 2.

8.2.6.4 Condition index (CI)

Two randomly selected oysters from each treatment group were sacrificed every month during the experiment. The oysters were cleaned of surface fouling, blotted dry and total wet weight determined using a balance. The oysters were dissected and the soft tissue, mantle and adductor muscle were separated and weighed to the nearest 0.01 g. Condition was calculated as outlined in Section 5.2.7.

8.2.7 Environmental monitoring

Environmental parameters were measured weekly from August 2001 to February 2002 according to the methods described in Section 3.5. As there was no growth sampling in December 2000, the corresponding environmental data for that month were excluded from the analysis.

8.2.8 Statistical Analyses

Graphical representation of sex and stages were used to compare gonad development between oysters from different treatments. The overall ratio of male to female oysters was tested with a χ^2 analysis to determine if the ratio differed significantly from an expected 1:1. A multivariate analysis of covariance (MANCOVA) was performed using indeterminate, male and female conditions the dependent variables, site, depth, age and size as fixed factors and time the covariate to determine if the occurrence of the different sex was different between treatments and over time. Interaction between the covariate and factors was tested to determine homogeneity of slopes. If the slopes were not significantly different, the use of ANCOVA was justified, i.e. the covariate did not have an effect on factors.

Correlation between G_T of length, height, thickness and wet weight was examined using pair-wise principal component analysis (PCA). Factors with eigenvalues greater than one (known as Kaiser's criterion) were retained and rotated by the Direct Oblimin method to produce two components. The new variables containing factor scores were entered into a dummy matrix with binomial values for site, depth, age and size, and set as the dependent variables for stepwise multiple regression analysis to establish which treatments were significant determinants of growth.

PCA was performed on G_{30} for oyster length, height, thickness and wet weight to produce one component. Factor score for the component was used to graph changes in monthly instantaneous growth rates. This eliminated the need to plot individual graphs for each growth parameter. G_{30} factor score was entered into univariate analysis of variance (ANOVA) to determine if site, depth, age and size had an effect on G_{30}.

Pearson's correlation analysis and stepwise linear regression analysis were applied to describe the relationships between CI and environmental parameters.

Relationships between the number of inactive, male or female oysters and length, height, thickness and weight as well as environmental parameters were tested using stepwise regression with the probability of F to enter set at ≤ 0.05 and the probability to remove ≥ 0.1. Significance of the regression was assessed with an ANOVA.

Prior to ANOVA, all data were tested for the homocedascity and normality using the Levene's and Shapiro-Wilk's test, respectively. In the event of a variable departing from the assumption of homocedascity, a $\log_{10}$ transformation was applied. When variance remained heteroscedastic after transformation, analysis of variance was performed nonetheless, as ANOVA is robust, operating well even within considerable heterogeneity of variances as long as all n are equal or nearly equal (Zar, 1984). The significance level (α) was set at 0.05 for all analysis of variance.

Discriminant analysis was performed according to the methods of Brown (1988) to determine if a classification scheme could be produced that might enable the sex of an oyster to be predicted based on the length, height, thickness and weight of the oyster, and the various environmental parameters. The degree of association between sex and environment was investigated using Pearson's correlation analysis and graphical representation of the data.

8.3 Results

8.3.1 Visual and histological inspection of gonads

Sagittal inspection of oysters established that gonads were not discrete organs. They were observed as gamete patches within the connective tissue around the digestive diverticulum and visible through the translucent epithelium. Histology confirmed that visual inspection of gonads corresponded to the various stages of gonad development (Table 8.1) described by Taylor (unpublished data, 2000) and an example of the early stages of oogenesis and spermatogenesis is given in Fig. 8.4.

Indeterminate, male and female oyster gonads were easily distinguished by their colour and appearance of the gonads. Indeterminate oysters did not display any obvious patchiness around the digestive diverticulum and the external surface appeared translucent when probed with a spatula (Fig. 8.5). In contrast, gonads of male oysters appeared as cloudy white patches clearly visible through the membrane of the digestive diverticulum (Fig. 8.6). Gonads of female oysters were similar in appearance to that of males with the exception that they were yellow orange in colour (Fig. 8.7)

8.3.2 Sex ratio

When the percentage of each sex was determined as a function of time, age and size, and site and depth, an overwhelming predominance of indeterminate and male oysters were observed. From 2,876 oyster examined throughout the experiment, 1,679 were indeterminate, 1,183 were males and only 14 were females. Females represented less than 1% of the total number sampled.

When sex ratio of oysters was investigated using a chi-squared (χ^2) test (Appendix B) to statistically determine if the occurrence of sexes were equal, results indicate that the overall ratio of males to females differed significantly from 1:1 ($\chi^2_{(0.05,\ 1)} = 1141.7$, $p < 0.05$). Sex was overwhelmingly skewed toward maleness. From the total samples analysed, the female: male sex ratio was 0.01:1. When the ratio was partitioned into age and size groups, the female: male sex ratios of Year 1 Large, Year 1 Small, Year 2 Large and Year 2 Small were 0.009, 0, 0.014 and 0.019 to 1, respectively, with marginally more females observed amongst older and larger oysters.

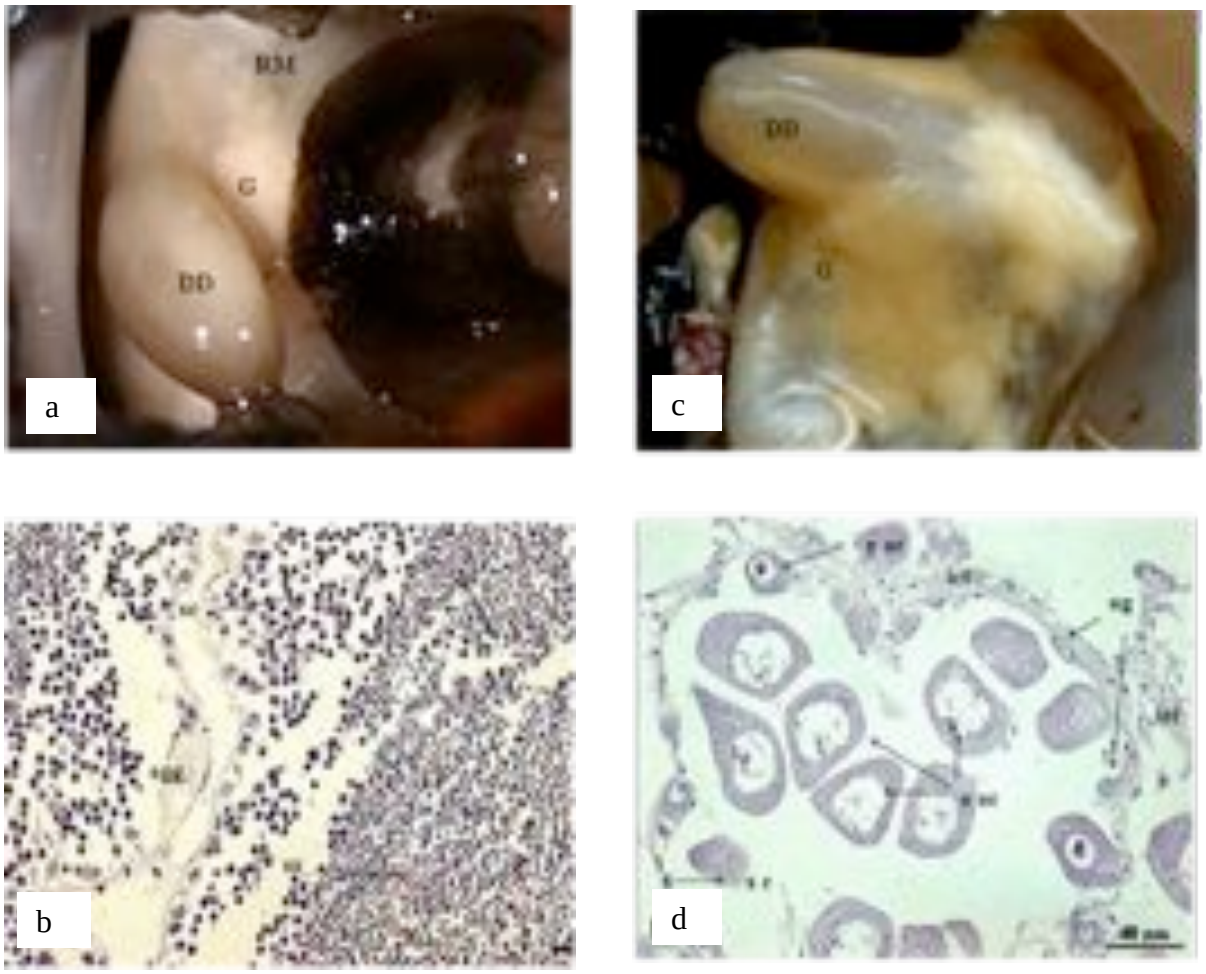


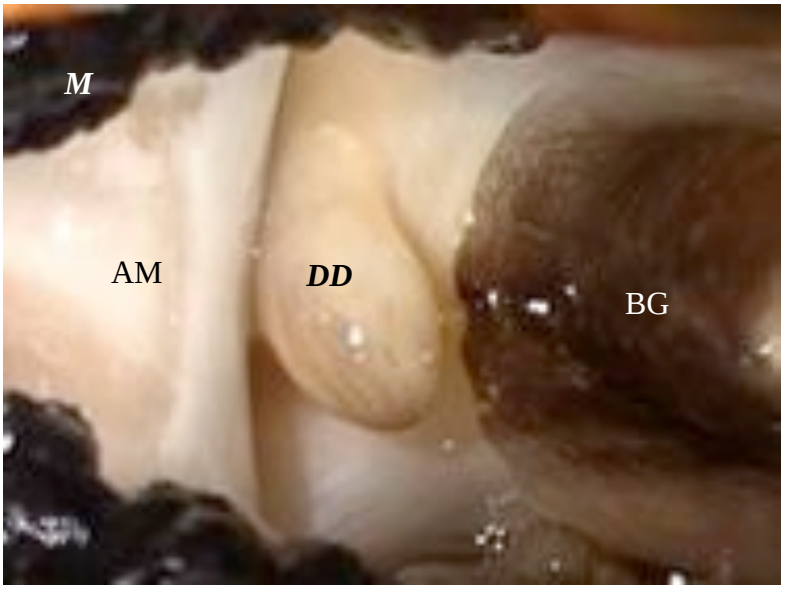
Fig. 8.4 Photo and micrographs of Stage 1 male (a) (b) and Stage 1 female (c) (d) *P. maxima.*

(a) Male gonad (G) is clearly visible as creamy white patches. Patches are visible at the base of the digestive diverticulum (DD) around the dorsal edge of the retractor muscle (RM).

(b) Early stages of gametogenesis. Large spermatogonia (sg) lining inner wall of acinus and sparse spermatocytes (sc) and greater number of spermatids (st) visible in the inner lumen. Tails of spermatozoa (sz) can be seen within the pockets of spermatids.

(c) Sex can be determined as female based on colour (yellowish-orange) but development is slight and patchy. The gonad (G) is still flaccid and the colour somewhat dull and development is only along the base of the digestive diverticulum (DD).

(d) Early oogenesis. Acinus show some stem cells (sc), oogonia (og) and young oocytes (y oc) developing along the inner wall. Larger and older oocytes (o oc) are found further within the acinar lumen. Interconnective tissue (ict) support area between acini.



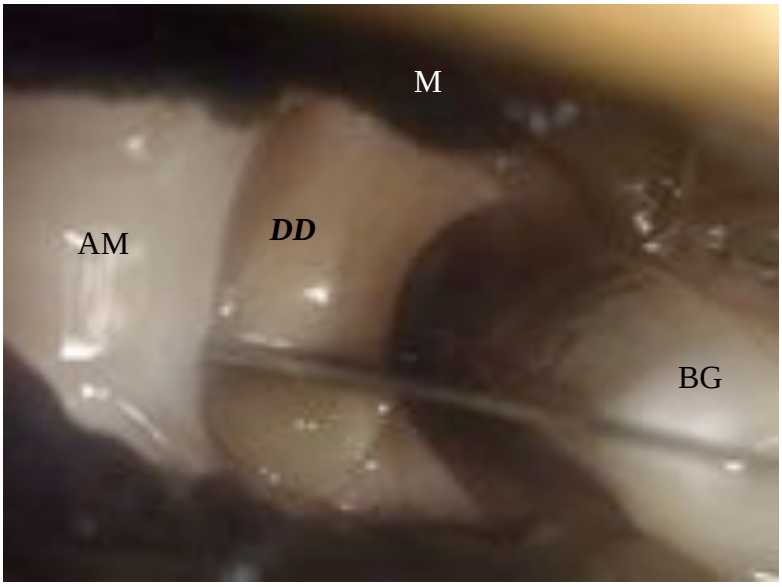
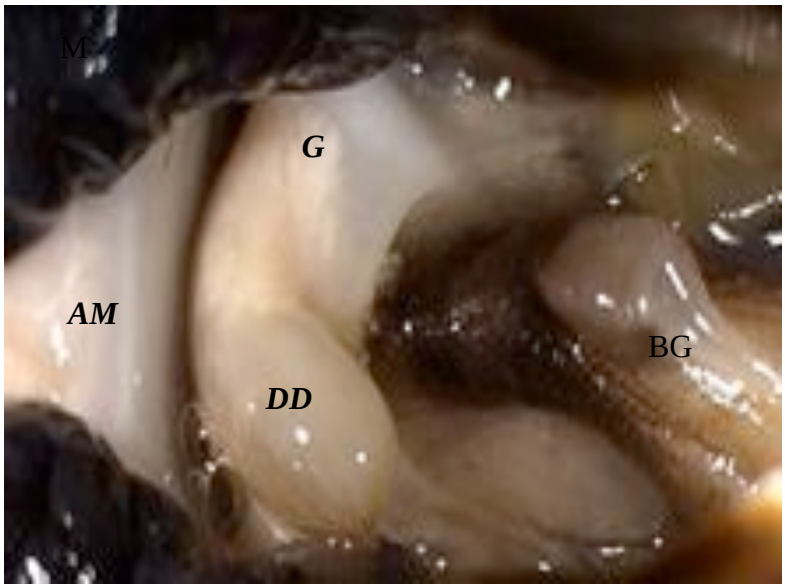
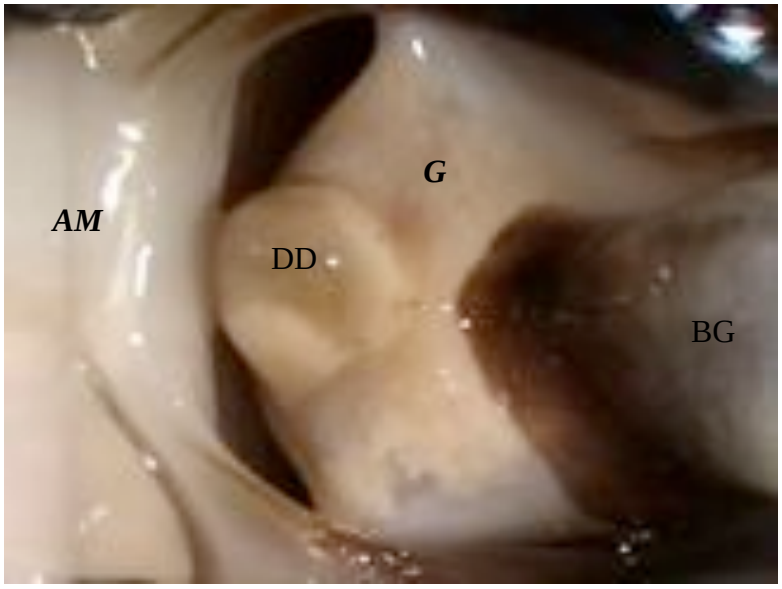


Fig. 8.5 Digestive diverticulum of an indeterminate oyster.
AM: adductor muscle; *DD*: digestive diverticulum; *M*: mantle; *BG*: byssal gland.



(a)



(b)

Fig. 8.6 Gonad of a Stage 1 (a) and Stage 4 (b) male oysters.
AM: adductor muscle; *DD*: digestive diverticulum; *G*: gonad; *M*: mantle; *BG*: byssal gland.

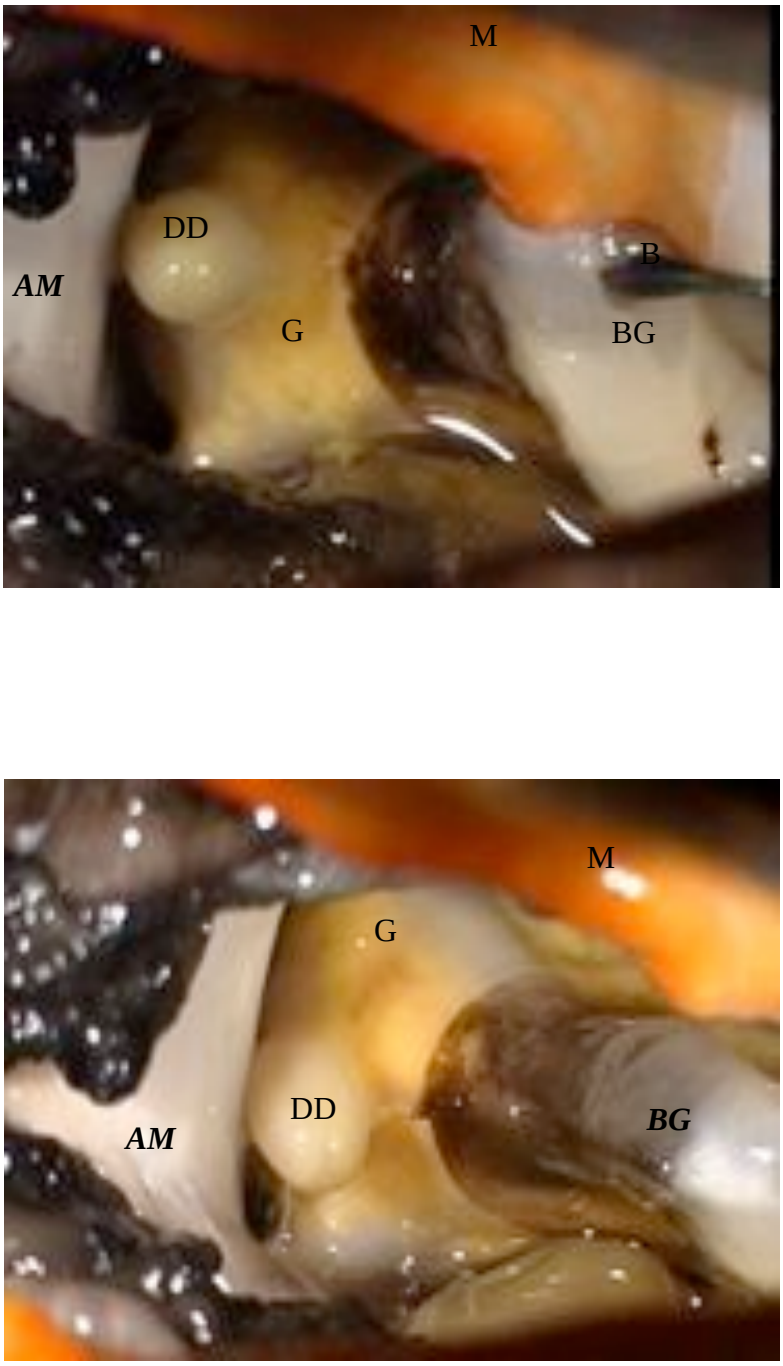


Fig. 8.7 Gonads of a Stage 4 female oyster.
AM: adductor muscle; *DD*: digestive diverticulum; *G*: gonad; *M*: mantle; *B*: byssus; *BG*: byssal gland.

8.3.2.1 Temporal effect

Multivariate analysis of variance (MANCOVA) results (Appendix 1.8.2) established that the number of indeterminate, male and female oysters differed significantly over time ($F_{(1,79)} = 5.371$, $p < 0.05$). While male and indeterminate oysters were present over the entire sampling period in varying proportions, female oysters were only observed from September onwards (Fig. 8.8). Overall, the percentage of indeterminate oysters was highest at the start of the experiment in August (77%). This number progressively decreased, with the lowest percentage observed in November (< 50%), after which the percentage began to increase again. In contrast, the number of male oysters was inversely proportional to indeterminate oysters, with the lowest proportion observed in August (22%) and largest observed in November (52%). No female oysters were observed in August while November recorded the highest percentage (1%).

8.3.2.2 Age and size effect

MANCOVA (Appendix 1.8.2) showed that age and size had an effect on the number of male, female and indeterminate oysters (Age: $F_{(1,79)} = 7.77$, $p < 0.05$; Size: $F_{(1,79)} = 32.52$, $p < 0.05$). A summary of the sex ratios from the four experimental age/size categories indicated that the greatest incidence of males and females occurred in large oysters older than 2 years (Fig. 8.9). The maximum number of indeterminate oysters was observed in the small classes of oysters from both age groups. The larger classes of both age groups had a higher proportion of males (46.3% and 69.4% for Year 1 and Year 2, respectively) than smaller oysters (21% and 28.1% for Year 1 and Year 2, respectively).

A breakdown of the different size classes showed that males were seen in oysters with shell lengths from 80 mm to > 170 mm, with the majority (67%) occurring between 130 to 160 mm shell length. Females, on the other hand never appeared in oysters with shell length under 100 mm. Most of the females observed in this experiment (65%) were found to have shell lengths of 140 mm or more. Indeterminate oysters were observed over the whole size range (Fig. 8.10). Males were present at a smaller size range while females only appeared as larger oysters.

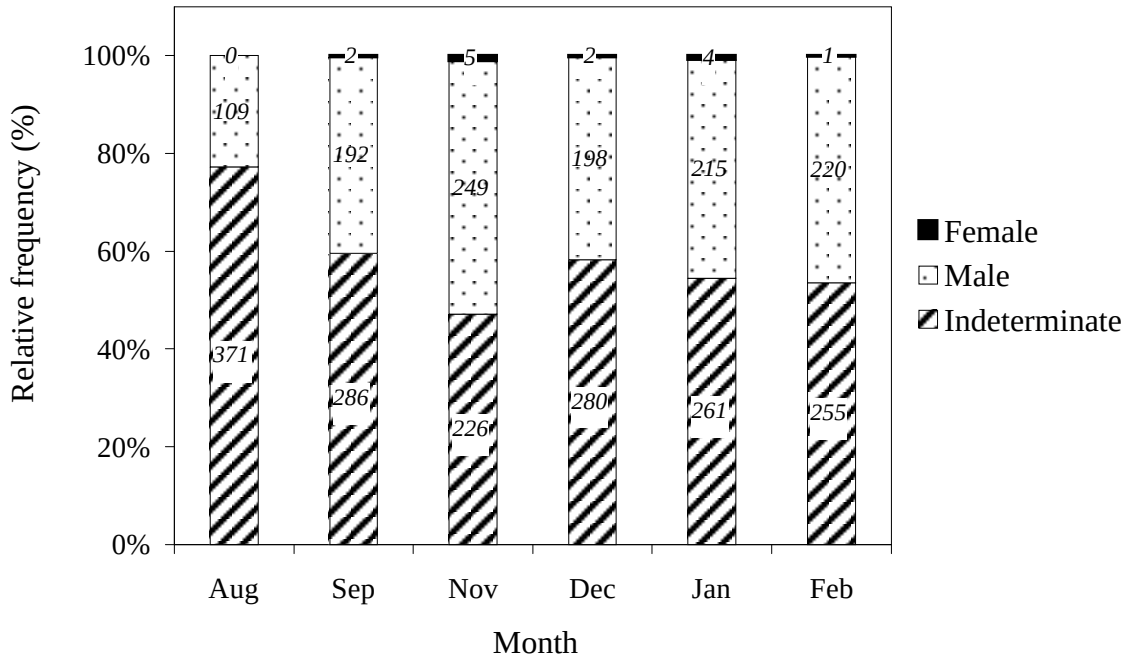


Fig. 8.8 Temporal sex ratio of oysters from all groups. Italic numerals in column represent the number of oysters of a particular sex.

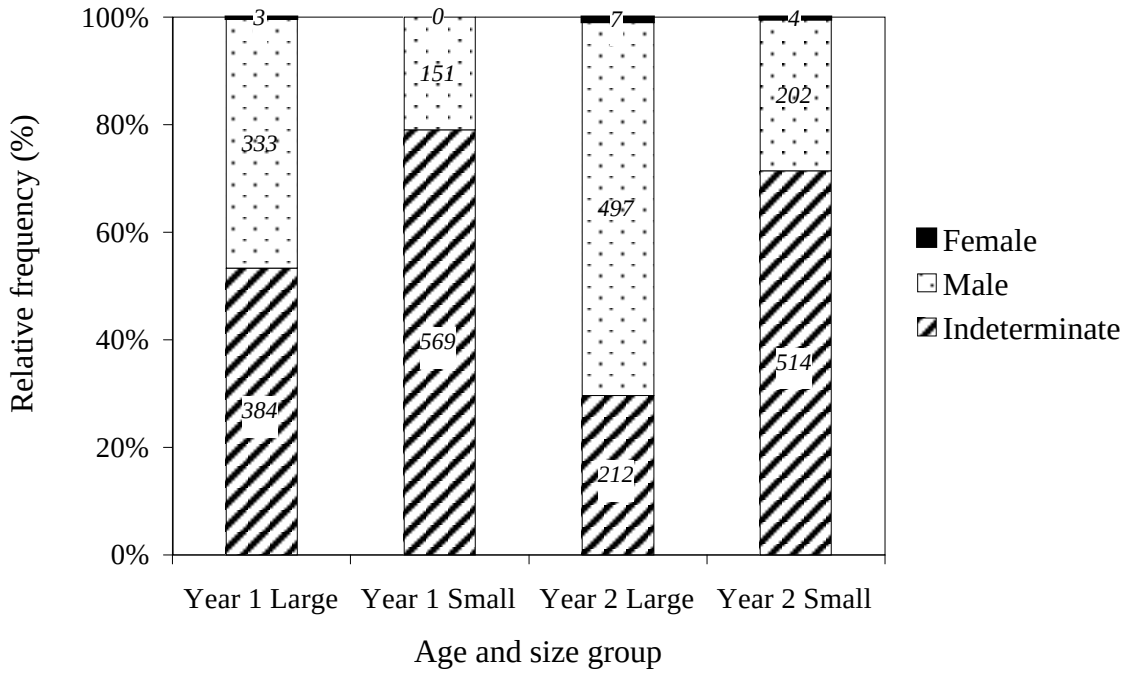


Fig. 8.9 Sex ratio of four categories of oysters from two sites and two depths. Italic numerals represent number of a particular sex in the category.

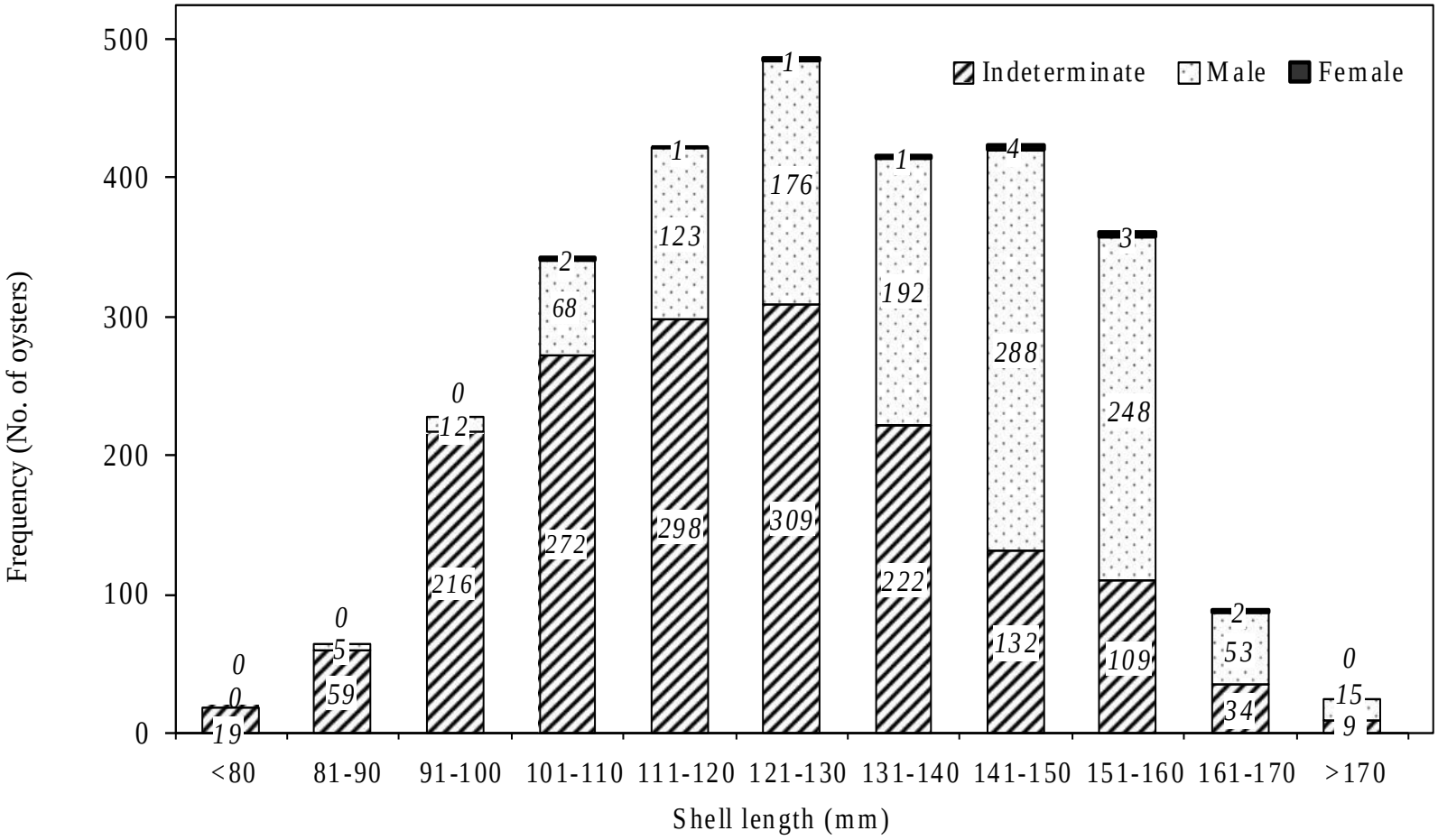


Fig. 8.10 Size frequencies and shell length-related sex ratios of all oysters sampled during the experiment. Italic numerals indicate number of oysters of a particular sex in the size class.

Both sexes and indeterminate oysters were present in the two age groups in varying proportions. Sex ratio as a function of age showed overall, Year 2 oysters accounted for 60% and 78% of all males and females observed during the experiment (Fig. 8.11). The opposite was true for indeterminate oysters, where the majority (57%) was observed in the oysters less than 2 years old. A high percentage of Year 1 oysters (66%) were indeterminate as compared to Year 2 oysters where only approximately 50% were indeterminate.

8.3.2.3 Spatial effect

Analysis of graphical data from all oysters sampled throughout the experiment indicated there was only a marginal difference in the sex ratio in oysters cultured at the two sites and depth (Fig. 8.12). MANCOVA (Appendix 1.8.2) revealed sex ratio was not significantly different between the Ganan and Batu Terio sites ($F_{(1,79)} = 2.14$, $p > 0.05$) or between depths of 5 m and 15 m ($F_{(1,79)} = 2.43$, $p > 0.05$).

The largest ratio of males and females to indeterminate oysters (male + female : indeterminate) was observed in oysters cultured at Batu Terio at a depth of 5 m (1 : 0.9) while the ratio of males and females to indeterminates were lowest in Ganan at a depth of 15 m (1 : 1.88).

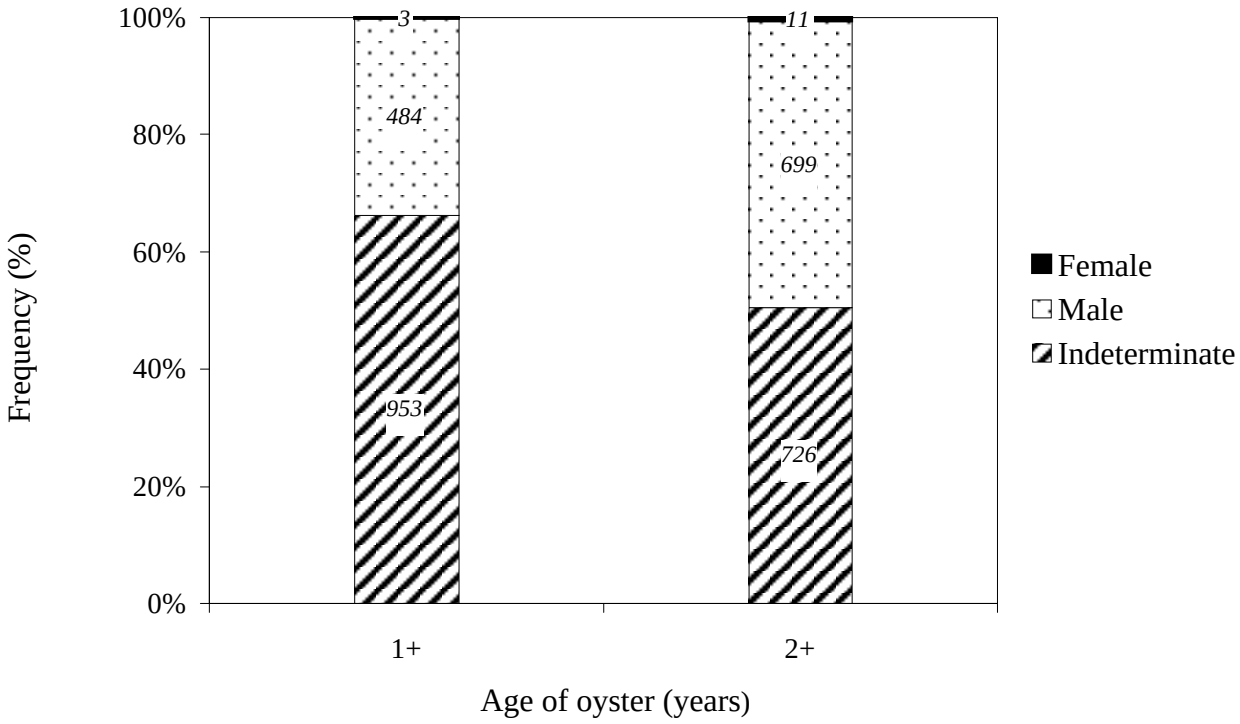


Fig. 8.11 Age-related sex ratio in oysters. Italic numerals in column indicate number of a particular sex in that age class.

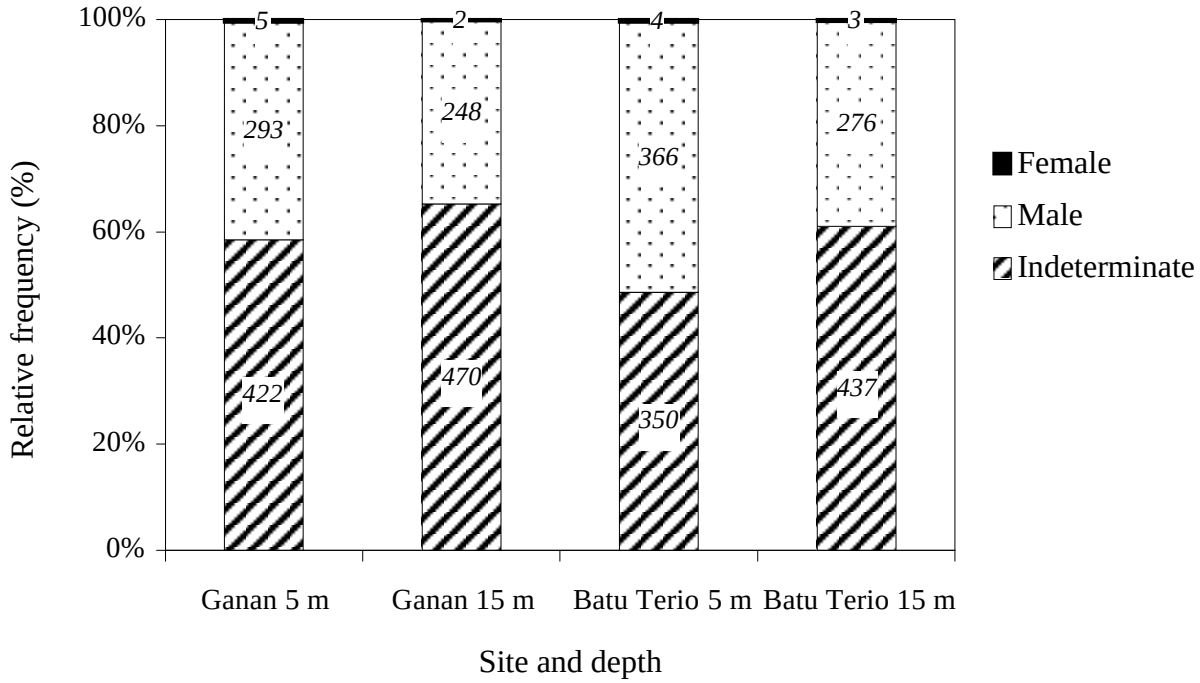


Fig. 8.12 Site and depth-related sex ratio in all oysters sampled during experiment. Italic numerals indicate number of a particular sex in the site and depth group

8.3.3 Gonad developmental stages and GI

The combined frequencies of male and female oysters in various gonad developmental stages are shown in Fig. 8.13. It was established that indeterminate oysters were highest in numbers in August. Stages 1, 2 and 3 were present early in the experiment and continued to be present throughout most of the sampling period. In contrast, Stages 4 and 5 were found mostly from December onwards, although a few were observed in September. Oysters in Stage 6 of development were only observed in December and January. No spawning activity was observed during sampling period. GI for each stage is shown in Table 8.2 . In addition, the mean GI for each sampling month was plotted and shown in Fig. 8.14. The GI rose in August and peaked in September before falling slightly in November. In December and January, the index increased again before falling in February.

Table 8.2 GI for the different stages of gonad development for each sampling interval.

	Gonad Stage						
	0	1	2	3	4	5	6
Aug	0	0.156	0.096	0.069	0.000	0.000	0.000
Sep	0	0.140	0.333	0.181	0.083	0.083	0.000
Nov	0	0.342	0.246	0.188	0.008	0.000	0.000
Dec	0	0.192	0.117	0.144	0.300	0.177	0.050
Jan	0	0.235	0.071	0.163	0.408	0.115	0.038
Feb	0	0.300	0.143	0.000	0.277	0.116	0.000

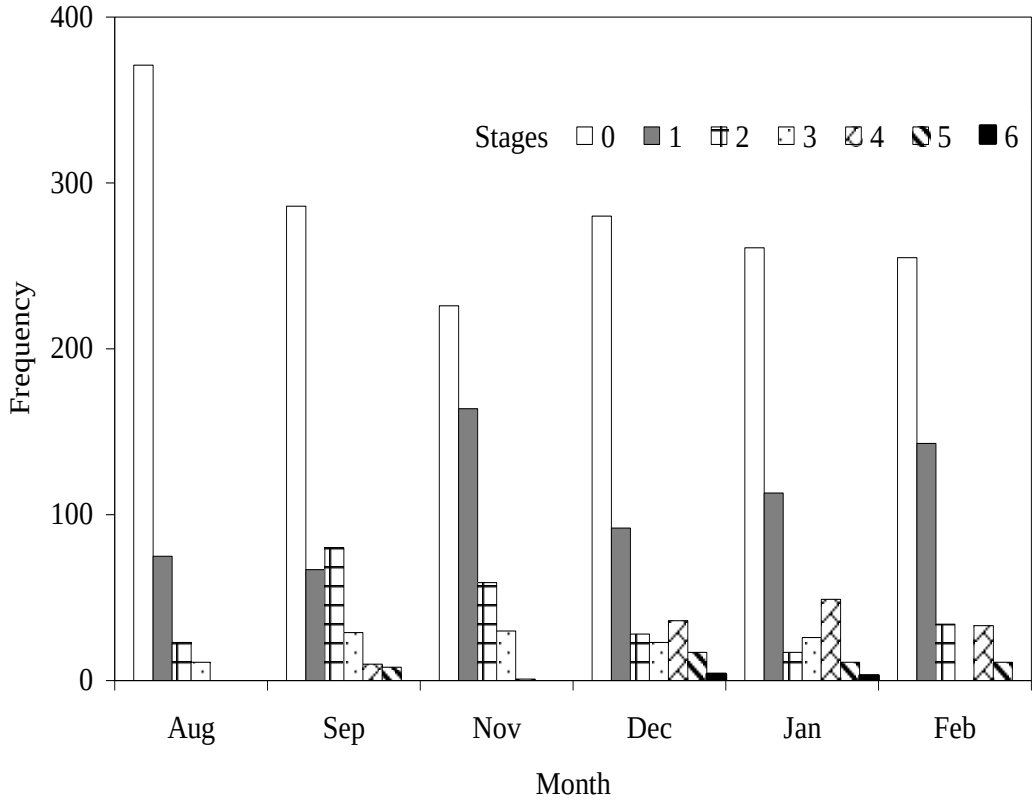


Fig. 8.13 Combined gametogenic stages in male and female oysters from August 2001 to February 2002.

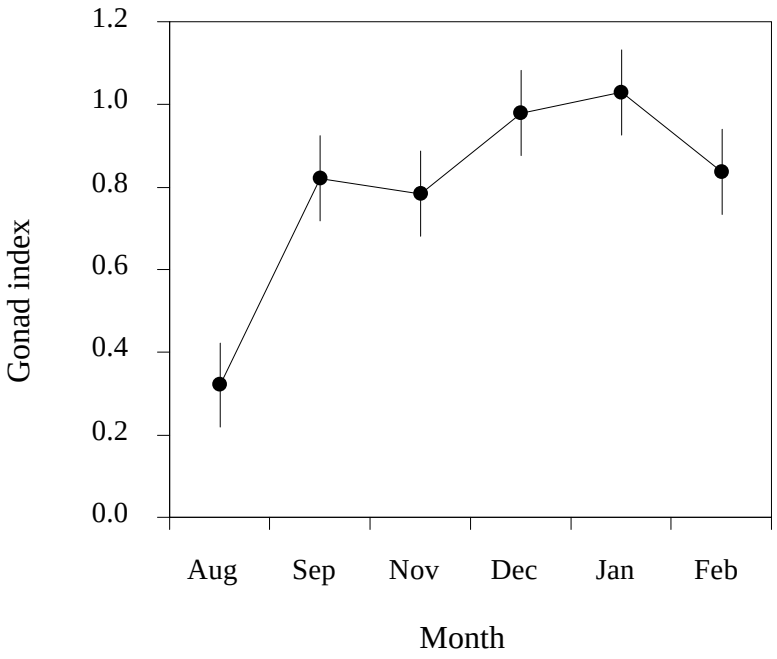


Fig. 8.14 Monthly mean (± SE) GI for *P. maxima* cultured at Ganan and Batu Terio at 5 m and 15 m depths.

8.3.4 Oyster growth

8.3.4.1 Total growth (G_T)

Overall, shell length, height, thickness and wet weight of all oysters increased over the sampling period. Total growth (G_T) was positive (Table 8.3). When G_T was subjected to PCA (Appendix 1.8.3), it was found that length and height were strongly correlated to each other ($p < 0.05$) and could be extracted as component 1, while thickness and weight, though not significantly correlated, were strongly loaded to component 2 (Fig. 8.15). Together the components accounted for 73% of the variance (Table 8.4). Factor scores were used to produce two new variables named LH (Length and height) and TW (Thickness and weight). When the new variables were introduced into a stepwise multiple regression analysis (Appendix 1.8.4) using binomial values for site, depth, age and size (Appendix 1.8.2), regression of LH yielded: LH = -0.680 – 1.361 (Year 1) – 0.981 (Large) with r = 0.86, while the regression of TW = -0.508 + 1.015 (Large) with r = 0.52.

The regression results indicate age and size accounted for variation in length and height but did not indicate what underlying processes may be responsible for this relationship. Shell thickness and weight appear to be less affected by size and not by age. Culture site and depth did not have an effect in the total growth of shell length, height, thickness and weight.

8.3.4.2 Growth rate (G_{30})

Growth rate of shell length, height and thickness was positive throughout the sampling period. Weight G_{30} was mostly positive with the exceptions being small Year 1 oysters cultured at a depth of 5 m at Ganan and large Year 1 oysters cultured at a depth of 15 m in Batu Terio during August, as well as small Year 2 oysters cultured at 5 m and 15 m in Batu Terio during February, when the G_{30} was negative.

Table 8.3 Mean (± S.D) initial and final length, height, thickness and weight of different age and size classes of oysters, and the total average growth over the experimental period.

	Large oysters				Small oyster			
	Length (mm)	Height (mm)	Thickness (mm)	Weight (g)	Length (mm)	Height (mm)	Thickness (mm)	Weight (g)
Year 1								
Initial	126.76	124.13	27.22	218.12	94.23	93.98	20.58	97.87
	(± 6.03)	(± 6.03)	(± 2.14)	(± 29.05)	(± 9.06)	(± 10.18)	(± 2.63)	(± 21.25)
Final	141.76	139.95	31.59	343.81	118.90	117.53	25.85	197.34
	(± 11.08)	(± 10.97)	(± 2.93)	(± 47.08)	(± 15.40)	(± 12.01)	(± 4.55)	(± 52.35)
T_G	15.00	15.82	4.37	125.69	24.67	23.55	5.28	99.47
Year 2								
Initial	148.27	144.91	32.59	381.27	108.83	111.42	25.45	179.77
	(± 6.89)	(± 8.59)	(± 2.95)	(± 41.89)	(± 11.59)	(± 10.56)	(± 2.55)	(± 40.46)
Final	154.23	152.74	35.33	483.25	121.38	123.30	28.96	252.90
	(± 7.31)	(± 7.95)	(± 6.20)	(± 75.92)	(± 16.75)	(± 14.07)	(± 6.68)	(± 64.12)
T_G	5.98	7.83	2.74	101.98	12.55	11.88	3.51	73.13

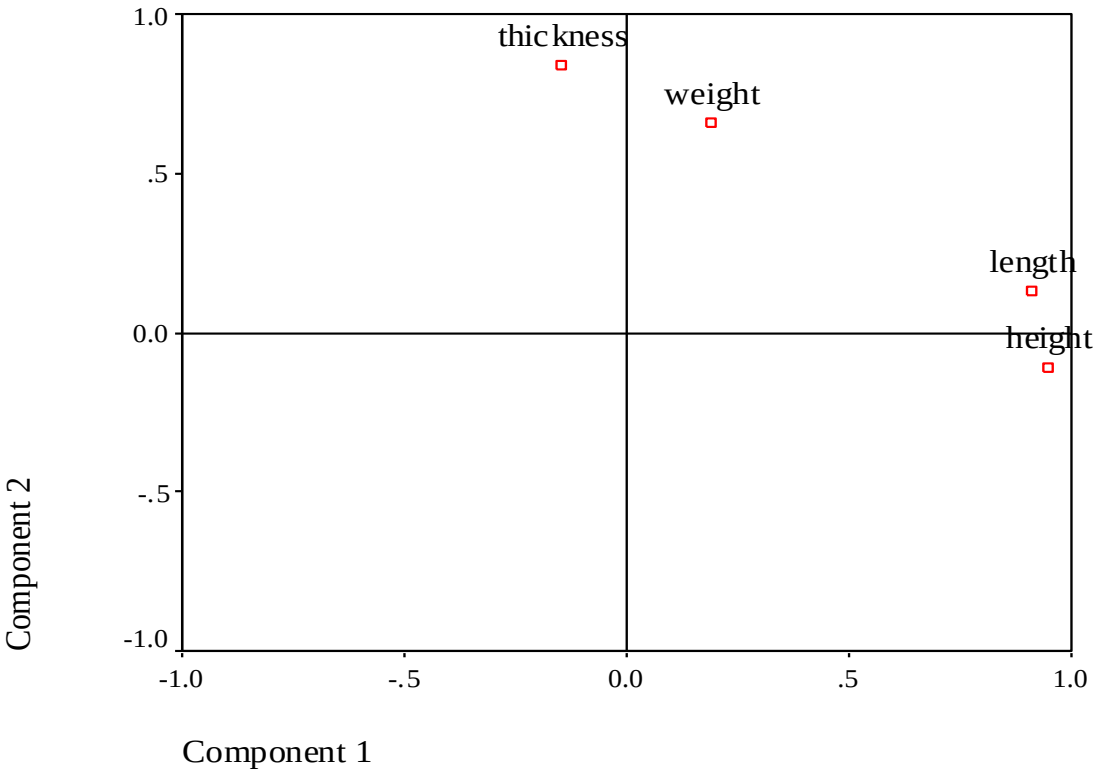


Fig. 8.15 Component plot of G_T parameters in rotated space.

Table 8.4 Rotated component matrix of PCA on G_T of height, length, thickness and weight. Rotation method: Promax with Kaiser normalisation

Growth parameter (G_T)	Principal components		Communality
	1	2	
Height	.937	.011	0.890
Length	.924	.246	0.870
Thickness	-.050	.821	0.677
Weight	.262	.683	0.498
Initial eigenvalues	1.83	1.12	
% Variance	45.84	28.06	
% Cumulative variance	45.84	73.91	

Table 8.5 Rotated component matrix of PCA on G_{30} of height, length, thickness and weight. Rotation method: Promax with Kaiser normalisation.

Growth parameter (G_{30})	Principal component	Communality
	1	
Height	.942	.882
Length	.939	.887
Thickness	.925	.522
Weight	.723	.856
Initial eigenvalues	3.14	
% Variance	78.63	
% Cumulative variance	78.63	

PCA of G_{30} (Appendix 8.5) resulted in one component being extracted that explained 78% of the variance in the factors (Table 8.5). The factor scores for the principal component were used to plot changes in G_{30} and to compare difference in growth of oysters. As only one component was extracted, G_{30} of shell length, height, thickness and weight was grouped and discussed as a single entity.

8.3.4.2 (a) Age, size and temporal effect

When an ANOVA (Appendix 1.8.6) was performed on G_{30} as the dependent variable, the results indicated that age, size and sampling time had a significant effect on growth rate (Age: $F_{(1,4)} = 78.92$, $p < 0.05$; Size: $F_{(1,4)} = 47.73$, $p < 0.05$; Sampling: $F_{(4,4)} = 54.8$, $p < 0.05$). There was significant interaction between age of oyster and time of sampling ($F_{(4,4)} = 27.81$, $p < 0.05$). Post-hoc test confirmed that G_{30} for September differed from all the other months.

With few exceptions, G_{30} of Year 1 oysters were consistently higher than Year 2 oysters (Fig. 8.16). For both age classes, smaller oysters appear to have higher G_{30} than larger

oysters. The temporal variation in G_{30} could be observed with maximum growth rates occurring in September for both age and size groups and minimum growth occurring in November (Fig. 8.16 and Fig. 8.17).

8.3.4.2 (b) Spatial effect

While G_{30} was not significantly different between oysters cultured at Ganan and Batu Terio and between the two depths when tested with an ANOVA (Appendix 1.8.6), the average G_{30} of oysters cultured at 5 m was higher than oysters cultured at 15 m at both sites in December and January. When G_{30} scores were plotted as a function of site and depth, growth rate for both sites were observed to peak in September (Fig. 8.17).

8.3.5 Condition index

CI for large and small Year 1 and Year 2 oysters peaked at the start of the experiment in August then dropped sharply in September, before gradually reaching a second peak in December (Fig. 8.16). Temporal changes in CI appeared to be reflected by inverse changes in monthly instantaneous growth rates - the decline in CI of oysters in September coincided with a peak in the monthly instantaneous growth, while the rise of the index in December was inversely reflected in a drop in monthly instantaneous growth rate during that month.

According to linear regression (Appendix 1.8.7) of $\log_{10}$ CI and environmental data, water temperature, salinity, pH, rainfall, SPM, POM, chlorophyll *a*, *b*, and *c* explained only 18% ($p < 0.05$) of the variation in the sampled oysters. Based on standardised partial regression coefficients (β) that indicate the relative importance of independent factors upon the dependent variable (Zar, 1984), chlorophyll *a* ($\beta = -1.04$) chlorophyll *b* ($\beta = -0.73$), chlorophyll *c* ($\beta = 1.77$), water temperature ($\beta = 0.47$) and salinity ($\beta = 0.34$) had the greatest influence on CI. Pearson's correlation analysis (Appendix 1.8.7) revealed that CI was correlated ($p < 0.05$) to water temperature ($r^2 = 0.204$) and salinity ($r^2 = 0.243$) but not to the other environmental variables.

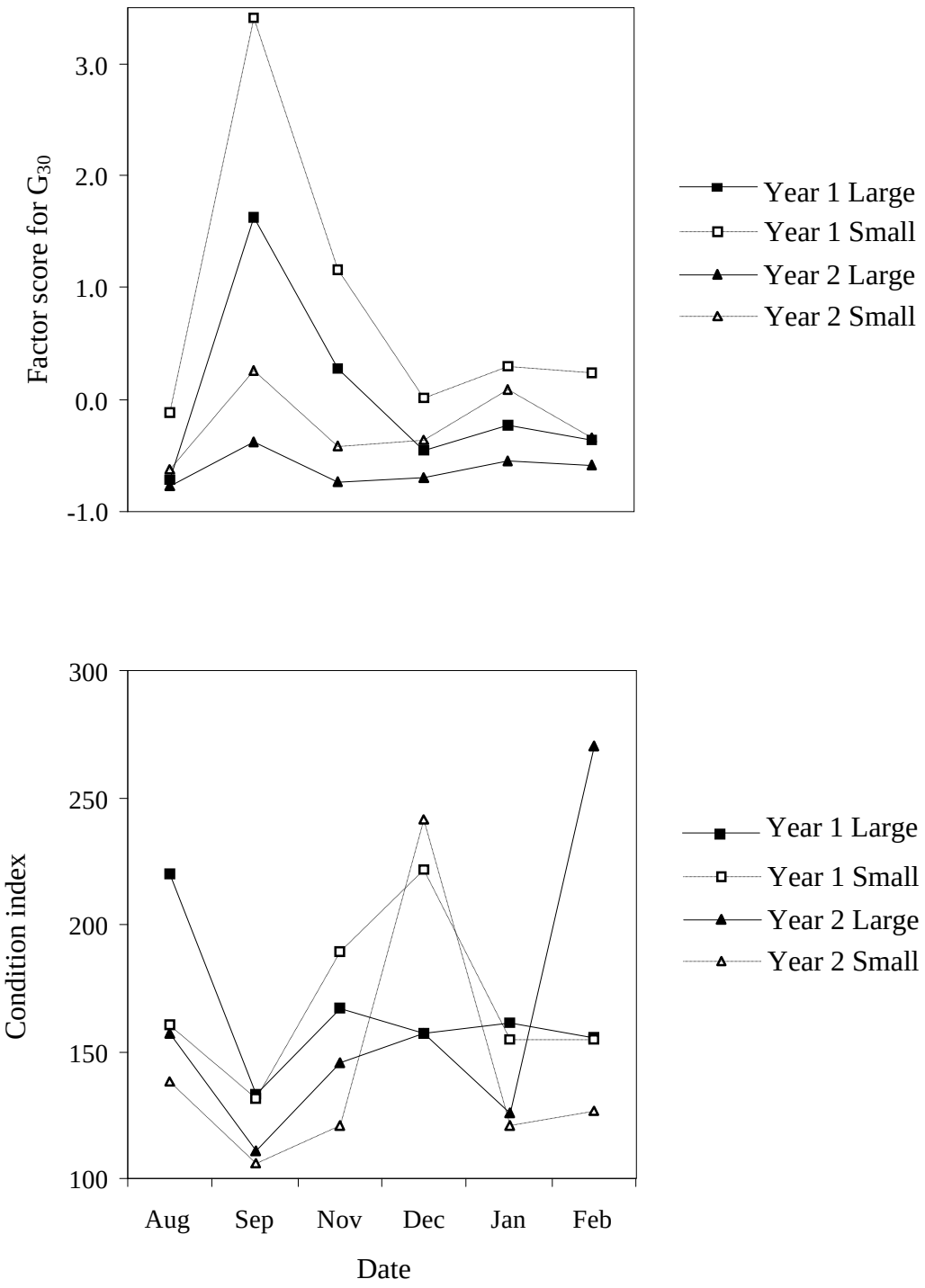


Fig. 8.16 Comparison between monthly G_{30} score (obtained by PCA of shell length, height, thickness and wet weight of oysters) and CI of oysters from two age and size classes.

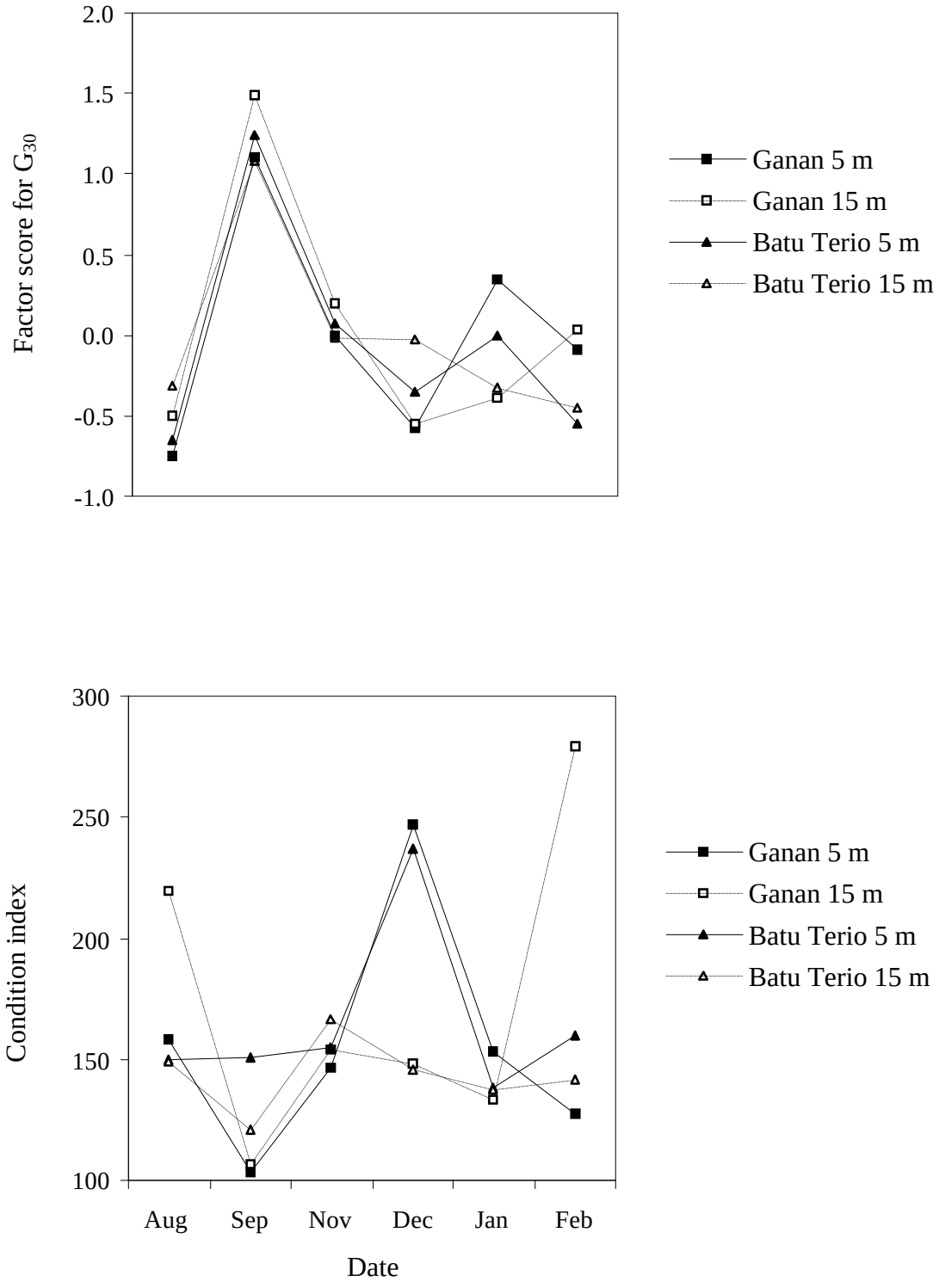


Fig. 8.17 Comparison between monthly G_{30} factor score and CI of oysters cultured at two sites and depths.

8.3.6 Environmental parameters

As environmental parameters were presented in detail in Chapter 4, changes in each parameter will not be repeated in this Chapter. However, as Chapter 4 explored environmental parameters from three sites and two depths over a 20 months period while in this study, two sites were compared and the period for the experiment was seven months, environmental data from the relevant time-frame were extracted from the overall data set to perform analyses. Environmental descriptors for the duration of this experiment at Ganan and Batu Terio are summarised in Table 8.6.

8.3.7 Relationship between sex, size and environmental parameters

The relationships between sex and size (as measured by length, height, thickness and weight), and sex and various environmental parameters were investigated using discriminant analysis.

8.3.7.1 Sex, size and weight

Mean length, height, thickness and wet weight of indeterminate, male and female oysters are given in Table 8.7. The mean length, height, thickness and weight of female oysters were greater than that of male oysters which were in turn larger than indeterminate oysters.

As there were three groups (indeterminate, male and female) to be classified, two discriminant functions were produced consisting of two canonical or discriminant variables (DV) (Appendix 1.8.10). Stepwise analysis established that weight, length and height were significant in separating the categories. DV1 appeared to distinguish indeterminate oysters from males and females by weight, while DV2 appeared to separate males from indeterminate oysters based on length (Fig. 8.18). However, there appeared to be a high degree of overlap between the different groups. In the classification matrix, indeterminate oysters were classified correctly 80.3% of the time while the number of oysters properly categorised as males and females were 42.8% and 35.7%, respectively.

Table 8.6 Means (± SD) of various environmental parameters at Ganan and Batu Terio at 5 m and 15 m depths.

Site	Month	Temperature (°C)	Salinity (‰)	PH	SPM (g L^{-1})	POM (g L^{-1})	Chlorophyll *a* (g L^{-1})	Chlorophyll *b* (g L^{-1})	Chlorophyll *c* (g L^{-1})
Ganan	Aug	27.58 (0.22)	34.4 (0.9)	7.4 (0.1)	0.0340 (0.0055)	0.0140 (0.0055)	0.8238 (0.8754)	1.2511 (1.3957)	3.3688 (3.7631)
5 m	Sep	27.73 (0.32)	34.0 (0.8)	7.5 (0.1)	0.3150 (0.0060)	0.0115 (0.0087)	0.3988 (0.1674)	0.5573 (0.2988)	1.5390 (0.7939)
	Nov	28.18 (0.37)	35.0 (0.8)	7.6 (0.1)	0.0350 (0.0058)	0.0075 (0.0050)	0.7867 (0.3039)	1.0045 (0.4319)	2.7434 (1.2078)
	Dec	29.21 (0.48)	35.0 (0.0)	7.7 (0.1)	0.0340 (0.0055)	0.0100 (0.0071)	0.2717 (0.3851)	0.4503 (0.6279)	1.2189 (1.8099)
	Jan	28.71 (0.24)	34.5 (0.6)	8.0 (0.0)	0.0225 (0.0050)	0.0075 (0.0050)	0.0219 (0.0155)	0.1375 (0.0750)	0.1188 (0.1048)
	Feb	28.50 (0.29)	35.0 (0.0)	8.0 (0.1)	0.0300 (0.0082)	0.0050 (0.0058)	0.0063 (0.0078)	0.0438 (0.0375)	0.0250 (0.0540)
Ganan	Aug	28.19 (0.17)	34.8 (0.8)	7.6 (0.1)	0.0360 (0.0055)	0.0060 (0.0055)	0.8700 (0.2405)	1.2439 (0.2877)	3.4218 (0.8117)
15 m	Sep	28.35 (0.24)	34.0 (0.8)	7.7 (0.1)	0.0350 (0.0058)	0.0050 (0.0058)	0.2546 (0.2233)	0.3261 (0.3439)	0.9534 (0.9287)
	Nov	28.80 (0.33)	35.0 (0.0)	7.7 (0.1)	0.0300 (0.0082)	0.0100 (0.0082)	0.5999 (0.5094)	0.8911 (0.7846)	2.5123 (2.1948)
	Dec	29.07 (0.29)	35.0 (0.0)	7.8 (0.1)	0.0300 (0.0000)	0.0040 (0.0055)	0.2101 (0.2639)	0.3534 (0.3591)	0.8275 (1.3317)
	Jan	28.70 (0.14)	35.0 (0.0)	8.0 (0.0)	0.0275 (0.0096)	0.0050 (0.0058)	0.0194 (0.0083)	0.1188 (0.0375)	0.1000 (0.0456)
	Feb	28.66 (0.18)	35.0 (0.0)	8.0 (0.0)	0.0350 (0.0058)	0.0100 (0.0000)	0.0013 (0.0127)	0.0188 (0.0688)	0.0250 (0.0957)
Batu Terio	Aug	28.56 (0.19)	34.2 (1.1)	7.6 (0.2)	0.0360 (0.0089)	0.0140 (0.0114)	0.4549 (0.2497	0.6324 (0.4046)	1.8248 (1.2168)
5 m	Sep	28.83 (0.19)	33.5 (0.6)	7.6 (0.2)	0.0325 (0.0050)	0.0075 (0.0050)	0.3271 (0.2413)	0.4555 (0.3135)	1.2971 (0.9456)
	Nov	29.34 (0.32)	34.5 (0.6)	7.8 (0.2)	0.0300 (0.0115)	0.0050 (0.0058)	0.3988 (0.1674)	0.5573 (0.2988)	1.5390 (0.7939)
	Dec	29.37 (0.35)	35.0 (0.0)	7.7 (0.1)	0.0300 (0.0071)	0.0060 (0.0089)	0.4135 (0.4356)	0.6131 (0.6912)	1.7499 (2.1337)
	Jan	28.94 (0.22)	34.8 (0.5)	8.0 (0.0)	0.0325 (0.0050)	0.0075 (0.0050)	0.0006 (0.0047)	0.0313 (0.0239)	0.0063 (0.0427)
	Feb	29.13 (0.22)	35.0 (0.0)	8.0 (0.0)	0.0275 (0.0050)	0.0075 (0.0050)	0.0063 (0.0060)	0.1438 (0.0375)	0.0250 (0.0456)
Batu Terio	Aug	28.15 (0.31)	34.0 (1.4)	7.6 (0.2)	0.0320 (0.0084)	0.0140 (0.0089)	0.4885 (0.2573)	0.7684 (0.3341)	2.0426 (0.8662)
15 m	Sep	28.35 (0.41)	33.3 (1.0)	7.5 (0.2)	0.0325 (0.0096)	0.0075 (0.0050)	0.1666 (0.1217)	0.1701 (0.2815)	0.6535 (0.7003)
	Nov	28.80 (0.45)	34.5 (0.6)	7.8 (0.2)	0.0325 (0.0050)	0.0100 (0.0000)	0.2999 (0.1692)	0.2913 (0.3958)	0.9045 (0.9338)
	Dec	29.22 (0.41)	35 (0.0)	7.7 (0.1)	0.0320 (0.0045)	0.0040 (0.0089)	0.2099 (0.2750)	0.3230 (0.3534)	0.8142 (1.1843)
	Jan	28.78 (0.31)	34.8 (0.5)	8.0 (0.0)	0.0300 (0.0082)	0.0025 (0.0050)	0.0050 (0.0061)	0.0500 (0.0289)	0.0250 (0.0354)
	Feb	28.74 (0.18)	35.0 (0.0)	8.0 (0.0)	0.0275 (0.0096)	0.0050 (0.0058)	0.0144 (0.0085)	0.0813 (0.0427)	0.0750 (0.0677)

Table 8.7 Mean (± SD) length, height, thickness and weight of indeterminate, male and female oysters in mm.

	Sex		
	Indeterminate	Male	Female
Length	119.9 (20.1)	137.5 (17.1)	138.6 (19.8)
Height	119.3 (19.0)	136.3 (16.5)	140.5 (14.9)
Thickness	26.9 (5.4)	30.7 (4.7)	32.4 (3.8)
Weight	222.1 (105.8)	326.0 (112.4)	367.6 (97.4)

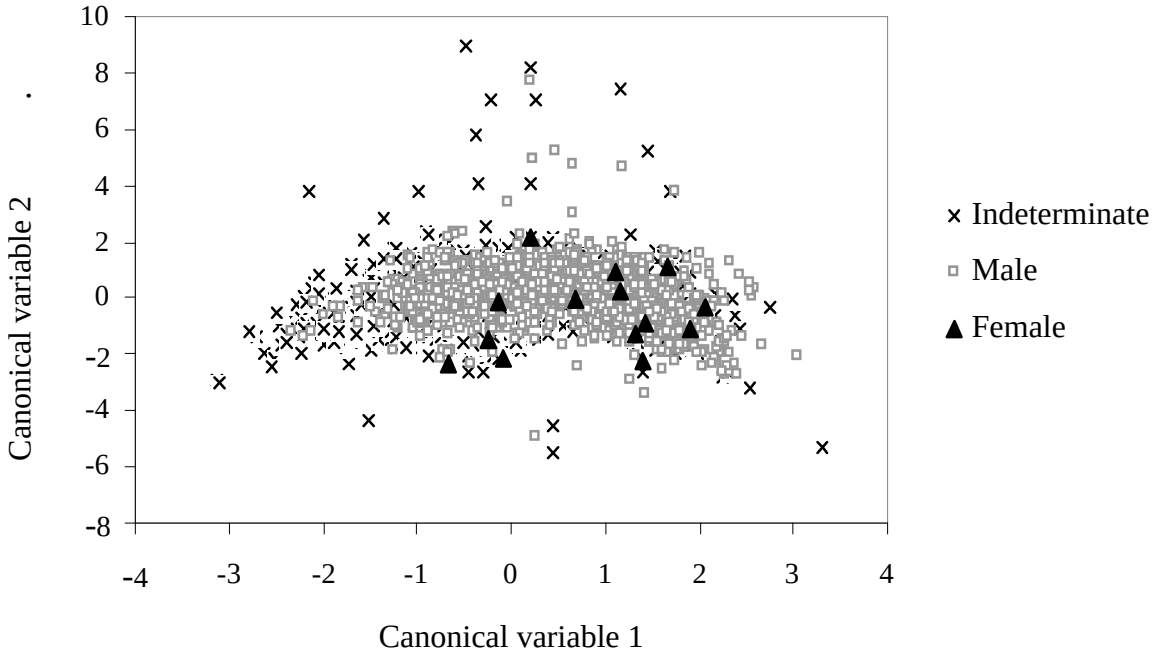


Fig. 8.18 Scatter plot showing sex groupings of oysters by two discriminant variables.

The correlation (r) of a particular variable to the discriminant functions may be used to determine the relative importance of the variable (Table 8.8). DV1 separated indeterminate oysters from males and females based on weight (r = 0.967), height (r = 0.948) and length (r = 0.933) while DV2 separated indeterminate oysters from males based mainly on length (r = 0.339). Thickness did not appear to be an important factor in discriminating between the sexes.

Although the analysis was significant in separating between the groups using the Wilks Lambda statistic (Weight: $F_{(2,2873)} = 324.2$, $p < 0.05$; Length: $F_{(4,5744)} = 162.1$, $p < 0.05$; Height: $F_{(6,5742)} = 110.0$, $p < 0.05$), size and weight were not reliable predictors of sex because of the low percentages of correctly classified cases as well the overlap in the scatter plot.

8.3.7.2 Sex and environmental parameters

There was an inverse correlation (Appendix 1.8.11) between the number of indeterminate oysters and pH ($r^2 = -0.209$, $p < 0.05$), water temperature ($r^2 = -0.261$, $p < 0.05$) and rainfall ($r^2 = -0.261$, $p < 0.05$) over the sampling period. A positive correlation was also observed between the number of male oysters and pH ($r^2 = 0.204$, $p < 0.05$), water temperature ($r^2 = 0.261$, $p < 0.05$) and rainfall ($r^2 = 0.217$, $p < 0.05$). No correlation was found between female oyster numbers and environmental parameters.

Graphical representation showed that as rainfall, water temperature and pH increased from August to December, there was a concomitant increase in the number of male oysters and a decrease in the number of indeterminate oysters (Fig. 8.23). However, when rainfall and temperature decreased from December to February, a decrease of the same magnitude was not observed in male oysters. The change in male and indeterminate numbers was relatively small from December to February.

While it appeared that pH, temperature and rainfall were significantly correlated to the number of indeterminate and male oysters, the correlations were relatively weak and did not fully account for the variation in the number of male and indeterminate oyster adequately.

Table 8.8 Coefficients of discriminant functions and correlation (r) of variables to the discriminant variables (DV).

Variable	DV1	DV2	Correlation to DV1	Correlation to DV2
Weight	0.479	-1.736	0.967	-0.210
Height	0.315	-0.083	0.948	0.045
Length	0.255	1.884	0.933	0.339
Thickness[a]			0.714	-0.084

a. Not used in analysis

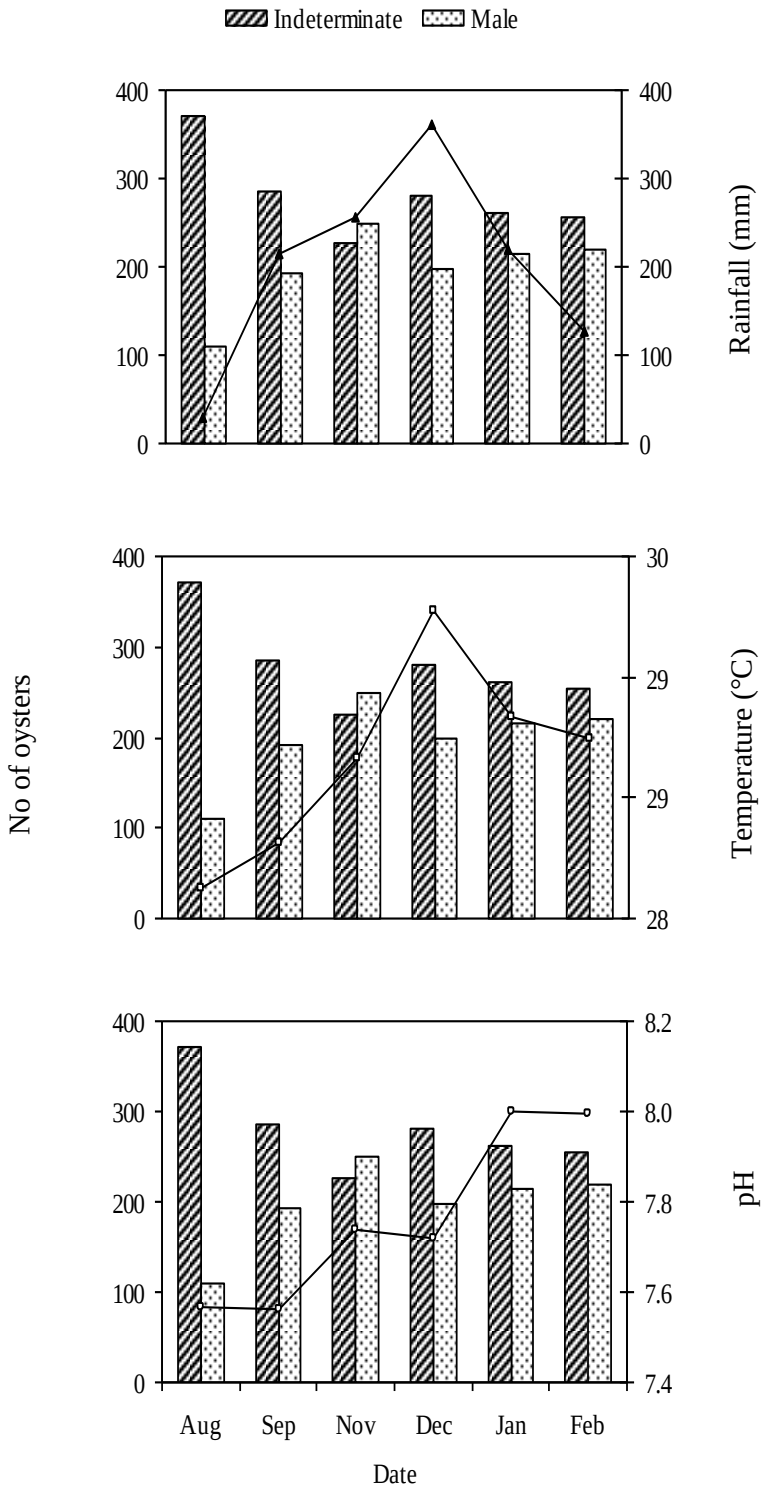


Fig. 8.19 Relationship between the number of indeterminate and male oysters and rainfall, water temperature and pH.

8.4 Discussion

The reproductive cycle in bivalve oysters involves several stages: growth and ripening of gametes, spawning and gonad redevelopment (Gosling, 2003). This study attempts to assess the course of the reproductive cycle in *P. maxima* through gross visual examination of the gonads. While this method does not provide detailed information on the development of gametes like that provided by histological or biopsy methods (Barber and Blake, 1991), it does offer a simple and quick method to estimate overall gonadal development in the field where access to more advanced equipment and technique are limited. It also has the advantage of not requiring the sacrifice of an animal.

Macroscopic investigation of gonads and comparison to histological data in this study support previous reports that gonad colour and appearance may be used to determine sex and stage of development in *P. maxima* (Rose *et al.,* 1986). The difference between the pale creamy patchiness in the digestive diverticula of male oysters, the bright yellow-orange patchiness in female oysters and the clear translucence of indeterminate oysters was very conspicuous in this species of pearl oyster. This fundamental difference in the colour and the area occupied by the developing gametes made it possible to distinguish between the gender and various stages of development of *P. maxima* oysters with relative ease, as demonstrated when visual observations were confirmed by histology (Appendix C). While gonad colour may be used to distinguish between sexes in *P. maxima* and *P. albina (*Tranter, 1958a), it is not a reliable criterion in other species of pearl oysters. For example, sex in *P. imbricata* could be distinguished by colour only in the later stages of development (O'Connor and Lawler, 2004b; Choi and Chang, 2003; Behzadi *et. al.,* 1997; Velayudhan and Gandhi, 1987; Tranter, 1959) while histological examination is often required to determine sex in *P. mazatlanica* (Saucedo and Monteforte, 1997a; Garcia-Dominguez *et. al.,* 1996) and *P. margaritifera* (Tranter, 1958d).

In this study, gametogenesis commenced immediately after pre-conditioned oysters were placed at experimental sites, as shown by the number of sexually distinct oysters observed at the first month of sampling. Proportion of indeterminate oysters fell to 77% from an initial 100% and oysters with distinct sex were observed as spermatogenesis

progressed, followed by oogenesis in a small number of oysters. In November, the proportion of indeterminate oysters was at its lowest (50%) after which the percentage of indeterminate oysters began to increase again. The percentage of indeterminate oysters observed was always inversely proportional to the percentage of sexually distinct oysters as oysters went through the gonadal cycle. The gonadal cycle began from an inactive state (forced by pre-conditioning in an unfavourable environment), progressed towards gonad maturity and finally culminated in a spawning event in September – November and again towards the end of the experiment in February. The subsequent increase in indeterminate oysters observed after those months represented spent oysters that had discharged gametes during spawning. Spawning was likely partial as male and female oysters with ripe gonads continued to be observed after November. Observation on the proportion of inactive and sexually mature oysters within a population of oysters could be used to interpret the gametogenic cycle of cultured *P. maxima*.

The timing of the gametogenic cycle was supported by observations of changes in the gonad index (GI). As GI increases during gametogenesis and decreases during spawning (Gosling, 2003), results could be interpreted as follows: gametogenesis commenced from August, followed by a partial spawning in September – November. It would appear that not all gametes were released, as the drop in the index was slight. In the following months, gametogenesis continued and resulted in a second spawning in February when the GI decreased for the second time.

Observation of gonad stages was consistent in supporting the findings that there was bimodal spawning in September - November and January - February. The number of Stage 0 oysters observed decreased progressively from August until November matched by a corresponding increase in Stage 1 and 2 oysters, indicative of the oysters undergoing the developing stage of gametogenesis when production of gametes was on the rise. A rise in the number of Stage 0 oysters was observed in December together with a corresponding fall in Stage 1 oysters. This was seen as oysters having spawned prior to the November sampling with the expulsion of gametes giving rise to the large number of Stage 0 spent oysters. Ripe oysters (Stages 4, 5 and 6) were not observed in November but were present in most of the other months, thus confirming that oysters had spawned before the November sampling. The number of Stage 1 plus oysters

increased until February, leading to the conclusion that a second stage of gametogenesis occurred to be followed by a second spawning.

Previous literature indicates that mature *P. maxima* oysters undergo an annual reproductive cycle which appear to vary in timing and duration according to location (Wada, 1953b; Minaur, 1969; Rose *et. al.,* 1990). This has also been reported in other pearl oysters (Wada *et al.*, 1995; O'Connor, 2002; Choi and Chang, 2003). Differences in the reproductive cycle of bivalves from the same species are thought to be due to critical water temperatures being achieved at different latitudinal ranges and at different times (Giese, 1959; Giese and Pearse, 1974) as water temperature has been reported to affect the onset of gametogenesis in pearl oysters (Araya-Nuñes *et. al.,* 1991; Behzadi *et. al.,* 1997; Saucedo and Monteforte, 1997a; O'Connor, 2002; Choi and Chang, 2003; O'Connor and Lawler, 2004b). For example, *P. maxima* populations in Queensland were observed to have two breeding peaks, October – November and February – March (Wada, 1953b; Minaur, 1969) while *P. maxima* in Western Australia were observed to breed from September to April with peaks at either ends (Rose *et. al.*, 1990).

Table 8.9 Spawning months of various species of pearl oysters found in different localities.

Pearl oyster species	Locality	Spawning months	References
P. maxima	Queensland, Australia	October - November February - March	Wada, 1953b Minaur, 1969
	Western Australia	September – April	Rose *et. al.*, 1990
	West Papua, Indonesia	September – November January - February	This study
P. fucata martensii	Japan	Continuous, with major peak in winter and minor peak in summer	Wada *et. al.,* 1995
	Korea	April – August, with peak in June – July	Choi and Chang, 2003
P. albino sugillata	New South Wales, Australia	Continuous, with peaks in Oct, Mar, Jan and April	O'Connor, 2002

Rose (1990) hypothesized that the difference in the timing was most likely due to the variation in surface water temperature at the two latitudes as *P. maxima* studied in Western Australia was approximately 7 to 11 degrees south from the Queensland populations and exposed to a lower minimum water temperature (18°C). Aljui Bay is situated close to the equator (Latitude 00° 11' S) and water temperature did not go below 27.6°C in this study. However, despite the mean water temperature at Aljui Bay being higher than the Australian sites, the timing for the reproductive cycles in *P. maxima* at Aljui Bay and Western Australia appear to occur around the same time of the year. It is likely that other factors, biological and environmental, also exert an influence on the gonadal cycle of *P. maxima* besides water temperature.

Visual inspection of gonad conditions has its limitations, as it was difficult to ascertain if indeterminate oysters were in an inactive or spent stage since the stages often overlapped. However, given what is known about the gametogenic cycle of pearl oysters in general (Rose *et. al.,* 1990; Garcia-Dominguez *et. al.,* 1996; Saucedo and Monteforte, 1997a; Choi and Chang, 2003; O'Connor and Lawler, 2004b), it is possible to approximate what state Stage 0 oysters were at during the experiment. Another disadvantage of visual examination is that classification tends to be subjective (Gosling, 2003). The problem can however be overcome in a farm situation if specifically trained workers were delegated the task of estimating gonad condition in preparation for artificial propagation. The advantage of not sacrificing a valuable pearl oyster outweighs the potential for possible errors in classifying gonad condition in cultured *P. maxima*.

GI estimated the proportion of developing, ripe, spawning and spent individuals by classifying sexual stages of the whole population of experimental oysters. CI, on the other hand, estimated the current metabolic and reproductive status of individual pearl oyster. A decrease in CI indicates a decrease in tissue weight of oysters likely caused when a major biological effort has been expended. The probable cause of this is loss of gonad tissue due to a recent spawning. From the results, low CI in September and January (Fig. 8.16 and Fig. 8.17) indicate that oysters spawned around the period and is consistent with other results obtained by visual examination of gonads. Condition indices assess physiological activity such as growth, reproduction or secretion in animals (Lucas and Beninger, 1985) and can indicate the nutritive state of the animal

(Brown and Hartwick, 1988c; Littlewood and Gordon, 1988). While CI is accurate in estimating the physiological state of an oyster, it represents only an individual. A larger sample is required to estimate with more accuracy the reproductive state of a population of oysters. Unfortunately, this is not economically viable in a farming situation. In this regard, GI is preferable as it has the advantage of not requiring the destruction of the animal.

Pearl oysters are protandric hermaphrodites and can reverse sex several times during their lifetimes (Galstoff, 1950; Wada, 1953a, 1953b; Chellam, 1987; Saucedo and Monteforte, 1997a; Vite-García and Saucedo, 2008). Pearl oysters in a natural population achieve a sex ratio of 1:1 with increasing age and size (Wada, 1953; Gervis and Sims, 1992). However, there have been reports that oysters in close proximity often result in a higher proportion of male in the population (Burkenroad, 1931; Menzel, 1951; Buroker, 1983; Taylor, 1999; Walker and Power, 2001; Kimani and Mavuti, 2002; Saucedo *et. al.*, 2002a, 2002b). This was shown to be the case here as the sex ratio of female to male was an overwhelming 0.01:1. Similarly, Saucedo and Monteforte (1997a) reported a strong male bias in small *P. mazatlanica* in Mexico, with a female to male sex ratio of 0.12:1, and increasing to 0.38:1 as oysters grew larger. Saucedo *et al.*, (2002b) also described a 0.35:1 female to male ratio in *P. mazatlanica* obtained from cultured conditions, but indicated that females outnumbered males in the wild. It has been proposed that the predominance of maleness is an indication of ambient stress and/or unsuitable culture condition with overcrowding playing a key role in greater expression of maleness (Saucedo and Southgate, 2008). Morton (1991) hypothesised that a male bias in juvenile bivalves allows for a more efficient allocation of resources into growth. Male gamete production is more cost effective since juvenile mortality in an unfavourable environment may be higher than for larger individuals. Older females, on the other hand with lower growth rates may divert more energy into ova production and with reduced mortality enhance the potential for fertilisation. The funnelling of energy into survival and growth by a protandrist in its male phase before taking advantage of the energy saved to become female, is an elaboration of the “size-advantage model” (Ghiselin, 1969), a theory to explain the evolution of protandry. This model predicts that when the reproductive success (RS, or number of viable offspring produced) of males are less sensitive to size and age than the RS of females, a protandric individual gains a reproductive advantage.

Sex change is reversible in several species of oysters and thought to be caused by changing environmental condition and stress (Cahn, 1949; Tranter, 1958a, b, c, d, 1959; Chellam 1987; Rose *et. al.,* 1990). Until recently, the prevailing view was that sex in various species of oysters was predominantly determined by environmental factors such as water temperature, acute water temperature changes (such as in an El Niño or La Niña event), salinity, nutritive conditions or food availability (Coe, 1943; Rao, 1956) or that hormone might be the major controlling mechanism (Kennedy, 1983; Thompson *et al.,* 1996). Results from this study showed that there was a correlation between pH, water temperature and rainfall and the number of male oysters observed. As rainfall, water temperature and pH increased from August to December, there was a concomitant increase in the number of male oysters. pH can significantly alter the physiology of marine invertebrates by having a synergistic effects with other environmental factors such as elevated temperatures (O'Donnell *et al.,* 2009), while rainfall could change the pH of seawater. That water temperature was correlated to spermatogenesis is not surprising, as it has been widely reported to affect gametogenesis in other pearl oysters (Wada, 1953b; Minaur, 1969; Rose *et al.,* 1990; Araya-Nuñes *et. al.,* 1991; Behzadi *et. al.,* 1997; Saucedo and Monteforte, 1997a; O'Connor, 2002; Choi and Chang, 2003; O'Connor and Lawler, 2004b). While there was positive correlation between environmental parameters and the expression of maleness, the correlations were relatively weak and did not fully account for the variation in the number of male oyster adequately. The occurrence of female oysters did not correlate to any environmental parameters. Given the persistently low number of female oysters observed in this study, the absence of any correlation between environmental parameters and number of female oysters should be interpreted with caution. The implications of these results are that environmental parameters affect the onset of spermatogenesis in *P. maxima* to a certain extent, but oogenesis is less governed by environmental parameters. Other factors, for example, biological or hormonal, are likely to play important roles in controlling gametogenesis.

Besides environmental parameters, a genetic basis of sex differentiation has been proposed by a number of authors. Tranter (1958c) hypothesized that a "weak hereditary sex-determining mechanism" may be responsible for sex change in *P. albina*, and more recently Guo *et al.* (1998) showed that sex in the rock oyster *Crassostrea gigas* was determined by a single gene locus with a dominant maleness allele and an allele for

protandric femaleness, and other genes and/or environmental factors such as temperature may regulate the rate of sex change. Bacci (1965) suggested that the duration of the male or female phase in oysters may result from simple Mendelian segregation of multiple sex genes whose action is additive. Haley (1975) proposed a three loci model for sex determination in the American oyster, *C. virginica*, with each locus segregating two additive alleles, m for maleness and f for femaleness, with the m:f ratio determining sex. Arnoud-Haond *et al.*, (2003) compared the geographical distribution of genetic variability at mitochondrial and nuclear loci among natural populations of *P. mazatlanica* in the tropical American Pacific coast and showed evidence for male-biased effective sex ratio. While there is probably a genetic basis for sex differentiation in *P. maxima*, it is likely that gene expression is mediated by external factors such as environmental parameters, with both endogenous and exogenous factors acting in concert to determine sex differentiation.

While the reproductive system in pearl oysters is relatively simple, consisting of branching tubules with an epithelial lining from which gametes bud off and are shed through an exhalant opening in the mantle (Gosling, 2003; Southgate and Lucas, 2008), gametogenesis is a complex process which is controlled by a multitude of factors encompassing biological, physical and chemical parameters. In this study, attempts to partition the relative influence of the various factors using statistical procedures produced outcomes, which can be interpreted in a meaningful biological context. Results showed that that spermatogenesis and oogenesis in *P. maxima* cultured at Aljui Bay were influenced by temporal (months of sampling as discussed earlier) and biological factors (age and size of oysters) but not by spatial factors (site or depth of culture). Results indicated that more males were found within faster growing oysters from a younger age (Year 1 Large) than slower growing oysters from an older age (Year 2 Small). Similarly, femaleness was also size-related. The implication for this is that size, and not age was more important in the expression of sex in *P. maxima*. Furthermore, discriminant analysis showed that indeterminate oysters could be separated from males and females based on wet weight ($r = 0.967$), shell height ($r = 0.948$) and shell length ($r = 0.933$) and indeterminate oysters could be distinguished from males based mainly on shell length ($r = 0.339$) while shell thickness was not important in discriminating between the sexes. In a farm situation, this information could be utilised by selecting and maintaining oysters with greater shell length or shell

height, regardless of age, for breeding stock and using smaller oysters for pearl seeding. By selecting larger oysters as broodstock, the likelihood of male and female expression is increased.

The overwhelming bias towards male oysters in this experiment may be due to other reasons besides those discussed. For instance, the duration of this experiment may not have been sufficiently long enough to enable conversion to the female state. Secondly, the majority of oysters used in this experiment fell between the 100 mm to 160 mm size range. Sex ratio in *P. maxima* from Western Australia was reported to approach parity only when oysters become larger and females reach a dorso-ventral measurement of 170 mm (Hart and Joll, 2006). Due to the scarcity of *P. maxima* with shell lengths > 170 mm, (only 12 out of the 480 oysters sampled had shell length > 170 mm), this theory could not be assessed in the study. However, given what is know about sex reversal in general in *P. maxima*, it is likely that the f:m sex ratio would be closer to parity if oysters from a larger size range was used in any future studies.

In summary, this study showed that gametogenesis in cultured *P. maxima* in Aljui Bay occurred between August to February, with spawning occurring twice during that period; once in October/November and again in February. Sex ratio was overwhelmingly biased towards maleness, and varied temporally for the duration of the experiment. There was no difference in sex ratio between oysters cultured at different sites and different depths. The expression of maleness was weakly correlated to water temperature, pH and rainfall, while there was no correlation between femaleness and environmental descriptors. Size, and not age, was more important in determining the sex of *P. maxima* in this study.

CHAPTER 9

General Discussion

9.1 Background to the study

The culture of *P. maxima* at P. T. Cendana encompasses the entire life cycle of oysters, starting with artificial propagation of broodstock to produce larvae, followed by grow-out of oysters from juvenile to adulthood in the ocean, and ending with using oysters to produce pearls or as broodstock. Hatchery techniques have improved in recent years and reliable production of *P. maxima* larvae in the hatchery is a feature of most pearl farms. However, the ocean grow-out phase of *P. maxima* culture is still frequently subjected to various uncertainties including slow growth of animals (Traithong *et al.*, 1997), high mortalities (Wu *et al.*, 2003b) and fouling and predation particularly in juvenile oysters (Gervis and Sims, 1992). This is due to the difficulty of creating a controlled growing environment out in the open ocean. However, it has been previously reported in other studies that growth variability in a population of molluscs living in the same locality has been reported to be related to differences in the microenvironments (Wilbur and Owen, 1964). Therefore, the selection of a particular culture site or culture depth within a farm is an indirect method of exerting control over the growth environment of cultured pearl oysters. Understanding how spatial difference affect growth and survival of *P. maxima* at different stages of growth would serve to assist management in refining grow-out techniques for optimal production of oysters.

This thesis aimed to address various growth aspects of farming *P. maxima* in a commercial farm in Indonesia with special emphasis on the influence of the environment, as bivalve growth is affected by a complex combination of biological and environmental factors.

9.2 Major findings of this study

The major findings in this study were:

1. Seawater parameters in the *P. maxima* growing area of Aljui Bay were not homogenous and environmental descriptors varied spatially between sites and depths, as well as temporally throughout the year.

2. Growth of *P. maxima* was affected by the age of the oysters, with growth slowing down with increasing age. Growth was also influenced by site and depth of culture. Further results showed differences in spatial growth rates was linked to the physico-chemical profile of the culture micro-environment, with variation in somatic growth of *P. maxima* governed by varying levels of pH, salinity and chlorophyll, while variation in gonad growth was influenced by variations in pH, SPM, POM and water temperature.

3. The Special VBGF was the mathematical model that best described the growth of *P. maxima* cultured in Aljui Bay.

4. Biofouling quantity and diversity affected growth of medium and small oysters. The spatial and temporal variation in quantity and diversity of six classes of biofouling was in turn affected by various environmental parameters, with chlorophyll levels, pH and salinity having a greater affect on biofouling settlement than SPM, POM and seawater temperature.

5. Gametogenesis in cultured *P. maxima* in Aljui Bay occurred between August to February, with spawning occurring twice during that period; once in October/November and again in February. Sex ratio in cultured *P. maxima* was overwhelmingly biased towards maleness, with no spatial difference in sex ratio between oysters cultured at various sites and depths. The expression of maleness was weakly correlated to water temperature, pH and rainfall, while there was no correlation between femaleness and environmental descriptors. Size, and not age, was more important in determining the sex of *P. maxima*.

9.3 Implications of these findings

1. The heterogeneous nature of environmental parameters at various sites around Aljui Bay means it is possible to culture *P. maxima* in various culture microenvironments within a farm, thus allowing culturist some level of control over the grow-out environment. Given the importance of environmental parameters in many aspects of *P. maxima* farming, it is recommended that some level of environmental monitoring be conducted as part of routine farming protocol. This is to ensure that any changes in the physico-chemical profile of a particular growing site are detected and grow-out management be adjusted accordingly. Identification of potential new sites should also take into consideration the environmental profile of the microenvironment.

2. It was found that culturing young *P. maxima* at the more favourable depth of 5 m favour faster somatic growth and shorten the time it takes for pearl oysters to reach operable size. The use of less rope for suspending oysters at 5 m reduces entanglement and saves on the cost of culture material. Conversely, as the quality of the resulting pearls has been reported to depend on slower growth and nacreous deposition (Wada, 1973; Pouvreau and Prasil, 2001), this can be achieved by suspending *P. maxima* at a depth of 15 m or greater during the pearl culture phase. Results of this study showed that *P. maxima* aged 1.5 to 3.5 years old grown at Batu Terio had the lowest survival rate. In light of this data, Ganan and Manselo should be selected for the culture of younger *P. maxima* in Aljui Bay, and Batu Terio be utilised for the culture of older and larger oysters.

3. Estimation of growth rate *K*, length at age t L_t and asymptotic size L_∞ using the VBGF showed that in the first two years of culture, the best site for grow-out would be Manselo at a depth of 5 m when fast growth, high survival and larger asymptotic size are essential to produce large numbers of oysters for grafting. Oysters grown at Ganan and at a depth of 15 m had lower *K* values and would be suitable to condition oysters prior to grafting and also for pearl grow-out.

4. Information on the effects of age, site, depth and month of sampling on quantity and diversity of biofouling may be utilised in management of biofouling control in

P. maxima culture. Cleaning approximately every 4 weeks was effective in keeping biofouling down, without detrimental effect to oysters. However, the scheduling of a cleaning regime according to oyster size may be of further benefit to ensure valuable resources are not wasted in superfluous cleaning, as smaller oysters require less cleaning than larger oysters. Bivalves contributed the greatest proportion of biofouling by weight - it would be judicious to avoid sites with high bivalve infestations that could potentially add excess weight to the suspended culture systems and impair floatation. A preliminary survey of the benthic composition at prospective sites would provide information on potential biofouling at those sites, as the settlement of biofouling organism is often a reflection of the predominant species composition of the surrounding habitat.

5. Preparation of oysters for spawning at Aljui Bay should take place in August, when gametogenesis commences. By selecting larger oysters as broodstock, the likelihood of male and female expression is increased, as maleness and femaleness in *P. maxima* are size-related. Indeterminate oysters could be separated from males and females based on wet weight ($r = 0.967$), shell height ($r = 0.948$) and shell length ($r = 0.933$) and indeterminate oysters could be distinguished from males based mainly on shell length ($r = 0.339$). In a farm situation, selecting and maintaining oysters with greater shell length or shell height, regardless of age for breeding stock and using smaller oysters for pearl seeding is recommended.

Table 9.1 summarises the various stages of grow-out of *P. maxima* at Aljui Bay and shows how the results from this study may be used to assist in the management of farming *P. maxima*. In summary, this research presented new data on growth of different age classes of *P. maxima* cultured in a farm situation in Indonesia. It has added to our knowledge the importance of various environmental factors and biofouling on somatic and gonadal growth of *P. maxima.* This information can be utilised to improve farming management practices through judicious selection of future culture sites. It is hoped that this will form a basis for further study into grow-out of *P. maxima* in the pearling industry in Indonesia and South-East Asia and lead to further improvement and expansion in the industry for the future.

Table 9.1 Summary of the findings and implications of the results obtained in this thesis.

	Thesis chapter	Findings	Implications and recommendations
Broodstock conditioning of oysters	8	Gametogenesis influenced by biological (age and size), but not spatial (site and depth) factors. Maleness and femaleness are size-related. Gametogenesis influenced by temporal (month) with two spawning events occurring between August – February.	Continued grading of oysters according to age and size to increase likelihood of male and female sex expressions. Depth or site does not affect gametogenesis; broodstock may be grown at the more cost effective depth of 5 m, and at any sites. Broodstock prepared for spawning from August. As two spawning peaks were observed, additional broodstock could be held in reserve for a second spawning later in the season.
Hatchery			
Juvenile oyster culture	5	Growth rate of different age classes of oysters influenced by site and depth.	Judicious selection of culture site and depth for different age classes of oysters to promote optimal growth so an operable size is achieved faster.
	6	Mathematical modelling for growth gives information on growth rate, *K*. Prediction on when an oyster achieves operable size. *K* is affected by site, depth and age of animal.	Information on *K* from various sites identified which sites were suitable for optimal growth. Planning and scheduling of seeding can be undertaken with size-at-age information available.
Cleaning	7	Biofouling levels and species affected by age of oysters, and spatial and temporal factors.	Identification of deleterious biofouling species at various sites and depths helps in selection of site with minimal fouling to reduce production costs. Scheduling of cleaning regime based on age of oysters.
Adult oyster culture	5, 6, 7		
Pearl seeding	5, 6		Oysters grown at a depth of 15 m to slow down growth so a better quality pearl is produced. Sites with low *K* selected for the same reason.
Pearl culture			

9.4 Strengths of this study

This study represents one of the most comprehensive studies on growth of cultured *P. maxima* in Indonesia. One advantage of conducting the research *in situ* is results need not be translated from laboratory to field. This is useful from an aquaculture point of view as it allows farm management to make decisions based directly on the results of this research as the sites and depths used as treatments in this thesis are actual culture sites and depths of farmed animals.

Access to farmed stocks of oysters ensured a large number of oysters was available for repeated measure and replication. Larger sample size increases the precision in estimates of growth parameters of the population. A dataset of this size would not be available should the study have been conducted within a research station or in a land-based facility.

Artificial propagation of *P. maxima* as a routine method of obtaining new oysters for pearl culture meant that the absolute age of all oysters used in experiments was known. This is particularly important in the mathematical modelling *P. maxima* growth and studies on the onset of gametogenesis.

9.5 Shortcomings of this study

Some of the limitations in this study include the time constraint imposed by the relatively short time frame for conducting field research for a PhD project. While it would have been ideal to sample a larger oyster age range for longer, this was not possible due to limited numbers of older oysters in farm stocks. To some degree, this shortfall was overcame by selecting staggered age groups of *P. maxima*, which allowed a wider age-span to be sampled over a shorter period.

Another disadvantage of this study was the remoteness of the farm site. The lack of access to more sophisticated laboratory equipment restricted certain experiments from being conducted. For instance, access to gas chromatography or high-performance liquid chromatography would have allowed the fatty acid composition or DNA:RNA ratio of *P. maxima* during different stages of growth to

be compiled. Preserving and getting samples back to James Cook University for analyses was initially considered for this study, but due to the logistics involved in passing Australian quarantine, this was not pursued. The absence of a facility to control ambient environmental parameters such as temperature and salinity meant the effects of a particular or precise variable on oyster growth could not be determined.

Inherent restrictions of working in a fully functional commercial farm include having to plan experiments around standard farm management practice. For example, only existing long-lines already deployed for culturing farm animals were available to grow experimental animals and no allowances were made for randomness in site selection. Due to the high cost of *P. maxima,* sacrifice of live animals was also kept to a minimum.

9.6 Future research

As hatchery culture is well established at P. T Cendana Indopearls, studies on selective breeding to improve growth in *P. maxima* could be a basis for future research. Previous work has shown that certain favourable traits such as shell shape, size and growth rate are inherited (Southgate *et. al.,* 2008). A better understanding of the genetic basis for growth and reproduction would likely lead to the improvement of oyster and pearl production.

While this research provided information on how various environmental parameters can affect growth of cultured *P. maxima* , it would be interesting to expand the research and conduct a similar growth study on wild *P. maxima* found within Indonesia, so comparison between wild and farmed oysters growth characteristics may be made.

Although the environmental impact of pearl farming has been studied in Japan, Australia and the Pacific (O'Connor and Gifford, 2008), there has not been any reported study of the impact of pearl culture in Indonesia. Pearl farming can effect the environmental in a number of ways. Translocation of oysters and artificial propagation might alter the gene pool of an indigenous population leading to

ecological damage. Habitat exclusion and modification of the local benthic biodiversity and carrying capacity due to farming activities would also be deleterious to the environment. This potential area of study is of national and global importance to ensure that best practice management plans might be implemented to reduce negative impact of pearl farming in Indonesia on the environment.

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APPENDIX A

Statistical Analyses

CHAPTER 4

4.1 Descriptive statistics for environmental parameters from various sites and depths

Ganan 5 m

	N	Range	Minimum	Maximum	Mean	Std. Deviation	Variance
Temp	89	2.07	27.43	29.50	28.3098	.48029	.231
Salinity	86	7.00	31.00	38.00	34.7907	.86930	.756
pH	79	1.90	6.40	8.30	7.6152	.43621	.190
SPM	86	.12	.01	.13	.0439	.02228	.000
POM	86	.11	.00	.11	.0210	.02076	.000
Chlorophyll a	77	1.09	.00	1.09	.5029	.25715	.066
Chlorophyll b	77	1.74	.00	1.74	.6987	.40990	.168
Chlorophyll c	77	5.15	.00	5.15	1.9753	1.15246	1.328
Valid N (listwise)	66						

Ganan 15 m

	N	Range	Minimum	Maximum	Mean	Std. Deviation	Variance
Temp	88	1.35	28.05	29.40	28.7534	.28278	.080
Salinity	86	4.00	33.00	37.00	34.8372	.71719	.514
pH	79	1.60	6.70	8.30	7.6949	.36158	.131
SPM	86	.12	.02	.14	.0437	.02191	.000
POM	86	.11	.00	.11	.0210	.02286	.001
Chlorophyll a	81	1.41	-.04	1.38	.4589	.26940	.073
Chlorophyll b	81	2.37	-.22	2.15	.6053	.45790	.210
Chlorophyll c	81	6.35	-.52	5.83	1.7365	1.22438	1.499
Valid N (listwise)	72						

Manselo 5 m

	N	Range	Minimum	Maximum	Mean	Std. Deviation	Variance
Temp	77	1.38	28.48	29.87	29.1828	.29978	.090
Salinity	73	5.00	31.00	36.00	34.3973	1.07677	1.159
pH	69	1.40	6.90	8.30	7.7464	.28880	.083
SPM	74	.15	.00	.15	.0457	.02433	.001
POM	74	.11	.00	.11	.0231	.02398	.001
Chlorophyll a	68	1.96	.00	1.96	.5661	.35155	.124
Chlorophyll b	68	3.18	.00	3.18	.7573	.59025	.348
Chlorophyll c	68	8.58	.00	8.58	2.1179	1.59147	2.533
Valid N (listwise)	62						

Manselo 15 m

	N	Range	Minimum	Maximum	Mean	Std. Deviation	Variance
Temp	76	1.41	28.04	29.45	28.7833	.30523	.093
Salinity	73	5.00	31.00	36.00	34.4521	1.01436	1.029
pH	69	1.40	6.90	8.30	7.7435	.29179	.085
SPM	74	.15	.00	.15	.0446	.02411	.001
POM	74	.13	-.01	.12	.0219	.02194	.000
Chlorophyll a	67	1.38	.00	1.38	.5730	.33673	.113
Chlorophyll b	67	2.26	.00	2.26	.7722	.56792	.323
Chlorophyll c	67	6.24	.00	6.24	2.1476	1.57032	2.466
Valid N (listwise)	60						

Batu Terio 5 m

	N	Range	Minimum	Maximum	Mean	Std. Deviation	Variance
Temp	86	1.59	28.28	29.87	29.0621	.30490	.093
Salinity	86	5.00	32.00	37.00	34.7791	.80295	.645
pH	79	1.60	6.70	8.30	7.7228	.32579	.106

SPM	86	.13	.02	.15	.0451	.02340	.001
POM	86	.12	.00	.12	.0220	.02330	.001
Chlorophyll a	81	1.67	.00	1.66	.5773	.35280	.124
Chlorophyll b	81	2.58	-.03	2.56	.7842	.56227	.316
Chlorophyll c	81	6.80	.00	6.80	2.2307	1.54629	2.391
Valid N (listwise)	67						

Batu Terio 15 m

	N	Range	Minimum	Maximum	Mean	Std. Deviation	Variance
Temp	91	1.57	27.88	29.45	28.8152	.30267	.092
Salinity	86	7.00	30.00	37.00	34.7209	.92864	.862
pH	79	1.40	6.90	8.30	7.7354	.30844	.095
SPM	86	.14	.00	.14	.0435	.02335	.001
POM	86	.10	.00	.10	.0209	.02294	.001
Chlorophyll a	81	1.89	.00	1.89	.5585	.36563	.134
Chlorophyll b	81	2.86	.00	2.86	.7609	.60902	.371
Chlorophyll c	81	7.94	.00	7.94	2.1384	1.67771	2.815
Valid N (listwise)	72						

4.2 Univariate ANOVA of environmental parameters

Dependent Variable: Temp

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	39.291(a)	6	6.549	57.743	.000
Intercept	2.164	1	2.164	19.079	.000
month	.518	1	.518	4.563	.033
depth	.582	1	.582	5.129	.024
site	20.718	2	10.359	91.342	.000
depth * site	17.120	2	8.560	75.482	.000
Error	56.704	500	.113		
Total	420875.934	507			
Corrected Total	95.995	506			

a R Squared = .409 (Adjusted R Squared = .402)

Dependent Variable: Salinity

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	15.280(a)	6	2.547	3.137	.005
Intercept	.099	1	.099	.121	.728
month	1.493	1	1.493	1.839	.176
depth	.025	1	.025	.031	.860
site	12.211	2	6.105	7.520	.001
depth * site	.330	2	.165	.203	.816
Error	392.126	483	.812		
Total	589579.000	490			
Corrected Total	407.406	489			

a R Squared = .038 (Adjusted R Squared = .026)

Dependent Variable: pH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.016(a)	6	.836	7.761	.000
Intercept	4.888	1	4.888	45.381	.000
month	4.056	1	4.056	37.661	.000
depth	.101	1	.101	.934	.334
site	.530	2	.265	2.459	.087
depth * site	.147	2	.073	.681	.506
Error	48.144	447	.108		
Total	27027.830	454			
Corrected Total	53.160	453			

a R Squared = .094 (Adjusted R Squared = .082)

Dependent Variable: SPM

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.022(a)	6	.004	7.392	.000
Intercept	.022	1	.022	44.439	.000
month	.022	1	.022	43.746	.000
depth	.000	1	.000	.230	.631
site	7.66E-005	2	3.83E-005	.077	.925
depth * site	4.55E-005	2	2.27E-005	.046	.955
Error	.240	485	.000		
Total	1.231	492			
Corrected Total	.262	491			

a R Squared = .084 (Adjusted R Squared = .072)

Dependent Variable: POM

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.023(a)	6	.004	8.143	.000
Intercept	.023	1	.023	48.615	.000
month	.023	1	.023	48.251	.000
depth	6.68E-005	1	6.68E-005	.143	.705
site	5.20E-005	2	2.60E-005	.056	.946
depth * site	3.90E-005	2	1.95E-005	.042	.959
Error	.227	485	.000		
Total	.479	492			
Corrected Total	.249	491			

a R Squared = .092 (Adjusted R Squared = .080)

Dependent Variable: Chlorophyll a

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.268(a)	6	.211	2.019	.062
Intercept	.355	1	.355	3.389	.066
month	.371	1	.371	3.544	.060
depth	.036	1	.036	.346	.556
site	.883	2	.442	4.221	.015
depth * site	.042	2	.021	.199	.820
Error	46.877	448	.105		
Total	179.828	455			
Corrected Total	48.145	454			

a R Squared = .026 (Adjusted R Squared = .013)

Dependent Variable: Chlorophyll b

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6.192(a)	6	1.032	3.706	.001
Intercept	4.298	1	4.298	15.432	.000
month	4.374	1	4.374	15.705	.000
depth	.111	1	.111	.399	.528
site	1.849	2	.924	3.320	.037
depth * site	.181	2	.091	.325	.723
Error	124.763	448	.278		
Total	372.061	455			
Corrected Total	130.955	454			

a R Squared = .047 (Adjusted R Squared = .035)

Dependent Variable: Chlorophyll c

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	46.775(a)	6	7.796	3.733	.001
Intercept	33.561	1	33.561	16.070	.000
month	34.162	1	34.162	16.358	.000
depth	.986	1	.986	.472	.492

site	12.669	2	6.335	3.033	.049
depth * site	1.047	2	.524	.251	.778
Error	935.612	448	2.088		
Total	2901.954	455			
Corrected Total	982.387	454			

a R Squared = .048 (Adjusted R Squared = .035)

Dependent Variable: Rainfall

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	279238.112(a)	17	16425.771	7.326	.000
Intercept	718997.491	1	718997.491	320.678	.000
SMONTH	279238.112	17	16425.771	7.326	.000
Error	959626.350	428	2242.118		
Total	2462488.500	446			
Corrected Total	1238864.462	445			

a R Squared = .225 (Adjusted R Squared = .195)

4.3 PCA of environmental parameters

Correlation Matrix

		Temp	Salinity	pH	SPM	POM	Chloro a	Chloro. b	Chloro c	Rain fall
Correlation	Temp	1.000	-.008	.229	.256	.264	.066	.014	.023	.107
	Salinity	-.008	1.000	.054	.023	.014	-.014	.020	.019	-.049
	pH	.229	.054	1.000	.128	.085	-.124	-.199	-.202	.248
	SPM	.256	.023	.128	1.000	.922	-.097	-.155	-.153	-.140
	POM	.264	.014	.085	.922	1.000	-.039	-.097	-.097	-.135
	Chlorophyll a	.066	-.014	-.124	-.097	-.039	1.000	.957	.956	.065
	Chlorophyll b	.014	.020	-.199	-.155	-.097	.957	1.000	.996	.027
	Chlorophyll c	.023	.019	-.202	-.153	-.097	.956	.996	1.000	.046
	Rainfall	.107	-.049	.248	-.140	-.135	.065	.027	.046	1.000
Sig. (1-tailed)	Temp		.428	.000	.000	.000	.082	.382	.316	.013
	Salinity	.428		.127	.305	.379	.380	.336	.346	.158
	pH	.000	.127		.003	.036	.006	.000	.000	.000
	SPM	.000	.305	.003		.000	.019	.000	.001	.002
	POM	.000	.379	.036	.000		.207	.020	.019	.003
	Chlorophyll a	.082	.380	.006	.019	.207		.000	.000	.103
	Chlorophyll b	.382	.336	.000	.000	.020	.000		.000	.297
	Chlorophyll c	.316	.346	.000	.001	.019	.000	.000		.185
	Rainfall	.013	.158	.000	.002	.003	.103	.297	.185	

Communalities

	Initial	Extraction
Temp	1.000	.475
Salinity	1.000	.973
pH	1.000	.628
SPM	1.000	.919
POM	1.000	.919
Chlorophyll a	1.000	.959
Chlorophyll b	1.000	.987
Chlorophyll c	1.000	.988
Rainfall	1.000	.615

Extraction Method: Principal Component Analysis.

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings(a)		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	3.063	34.035	34.035	3.063	34.035	34.035	3.003		
2	2.027	22.525	56.561	2.027	22.525	56.561	2.113		
3	1.358	15.092	71.653	1.358	15.092	71.653	1.395		

4	1.014	11.271	82.924	1.014	11.271	82.924	1.029		
5	.749	8.325	91.249						
6	.658	7.314	98.563						
7	7.614E-02	.846	99.409						
8	4.975E-02	.553	99.962						
9	3.395E-03	3.773E-02	100.000						

Extraction Method: Principal Component Analysis.
a When components are correlated, sums of squared loadings cannot be added to obtain a total variance.

Pattern Matrix(a)

	Component			
	1	2	3	4
Chlorophyll c	.992			
Chlorophyll b	.991			
Chlorophyll a	.981			
POM		.963		
SPM		.954		
pH	-.179		.744	.160
Rainfall		-.268	.742	-.119
Temp	.115	.404	.519	
Salinity				.990

Extraction Method: Principal Component Analysis.
Rotation Method: Oblimin with Kaiser Normalization.
a Rotation converged in 5 iterations.

4.4 Multivariate analysis of variance to determine if environmental parameters differed between sites, depths and sampling month.

Between-Subjects Factors

		Value Label	N
Site	1	Ganan	138
	2	Manselo	122
	3	Batu Terio	139
Depth	1	5m	195
	2	15m	204

Multivariate Tests(c)

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Intercept	Pillai's Trace	1.000	98606.866(a)	8.000	380.000	.000	1.000
	Wilks' Lambda	.000	98606.866(a)	8.000	380.000	.000	1.000
	Hotelling's Trace	2075.934	98606.866(a)	8.000	380.000	.000	1.000
	Roy's Largest Root	2075.934	98606.866(a)	8.000	380.000	.000	1.000
SMONTH	Pillai's Trace	.213	12.861(a)	8.000	380.000	.000	.213
	Wilks' Lambda	.787	12.861(a)	8.000	380.000	.000	.213
	Hotelling's Trace	.271	12.861(a)	8.000	380.000	.000	.213
	Roy's Largest Root	.271	12.861(a)	8.000	380.000	.000	.213
SITE	Pillai's Trace	.138	3.528	16.000	762.000	.000	.069
	Wilks' Lambda	.864	3.613(a)	16.000	760.000	.000	.071
	Hotelling's Trace	.156	3.698	16.000	758.000	.000	.072
	Roy's Largest Root	.144	6.839(b)	8.000	381.000	.000	.126
DEPTH	Pillai's Trace	.010	.487(a)	8.000	380.000	.866	.010
	Wilks' Lambda	.990	.487(a)	8.000	380.000	.866	.010
	Hotelling's Trace	.010	.487(a)	8.000	380.000	.866	.010
	Roy's Largest Root	.010	.487(a)	8.000	380.000	.866	.010
SITE * DEPTH	Pillai's Trace	.091	2.263	16.000	762.000	.003	.045
	Wilks' Lambda	.910	2.302(a)	16.000	760.000	.003	.046
	Hotelling's Trace	.099	2.340	16.000	758.000	.002	.047

	Roy's Largest Root	.094	4.470(b)	8.000	381.000	.000	.086
SITE * SMONTH	Pillai's Trace	.030	.727	16.000	762.000	.768	.015
	Wilks' Lambda	.970	.729(a)	16.000	760.000	.766	.015
	Hotelling's Trace	.031	.730	16.000	758.000	.764	.015
	Roy's Largest Root	.028	1.321(b)	8.000	381.000	.231	.027
DEPTH * SMONTH	Pillai's Trace	.018	.858(a)	8.000	380.000	.552	.018
	Wilks' Lambda	.982	.858(a)	8.000	380.000	.552	.018
	Hotelling's Trace	.018	.858(a)	8.000	380.000	.552	.018
	Roy's Largest Root	.018	.858(a)	8.000	380.000	.552	.018
SITE * DEPTH * SMONTH	Pillai's Trace	.024	.573	16.000	762.000	.905	.012
	Wilks' Lambda	.976	.572(a)	16.000	760.000	.906	.012
	Hotelling's Trace	.024	.571	16.000	758.000	.906	.012
	Roy's Largest Root	.018	.874(b)	8.000	381.000	.538	.018

a Exact statistic

b The statistic is an upper bound on F that yields a lower bound on the significance level.

c Design: Intercept+SMONTH+SITE+DEPTH+SITE * DEPTH+SITE * SMONTH+DEPTH * SMONTH+SITE * DEPTH * SMONTH

Levene's Test of Equality of Error Variances(a)

	F	df1	df2	Sig.
Temp	8.014	5	393	.000
Salinity	2.197	5	393	.054
pH	1.844	5	393	.103
SPM	.637	5	393	.672
POM	.990	5	393	.423
Chlorophyll a	2.136	5	393	.060
Chlorophyll b	2.327	5	393	.042
Chlorophyll c	2.477	5	393	.032

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a Design: Intercept+SMONTH+SITE+DEPTH+SITE * DEPTH+SITE * SMONTH+DEPTH * SMONTH+SITE * DEPTH * SMONTH

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	Temp	30.713(a)	11	2.792	22.454	.000	.390
	Salinity	20.607(b)	11	1.873	2.126	.018	.057
	pH	3.961(c)	11	.360	3.358	.000	.087
	SPM	1.929E-02(d)	11	1.754E-03	3.302	.000	.086
	POM	2.051E-02(e)	11	1.865E-03	3.693	.000	.095
	Chlorophyll a	1.265(f)	11	.115	1.133	.334	.031
	Chlorophyll b	5.199(g)	11	.473	1.758	.060	.048
	Chlorophyll c	39.366(g)	11	3.579	1.765	.058	.048
Intercept	Temp	77784.362	1	77784.362	625543.034	.000	.999
	Salinity	110417.315	1	110417.315	125311.610	.000	.997
	pH	5761.738	1	5761.738	53720.235	.000	.993
	SPM	.298	1	.298	561.284	.000	.592
	POM	.112	1	.112	221.003	.000	.363
	Chlorophyll a	22.373	1	22.373	220.531	.000	.363
	Chlorophyll b	28.541	1	28.541	106.147	.000	.215
	Chlorophyll c	227.657	1	227.657	112.289	.000	.225
SMONTH	Temp	.801	1	.801	6.445	.012	.016
	Salinity	4.898	1	4.898	5.558	.019	.014
	pH	2.937	1	2.937	27.384	.000	.066
	SPM	1.640E-02	1	1.640E-02	30.877	.000	.074
	POM	1.796E-02	1	1.796E-02	35.557	.000	.084
	Chlorophyll a	.233	1	.233	2.294	.131	.006
	Chlorophyll b	3.261	1	3.261	12.127	.001	.030
	Chlorophyll c	25.466	1	25.466	12.561	.000	.031
SITE	Temp	5.407	2	2.704	21.743	.000	.101
	Salinity	5.694	2	2.847	3.231	.041	.016

	pH	.273	2	.137	1.273	.281	.007
	SPM	2.197E-04	2	1.099E-04	.207	.813	.001
	POM	1.332E-04	2	6.662E-05	.132	.876	.001
	Chlorophyll a	9.444E-02	2	4.722E-02	.465	.628	.002
	Chlorophyll b	.182	2	9.096E-02	.338	.713	.002
	Chlorophyll c	1.536	2	.768	.379	.685	.002
DEPTH	Temp	8.569E-02	1	8.569E-02	.689	.407	.002
	Salinity	3.243E-04	1	3.243E-04	.000	.985	.000
	pH	5.585E-03	1	5.585E-03	.052	.820	.000
	SPM	8.112E-06	1	8.112E-06	.015	.902	.000
	POM	1.127E-04	1	1.127E-04	.223	.637	.001
	Chlorophyll a	2.615E-02	1	2.615E-02	.258	.612	.001
	Chlorophyll b	.127	1	.127	.474	.492	.001
	Chlorophyll c	1.253	1	1.253	.618	.432	.002
SITE * DEPTH	Temp	3.319	2	1.660	13.347	.000	.065
	Salinity	.900	2	.450	.511	.600	.003
	pH	3.182E-03	2	1.591E-03	.015	.985	.000
	SPM	7.618E-05	2	3.809E-05	.072	.931	.000
	POM	4.500E-06	2	2.250E-06	.004	.996	.000
	Chlorophyll a	4.551E-02	2	2.276E-02	.224	.799	.001
	Chlorophyll b	9.706E-02	2	4.853E-02	.180	.835	.001
	Chlorophyll c	.402	2	.201	.099	.906	.001
SITE * SMONTH	Temp	.526	2	.263	2.116	.122	.011
	Salinity	.737	2	.369	.418	.659	.002
	pH	.251	2	.125	1.169	.312	.006
	SPM	3.942E-04	2	1.971E-04	.371	.690	.002
	POM	2.643E-04	2	1.322E-04	.262	.770	.001
	Chlorophyll a	9.209E-02	2	4.605E-02	.454	.636	.002
	Chlorophyll b	.187	2	9.333E-02	.347	.707	.002
	Chlorophyll c	1.150	2	.575	.284	.753	.001
DEPTH * SMONTH	Temp	.480	1	.480	3.863	.050	.010
	Salinity	1.133E-02	1	1.133E-02	.013	.910	.000
	pH	1.038E-02	1	1.038E-02	.097	.756	.000
	SPM	3.838E-05	1	3.838E-05	.072	.788	.000
	POM	1.446E-04	1	1.446E-04	.286	.593	.001
	Chlorophyll a	1.510E-03	1	1.510E-03	.015	.903	.000
	Chlorophyll b	3.003E-02	1	3.003E-02	.112	.738	.000
	Chlorophyll c	.351	1	.351	.173	.677	.000
SITE * DEPTH * SMONTH	Temp	4.468E-02	2	2.234E-02	.180	.836	.001
	Salinity	1.325	2	.663	.752	.472	.004
	pH	2.028E-02	2	1.014E-02	.095	.910	.000
	SPM	1.550E-05	2	7.750E-06	.015	.986	.000
	POM	6.124E-05	2	3.062E-05	.061	.941	.000
	Chlorophyll a	2.533E-02	2	1.266E-02	.125	.883	.001
	Chlorophyll b	1.463E-02	2	7.316E-03	.027	.973	.000
	Chlorophyll c	6.658E-02	2	3.329E-02	.016	.984	.000
Error	Temp	48.122	387	.124			
	Salinity	341.002	387	.881			
	pH	41.508	387	.107			
	SPM	.206	387	5.312E-04			
	POM	.195	387	5.050E-04			
	Chlorophyll a	39.261	387	.101			
	Chlorophyll b	104.057	387	.269			
	Chlorophyll c	784.610	387	2.027			
Total	Temp	331236.736	399				
	Salinity	478693.000	399				
	pH	23746.860	399				
	SPM	1.022	399				
	POM	.413	399				
	Chlorophyll a	152.462	399				
	Chlorophyll b	311.525	399				

	Chlorophyll c	2433.493	399				
Corrected Total	Temp	78.836	398				
	Salinity	361.609	398				
	pH	45.469	398				
	SPM	.225	398				
	POM	.216	398				
	Chlorophyll a	40.525	398				
	Chlorophyll b	109.257	398				
	Chlorophyll c	823.977	398				

a R Squared = .390 (Adjusted R Squared = .372)
b R Squared = .057 (Adjusted R Squared = .030)
c R Squared = .087 (Adjusted R Squared = .061)
d R Squared = .086 (Adjusted R Squared = .060)
e R Squared = .095 (Adjusted R Squared = .069)
f R Squared = .031 (Adjusted R Squared = .004)
g R Squared = .048 (Adjusted R Squared = .021)

CHAPTER 5

5.1 Multivariate analysis of variance of length and weight G_T

Between-Subjects Factors

		Value Label	N
Site	1	Ganan	109
	2	Manselo	128
	3	Batu Terio	70
Depth	5		156
	15		151
Size	1	Large	124
	2	Medium	107
	3	Small	76

Multivariate Tests(c)

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.949	2657.022(a)	2.000	288.000	.000
	Wilks' Lambda	.051	2657.022(a)	2.000	288.000	.000
	Hotelling's Trace	18.452	2657.022(a)	2.000	288.000	.000
	Roy's Largest Root	18.452	2657.022(a)	2.000	288.000	.000
Site	Pillai's Trace	.082	6.194	4.000	578.000	.000
	Wilks' Lambda	.918	6.292(a)	4.000	576.000	.000
	Hotelling's Trace	.089	6.390	4.000	574.000	.000
	Roy's Largest Root	.086	12.463(b)	2.000	289.000	.000
Depth	Pillai's Trace	.039	5.922(a)	2.000	288.000	.003
	Wilks' Lambda	.961	5.922(a)	2.000	288.000	.003
	Hotelling's Trace	.041	5.922(a)	2.000	288.000	.003
	Roy's Largest Root	.041	5.922(a)	2.000	288.000	.003
Size	Pillai's Trace	.926	124.506	4.000	578.000	.000
	Wilks' Lambda	.123	265.975(a)	4.000	576.000	.000
	Hotelling's Trace	6.708	481.311	4.000	574.000	.000
	Roy's Largest Root	6.648	960.691(b)	2.000	289.000	.000
Site * Depth	Pillai's Trace	.026	1.924	4.000	578.000	.105
	Wilks' Lambda	.974	1.923(a)	4.000	576.000	.105
	Hotelling's Trace	.027	1.923	4.000	574.000	.105
	Roy's Largest Root	.023	3.296(b)	2.000	289.000	.038
Site * Size	Pillai's Trace	.116	4.465	8.000	578.000	.000
	Wilks' Lambda	.885	4.514(a)	8.000	576.000	.000
	Hotelling's Trace	.127	4.562	8.000	574.000	.000
	Roy's Largest Root	.107	7.739(b)	4.000	289.000	.000
Depth * Size	Pillai's Trace	.024	1.744	4.000	578.000	.139
	Wilks' Lambda	.976	1.738(a)	4.000	576.000	.140
	Hotelling's Trace	.024	1.733	4.000	574.000	.141
	Roy's Largest Root	.015	2.130(b)	2.000	289.000	.121
Site * Depth * Size	Pillai's Trace	.059	2.199	8.000	578.000	.026
	Wilks' Lambda	.942	2.192(a)	8.000	576.000	.027
	Hotelling's Trace	.061	2.185	8.000	574.000	.027
	Roy's Largest Root	.035	2.514(b)	4.000	289.000	.042

a Exact statistic
b The statistic is an upper bound on F that yields a lower bound on the significance level.
c Design: Intercept+Site+Depth+Size+Site * Depth+Site * Size+Depth * Size+Site * Depth * Size

Levene's Test of Equality of Error Variances(a)

	F	df1	df2	Sig.
GTLength	2.116	17	289	.007
GTWeight	2.074	17	289	.008

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.
a Design: Intercept+Site+Depth+Size+Site * Depth+Site * Size+Depth * Size+Site * Depth * Size

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	GTLength	329226.060(a)	17	19366.239	113.331	.000
	GTWeight	1557445.808(b)	17	91614.459	12.096	.000
Intercept	GTLength	870019.879	1	870019.879	5091.345	.000
	GTWeight	21407780.647	1	21407780.647	2826.616	.000
Site	GTLength	383.072	2	191.536	1.121	.327
	GTWeight	166695.217	2	83347.609	11.005	.000
Depth	GTLength	1905.773	1	1905.773	11.153	.001
	GTWeight	10609.783	1	10609.783	1.401	.238
Size	GTLength	286883.599	2	143441.800	839.420	.000
	GTWeight	920892.060	2	460446.030	60.796	.000
Site * Depth	GTLength	1125.830	2	562.915	3.294	.038
	GTWeight	20957.704	2	10478.852	1.384	.252
Site * Size	GTLength	3111.060	4	777.765	4.551	.001
	GTWeight	228496.155	4	57124.039	7.542	.000
Depth * Size	GTLength	696.893	2	348.446	2.039	.132
	GTWeight	21314.561	2	10657.280	1.407	.247
Site * Depth * Size	GTLength	1461.227	4	365.307	2.138	.076
	GTWeight	58641.930	4	14660.482	1.936	.105
Error	GTLength	49384.933	289	170.882		
	GTWeight	2188782.746	289	7573.643		
Total	GTLength	1185447.503	307			
	GTWeight	27264263.905	307			
Corrected Total	GTLength	378610.993	306			
	GTWeight	3746228.554	306			

a R Squared = .870 (Adjusted R Squared = .862)
b R Squared = .416 (Adjusted R Squared = .381)

Post hoc Multiple Comparisons
Site
Bonferroni

Dependent Variable	(I) Site	(J) Site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
GTLength	Ganan	Manselo	-3.472	1.7037	.127	-7.574	.631
		Batu Terio	-2.553	2.0022	.610	-7.374	2.269
	Manselo	Ganan	3.472	1.7037	.127	-.631	7.574
		Batu Terio	.919	1.9432	1.000	-3.760	5.598
	Batu Terio	Ganan	2.553	2.0022	.610	-2.269	7.374
		Manselo	-.919	1.9432	1.000	-5.598	3.760
GTWeight	Ganan	Manselo	-50.0454(*)	11.34249	.000	-77.3581	-22.7327
		Batu Terio	-27.3528	13.32959	.123	-59.4504	4.7448
	Manselo	Ganan	50.0454(*)	11.34249	.000	22.7327	77.3581
		Batu Terio	22.6926	12.93692	.241	-8.4594	53.8447
	Batu Terio	Ganan	27.3528	13.32959	.123	-4.7448	59.4504
		Manselo	-22.6926	12.93692	.241	-53.8447	8.4594

Based on observed means.
* The mean difference is significant at the .05 level.

Size
Bonferroni

Dependent Variable	(I) Size	(J) Size	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
GTLength	Large	Medium	-40.317(*)	1.7249	.000	-44.471	-36.164
		Small	-81.495(*)	1.9043	.000	-86.081	-76.909
	Medium	Large	40.317(*)	1.7249	.000	36.164	44.471
		Small	-41.177(*)	1.9610	.000	-45.900	-36.455
	Small	Large	81.495(*)	1.9043	.000	76.909	86.081
		Medium	41.177(*)	1.9610	.000	36.455	45.900
GTWeight	Large	Medium	-107.4357(*)	11.48301	.000	-135.0867	-79.7846

		Small	-133.8757(*)	12.67797	.000	-164.4043	-103.3472
	Medium	Large	107.4357(*)	11.48301	.000	79.7846	135.0867
		Small	-26.4401	13.05507	.131	-57.8766	4.9965
	Small	Large	133.8757(*)	12.67797	.000	103.3472	164.4043
		Medium	26.4401	13.05507	.131	-4.9965	57.8766

Based on observed means.
* The mean difference is significant at the .05 level.

5.2 ANOVA of G_{30} length and weight with repeated measures

Within-Subjects Factors

Measure	month	Dependent Variable
length	1	ljun00
	2	ljul00
	3	laug00
	4	lsep00
	5	loct00
	6	lnov00
	7	ljan01
	8	lfeb01
	9	lmar01
	10	lapr01
	11	lmay01
	12	ljun01
	13	ljul01
	14	laug01
	15	lsep01
	16	loct01
	17	lnov01
weight	1	wjun00
	2	wjul00
	3	waug00
	4	wsep00
	5	woct00
	6	wnov00
	7	wjan01
	8	wfeb01
	9	wmar01
	10	wapr01
	11	wmay01
	12	wjun01
	13	wjul01
	14	waug01
	15	wsep01
	16	woct01
	17	wnov01

Between-Subjects Factors

		Value Label	N
SITE	1	Ganan	108
	2	Manselo	124
	3	Batu Terio	70
DEPTH	1	5m	152
	2	15m	150
SIZE(AGE)	1	Large	119
	2	Medium	107
	3	Small	76

Multivariate Tests(c)

Effect			Value	F	Hypothesis df	Error df	Sig.
Between Subjects	Intercept	Pillai's Trace	.958	3222.892(a)	2.000	282.000	.000
		Wilks' Lambda	.042	3222.892(a)	2.000	282.000	.000

		Hotelling's Trace	22.857	3222.892(a)	2.000	282.000	.000
		Roy's Largest Root	22.857	3222.892(a)	2.000	282.000	.000
	site	Pillai's Trace	.028	2.028	4.000	566.000	.089
		Wilks' Lambda	.972	2.033(a)	4.000	564.000	.088
		Hotelling's Trace	.029	2.038	4.000	562.000	.088
		Roy's Largest Root	.028	3.933(b)	2.000	283.000	.021
	depth	Pillai's Trace	.059	8.846(a)	2.000	282.000	.000
		Wilks' Lambda	.941	8.846(a)	2.000	282.000	.000
		Hotelling's Trace	.063	8.846(a)	2.000	282.000	.000
		Roy's Largest Root	.063	8.846(a)	2.000	282.000	.000
	size	Pillai's Trace	.921	120.682	4.000	566.000	.000
		Wilks' Lambda	.088	335.168(a)	4.000	564.000	.000
		Hotelling's Trace	10.310	724.293	4.000	562.000	.000
		Roy's Largest Root	10.301	1457.599(b)	2.000	283.000	.000
	site * depth	Pillai's Trace	.024	1.742	4.000	566.000	.139
		Wilks' Lambda	.976	1.745(a)	4.000	564.000	.139
		Hotelling's Trace	.025	1.748	4.000	562.000	.138
		Roy's Largest Root	.024	3.408(b)	2.000	283.000	.034
	site * size	Pillai's Trace	.060	2.185	8.000	566.000	.027
		Wilks' Lambda	.940	2.198(a)	8.000	564.000	.026
		Hotelling's Trace	.063	2.211	8.000	562.000	.025
		Roy's Largest Root	.056	3.974(b)	4.000	283.000	.004
	depth * size	Pillai's Trace	.038	2.743	4.000	566.000	.028
		Wilks' Lambda	.962	2.758(a)	4.000	564.000	.027
		Hotelling's Trace	.039	2.773	4.000	562.000	.027
		Roy's Largest Root	.039	5.475(b)	2.000	283.000	.005
	site * depth * size	Pillai's Trace	.056	2.050	8.000	566.000	.039
		Wilks' Lambda	.944	2.067(a)	8.000	564.000	.037
		Hotelling's Trace	.059	2.083	8.000	562.000	.036
		Roy's Largest Root	.056	3.975(b)	4.000	283.000	.004
Within Subjects	month	Pillai's Trace	.973	280.759(a)	32.000	252.000	.000
		Wilks' Lambda	.027	280.759(a)	32.000	252.000	.000
		Hotelling's Trace	35.652	280.759(a)	32.000	252.000	.000
		Roy's Largest Root	35.652	280.759(a)	32.000	252.000	.000
	month * site	Pillai's Trace	1.157	10.861	64.000	506.000	.000
		Wilks' Lambda	.173	11.054(a)	64.000	504.000	.000
		Hotelling's Trace	2.868	11.248	64.000	502.000	.000
		Roy's Largest Root	1.817	14.364(b)	32.000	253.000	.000
	month * depth	Pillai's Trace	.198	1.943(a)	32.000	252.000	.003
		Wilks' Lambda	.802	1.943(a)	32.000	252.000	.003
		Hotelling's Trace	.247	1.943(a)	32.000	252.000	.003
		Roy's Largest Root	.247	1.943(a)	32.000	252.000	.003
	month * size	Pillai's Trace	1.369	17.137	64.000	506.000	.000
		Wilks' Lambda	.033	35.633(a)	64.000	504.000	.000
		Hotelling's Trace	17.273	67.742	64.000	502.000	.000
		Roy's Largest Root	16.532	130.705(b)	32.000	253.000	.000
	month * site * depth	Pillai's Trace	.351	1.683	64.000	506.000	.001
		Wilks' Lambda	.678	1.692(a)	64.000	504.000	.001
		Hotelling's Trace	.434	1.700	64.000	502.000	.001
		Roy's Largest Root	.285	2.255(b)	32.000	253.000	.000
	month * site * size	Pillai's Trace	1.229	3.535	128.000	1020.000	.000
		Wilks' Lambda	.217	3.674	128.000	1005.147	.000
		Hotelling's Trace	1.950	3.817	128.000	1002.000	.000
		Roy's Largest Root	.902	7.189(b)	32.000	255.000	.000
	month * depth * size	Pillai's Trace	.463	2.385	64.000	506.000	.000
		Wilks' Lambda	.582	2.444(a)	64.000	504.000	.000
		Hotelling's Trace	.639	2.504	64.000	502.000	.000
		Roy's Largest Root	.472	3.731(b)	32.000	253.000	.000
	month * site * depth * size	Pillai's Trace	.705	1.704	128.000	1020.000	.000
		Wilks' Lambda	.448	1.756	128.000	1005.147	.000

	Hotelling's Trace	.926	1.812	128.000	1002.000	.000
	Roy's Largest Root	.496	3.952(b)	32.000	255.000	.000

a Exact statistic
b The statistic is an upper bound on F that yields a lower bound on the significance level.
c Design: Intercept+site+depth+size+site * depth+site * size+depth * size+site * depth * size
Within Subjects Design: month

Mauchly's Test of Sphericity(b)

Within Subjects Effect	Measure	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon(a) Greenhouse-Geisser	Huynh-Feldt	Lower-bound
month	length	.001	1871.594	135	.000	.537	.588	.063
	weight	.000	7367.849	135	.000	.154	.164	.063

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.
a May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.
b Design: Intercept+site+depth+size+site * depth+site * size+depth * size+site * depth * size
Within Subjects Design: month

Tests of Within-Subjects Effects
Multivariate(c,d)

Within Subjects Effect		Value	F	Hypothesis df	Error df	Sig.
month	Pillai's Trace	.549	107.004	32.000	9056.000	.000
	Wilks' Lambda	.479	125.909(a)	32.000	9054.000	.000
	Hotelling's Trace	1.030	145.723	32.000	9052.000	.000
	Roy's Largest Root	.971	274.743(b)	16.000	4528.000	.000
month * site	Pillai's Trace	.087	6.431	64.000	9056.000	.000
	Wilks' Lambda	.915	6.443(a)	64.000	9054.000	.000
	Hotelling's Trace	.091	6.456	64.000	9052.000	.000
	Roy's Largest Root	.060	8.516(b)	32.000	4528.000	.000
month * depth	Pillai's Trace	.012	1.660	32.000	9056.000	.011
	Wilks' Lambda	.988	1.662(a)	32.000	9054.000	.011
	Hotelling's Trace	.012	1.664	32.000	9052.000	.011
	Roy's Largest Root	.010	2.760(b)	16.000	4528.000	.000
month * size	Pillai's Trace	.411	36.580	64.000	9056.000	.000
	Wilks' Lambda	.599	41.298(a)	64.000	9054.000	.000
	Hotelling's Trace	.652	46.139	64.000	9052.000	.000
	Roy's Largest Root	.626	88.560(b)	32.000	4528.000	.000
month * site * depth	Pillai's Trace	.024	1.683	64.000	9056.000	.001
	Wilks' Lambda	.977	1.687(a)	64.000	9054.000	.001
	Hotelling's Trace	.024	1.692	64.000	9052.000	.000
	Roy's Largest Root	.020	2.897(b)	32.000	4528.000	.000
month * site * size	Pillai's Trace	.066	2.404	128.000	9056.000	.000
	Wilks' Lambda	.935	2.414(a)	128.000	9054.000	.000
	Hotelling's Trace	.069	2.424	128.000	9052.000	.000
	Roy's Largest Root	.052	3.692(b)	64.000	4528.000	.000
month * depth * size	Pillai's Trace	.031	2.250	64.000	9056.000	.000
	Wilks' Lambda	.969	2.256(a)	64.000	9054.000	.000
	Hotelling's Trace	.032	2.261	64.000	9052.000	.000
	Roy's Largest Root	.025	3.594(b)	32.000	4528.000	.000
month * site * depth * size	Pillai's Trace	.055	1.998	128.000	9056.000	.000
	Wilks' Lambda	.946	2.002(a)	128.000	9054.000	.000
	Hotelling's Trace	.057	2.007	128.000	9052.000	.000
	Roy's Largest Root	.040	2.846(b)	64.000	4528.000	.000

a Exact statistic
b The statistic is an upper bound on F that yields a lower bound on the significance level.
c Design: Intercept+site+depth+size+site * depth+site * size+depth * size+site * depth * size
Within Subjects Design: month
d Tests are based on averaged variables.

Univariate Tests

Source	Measure	Type III Sum of Squares	df	Mean Square	F	Sig.

month	length	Sphericity Assumed	187.634	16	11.727	253.560	.000
		Greenhouse-Geisser	187.634	8.586	21.853	253.560	.000
	weight	Sphericity Assumed	975.511	16	60.969	51.147	.000
		Greenhouse-Geisser	975.511	2.456	397.170	51.147	.000
month * site	length	Sphericity Assumed	12.501	32	.391	8.447	.000
		Greenhouse-Geisser	12.501	17.173	.728	8.447	.000
	weight	Sphericity Assumed	168.281	32	5.259	4.412	.000
		Greenhouse-Geisser	168.281	4.912	34.257	4.412	.001
month * depth	length	Sphericity Assumed	1.993	16	.125	2.693	.000
		Greenhouse-Geisser	1.993	8.586	.232	2.693	.005
	weight	Sphericity Assumed	13.570	16	.848	.711	.785
		Greenhouse-Geisser	13.570	2.456	5.525	.711	.519
month * size	length	Sphericity Assumed	121.437	32	3.795	82.053	.000
		Greenhouse-Geisser	121.437	17.173	7.072	82.053	.000
	weight	Sphericity Assumed	552.116	32	17.254	14.474	.000
		Greenhouse-Geisser	552.116	4.912	112.394	14.474	.000
month * site * depth	length	Sphericity Assumed	4.216	32	.132	2.849	.000
		Greenhouse-Geisser	4.216	17.173	.245	2.849	.000
	weight	Sphericity Assumed	23.221	32	.726	.609	.959
		Greenhouse-Geisser	23.221	4.912	4.727	.609	.690
month * site * size	length	Sphericity Assumed	10.829	64	.169	3.658	.000
		Greenhouse-Geisser	10.829	34.345	.315	3.658	.000
	weight	Sphericity Assumed	88.632	64	1.385	1.162	.179
		Greenhouse-Geisser	88.632	9.825	9.021	1.162	.314
month * depth * size	length	Sphericity Assumed	5.129	32	.160	3.466	.000
	weight	Sphericity Assumed	44.606	32	1.394	1.169	.235
		Greenhouse-Geisser	44.606	4.912	9.080	1.169	.323
month * site * depth * size	length	Sphericity Assumed	8.401	64	.131	2.838	.000
		Greenhouse-Geisser	8.401	34.345	.245	2.838	.000
	weight	Sphericity Assumed	89.250	64	1.395	1.170	.168
		Greenhouse-Geisser	89.250	9.825	9.084	1.170	.309
Error(month)	length	Sphericity Assumed	209.419	4528	.046		
		Greenhouse-Geisser	209.419	2429.938	.086		
	weight	Sphericity Assumed	5397.611	4528	1.192		
		Greenhouse-Geisser	5397.611	695.091	7.765		

Tests of Between-Subjects Effects
Transformed Variable: Average

Source	Measure	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	length	353.041	1	353.041	6158.220	.000
	weight	2584.776	1	2584.776	3988.138	.000
site	length	.432	2	.216	3.764	.024
	weight	1.218	2	.609	.940	.392
depth	length	.969	1	.969	16.898	.000
	weight	2.274	1	2.274	3.509	.062
size	length	157.507	2	78.754	1373.727	.000
	weight	1204.761	2	602.381	929.433	.000
site * depth	length	.346	2	.173	3.017	.051
	weight	3.300	2	1.650	2.546	.080
site * size	length	.572	4	.143	2.495	.043
	weight	10.010	4	2.503	3.861	.005
depth * size	length	.575	2	.287	5.012	.007
	weight	1.120	2	.560	.864	.423
site * depth * size	length	.897	4	.224	3.912	.004
	weight	5.691	4	1.423	2.195	.070
Error	length	16.224	283	.057		
	weight	183.417	283	.648		

Post Hoc Tests

Site
Bonferroni

Measure	(I) site	(J) site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
length	Ganan	Manselo	-.03808(*)	.007658	.000	-.05652	-.01964
		Batu Terio	-.01730	.008911	.160	-.03876	.00416
	Manselo	Ganan	.03808(*)	.007658	.000	.01964	.05652
		Batu Terio	.02078	.008694	.052	-.00016	.04172
	Batu Terio	Ganan	.01730	.008911	.160	-.00416	.03876
		Manselo	-.02078	.008694	.052	-.04172	.00016
weight	Ganan	Manselo	-.07706(*)	.025748	.009	-.13907	-.01506
		Batu Terio	-.03998	.029961	.550	-.11213	.03218
	Manselo	Ganan	.07706(*)	.025748	.009	.01506	.13907
		Batu Terio	.03709	.029233	.617	-.03332	.10749
	Batu Terio	Ganan	.03998	.029961	.550	-.03218	.11213
		Manselo	-.03709	.029233	.617	-.10749	.03332

Based on observed means.
* The mean difference is significant at the .05 level.

Size
Bonferroni

Measure	(I) size	(J) size	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
length	Large	Medium	-.18040(*)	.007756	.000	-.19908	-.16173
		Small	-.47129(*)	.008527	.000	-.49182	-.45075
	Medium	Large	.18040(*)	.007756	.000	.16173	.19908
		Small	-.29088(*)	.008728	.000	-.31190	-.26986
	Small	Large	.47129(*)	.008527	.000	.45075	.49182
		Medium	.29088(*)	.008728	.000	.26986	.31190
weight	Large	Medium	-.53042(*)	.026078	.000	-.59322	-.46762
		Small	-1.29954(*)	.028671	.000	-1.36859	-1.23050
	Medium	Large	.53042(*)	.026078	.000	.46762	.59322
		Small	-.76912(*)	.029348	.000	-.83980	-.69845
	Small	Large	1.29954(*)	.028671	.000	1.23050	1.36859
		Medium	.76912(*)	.029348	.000	.69845	.83980

Based on observed means.
* The mean difference is significant at the .05 level.

5.3 ANOVA of G_{30} length and G_{30} weight partitioned by size

G_{30} length

SIZE = Small
Between-Subjects Factors[a]

		Value Label	N
DEPTH	5		985
	15		926
SITE	1	Ganan	680
	2	Manselo	642
	3	Batu Terio	589

a. SIZE = Small

Levene's Test of Equality of Error Variances[a,b]
Dependent Variable:MIGR_Length

F	df1	df2	Sig.

6.489	5	1905	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. SIZE = Small

b. Design: Intercept + DEPTH + SITE + DEPTH * SITE

Tests of Between-Subjects Effects[b]

Dependent Variable:MIGR_Length

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7.736[a]	5	1.547	4.839	.000
Intercept	700.591	1	700.591	2191.309	.000
DEPTH	.248	1	.248	.775	.379
SITE	4.366	2	2.183	6.829	.001
DEPTH * SITE	3.295	2	1.647	5.153	.006
Error	609.054	1905	.320		
Total	1312.474	1911			
Corrected Total	616.790	1910			

a. R Squared = .013 (Adjusted R Squared = .010)

b. SIZE = Small

SIZE = Medium

Between-Subjects Factors[a]

		Value Label	N
DEPTH	5		1106
	15		1072
SITE	1	Ganan	767
	2	Manselo	815
	3	Batu Terio	596

a. SIZE = Medium

Levene's Test of Equality of Error Variances[a,b]

Dependent Variable:MIGR_Length

F	df1	df2	Sig.
7.106	5	2172	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. SIZE = Medium

b. Design: Intercept + DEPTH + SITE + DEPTH * SITE

Tests of Between-Subjects Effects[b]

Dependent Variable:MIGR_Length

Source	Type III Sum of Squares	df	Mean Square	F	Sig.

Corrected Model	1.357[a]	5	.271	3.117	.008
Intercept	150.572	1	150.572	1729.974	.000
DEPTH	.006	1	.006	.064	.801
SITE	.991	2	.495	5.692	.003
DEPTH * SITE	.328	2	.164	1.886	.152
Error	189.045	2172	.087		
Total	341.769	2178			
Corrected Total	190.402	2177			

a. R Squared = .007 (Adjusted R Squared = .005)

b. SIZE = Medium

SIZE = Large

Between-Subjects Factors[a]

		Value Label	N
DEPTH	5		1151
	15		1217
SITE	1	Ganan	794
	2	Manselo	833
	3	Batu Terio	741

a. SIZE = Large

Levene's Test of Equality of Error Variances[a,b]

Dependent Variable:MIGR_Length

F	df1	df2	Sig.
19.815	5	2362	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. SIZE = Large

b. Design: Intercept + DEPTH + SITE + DEPTH * SITE

Tests of Between-Subjects Effects[b]

Dependent Variable:MIGR_Length

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.902[a]	5	.180	10.876	.000
Intercept	11.224	1	11.224	677.015	.000
DEPTH	.706	1	.706	42.601	.000
SITE	.081	2	.041	2.447	.087
DEPTH * SITE	.088	2	.044	2.649	.071
Error	39.159	2362	.017		
Total	51.290	2368			
Corrected Total	40.060	2367			

a. R Squared = .023 (Adjusted R Squared = .020)

b. SIZE = Large

G_{30} weight

SIZE = Small

Between-Subjects Factors[a]

		Value Label	N
DEPTH	5		984
	15		921
SITE	1	Ganan	680
	2	Manselo	640
	3	Batu Terio	585

a. SIZE = Small

Levene's Test of Equality of Error Variances[a,b]

Dependent Variable:MIGR_Weight

F	df1	df2	Sig.
2.356	5	1899	.038

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. SIZE = Small

b. Design: Intercept + DEPTH + SITE + DEPTH * SITE

Tests of Between-Subjects Effects[b]

Dependent Variable:MIGR_Weight

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	44.548[a]	5	8.910	3.992	.001
Intercept	4883.894	1	4883.894	2188.307	.000
DEPTH	.021	1	.021	.009	.922
SITE	25.240	2	12.620	5.655	.004
DEPTH * SITE	21.872	2	10.936	4.900	.008
Error	4238.214	1899	2.232		
Total	9133.924	1905			
Corrected Total	4282.763	1904			

a. R Squared = .010 (Adjusted R Squared = .008)

b. SIZE = Small

SIZE = Medium

Between-Subjects Factorsa

		Value Label	N
DEPTH	5		1098
	15		1070
SITE	1	Ganan	767
	2	Manselo	813
	3	Batu Terio	588

Between-Subjects Factorsa

		Value Label	N
DEPTH	5		1098
	15		1070
SITE	1	Ganan	767
	2	Manselo	813
	3	Batu Terio	588

a. SIZE = Medium

Levene's Test of Equality of Error Variances[a,b]

Dependent Variable:MIGR_Weight

F	df1	df2	Sig.
2.038	5	2162	.071

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. SIZE = Medium

b. Design: Intercept + DEPTH + SITE + DEPTH * SITE

Tests of Between-Subjects Effects[b]

Dependent Variable:MIGR_Weight

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	12.897[a]	5	2.579	4.602	.000
Intercept	1074.662	1	1074.662	1917.468	.000
DEPTH	.174	1	.174	.311	.577
SITE	11.029	2	5.514	9.839	.000
DEPTH * SITE	1.587	2	.794	1.416	.243
Error	1211.713	2162	.560		
Total	2310.166	2168			
Corrected Total	1224.609	2167			

a. R Squared = .011 (Adjusted R Squared = .008)

b. SIZE = Medium

SIZE = Large

Between-Subjects Factors[a]

		Value Label	N
DEPTH	5		1153
	15		1216
SITE	1	Ganan	793
	2	Manselo	835
	3	Batu Terio	741

a. SIZE = Large

Levene's Test of Equality of Error Variances[a,b]

Dependent Variable:MIGR_Weight

F	df1	df2	Sig.
4.040	5	2363	.001

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. SIZE = Large

b. Design: Intercept + DEPTH + SITE + DEPTH * SITE

Tests of Between-Subjects Effects[b]

Dependent Variable:MIGR_Weight

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8.570[a]	5	1.714	1.221	.297
Intercept	62.074	1	62.074	44.210	.000
DEPTH	3.568	1	3.568	2.541	.111
SITE	3.141	2	1.571	1.119	.327
DEPTH * SITE	1.802	2	.901	.642	.527
Error	3317.835	2363	1.404		
Total	3387.651	2369			
Corrected Total	3326.405	2368			

a. R Squared = .003 (Adjusted R Squared = .000)

b. SIZE = Large

5.4 ANOVA of oyster mortality (3-way factorial)

Tests of Between-Subjects Effects

Dependent Variable: Mortality

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	471.352(a)	255	1.848	2.310	.000
Intercept	62.235	1	62.235	77.774	.000
Month	45.432	17	2.672	3.340	.000
Site	16.321	2	8.160	10.198	.000
Size	11.062	2	5.531	6.912	.002
Depth	.049	1	.049	.062	.805
Month * Site	106.012	34	3.118	3.897	.000
Month * Size	55.605	34	1.635	2.044	.006
Month * Depth	30.951	17	1.821	2.275	.009
Site * Size	1.994	4	.498	.623	.648
Site * Depth	.321	2	.160	.201	.819
Size * Depth	1.210	2	.605	.756	.473
Month * Site * Size	130.340	68	1.917	2.395	.000
Month * Site * Depth	40.012	34	1.177	1.471	.089
Month * Size * Depth	29.457	34	.866	1.083	.382
Site * Size * Depth	2.586	4	.647	.808	.524
Error	54.414	68	.800		
Total	588.000	324			
Corrected Total	525.765	323			

a R Squared = .897 (Adjusted R Squared = .508)

Multiple Comparisons
Dependent Variable: Mortality
Bonferroni

(I) Site	(J) Site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ganan	Manselo	.17	.122	.526	-.13	.47
	Batu Terio	-.37(*)	.122	.010	-.67	-.07
Manselo	Ganan	-.17	.122	.526	-.47	.13
	Batu Terio	-.54(*)	.122	.000	-.84	-.24
Batu Terio	Ganan	.37(*)	.122	.010	.07	.67
	Manselo	.54(*)	.122	.000	.24	.84

Based on observed means.
* The mean difference is significant at the .05 level.

Multiple Comparisons
Dependent Variable: Mortality
Bonferroni

(I) Size	(J) Size	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Large	Medium	-.15	.122	.683	-.45	.15
	Small	-.44(*)	.122	.002	-.74	-.15
Medium	Large	.15	.122	.683	-.15	.45
	Small	-.30	.122	.053	-.60	.00
Small	Large	.44(*)	.122	.002	.15	.74
	Medium	.30	.122	.053	.00	.60

Based on observed means.
* The mean difference is significant at the .05 level.

5.5 ANOVA of Condition Index (3-way factorial)

Tests of Between-Subjects Effects
Dependent Variable: CI

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	82328.921(a)	255	322.859	6.600	.000
Intercept	727005.626	1	727005.626	14862.453	.000
Month	29518.993	17	1736.411	35.498	.000
Site	1733.447	2	866.723	17.719	.000
Depth	794.919	1	794.919	16.251	.000
Size	23263.346	2	11631.673	237.791	.000
Month * Site	3447.784	34	101.405	2.073	.001
Month * Depth	1780.279	17	104.722	2.141	.005
Month * Size	9130.809	34	268.553	5.490	.000
Site * Depth	296.140	2	148.070	3.027	.050
Site * Size	1001.554	4	250.388	5.119	.000
Depth * Size	8.989	2	4.495	.092	.912
Month * Site * Depth	2755.996	34	81.059	1.657	.014
Month * Site * Size	5969.300	68	87.784	1.795	.000
Month * Depth * Size	1930.625	34	56.783	1.161	.251
Site * Depth * Size	696.739	4	174.185	3.561	.007
Error	19174.910	392	48.916		
Total	828509.457	648			
Corrected Total	101503.831	647			

a R Squared = .811 (Adjusted R Squared = .688)

Multiple Comparisons
Dependent Variable: CI
Bonferroni

(I) Site	(J) Site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ganan	Manselo	-2.9350(*)	.67299	.000	-4.5530	-1.3169
	Batu Terio	-3.8291(*)	.67299	.000	-5.4472	-2.2110
Manselo	Ganan	2.9350(*)	.67299	.000	1.3169	4.5530
	Batu Terio	-.8942	.67299	.554	-2.5122	.7239
Batu Terio	Ganan	3.8291(*)	.67299	.000	2.2110	5.4472
	Manselo	.8942	.67299	.554	-.7239	2.5122

Based on observed means.
* The mean difference is significant at the .05 level.

Multiple Comparisons
Dependent Variable: CI
Bonferroni

(I) Size	(J) Size	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Large	Medium	-6.4347(*)	.67299	.000	-8.0528	-4.8166
	Small	-14.6409(*)	.67299	.000	-16.2590	-13.0228
Medium	Large	6.4347(*)	.67299	.000	4.8166	8.0528
	Small	-8.2062(*)	.67299	.000	-9.8242	-6.5881
Small	Large	14.6409(*)	.67299	.000	13.0228	16.2590
	Medium	8.2062(*)	.67299	.000	6.5881	9.8242

Based on observed means.
* The mean difference is significant at the .05 level.

5.6 Stepwise regression of G_{30} length with environmental parameters

All sizes, sites and depths
Variables Entered/Removed(a)

Model	Variables Entered	Variables Removed	Method
1	pH	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
2	Salinity	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: G30Length

Model Summary(c)

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.215(a)	.046	.043	.3349415	.046	13.727	1	283	.000
2	.271(b)	.073	.067	.3307334	.027	8.247	1	282	.004

a Predictors: (Constant), pH
b Predictors: (Constant), pH, Salinity
c Dependent Variable: G30Length

ANOVA©

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.540	1	1.540	13.727	.000(a)
	Residual	31.749	283	.112		
	Total	33.289	284			
2	Regression	2.442	2	1.221	11.163	.000(b)

Residual	30.846	282	.109			
Total	33.289	284				

a Predictors: (Constant), pH
b Predictors: (Constant), pH, Salinity
c Dependent Variable: G30Length

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-1.540	.493		-3.121	.002					
	pH	.238	.064	.215	3.705	.000	.215	.215	.215	1.000	1.000
2	(Constant)	1.426	1.142		1.249	.213					
	pH	.257	.064	.232	4.028	.000	.215	.233	.231	.989	1.011
	Salinity	-.090	.031	-.166	-2.872	.004	-.142	-.169	-.165	.989	1.011

a Dependent Variable: G30Length

Excluded Variables(c)

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics		
						Tolerance	VIF	Minimum Tolerance
1	SPM	.033(a)	.560	.576	.033	.956	1.046	.956
	POM	.052(a)	.882	.378	.052	.985	1.015	.985
	Salinity	-.166(a)	-2.872	.004	-.169	.989	1.011	.989
	Temperature	.022(a)	.370	.712	.022	.945	1.059	.945
	a	.127(a)	2.097	.037	.124	.903	1.107	.903
	b	.035(a)	.545	.586	.032	.799	1.252	.799
	c	.041(a)	.621	.535	.037	.787	1.270	.787
	Rainfall	-.014(a)	-.231	.817	-.014	.966	1.035	.966
2	SPM	.047(b)	.805	.421	.048	.950	1.053	.950
	POM	.060(b)	1.038	.300	.062	.983	1.017	.976
	Temperature	.009(b)	.150	.881	.009	.939	1.065	.931
	a	.089(b)	1.428	.154	.085	.844	1.184	.844
	b	.007(b)	.115	.908	.007	.780	1.282	.780
	c	.010(b)	.149	.881	.009	.765	1.307	.765
	Rainfall	-.023(b)	-.401	.689	-.024	.963	1.038	.954

a Predictors in the Model: (Constant), pH
b Predictors in the Model: (Constant), pH, Salinity
c Dependent Variable: G30Length

Site = Manselo

Variables Entered/Removed(a,b)

Model	Variables Entered	Variables Removed	Method
1	pH	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: G30Length
b Models are based only on cases for which site = Manselo

Model Summary(b,c)

Model	R		R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
	site = Manselo (Selected)	site ~= Manselo (Unselected)				R Square Change	F Change	df1	df2	Sig. F Change
1	.249(a)	.206	.062	.052	.3239461	.062	6.224	1	94	.014

a Predictors: (Constant), pH
b Unless noted otherwise, statistics are based only on cases for which site = Manselo.
c Dependent Variable: G30Length

Coefficients(a,b)

Model	Unstandardized	Standardized	t	Sig.	Correlations	Collinearity

		Coefficients		Coefficients						Statistics	
		B	Std. Error	Beta			Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-2.378	1.071		-2.220	.029					
	pH	.345	.138	.249	2.495	.014	.249	.249	.249	1.000	1.000

a Dependent Variable: G30Length
b Selecting only cases for which site = Manselo

Excluded Variables(b)

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics		
						Tolerance	VIF	Minimum Tolerance
1	SPM	-.077(a)	-.770	.443	-.080	.994	1.006	.994
	POM	-.052(a)	-.514	.608	-.053	.996	1.004	.996
	Salinity	-.174(a)	-1.730	.087	-.177	.966	1.036	.966
	Temperature	.024(a)	.234	.816	.024	.943	1.060	.943
	a	-.031(a)	-.302	.763	-.031	.959	1.043	.959
	b	-.120(a)	-1.137	.258	-.117	.894	1.119	.894
	c	-.113(a)	-1.064	.290	-.110	.890	1.123	.890
	Rainfall	.014(a)	.137	.891	.014	.956	1.046	.956

a Predictors in the Model: (Constant), pH
b Dependent Variable: G30Length

Site = Batu Terio

Variables Entered/Removed(a,b)

Model	Variables Entered	Variables Removed	Method
1	pH	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
2	a	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
3	Salinity	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: G30Length
b Models are based only on cases for which site = Batu Terio

Model Summary(d,e)

Model	R		R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
	site = Batu Terio (Selected)	site ~= Batu Terio (Unselected)				R Square Change	F Change	df1	df2	Sig. F Change
1	.250(a)		.062	.052	.3568221	.062	6.255	1	94	.014
2	.352(b)		.124	.105	.3467374	.062	6.547	1	93	.012
3	.403(c)	.245	.163	.135	.3408246	.039	4.255	1	92	.042

a Predictors: (Constant), pH
b Predictors: (Constant), pH, a
c Predictors: (Constant), pH, a, Salinity
d Unless noted otherwise, statistics are based only on cases for which site = Batu Terio.
e Dependent Variable: G30Length

ANOVA(d,e)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.796	1	.796	6.255	.014(a)
	Residual	11.968	94	.127		
	Total	12.765	95			
2	Regression	1.584	2	.792	6.586	.002(b)
	Residual	11.181	93	.120		
	Total	12.765	95			
3	Regression	2.078	3	.693	5.962	.001(c)
	Residual	10.687	92	.116		
	Total	12.765	95			

a Predictors: (Constant), pH
b Predictors: (Constant), pH, a
c Predictors: (Constant), pH, a, Salinity

d Dependent Variable: G30Length
e Selecting only cases for which site = Batu Terio

Coefficients(a,b)

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-2.202	.996		-2.210	.030					
	pH	.324	.129	.250	2.501	.014	.250	.250	.250	1.000	1.000
2	(Constant)	-3.902	1.174		-3.323	.001					
	pH	.504	.144	.389	3.496	.001	.250	.341	.339	.761	1.314
	a	.586	.229	.285	2.559	.012	.095	.256	.248	.761	1.314
3	(Constant)	.574	2.458		.234	.816					
	pH	.556	.144	.430	3.867	.000	.250	.374	.369	.737	1.357
	a	.519	.227	.252	2.284	.025	.095	.232	.218	.746	1.341
	Salinity	-.140	.068	-.207	-2.063	.042	-.153	-.210	-.197	.906	1.103

a Dependent Variable: G30Length
b Selecting only cases for which site = Batu Terio

Excluded Variables(d)

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics		
						Tolerance	VIF	Minimum Tolerance
1	SPM	.054(a)	.514	.608	.053	.928	1.078	.928
	POM	.066(a)	.654	.515	.068	.980	1.021	.980
	Salinity	-.239(a)	-2.359	.020	-.238	.925	1.081	.925
	Temperature	.088(a)	.880	.381	.091	.990	1.010	.990
	a	.285(a)	2.559	.012	.256	.761	1.314	.761
	b	.250(a)	1.943	.055	.198	.587	1.705	.587
	c	.254(a)	1.965	.052	.200	.582	1.719	.582
	Rainfall	-.010(a)	-.100	.921	-.010	.969	1.032	.969
2	SPM	.043(b)	.429	.669	.045	.926	1.079	.712
	POM	.049(b)	.496	.621	.052	.975	1.026	.742
	Salinity	-.207(b)	-2.063	.042	-.210	.906	1.103	.737
	Temperature	.023(b)	.222	.825	.023	.917	1.090	.706
	b	-.304(b)	-.951	.344	-.099	.092	10.819	.092
	c	-.299(b)	-.921	.359	-.096	.089	11.174	.089
	Rainfall	.015(b)	.146	.884	.015	.960	1.042	.752
3	SPM	.083(c)	.818	.416	.085	.897	1.115	.700
	POM	.073(c)	.751	.454	.079	.961	1.040	.723
	Temperature	.078(c)	.757	.451	.079	.862	1.161	.675
	b	-.049(c)	-.142	.887	-.015	.077	12.954	.077
	c	-.078(c)	-.228	.820	-.024	.079	12.739	.079
	Rainfall	-.008(c)	-.077	.939	-.008	.948	1.054	.725

a Predictors in the Model: (Constant), pH
b Predictors in the Model: (Constant), pH, a
c Predictors in the Model: (Constant), pH, a, Salinity
d Dependent Variable: G30Length

Size: Medium
Variables Entered/Removed(a,b)

Model	Variables Entered	Variables Removed	Method
1	pH	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
2	Salinity	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: G30Length
b Models are based only on cases for which size = Medium

Model Summary(c,d)

Model	R			Adjusted R Square	Std. Error of the Estimate	Change Statistics				
	size = Medium (Selected)	size ~= Medium (Unselected)	R Square			R Square Change	F Change	df1	df2	Sig. F Change

1	.287(a)		.083	.073	.1833946	.083	8.370	1	93	.005
2	.392(b)	.243	.153	.135	.1771299	.071	7.695	1	92	.007

a Predictors: (Constant), pH
b Predictors: (Constant), pH, Salinity
c Unless noted otherwise, statistics are based only on cases for which size = Medium.
d Dependent Variable: G30Length

ANOVA(c,d)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.282	1	.282	8.370	.005(a)
	Residual	3.128	93	.034		
	Total	3.409	94			
2	Regression	.523	2	.261	8.334	.000(b)
	Residual	2.887	92	.031		
	Total	3.409	94			

a Predictors: (Constant), pH
b Predictors: (Constant), pH, Salinity
c Dependent Variable: G30Length
d Selecting only cases for which size = Medium

Coefficients(a,b)

		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
Model		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-1.103	.468		-2.358	.020					
	pH	.176	.061	.287	2.893	.005	.287	.287	.287	1.000	1.000
2	(Constant)	1.554	1.059		1.467	.146					
	pH	.193	.059	.315	3.265	.002	.287	.322	.313	.989	1.011
	Salinity	-.080	.029	-.268	-2.774	.007	-.235	-.278	-.266	.989	1.011

a Dependent Variable: G30Length
b Selecting only cases for which size = Medium

Excluded Variables(c)

						Collinearity Statistics		
Model		Beta In	t	Sig.	Partial Correlation	Tolerance	VIF	Minimum Tolerance
1	SPM	.049(a)	.477	.634	.050	.956	1.046	.956
	POM	.072(a)	.722	.472	.075	.985	1.015	.985
	Salinity	-.268(a)	-2.774	.007	-.278	.989	1.011	.989
	Temperature	.109(a)	1.069	.288	.111	.945	1.059	.945
	a	.205(a)	1.988	.050	.203	.903	1.107	.903
	b	.028(a)	.253	.801	.026	.799	1.252	.799
	c	.032(a)	.281	.779	.029	.787	1.270	.787
	Rainfall	-.041(a)	-.399	.691	-.042	.966	1.035	.966
2	SPM	.071(b)	.724	.471	.076	.950	1.053	.950
	POM	.086(b)	.888	.377	.093	.983	1.017	.976
	Temperature	.088(b)	.890	.376	.093	.939	1.065	.931
	a	.142(b)	1.370	.174	.142	.844	1.184	.844
	b	-.018(b)	-.162	.871	-.017	.780	1.282	.780
	c	-.019(b)	-.174	.862	-.018	.765	1.307	.765
	Rainfall	-.056(b)	-.575	.567	-.060	.963	1.038	.954

a Predictors in the Model: (Constant), pH
b Predictors in the Model: (Constant), pH, Salinity
c Dependent Variable: G30Length

Size: Small
Variables Entered/Removed(a,b)

Model	Variables Entered	Variables Removed	Method
1	pH	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
2	Salinity	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: G30Length

b Models are based only on cases for which size = Small

Model Summary(c,d)

Model	R		R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
	size = Small (Selected)	size ~= Small (Unselected)				R Square Change	F Change	df1	df2	Sig. F Change
1	.367(a)		.135	.125	.4166422	.135	14.472	1	93	.000
2	.449(b)	.226	.201	.184	.4024402	.067	7.680	1	92	.007

a Predictors: (Constant), pH
b Predictors: (Constant), pH, Salinity
c Unless noted otherwise, statistics are based only on cases for which size = Small.
d Dependent Variable: G30Length

ANOVA(c,d)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2.512	1	2.512	14.472	.000(a)
	Residual	16.144	93	.174		
	Total	18.656	94			
2	Regression	3.756	2	1.878	11.596	.000(b)
	Residual	14.900	92	.162		
	Total	18.656	94			

a Predictors: (Constant), pH
b Predictors: (Constant), pH, Salinity
c Dependent Variable: G30Length
d Selecting only cases for which size = Small

Coefficients(a,b)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-3.497	1.063		-3.290	.001					
	pH	.526	.138	.367	3.804	.000	.367	.367	.367	1.000	1.000
2	(Constant)	2.536	2.407		1.054	.295					
	pH	.564	.134	.394	4.203	.000	.367	.401	.392	.989	1.011
	Salinity	-.183	.066	-.260	-2.771	.007	-.219	-.278	-.258	.989	1.011

a Dependent Variable: G30Length
b Selecting only cases for which size = Small

Excluded Variables(c)

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics		
						Tolerance	VIF	Minimum Tolerance
1	SPM	.071(a)	.722	.472	.075	.956	1.046	.956
	POM	.104(a)	1.073	.286	.111	.985	1.015	.985
	Salinity	-.260(a)	-2.771	.007	-.278	.989	1.011	.989
	Temperature	.003(a)	.033	.973	.003	.945	1.059	.945
	a	.181(a)	1.808	.074	.185	.903	1.107	.903
	b	.053(a)	.490	.625	.051	.799	1.252	.799
	c	.063(a)	.578	.565	.060	.787	1.270	.787
	Rainfall	-.030(a)	-.300	.765	-.031	.966	1.035	.966
2	SPM	.094(b)	.980	.330	.102	.950	1.053	.950
	POM	.117(b)	1.253	.214	.130	.983	1.017	.976
	Temperature	-.018(b)	-.183	.856	-.019	.939	1.065	.931
	a	.120(b)	1.184	.240	.123	.844	1.184	.844
	b	.009(b)	.086	.932	.009	.780	1.282	.780
	c	.015(b)	.137	.891	.014	.765	1.307	.765
	Rainfall	-.045(b)	-.472	.638	-.049	.963	1.038	.954

a Predictors in the Model: (Constant), pH
b Predictors in the Model: (Constant), pH, Salinity
c Dependent Variable: G30Length

Depth = 5 m

Variables Entered/Removed(a,b)

Model	Variables Entered	Variables Removed	Method
1	pH	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
2	Salinity	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: G30Length

b Models are based only on cases for which depth = 5m

Model Summary(c,d)

Model	R		R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
	depth = 5m (Selected)	depth ~= 5m (Unselected)				R Square Change	F Change	df1	df2	Sig. F Change
1	.211(a)		.045	.038	.3521443	.045	6.486	1	139	.012
2	.277(b)	.253	.077	.063	.3474214	.032	4.805	1	138	.030

a Predictors: (Constant), pH

b Predictors: (Constant), pH, Salinity

c Unless noted otherwise, statistics are based only on cases for which depth = 5m.

d Dependent Variable: G30Length

ANOVA(c,d)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.804	1	.804	6.486	.012(a)
	Residual	17.237	139	.124		
	Total	18.041	140			
2	Regression	1.384	2	.692	5.734	.004(b)
	Residual	16.657	138	.121		
	Total	18.041	140			

a Predictors: (Constant), pH

b Predictors: (Constant), pH, Salinity

c Dependent Variable: G30Length

d Selecting only cases for which depth = 5m

Coefficients(a,b)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-1.434	.682		-2.103	.037					
	pH	.226	.089	.211	2.547	.012	.211	.211	.211	1.000	1.000
2	(Constant)	1.862	1.647		1.130	.260					
	pH	.239	.088	.223	2.725	.007	.211	.226	.223	.995	1.005
	Salinity	-.098	.045	-.180	-2.192	.030	-.165	-.183	-.179	.995	1.005

a Dependent Variable: G30Length

b Selecting only cases for which depth = 5m

Excluded Variables(c)

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics		
						Tolerance	VIF	Minimum Tolerance
1	SPM	.003(a)	.033	.974	.003	.972	1.029	.972
	POM	.030(a)	.362	.718	.031	.993	1.007	.993
	Salinity	-.180(a)	-2.192	.030	-.183	.995	1.005	.995
	Temperature	-.015(a)	-.179	.858	-.015	.944	1.060	.944
	a	.099(a)	1.125	.263	.095	.885	1.130	.885
	b	.009(a)	.095	.924	.008	.771	1.298	.771
	c	.025(a)	.261	.795	.022	.764	1.310	.764
	Rainfall	.001(a)	.013	.990	.001	.972	1.029	.972
2	SPM	.011(b)	.137	.892	.012	.970	1.031	.968
	POM	.025(b)	.308	.759	.026	.993	1.008	.988
	Temperature	-.045(b)	-.530	.597	-.045	.921	1.086	.921
	a	.054(b)	.598	.551	.051	.827	1.209	.827

b	-.025(b)	-.268	.789	-.023	.750	1.334	.750
c	-.013(b)	-.136	.892	-.012	.738	1.354	.738
Rainfall	-.006(b)	-.074	.941	-.006	.970	1.030	.967

a Predictors in the Model: (Constant), pH
b Predictors in the Model: (Constant), pH, Salinity
c Dependent Variable: G30Length

Depth = 15 m

Variables Entered/Removed(a,b)

Model	Variables Entered	Variables Removed	Method
1	pH	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: G30Length
b Models are based only on cases for which depth = 15m

Model Summary(b,c)

Model	R		R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
	depth = 15m (Selected)	depth ~= 15m (Unselected)				R Square Change	F Change	df1	df2	Sig. F Change
1	.227(a)	.211	.052	.045	.3180816	.052	7.734	1	142	.006

a Predictors: (Constant), pH
b Unless noted otherwise, statistics are based only on cases for which depth = 15m.
c Dependent Variable: G30Length

ANOVA(b,c)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.783	1	.783	7.734	.006(a)
	Residual	14.367	142	.101		
	Total	15.150	143			

a Predictors: (Constant), pH
b Dependent Variable: G30Length
c Selecting only cases for which depth = 15m

Coefficients(a,b)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-1.744	.726		-2.403	.018					
	pH	.262	.094	.227	2.781	.006	.227	.227	.227	1.000	1.000

a Dependent Variable: G30Length
b Selecting only cases for which depth = 15m

Excluded Variables(b)

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics		
						Tolerance	VIF	Minimum Tolerance
1	SPM	.061(a)	.714	.476	.060	.930	1.075	.930
	POM	.072(a)	.862	.390	.072	.970	1.031	.970
	Salinity	-.152(a)	-1.850	.066	-.154	.979	1.022	.979
	Temperature	.110(a)	1.298	.196	.109	.930	1.076	.930
	a	.153(a)	1.818	.071	.151	.922	1.084	.922
	b	.060(a)	.665	.507	.056	.822	1.217	.822
	c	.056(a)	.616	.539	.052	.805	1.242	.805
	Rainfall	-.033(a)	-.390	.697	-.033	.958	1.044	.958

a Predictors in the Model: (Constant), pH
b Dependent Variable: G30Length

5.7 Stepwise regression of G_{30} weight with environmental parameters

All sites, sizes and depths

Variables Entered/Removed(a)

Model	Variables Entered	Variables Removed	Method
1	SPM	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
2	pH	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: G30Weight

Model Summary(c)

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.208(a)	.043	.040	.8398439	.043	12.855	1	283	.000
2	.247(b)	.061	.054	.8336076	.017	5.250	1	282	.023

a Predictors: (Constant), SPM
b Predictors: (Constant), SPM, pH
c Dependent Variable: G30Weight

ANOVA(c)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	9.067	1	9.067	12.855	.000(a)
	Residual	199.611	283	.705		
	Total	208.677	284			
2	Regression	12.715	2	6.358	9.149	.000(b)
	Residual	195.962	282	.695		
	Total	208.677	284			

a Predictors: (Constant), SPM
b Predictors: (Constant), SPM, pH
c Dependent Variable: G30Weight

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	.196	.168		1.168	.244					
	SPM	12.820	3.576	.208	3.585	.000	.208	.208	.208	1.000	1.000
2	(Constant)	-2.601	1.232		-2.112	.036					
	SPM	11.083	3.629	.180	3.054	.002	.208	.179	.176	.956	1.046
	pH	.374	.163	.135	2.291	.023	.173	.135	.132	.956	1.046

a Dependent Variable: G30Weight

Excluded Variables(c)

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics		
						Tolerance	VIF	Minimum Tolerance
1	POM	.030(a)	.151	.880	.009	.088	11.356	.088
	pH	.135(a)	2.291	.023	.135	.956	1.046	.956
	Salinity	-.025(a)	-.432	.666	-.026	.990	1.010	.990
	Temperature	.045(a)	.706	.481	.042	.842	1.187	.842
	a	-.006(a)	-.095	.925	-.006	.998	1.002	.998
	b	-.057(a)	-.962	.337	-.057	.966	1.035	.966
	c	-.054(a)	-.911	.363	-.054	.967	1.034	.967
	Rainfall	-.026(a)	-.438	.662	-.026	.948	1.055	.948
2	POM	.161(b)	.798	.425	.048	.082	12.242	.079
	Salinity	-.037(b)	-.630	.529	-.038	.983	1.018	.950
	Temperature	.021(b)	.327	.744	.020	.818	1.223	.818
	a	.039(b)	.645	.519	.038	.903	1.108	.865
	b	.001(b)	.009	.993	.001	.791	1.265	.782

c	.007(b)	.101	.920	.006	.780	1.283	.771
Rainfall	-.063(b)	-1.029	.304	-.061	.892	1.121	.883

a Predictors in the Model: (Constant), SPM
b Predictors in the Model: (Constant), SPM, pH
c Dependent Variable: G30Weight

Site = Ganan

Variables Entered/Removed(a,b)

Model	Variables Entered	Variables Removed	Method
1	SPM	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: G30Weight
b Models are based only on cases for which site = Ganan

Model Summary(b,c)

Model	R					Change Statistics				
	site = Ganan (Selected)	site ~= Ganan (Unselected)	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change
1	.267(a)	.182	.071	.061	.8171760	.071	7.002	1	91	.010

a Predictors: (Constant), SPM
b Unless noted otherwise, statistics are based only on cases for which site = Ganan.
c Dependent Variable: G30Weight

ANOVA(b,c)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	4.675	1	4.675	7.002	.010(a)
	Residual	60.768	91	.668		
	Total	65.443	92			

a Predictors: (Constant), SPM
b Dependent Variable: G30Weight
c Selecting only cases for which site = Ganan

Coefficients(a,b)

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-.012	.300		-.039	.969					
	SPM	17.088	6.458	.267	2.646	.010	.267	.267	.267	1.000	1.000

a Dependent Variable: G30Weight
b Selecting only cases for which site = Ganan

Excluded Variables(b)

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics		
						Tolerance	VIF	Minimum Tolerance
1	POM	-.008(a)	-.027	.978	-.003	.128	7.831	.128
	pH	.064(a)	.602	.549	.063	.916	1.092	.916
	Salinity	.122(a)	1.211	.229	.127	.999	1.001	.999
	Temperature	.016(a)	.141	.888	.015	.765	1.307	.765
	a	.120(a)	1.148	.254	.120	.937	1.067	.937
	b	.018(a)	.181	.857	.019	.997	1.003	.997
	c	.009(a)	.091	.927	.010	.996	1.004	.996
	Rainfall	-.114(a)	-1.113	.269	-.116	.964	1.037	.964

a Predictors in the Model: (Constant), SPM
b Dependent Variable: G30Weight

SITE = Manselo

Variables Entered/Removed(a,b)

Model	Variables Entered	Variables Removed	Method
1	pH	.	Stepwise (Criteria: Probability-of-F-to-enter <=

			.050, Probability-of-F-to-remove >= .100).

a Dependent Variable: G30Weight
b Models are based only on cases for which site = Manselo

\Model Summary(b,c)

Model	R					Change Statistics				
	site = Manselo (Selected)	site ~= Manselo (Unselected)	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change
1	.214(a)	.162	.046	.036	.8398719	.046	4.507	1	94	.036

a Predictors: (Constant), pH
b Unless noted otherwise, statistics are based only on cases for which site = Manselo.
c Dependent Variable: G30Weight

ANOVA(b,c)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	3.179	1	3.179	4.507	.036(a)
	Residual	66.306	94	.705		
	Total	69.485	95			

a Predictors: (Constant), pH
b Dependent Variable: G30Weight
c Selecting only cases for which site = Manselo

Coefficients(a,b)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-5.118	2.776		-1.844	.068					
	pH	.762	.359	.214	2.123	.036	.214	.214	.214	1.000	1.000

a Dependent Variable: G30Weight
b Selecting only cases for which site = Manselo

Excluded Variables(b)

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics		
						Tolerance	VIF	Minimum Tolerance
1	SPM	.124(a)	1.234	.220	.127	.994	1.006	.994
	POM	.149(a)	1.484	.141	.152	.996	1.004	.996
	Salinity	-.138(a)	-1.350	.180	-.139	.966	1.036	.966
	Temperature	.125(a)	1.205	.231	.124	.943	1.060	.943
	a	-.060(a)	-.585	.560	-.061	.959	1.043	.959
	b	-.104(a)	-.972	.333	-.100	.894	1.119	.894
	c	-.092(a)	-.858	.393	-.089	.890	1.123	.890
	Rainfall	-.047(a)	-.455	.650	-.047	.956	1.046	.956

a Predictors in the Model: (Constant), pH
b Dependent Variable: G30Weight

Site = Batu Terio

Variables Entered/Removed(a,b)

Model	Variables Entered	Variables Removed	Method
1	SPM	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: G30Weight
b Models are based only on cases for which site = Batu Terio

Model Summary(b,c)

Model	R					Change Statistics				
	site = Batu Terio (Selected)	site ~= Batu Terio (Unselected)	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change
1	.225(a)	.199	.051	.041	.8625230	.051	5.030	1	94	.027

a Predictors: (Constant), SPM

b Unless noted otherwise, statistics are based only on cases for which site = Batu Terio.
c Dependent Variable: G30Weight

ANOVA(b,c)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	3.742	1	3.742	5.030	.027(a)
	Residual	69.931	94	.744		
	Total	73.673	95			

a Predictors: (Constant), SPM
b Dependent Variable: G30Weight
c Selecting only cases for which site = Batu Terio

Coefficients(a,b)

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	.135	.303		.447	.656					
	SPM	14.227	6.343	.225	2.243	.027	.225	.225	.225	1.000	1.000

a Dependent Variable: G30Weight
b Selecting only cases for which site = Batu Terio

Excluded Variables(b)

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics		
						Tolerance	VIF	Minimum Tolerance
1	POM	-.241(a)	-.660	.511	-.068	.076	13.143	.076
	pH	.147(a)	1.413	.161	.145	.928	1.078	.928
	Salinity	-.051(a)	-.488	.627	-.051	.946	1.057	.946
	Temperature	-.027(a)	-.225	.823	-.023	.717	1.395	.717
	a	-.053(a)	-.527	.600	-.055	.990	1.010	.990
	b	-.077(a)	-.738	.462	-.076	.937	1.067	.937
	c	-.074(a)	-.708	.481	-.073	.942	1.062	.942
	Rainfall	-.008(a)	-.073	.942	-.008	.949	1.054	.949

a Predictors in the Model: (Constant), SPM
b Dependent Variable: G30Weight

Size = Large

Variables Entered/Removed(a,b)

Model	Variables Entered	Variables Removed	Method
1	POM	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: G30Weight
b Models are based only on cases for which size = Large

Model Summary(b,c)

Model	R			Adjusted R Square	Std. Error of the Estimate	Change Statistics				
	size = Large (Selected)	size ~= Large (Unselected)	R Square			R Square Change	F Change	df1	df2	Sig. F Change
1	.223(a)	.254	.050	.040	.3357716	.050	4.885	1	93	.030

a Predictors: (Constant), POM
b Unless noted otherwise, statistics are based only on cases for which size = Large.
c Dependent Variable: G30Weight

ANOVA(b,c)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.551	1	.551	4.885	.030(a)
	Residual	10.485	93	.113		
	Total	11.036	94			

a Predictors: (Constant), POM
b Dependent Variable: G30Weight

c Selecting only cases for which size = Large

Coefficients(a,b)

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	.038	.063		.604	.547					
	POM	5.289	2.393	.223	2.210	.030	.223	.223	.223	1.000	1.000

a Dependent Variable: G30Weight
b Selecting only cases for which size = Large

Excluded Variables(b)

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics		
						Tolerance	VIF	Minimum Tolerance
1	SPM	.100(a)	.293	.770	.031	.088	11.356	.088
	pH	.048(a)	.470	.639	.049	.985	1.015	.985
	Salinity	-.025(a)	-.249	.804	-.026	.996	1.004	.996
	Temperature	.009(a)	.083	.934	.009	.829	1.207	.829
	a	.005(a)	.046	.963	.005	.999	1.001	.999
	b	.000(a)	-.004	.997	.000	.991	1.009	.991
	c	.000(a)	.005	.996	.000	.992	1.009	.992
	Rainfall	-.137(a)	-1.326	.188	-.137	.947	1.056	.947

a Predictors in the Model: (Constant), POM
b Dependent Variable: G30Weight

Size = Medium

Variables Entered/Removed(a,b)

Model	Variables Entered	Variables Removed	Method
1	SPM	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: G30Weight
b Models are based only on cases for which size = Medium

Model Summary(b,c)

Model	R		R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
	size = Medium (Selected)	size ~= Medium (Unselected)				R Square Change	F Change	df1	df2	Sig. F Change
1	.397(a)	.185	.158	.148	.3815877	.158	17.390	1	93	.000

a Predictors: (Constant), SPM
b Unless noted otherwise, statistics are based only on cases for which size = Medium.
c Dependent Variable: G30Weight

ANOVA(b,c)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2.532	1	2.532	17.390	.000(a)
	Residual	13.542	93	.146		
	Total	16.074	94			

a Predictors: (Constant), SPM
b Dependent Variable: G30Weight
c Selecting only cases for which size = Medium

Coefficients(a,b)

Model		Unstandardized Coefficients		Standardized Coefficients			Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	.158	.132		1.201	.233					

SPM	11.737	2.815	.397	4.170	.000	.397	.397	.397	1.000	1.000

a Dependent Variable: G30Weight
b Selecting only cases for which size = Medium

Excluded Variables(b

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics		
						Tolerance	VIF	Minimum Tolerance
1	POM	.066(a)	.204	.839	.021	.088	11.356	.088
	pH	.107(a)	1.099	.275	.114	.956	1.046	.956
	Salinity	.053(a)	.552	.582	.057	.990	1.010	.990
	Temperature	.161(a)	1.562	.122	.161	.842	1.187	.842
	a	.034(a)	.354	.724	.037	.998	1.002	.998
	b	-.025(a)	-.258	.797	-.027	.966	1.035	.966
	c	-.021(a)	-.217	.829	-.023	.967	1.034	.967
	Rainfall	-.070(a)	-.719	.474	-.075	.948	1.055	.948

a Predictors in the Model: (Constant), SPM
b Dependent Variable: G30Weight

Size = Small

Variables Entered/Removed(a,b)

Model	Variables Entered	Variables Removed	Method
1	pH	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
2	POM	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: G30Weight
b Models are based only on cases for which size = Small

Model Summary(c,d)

Model	R					Change Statistics				
	size = Small (Selected)	size ~= Small (Unselected)	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change
1	.339(a)		.115	.105	.9679286	.115	12.079	1	93	.001
2	.415(b)	.230	.172	.154	.9413128	.057	6.334	1	92	.014

a Predictors: (Constant), pH
b Predictors: (Constant), pH, POM
c Unless noted otherwise, statistics are based only on cases for which size = Small.
d Dependent Variable: G30Weight

ANOVA(c,d)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	11.317	1	11.317	12.079	.001(a)
	Residual	87.130	93	.937		
	Total	98.447	94			
2	Regression	16.929	2	8.464	9.553	.000(b)
	Residual	81.518	92	.886		
	Total	98.447	94			

a Predictors: (Constant), pH
b Predictors: (Constant), pH, POM
c Dependent Variable: G30Weight
d Selecting only cases for which size = Small

Coefficients(a,b)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-7.106	2.469		-2.878	.005					
	pH	1.116	.321	.339	3.476	.001	.339	.339	.339	1.000	1.000

2	(Constant)	-6.744	2.406		-2.803	.006					
	pH	1.020	.315	.310	3.242	.002	.339	.320	.308	.985	1.015
	POM	17.008	6.758	.241	2.517	.014	.278	.254	.239	.985	1.015

a Dependent Variable: G30Weight
b Selecting only cases for which size = Small

Excluded Variables(c)

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics		
						Tolerance	VIF	Minimum Tolerance
1	SPM	.230(a)	2.361	.020	.239	.956	1.046	.956
	POM	.241(a)	2.517	.014	.254	.985	1.015	.985
	Salinity	-.079(a)	-.806	.422	-.084	.989	1.011	.989
	Temperature	.076(a)	.759	.450	.079	.945	1.059	.945
	a	.065(a)	.636	.526	.066	.903	1.107	.903
	b	-.045(a)	-.410	.682	-.043	.799	1.252	.799
	c	-.033(a)	-.297	.767	-.031	.787	1.270	.787
	Rainfall	-.125(a)	-1.261	.210	-.130	.966	1.035	.966
2	SPM	-.046(b)	-.136	.892	-.014	.079	12.612	.079
	Salinity	-.091(b)	-.956	.342	-.100	.987	1.013	.976
	Temperature	-.026(b)	-.242	.809	-.025	.794	1.259	.794
	a	.048(b)	.474	.636	.050	.899	1.113	.886
	b	-.033(b)	-.305	.761	-.032	.797	1.254	.793
	c	-.022(b)	-.202	.840	-.021	.786	1.272	.781
	Rainfall	-.066(b)	-.661	.510	-.069	.902	1.109	.902

a Predictors in the Model: (Constant), pH
b Predictors in the Model: (Constant), pH, POM
c Dependent Variable: G30Weight

Depth = 5 m

Variables Entered/Removed(a,b)

Model	Variables Entered	Variables Removed	Method
1	POM	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: G30Weight
b Models are based only on cases for which depth = 5m

Model Summary(b,c)

Model	R			Adjusted R Square	Std. Error of the Estimate	Change Statistics				
	depth = 5m (Selected)	depth ~= 5m (Unselected)	R Square			R Square Change	F Change	df1	df2	Sig. F Change
1	.194(a)	.210	.038	.031	.8746686	.038	5.426	1	139	.021

a Predictors: (Constant), POM
b Unless noted otherwise, statistics are based only on cases for which depth = 5m.
c Dependent Variable: G30Weight

ANOVA(b,c)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	4.151	1	4.151	5.426	.021(a)
	Residual	106.341	139	.765		
	Total	110.492	140			

a Predictors: (Constant), POM
b Dependent Variable: G30Weight
c Selecting only cases for which depth = 5m

Coefficients(a,b)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	.524	.135		3.869	.000					

	POM	11.838	5.082	.194	2.329	.021	.194	.194	.194	1.000	1.000

a Dependent Variable: G30Weight
b Selecting only cases for which depth = 5m

Excluded Variables(b)

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics		
						Tolerance	VIF	Minimum Tolerance
1	SPM	.050(a)	.169	.866	.014	.081	12.415	.081
	pH	.162(a)	1.956	.053	.164	.993	1.007	.993
	Salinity	.002(a)	.026	.980	.002	1.000	1.000	1.000
	Temperature	.007(a)	.081	.936	.007	.864	1.158	.864
	a	-.067(a)	-.799	.426	-.068	.993	1.007	.993
	b	-.112(a)	-1.348	.180	-.114	.999	1.001	.999
	c	-.101(a)	-1.217	.226	-.103	1.000	1.000	1.000
	Rainfall	-.054(a)	-.622	.535	-.053	.935	1.069	.935

a Predictors in the Model: (Constant), POM
b Dependent Variable: G30Weight

Depth = 15 m

Variables Entered/Removed(a,b)

Model	Variables Entered	Variables Removed	Method
1	Temperature	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: G30Weight
b Models are based only on cases for which depth = 15m

Model Summary(b,c)

Model	R		R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
	depth = 15m (Selected)	depth ~= 15m (Unselected)				R Square Change	F Change	df1	df2	Sig. F Change
1	.236(a)	.078	.056	.049	.8067297	.056	8.375	1	142	.004

a Predictors: (Constant), Temperature
b Unless noted otherwise, statistics are based only on cases for which depth = 15m.
c Dependent Variable: G30Weight

ANOVA(b,c)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	5.450	1	5.450	8.375	.004(a)
	Residual	92.415	142	.651		
	Total	97.866	143			

a Predictors: (Constant), Temperature
b Dependent Variable: G30Weight
c Selecting only cases for which depth = 15m

Coefficients(a,b)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-22.758	8.123		-2.802	.006					
	Temperature	.817	.282	.236	2.894	.004	.236	.236	.236	1.000	1.000

a Dependent Variable: G30Weight
b Selecting only cases for which depth = 15m

Excluded Variables(b)

Model	Beta In	t	Sig.	Partial	Collinearity Statistics

					Correlation	Tolerance	VIF	Minimum Tolerance
1	SPM	.134(a)	1.328	.186	.111	.645	1.550	.645
	POM	.108(a)	1.072	.286	.090	.653	1.532	.653
	pH	.118(a)	1.399	.164	.117	.930	1.076	.930
	Salinity	-.039(a)	-.467	.641	-.039	.982	1.018	.982
	a	.004(a)	.052	.958	.004	.994	1.006	.994
	b	-.050(a)	-.607	.545	-.051	.990	1.010	.990
	c	-.055(a)	-.665	.507	-.056	.989	1.011	.989
	Rainfall	-.086(a)	-1.036	.302	-.087	.972	1.029	.972

a Predictors in the Model: (Constant), Temperature

b Dependent Variable: G30Weight

CHAPTER 6 (NO STATS)

CHAPTER 7

7.1 MANOVA of biofouling to test the effect of size, site, depth and month. Factorial design with no 4-way interaction

Between-Subjects Factors

		Value Label	N
month	AUG 00		18
	SEP 00		18
	OCT 00		18
	NOV 00		18
	JAN 01		18
	FEB 01		15
	APR 01		18
	MAY 01		14
	JUN 01		18
	JUL 01		18
	AUG 01		18
	SEP 01		18
	OCT 01		18
	NOV 01		18
site	1	Ganan	83
	2	Manselo	80
	3	Batu Terio	82
size	1	Large	82
	2	Medium	79
	3	Small	84
depth	5		123
	15		122

Multivariate Tests(c)

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.997	2566.906(a)	6.000	43.000	.000
	Wilks' Lambda	.003	2566.906(a)	6.000	43.000	.000
	Hotelling's Trace	358.173	2566.906(a)	6.000	43.000	.000
	Roy's Largest Root	358.173	2566.906(a)	6.000	43.000	.000
month	Pillai's Trace	4.333	9.595	78.000	288.000	.000
	Wilks' Lambda	.000	23.140	78.000	243.195	.000
	Hotelling's Trace	88.632	46.967	78.000	248.000	.000
	Roy's Largest Root	47.759	176.339(b)	13.000	48.000	.000
site	Pillai's Trace	1.609	30.177	12.000	88.000	.000
	Wilks' Lambda	.030	34.126(a)	12.000	86.000	.000
	Hotelling's Trace	10.981	38.432	12.000	84.000	.000
	Roy's Largest Root	8.478	62.171(b)	6.000	44.000	.000
size	Pillai's Trace	1.177	10.478	12.000	88.000	.000
	Wilks' Lambda	.083	17.729(a)	12.000	86.000	.000
	Hotelling's Trace	7.937	27.779	12.000	84.000	.000
	Roy's Largest	7.521	55.151(b)	6.000	44.000	.000

	Root					
depth	Pillai's Trace	.817	31.913(a)	6.000	43.000	.000
	Wilks' Lambda	.183	31.913(a)	6.000	43.000	.000
	Hotelling's Trace	4.453	31.913(a)	6.000	43.000	.000
	Roy's Largest Root	4.453	31.913(a)	6.000	43.000	.000
month * site	Pillai's Trace	4.236	4.433	156.000	288.000	.000
	Wilks' Lambda	.000	5.627	156.000	260.324	.000
	Hotelling's Trace	25.568	6.774	156.000	248.000	.000
	Roy's Largest Root	10.627	19.620(b)	26.000	48.000	.000
month * size	Pillai's Trace	3.112	1.989	156.000	288.000	.000
	Wilks' Lambda	.007	2.253	156.000	260.324	.000
	Hotelling's Trace	9.486	2.513	156.000	248.000	.000
	Roy's Largest Root	3.709	6.848(b)	26.000	48.000	.000
month * depth	Pillai's Trace	2.384	2.435	78.000	288.000	.000
	Wilks' Lambda	.024	3.034	78.000	243.195	.000
	Hotelling's Trace	7.305	3.871	78.000	248.000	.000
	Roy's Largest Root	4.475	16.523(b)	13.000	48.000	.000
site * size	Pillai's Trace	1.204	3.302	24.000	184.000	.000
	Wilks' Lambda	.212	3.530	24.000	151.219	.000
	Hotelling's Trace	2.084	3.604	24.000	166.000	.000
	Roy's Largest Root	1.115	8.546(b)	6.000	46.000	.000
site * depth	Pillai's Trace	.640	3.451	12.000	88.000	.000
	Wilks' Lambda	.422	3.871(a)	12.000	86.000	.000
	Hotelling's Trace	1.226	4.291	12.000	84.000	.000
	Roy's Largest Root	1.092	8.009(b)	6.000	44.000	.000
size * depth	Pillai's Trace	.440	2.071	12.000	88.000	.027
	Wilks' Lambda	.598	2.100(a)	12.000	86.000	.025
	Hotelling's Trace	.607	2.125	12.000	84.000	.023
	Roy's Largest Root	.470	3.444(b)	6.000	44.000	.007
month * site * size	Pillai's Trace	3.835	1.735	294.000	288.000	.000
	Wilks' Lambda	.002	1.748	294.000	265.343	.000
	Hotelling's Trace	12.357	1.737	294.000	248.000	.000
	Roy's Largest Root	3.423	3.353(b)	49.000	48.000	.000
month * site * depth	Pillai's Trace	2.609	1.420	156.000	288.000	.005
	Wilks' Lambda	.025	1.458	156.000	260.324	.004
	Hotelling's Trace	5.664	1.501	156.000	248.000	.002
	Roy's Largest	2.352	4.342(b)	26.000	48.000	.000

	Root					
month * size * depth	Pillai's Trace	2.356	1.193	156.000	288.000	.100
	Wilks' Lambda	.043	1.179	156.000	260.324	.121
	Hotelling's Trace	4.369	1.158	156.000	248.000	.152
	Roy's Largest Root	1.325	2.446(b)	26.000	48.000	.004
site * size * depth	Pillai's Trace	1.090	2.872	24.000	184.000	.000
	Wilks' Lambda	.249	3.089	24.000	151.219	.000
	Hotelling's Trace	1.860	3.217	24.000	166.000	.000
	Roy's Largest Root	1.142	8.752(b)	6.000	46.000	.000

a Exact statistic
b The statistic is an upper bound on F that yields a lower bound on the significance level.
c Design: Intercept+month+site+size+depth+month * site+month * size+month * depth+site * size+site * depth+size * depth+month * site * size+month * site * depth+month * size * depth+site * size * depth

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Maxillopoda	9731.931(a)	196	49.653	9.830	.000
	Polychaeta	5821.287(b)	196	29.700	3.767	.000
	Bivalvia	9303.771(c)	196	47.468	7.334	.000
	Demospongiae	3240.016(d)	196	16.531	4.876	.000
	Foraminifera	10668.451(e)	196	54.431	6.113	.000
	Ascidicea	9366.896(f)	196	47.790	8.692	.000
Intercept	Maxillopoda	6434.534	1	6434.534	1273.891	.000
	Polychaeta	79524.165	1	79524.165	10087.350	.000
	Bivalvia	11313.548	1	11313.548	1747.950	.000
	Demospongiae	2311.284	1	2311.284	681.802	.000
	Foraminifera	57598.779	1	57598.779	6468.711	.000
	Ascidicea	7851.151	1	7851.151	1428.001	.000
month	Maxillopoda	6200.033	13	476.926	94.420	.000
	Polychaeta	674.636	13	51.895	6.583	.000
	Bivalvia	5756.432	13	442.802	68.413	.000
	Demospongiae	921.686	13	70.899	20.914	.000
	Foraminifera	2314.378	13	178.029	19.994	.000
	Ascidicea	3620.145	13	278.473	50.650	.000
site	Maxillopoda	248.404	2	124.202	24.589	.000
	Polychaeta	346.142	2	173.071	21.953	.000
	Bivalvia	40.167	2	20.084	3.103	.054
	Demospongiae	14.998	2	7.499	2.212	.121
	Foraminifera	941.819	2	470.909	52.886	.000
	Ascidicea	1756.470	2	878.235	159.737	.000
size	Maxillopoda	396.558	2	198.279	39.255	.000
	Polychaeta	745.409	2	372.705	47.276	.000
	Bivalvia	11.499	2	5.750	.888	.418
	Demospongiae	211.641	2	105.820	31.216	.000
	Foraminifera	1283.982	2	641.991	72.100	.000
	Ascidicea	339.299	2	169.650	30.857	.000
depth	Maxillopoda	108.568	1	108.568	21.494	.000
	Polychaeta	21.510	1	21.510	2.728	.105
	Bivalvia	90.175	1	90.175	13.932	.001
	Demospongiae	8.461	1	8.461	2.496	.121
	Foraminifera	1337.689	1	1337.689	150.231	.000
	Ascidicea	97.881	1	97.881	17.803	.000
month * site	Maxillopoda	1126.704	26	43.335	8.579	.000
	Polychaeta	1537.986	26	59.153	7.503	.000
	Bivalvia	696.460	26	26.787	4.139	.000
	Demospongiae	667.368	26	25.668	7.572	.000
	Foraminifera	519.679	26	19.988	2.245	.008
	Ascidicea	1632.557	26	62.791	11.421	.000

month * size	Maxillopoda	372.095	26	14.311	2.833	.001
	Polychaeta	474.789	26	18.261	2.316	.006
	Bivalvia	315.666	26	12.141	1.876	.029
	Demospongiae	292.551	26	11.252	3.319	.000
	Foraminifera	348.529	26	13.405	1.505	.109
	Ascidicea	338.162	26	13.006	2.366	.005
month * depth	Maxillopoda	220.671	13	16.975	3.361	.001
	Polychaeta	146.314	13	11.255	1.428	.182
	Bivalvia	65.705	13	5.054	.781	.676
	Demospongiae	117.166	13	9.013	2.659	.007
	Foraminifera	1217.089	13	93.622	10.514	.000
	Ascidicea	130.978	13	10.075	1.833	.065
site * size	Maxillopoda	48.060	4	12.015	2.379	.065
	Polychaeta	237.236	4	59.309	7.523	.000
	Bivalvia	29.088	4	7.272	1.124	.357
	Demospongiae	55.840	4	13.960	4.118	.006
	Foraminifera	303.544	4	75.886	8.522	.000
	Ascidicea	97.980	4	24.495	4.455	.004
site * depth	Maxillopoda	25.932	2	12.966	2.567	.087
	Polychaeta	84.567	2	42.283	5.363	.008
	Bivalvia	35.526	2	17.763	2.744	.074
	Demospongiae	7.323	2	3.662	1.080	.348
	Foraminifera	23.869	2	11.935	1.340	.271
	Ascidicea	90.694	2	45.347	8.248	.001
size * depth	Maxillopoda	42.939	2	21.470	4.251	.020
	Polychaeta	68.253	2	34.127	4.329	.019
	Bivalvia	14.532	2	7.266	1.123	.334
	Demospongiae	8.703	2	4.351	1.284	.286
	Foraminifera	93.270	2	46.635	5.237	.009
	Ascidicea	7.739	2	3.869	.704	.500
month * site * size	Maxillopoda	393.345	49	8.027	1.589	.055
	Polychaeta	594.377	49	12.130	1.539	.069
	Bivalvia	597.453	49	12.193	1.884	.015
	Demospongiae	413.970	49	8.448	2.492	.001
	Foraminifera	656.816	49	13.404	1.505	.079
	Ascidicea	531.374	49	10.844	1.972	.010
month * site * depth	Maxillopoda	113.026	26	4.347	.861	.654
	Polychaeta	289.908	26	11.150	1.414	.147
	Bivalvia	227.627	26	8.755	1.353	.180
	Demospongiae	168.274	26	6.472	1.909	.026
	Foraminifera	595.988	26	22.923	2.574	.002
	Ascidicea	227.937	26	8.767	1.595	.080
month * size * depth	Maxillopoda	192.991	26	7.423	1.470	.123
	Polychaeta	174.327	26	6.705	.850	.666
	Bivalvia	179.741	26	6.913	1.068	.411
	Demospongiae	94.338	26	3.628	1.070	.409
	Foraminifera	287.684	26	11.065	1.243	.252
	Ascidicea	238.176	26	9.161	1.666	.062
site * size * depth	Maxillopoda	94.992	4	23.748	4.702	.003
	Polychaeta	187.034	4	46.758	5.931	.001
	Bivalvia	42.433	4	10.608	1.639	.180
	Demospongiae	46.893	4	11.723	3.458	.015
	Foraminifera	214.598	4	53.649	6.025	.001
	Ascidicea	70.374	4	17.593	3.200	.021
Error	Maxillopoda	242.452	48	5.051		
	Polychaeta	378.411	48	7.884		
	Bivalvia	310.678	48	6.472		
	Demospongiae	162.718	48	3.390		
	Foraminifera	427.402	48	8.904		
	Ascidicea	263.904	48	5.498		
Total	Maxillopoda	17097.000	245			
	Polychaeta	90774.000	245			
	Bivalvia	21272.000	245			
	Demospongiae	5886.000	245			
	Foraminifera	73624.000	245			
	Ascidicea	18118.000	245			

Corrected Total	Maxillopoda	9974.384	244			
	Polychaeta	6199.698	244			
	Bivalvia	9614.449	244			
	Demospongiae	3402.735	244			
	Foraminifera	11095.853	244			
	Ascidicea	9630.800	244			

a R Squared = .976 (Adjusted R Squared = .876)
b R Squared = .939 (Adjusted R Squared = .690)
c R Squared = .968 (Adjusted R Squared = .836)
d R Squared = .952 (Adjusted R Squared = .757)
e R Squared = .961 (Adjusted R Squared = .804)
f R Squared = .973 (Adjusted R Squared = .861)

7.2 Univariate analysis of variance for dry weight of fouling

Tests of Between-Subjects Effects

Dependent Variable:weight

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	27235.931[a]	181	150.475	7.119	.000
Intercept	57438.817	1	57438.817	2717.378	.000
month	7447.970	12	620.664	29.363	.000
site	597.590	2	298.795	14.136	.000
size	9079.374	2	4539.687	214.768	.000
depth	928.207	1	928.207	43.913	.000
month * site	3312.634	23	144.028	6.814	.000
month * size	1994.618	24	83.109	3.932	.000
month * depth	384.184	12	32.015	1.515	.153
site * size	51.841	4	12.960	.613	.655
site * depth	4.880	2	2.440	.115	.891
size * depth	87.080	2	43.540	2.060	.139
month * site * size	1720.511	46	37.402	1.769	.028
month * site * depth	1112.190	23	48.356	2.288	.008
month * size * depth	421.357	24	17.557	.831	.682
site * size * depth	35.502	4	8.876	.420	.793
Error	972.329	46	21.138		
Total	86757.200	228			
Corrected Total	28208.260	227			

a. R Squared = .966 (Adjusted R Squared = .830)

Multiple Comparisons

weight

Bonferroni

					95% Confidence Interval	
(I) site	(J) site	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Ganan	Manselo	-.3412	.73620	1.000	-2.1704	1.4881
	Batu Terio	3.1671*	.75138	.000	1.3001	5.0340
Manselo	Ganan	.3412	.73620	1.000	-1.4881	2.1704
	Batu Terio	3.5082*	.75138	.000	1.6413	5.3752

Batu Terio	Ganan	-3.1671*	.75138	.000	-5.0340	-1.3001
	Manselo	-3.5082*	.75138	.000	-5.3752	-1.6413

Based on observed means.
The error term is Mean Square(Error) = 21.138.

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons

weight
Bonferroni

					95% Confidence Interval	
(I) size	(J) size	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Large	Medium	11.1941*	.74582	.000	9.3409	13.0472
	Small	14.8699*	.74582	.000	13.0167	16.7230
Medium	Large	-11.1941*	.74582	.000	-13.0472	-9.3409
	Small	3.6758*	.74582	.000	1.8226	5.5289
Small	Large	-14.8699*	.74582	.000	-16.7230	-13.0167
	Medium	-3.6758*	.74582	.000	-5.5289	-1.8226

Based on observed means.
The error term is Mean Square(Error) = 21.138.

*. The mean difference is significant at the 0.05 level.

7.3 PCA of biofouling species and environmental parameters

Correlation Matrix

		Maxillopoda	Polychaeta	Bivalvia	Demospongiae	Foraminifera	Ascidicea	temp	sal	ph	spm	pom	a	b	c
Correlation	Maxillopoda	1.000	.232	-.068	.007	.340	.455	-.075	-.274	-.206	-.241	-.024	.396	.464	.427
	Polychaeta	.232	1.000	.057	.204	.530	.205	.098	-.014	.184	-.024	-.060	-.116	-.123	-.124
	Bivalvia	-.068	.057	1.000	.173	-.270	-.031	.011	.197	.279	.367	.350	-.100	-.078	-.115
	Demospongiae	.007	.204	.173	1.000	.084	-.042	.185	-.013	.299	.160	.104	-.088	-.108	-.123
	Foraminifera	.340	.530	-.270	.084	1.000	.168	-.118	-.180	-.054	-.216	-.242	-.024	.033	-.024
	Ascidicea	.455	.205	-.031	-.042	.168	1.000	.204	-.307	-.051	-.203	-.200	.465	.476	.438
	temp	-.075	.098	.011	.185	-.118	.204	1.000	.013	.249	.418	.441	.233	.091	.121
	sal	-.274	-.014	.197	-.013	-.180	-.307	.013	1.000	.121	.034	.054	-.238	-.243	-.200
	ph	-.206	.184	.279	.299	-.054	-.051	.249	.121	1.000	.244	.159	-.359	-.482	-.496
	spm	-.241	-.024	.367	.160	-.216	-.203	.41	.03	.24	1.0	.92	.01	-	-

								8	4	4	00	4	3	.146	.161
	pom	-.242	-.060	.350	.104	-.242	-.200	.441	.054	.159	.924	1.000	.113	-.071	-.040
	a	.396	-.116	-.100	-.088	-.024	.465	.233	-.238	-.359	.013	.113	1.000	.846	.938
	b	.464	-.123	-.078	-.108	.033	.476	.091	-.243	-.482	-.146	-.071	.846	1.000	.898
	c	.427	-.124	-.115	-.123	-.024	.438	.121	-.200	-.496	-.161	-.040	.938	.898	1.000
Sig. (1-tailed)	Maxillopoda		.000	.144	.454	.000	.000	.120	.000	.001	.000	.000	.000	.000	.000
	Polychaeta	.000		.189	.001	.000	.001	.064	.416	.003	.353	.176	.035	.027	.027
	Bivalvia	.144	.189		.003	.000	.312	.432	.001	.000	.000	.000	.060	.113	.036
	Demospongiae	.454	.001	.003		.096	.255	.002	.419	.000	.006	.052	.086	.046	.027
	Foraminifera	.000	.000	.000	.096		.004	.033	.002	.209	.000	.000	.354	.301	.356
	Ascidicea	.000	.001	.312	.255	.004		.001	.000	.222	.001	.001	.000	.000	.000
	temp	.120	.064	.432	.002	.033	.001		.422	.000	.000	.000	.000	.075	.027
	sal	.000	.416	.001	.419	.002	.000	.422		.032	.297	.196	.000	.000	.001
	ph	.001	.003	.000	.000	.209	.222	.000	.032		.000	.007	.000	.000	.000
	spm	.000	.353	.000	.006	.000	.001	.000	.297	.000		.000	.420	.010	.005
	pom	.000	.176	.000	.052	.000	.001	.000	.196	.007	.000		.037	.131	.265
	a	.000	.035	.060	.086	.354	.000	.000	.000	.000	.420	.037		.000	.000
	b	.000	.027	.113	.046	.301	.000	.075	.000	.000	.010	.131	.000		.000
	c	.000	.027	.036	.027	.356	.000	.027	.001	.000	.005	.265	.000	.000	

Communalities

	Initial	Extraction
Maxillopoda	1.000	.594
Polychaeta	1.000	.659
Bivalvia	1.000	.729
Demospongiae	1.000	.355
Foraminifera	1.000	.689
Ascidicea	1.000	.577
temp	1.000	.544
sal	1.000	.390
ph	1.000	.574
spm	1.000	.862
pom	1.000	.872
a	1.000	.894
b	1.000	.879
c	1.000	.915

Extraction Method: Principal Component Analysis.

Total Variance Explained

Component	Initial Eigenvalues	Extraction Sums of Squared Loadings	Rotation Sums of Squared Loadings(a)

	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total
1	3.942	28.159	28.159	3.942	28.159	28.159	3.752
2	2.586	18.474	46.634	2.586	18.474	46.634	2.578
3	1.934	13.812	60.445	1.934	13.812	60.445	1.938
4	1.071	7.652	68.097	1.071	7.652	68.097	1.962
5	.959	6.850	74.947				
6	.891	6.365	81.312				
7	.823	5.881	87.193				
8	.487	3.481	90.674				
9	.421	3.009	93.682				
10	.354	2.528	96.210				
11	.302	2.154	98.364				
12	.134	.957	99.322				
13	.064	.458	99.779				
14	.031	.221	100.000				

Extraction Method: Principal Component Analysis.
a When components are correlated, sums of squared loadings cannot be added to obtain a total variance.

Pattern Matrix(a)

	Component			
	1	2	3	4
Maxillopoda	.598	-.245	.381	-.035
Polychaeta	-.051	-.020	.803	-.111
Bivalvia	.147	.102	.155	.836
Demospongiae	-.021	.163	.499	.239
Foraminifera	-.066	-.154	.604	-.520
Ascidicea	.660	-.084	.366	.030
temp	.161	.711	.170	-.091
sal	-.190	-.172	-.129	.546
ph	-.361	.190	.468	.320
spm	-.124	.905	-.033	.034
pom	-.042	.919	-.117	.028
a	.910	.214	-.167	-.040
b	.928	-.001	-.164	.005
c	.936	.027	-.218	-.001

Extraction Method: Principal Component Analysis.
Rotation Method: Oblimin with Kaiser Normalization.
a Rotation converged in 7 iterations.

Multiple Comparisons
Bonferroni

Dependent Variable	(I) site	(J) site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Maxillopoda	Ganan	Manselo	-.19	.352	1.000	-1.06	.69
		Batu Terio	2.07(*)	.350	.000	1.20	2.94
	Manselo	Ganan	.19	.352	1.000	-.69	1.06
		Batu Terio	2.26(*)	.353	.000	1.39	3.14
	Batu Terio	Ganan	-2.07(*)	.350	.000	-2.94	-1.20
		Manselo	-2.26(*)	.353	.000	-3.14	-1.39
Polychaeta	Ganan	Manselo	-2.63(*)	.440	.000	-3.73	-1.54
		Batu Terio	.59	.437	.558	-.50	1.67
	Manselo	Ganan	2.63(*)	.440	.000	1.54	3.73
		Batu Terio	3.22(*)	.441	.000	2.13	4.32
	Batu Terio	Ganan	-.59	.437	.558	-1.67	.50
		Manselo	-3.22(*)	.441	.000	-4.32	-2.13

Bivalvia	Ganan	Manselo	.19	.399	1.000	-.80	1.18
		Batu Terio	1.17(*)	.396	.015	.18	2.15
	Manselo	Ganan	-.19	.399	1.000	-1.18	.80
		Batu Terio	.98	.400	.054	-.01	1.97
	Batu Terio	Ganan	-1.17(*)	.396	.015	-2.15	-.18
		Manselo	-.98	.400	.054	-1.97	.01
Demospongiae	Ganan	Manselo	.09	.288	1.000	-.63	.80
		Batu Terio	-.56	.287	.168	-1.27	.15
	Manselo	Ganan	-.09	.288	1.000	-.80	.63
		Batu Terio	-.65	.289	.089	-1.37	.07
	Batu Terio	Ganan	.56	.287	.168	-.15	1.27
		Manselo	.65	.289	.089	-.07	1.37
Foraminifera	Ganan	Manselo	-.06	.468	1.000	-1.22	1.10
		Batu Terio	4.06(*)	.465	.000	2.90	5.21
	Manselo	Ganan	.06	.468	1.000	-1.10	1.22
		Batu Terio	4.12(*)	.469	.000	2.96	5.28
	Batu Terio	Ganan	-4.06(*)	.465	.000	-5.21	-2.90
		Manselo	-4.12(*)	.469	.000	-5.28	-2.96
Ascidicea	Ganan	Manselo	-6.69(*)	.367	.000	-7.61	-5.78
		Batu Terio	-4.50(*)	.365	.000	-5.41	-3.60
	Manselo	Ganan	6.69(*)	.367	.000	5.78	7.61
		Batu Terio	2.19(*)	.368	.000	1.28	3.11
	Batu Terio	Ganan	4.50(*)	.365	.000	3.60	5.41
		Manselo	-2.19(*)	.368	.000	-3.11	-1.28

Based on observed means.
* The mean difference is significant at the .05 level.

Multiple Comparisons
Bonferroni

Dependent Variable	(I) size	(J) size	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Maxillopoda	Large	Medium	1.13(*)	.354	.007	.25	2.01
		Small	3.20(*)	.349	.000	2.33	4.06
	Medium	Large	-1.13(*)	.354	.007	-2.01	-.25
		Small	2.07(*)	.352	.000	1.19	2.94
	Small	Large	-3.20(*)	.349	.000	-4.06	-2.33
		Medium	-2.07(*)	.352	.000	-2.94	-1.19
Polychaeta	Large	Medium	1.87(*)	.443	.000	.77	2.97
		Small	4.37(*)	.436	.000	3.29	5.45
	Medium	Large	-1.87(*)	.443	.000	-2.97	-.77
		Small	2.50(*)	.440	.000	1.41	3.59
	Small	Large	-4.37(*)	.436	.000	-5.45	-3.29
		Medium	-2.50(*)	.440	.000	-3.59	-1.41
Bivalvia	Large	Medium	.97	.401	.058	-.02	1.97
		Small	.59	.395	.419	-.39	1.57
	Medium	Large	-.97	.401	.058	-1.97	.02
		Small	-.38	.399	1.000	-1.37	.61
	Small	Large	-.59	.395	.419	-1.57	.39
		Medium	.38	.399	1.000	-.61	1.37
Demospongiae	Large	Medium	1.82(*)	.290	.000	1.10	2.54
		Small	2.02(*)	.286	.000	1.31	2.73

	Medium	Large	-1.82(*)	.290	.000	-2.54	-1.10
		Small	.21	.289	1.000	-.51	.92
	Small	Large	-2.02(*)	.286	.000	-2.73	-1.31
		Medium	-.21	.289	1.000	-.92	.51
Foraminifera	Large	Medium	2.99(*)	.470	.000	1.82	4.16
		Small	5.65(*)	.463	.000	4.50	6.80
	Medium	Large	-2.99(*)	.470	.000	-4.16	-1.82
		Small	2.66(*)	.468	.000	1.50	3.82
	Small	Large	-5.65(*)	.463	.000	-6.80	-4.50
		Medium	-2.66(*)	.468	.000	-3.82	-1.50
Ascidicea	Large	Medium	1.30(*)	.370	.003	.39	2.22
		Small	2.95(*)	.364	.000	2.05	3.85
	Medium	Large	-1.30(*)	.370	.003	-2.22	-.39
		Small	1.64(*)	.367	.000	.73	2.56
	Small	Large	-2.95(*)	.364	.000	-3.85	-2.05
		Medium	-1.64(*)	.367	.000	-2.56	-.73

Based on observed means.
*The mean difference is significant at the .05 level.

7.4 Simple linear regression of different classes of biofouling and environmental parameters

Maxillopoda
Variables Entered/Removed(a)

Model	Variables Entered	Variables Removed	Method
1	b		Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100)
2	pom	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
3	sal	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: Maxillopoda

Model Summary

					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change
1	.464(a)	.215	.211	5.678	.215	61.565	1	225	.000
2	.509(b)	.259	.252	5.529	.044	13.294	1	224	.000
3	.533(c)	.284	.274	5.447	.025	7.795	1	223	.006

a Predictors: (Constant), b
b Predictors: (Constant), b, pom
c Predictors: (Constant), b, pom, sal

ANOVA(d)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1984.802	1	1984.802	61.565	.000(a)
	Residual	7253.766	225	32.239		
	Total	9238.568	226			
2	Regression	2391.172	2	1195.586	39.111	.000(b)
	Residual	6847.397	224	30.569		
	Total	9238.568	226			
3	Regression	2622.454	3	874.151	29.464	.000(c)
	Residual	6616.115	223	29.669		
	Total	9238.568	226			

a Predictors: (Constant), b
b Predictors: (Constant), b, pom
c Predictors: (Constant), b, pom, sal
d Dependent Variable: Maxillopoda

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-1.032	.901		-1.145	.253
	b	9.390	1.197	.464	7.846	.000
2	(Constant)	1.174	1.066		1.101	.272
	b	9.088	1.168	.449	7.779	.000
	pom	-86.313	23.673	-.210	-3.646	.000
3	(Constant)	64.298	22.633		2.841	.005
	b	8.292	1.186	.409	6.993	.000
	pom	-83.834	23.339	-.204	-3.592	.000
	sal	-1.804	.646	-.163	-2.792	.006

a Dependent Variable: Maxillopoda

Variables Entered/Removed(a)

Model	Variables Entered	Variables Removed	Method
1	ph	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: Polychaeta

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.184(a)	.034	.029	4.966	.034	7.850	1	225	.006

a Predictors: (Constant), ph

ANOVA(b)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	193.580	1	193.580	7.850	.006(a)
	Residual	5548.763	225	24.661		
	Total	5742.343	226			

a Predictors: (Constant), ph
b Dependent Variable: Polychaeta

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-3.002	7.710		-.389	.697
	ph	2.819	1.006	.184	2.802	.006

a Dependent Variable: Polychaeta

Bivalvia
Variables Entered/Removed(a)

Model	Variables Entered	Variables Removed	Method

1	spm		Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
2	ph	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
3	temp	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
4	sal	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
5	b	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
6	a	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
7	c	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: Bivalvia

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.367(a)	.135	.131	5.851	.135	35.086	1	225	.000
2	.416(b)	.173	.166	5.734	.038	10.340	1	224	.001
3	.458(c)	.210	.199	5.617	.037	10.405	1	223	.001
4	.485(d)	.235	.222	5.538	.025	7.378	1	222	.007
5	.515(e)	.265	.249	5.441	.030	9.028	1	221	.003
6	.528(f)	.279	.259	5.402	.014	4.203	1	220	.042
7	.549(g)	.301	.279	5.331	.022	6.851	1	219	.009

a Predictors: (Constant), spm
b Predictors: (Constant), spm, ph
c Predictors: (Constant), spm, ph, temp
d Predictors: (Constant), spm, ph, temp, sal
e Predictors: (Constant), spm, ph, temp, sal, b
f Predictors: (Constant), spm, ph, temp, sal, b, a
g Predictors: (Constant), spm, ph, temp, sal, b, a, c

ANOVA(h)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1201.323	1	1201.323	35.086	.000(a)
	Residual	7703.863	225	34.239		
	Total	8905.186	226			
2	Regression	1541.233	2	770.616	23.441	.000(b)
	Residual	7363.954	224	32.875		
	Total	8905.186	226			
3	Regression	1869.523	3	623.174	19.752	.000(c)
	Residual	7035.663	223	31.550		
	Total	8905.186	226			
4	Regression	2095.819	4	523.955	17.082	.000(d)
	Residual	6809.368	222	30.673		
	Total	8905.186	226			
5	Regression	2363.055	5	472.611	15.965	.000(e)
	Residual	6542.132	221	29.602		
	Total	8905.186	226			

6	Regression	2485.692	6	414.282	14.198	.000(f)
	Residual	6419.495	220	29.180		
	Total	8905.186	226			
7	Regression	2680.429	7	382.918	13.472	.000(g)
	Residual	6224.757	219	28.424		
	Total	8905.186	226			

a Predictors: (Constant), spm
b Predictors: (Constant), spm, ph
c Predictors: (Constant), spm, ph, temp
d Predictors: (Constant), spm, ph, temp, sal
e Predictors: (Constant), spm, ph, temp, sal, b
f Predictors: (Constant), spm, ph, temp, sal, b, a
g Predictors: (Constant), spm, ph, temp, sal, b, a, c
h Dependent Variable: Bivalvia

Coefficients(a)

		Unstandardized Coefficients		Standardized Coefficients		
Model		B	Std. Error	Beta	t	Sig.
1	(Constant)	-.085	1.241		-.068	.946
	spm	154.516	26.086	.367	5.923	.000
2	(Constant)	-28.640	8.963		-3.195	.002
	spm	133.797	26.360	.318	5.076	.000
	ph	3.852	1.198	.201	3.216	.001
3	(Constant)	58.904	28.525		2.065	.040
	spm	167.994	27.915	.399	6.018	.000
	ph	4.493	1.190	.235	3.775	.000
	temp	-3.267	1.013	-.214	-3.226	.001
4	(Constant)	-.340	35.592		-.010	.992
	spm	167.087	27.527	.397	6.070	.000
	ph	4.113	1.182	.215	3.480	.001
	temp	-3.209	.999	-.210	-3.212	.002
	sal	1.743	.642	.161	2.716	.007
5	(Constant)	-8.401	35.068		-.240	.811
	spm	178.811	27.322	.425	6.545	.000
	ph	6.112	1.338	.320	4.567	.000
	temp	-4.083	1.024	-.268	-3.989	.000
	sal	2.161	.645	.199	3.348	.001
	b	4.200	1.398	.211	3.005	.003
6	(Constant)	-16.381	35.033		-.468	.641
	spm	187.205	27.433	.445	6.824	.000
	ph	6.098	1.329	.319	4.590	.000
	temp	-3.649	1.038	-.239	-3.515	.001
	sal	2.062	.643	.190	3.209	.002
	b	8.076	2.346	.406	3.443	.001
	a	-7.726	3.768	-.233	-2.050	.042
7	(Constant)	-13.704	34.592		-.396	.692
	spm	216.280	29.266	.514	7.390	.000
	ph	7.321	1.392	.383	5.259	.000
	temp	-3.729	1.025	-.245	-3.637	.000
	sal	1.773	.644	.163	2.753	.006
	b	4.999	2.596	.251	1.925	.055
	a	-21.946	6.584	-.662	-3.333	.001
	c	4.276	1.634	.615	2.617	.009

a Dependent Variable: Bivalvia

Variables Entered/Removed(a)

Model	Variables Entered	Variables Removed	Method
1	ph	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: Demospongiae

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.299(a)	.089	.085	3.571	.089	22.091	1	225	.000

a Predictors: (Constant), ph

ANOVA(b)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	281.773	1	281.773	22.091	.000(a)
	Residual	2869.941	225	12.755		
	Total	3151.713	226			

a Predictors: (Constant), ph
b Dependent Variable: Demospongiae

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-22.853	5.545		-4.122	.000
	ph	3.401	.724	.299	4.700	.000

a Dependent Variable: Demospongiae

Variables Entered/Removed(a)

Model	Variables Entered	Variables Removed	Method
1	pom	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
2	sal	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: Foraminifera

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.242(a)	.058	.054	6.558	.058	13.978	1	225	.000
2	.294(b)	.087	.078	6.474	.028	6.889	1	224	.009

a Predictors: (Constant), pom
b Predictors: (Constant), pom, sal

ANOVA(c)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	601.134	1	601.134	13.978	.000(a)

	Residual	9676.173	225	43.005		
	Total	10277.307	226			
2	Regression	889.855	2	444.927	10.617	.000(b)
	Residual	9387.452	224	41.908		
	Total	10277.307	226			

a Predictors: (Constant), pom
b Predictors: (Constant), pom, sal
c Dependent Variable: Foraminifera

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	18.401	.781		23.552	.000
	pom	-104.714	28.008	-.242	-3.739	.000
2	(Constant)	86.238	25.857		3.335	.001
	pom	-100.785	27.689	-.233	-3.640	.000
	sal	-1.956	.745	-.168	-2.625	.009

a Dependent Variable: Foraminifera

Variables Entered/Removed(a)

Model	Variables Entered	Variables Removed	Method
1	b	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
2	ph	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
3	sal	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
4	pom	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
5	temp	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).
6	a	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

a Dependent Variable: Ascidicea

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.476(a)	.226	.223	5.539	.226	65.774	1	225	.000
2	.517(b)	.267	.261	5.401	.041	12.610	1	224	.000
3	.554(c)	.307	.297	5.266	.039	12.603	1	223	.000
4	.586(d)	.343	.331	5.138	.036	12.264	1	222	.001
5	.622(e)	.387	.373	4.975	.044	15.782	1	221	.000
6	.633(f)	.401	.385	4.928	.014	5.288	1	220	.022

a Predictors: (Constant), b
b Predictors: (Constant), b, ph
c Predictors: (Constant), b, ph, sal
d Predictors: (Constant), b, ph, sal, pom
e Predictors: (Constant), b, ph, sal, pom, temp
f Predictors: (Constant), b, ph, sal, pom, temp, a

ANOVA(g)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2017.807	1	2017.807	65.774	.000(a)
	Residual	6902.524	225	30.678		
	Total	8920.331	226			
2	Regression	2385.663	2	1192.831	40.889	.000(b)
	Residual	6534.668	224	29.173		
	Total	8920.331	226			
3	Regression	2735.213	3	911.738	32.872	.000(c)
	Residual	6185.118	223	27.736		
	Total	8920.331	226			
4	Regression	3059.009	4	764.752	28.965	.000(d)
	Residual	5861.322	222	26.402		
	Total	8920.331	226			
5	Regression	3449.678	5	689.936	27.872	.000(e)
	Residual	5470.653	221	24.754		
	Total	8920.331	226			
6	Regression	3578.097	6	596.350	24.558	.000(f)
	Residual	5342.234	220	24.283		
	Total	8920.331	226			

a Predictors: (Constant), b
b Predictors: (Constant), b, ph
c Predictors: (Constant), b, ph, sal
d Predictors: (Constant), b, ph, sal, pom
e Predictors: (Constant), b, ph, sal, pom, temp
f Predictors: (Constant), b, ph, sal, pom, temp, a
g Dependent Variable: Ascidicea

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-.591	.879		-.673	.502
	b	9.468	1.167	.476	8.110	.000
2	(Constant)	-36.060	10.025		-3.597	.000
	b	11.690	1.299	.587	8.999	.000
	ph	4.434	1.249	.232	3.551	.000
3	(Constant)	41.415	23.913		1.732	.085
	b	10.710	1.296	.538	8.262	.000
	ph	4.453	1.218	.233	3.657	.000
	sal	-2.216	.624	-.204	-3.550	.000
4	(Constant)	35.783	23.386		1.530	.127
	b	10.774	1.265	.541	8.517	.000
	ph	5.053	1.200	.264	4.210	.000
	sal	-2.135	.609	-.197	-3.504	.001
	pom	-77.899	22.244	-.193	-3.502	.001
5	(Constant)	-57.712	32.660		-1.767	.079
	b	9.413	1.272	.473	7.401	.000
	ph	3.562	1.221	.186	2.917	.004
	sal	-2.188	.590	-.202	-3.707	.000
	pom	-118.790	23.872	-.294	-4.976	.000
	temp	3.775	.950	.247	3.973	.000
6	(Constant)	-52.167	32.437		-1.608	.109
	b	5.378	2.160	.270	2.490	.014
	ph	3.543	1.210	.185	2.929	.004
	sal	-2.074	.587	-.191	-3.534	.000

pom	-131.502	24.281	-.326	-5.416	.000
temp	3.411	.955	.224	3.573	.000
a	8.028	3.491	.242	2.300	.022

a Dependent Variable: Ascidicea

CHAPTER 8

8.1 χ^2 test to determine if the overall proportion of male and female oysters differed from 1 : 1

SEX

	Observed N	Expected N	Residual
1.00	1183	598.5	584.5
2.00	14	598.5	-584.5
Total	1197		

Test Statistics

	SEX
Chi-Square	1141.655
df	1
Asymp. Sig.	.000

a 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 598.5.

8.2 Multivariate analysis of covariance to determine if the number of inactive, male and female oysters are different between site, depth, age and size over the sampling period

Levenes Test for Equality of variance

	F	df1	df2	Sig.
INACTIVE	.743	15	80	.734
MALE	.759	15	80	.718
FEMALE	6.160	15	80	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.999	34930.766	3.000	77.000	.000
	Wilks' Lambda	.001	34930.766	3.000	77.000	.000
	Hotelling's Trace	1360.939	34930.766	3.000	77.000	.000
	Roy's Largest Root	1360.939	34930.766	3.000	77.000	.000
SITE	Pillai's Trace	.077	2.144	3.000	77.000	.101
	Wilks' Lambda	.923	2.144	3.000	77.000	.101
	Hotelling's Trace	.084	2.144	3.000	77.000	.101
	Roy's Largest Root	.084	2.144	3.000	77.000	.101
DEPTH	Pillai's Trace	.087	2.431	3.000	77.000	.072
	Wilks' Lambda	.913	2.431	3.000	77.000	.072
	Hotelling's Trace	.095	2.431	3.000	77.000	.072
	Roy's Largest Root	.095	2.431	3.000	77.000	.072
YEAR	Pillai's Trace	.232	7.772	3.000	77.000	.000

	Wilks' Lambda	.768	7.772	3.000	77.000	.000
	Hotelling's Trace	.303	7.772	3.000	77.000	.000
	Roy's Largest Root	.303	7.772	3.000	77.000	.000
SIZE	Pillai's Trace	.559	32.524	3.000	77.000	.000
	Wilks' Lambda	.441	32.524	3.000	77.000	.000
	Hotelling's Trace	1.267	32.524	3.000	77.000	.000
	Roy's Largest Root	1.267	32.524	3.000	77.000	.000
SAMPLING	Pillai's Trace	.173	5.371	3.000	77.000	.002
	Wilks' Lambda	.827	5.371	3.000	77.000	.002
	Hotelling's Trace	.209	5.371	3.000	77.000	.002
	Roy's Largest Root	.209	5.371	3.000	77.000	.002
SITE * DEPTH	Pillai's Trace	.024	.636	3.000	77.000	.594
	Wilks' Lambda	.976	.636	3.000	77.000	.594
	Hotelling's Trace	.025	.636	3.000	77.000	.594
	Roy's Largest Root	.025	.636	3.000	77.000	.594
SITE * YEAR	Pillai's Trace	.017	.431	3.000	77.000	.731
	Wilks' Lambda	.983	.431	3.000	77.000	.731
	Hotelling's Trace	.017	.431	3.000	77.000	.731
	Roy's Largest Root	.017	.431	3.000	77.000	.731
DEPTH * YEAR	Pillai's Trace	.072	1.991	3.000	77.000	.122
	Wilks' Lambda	.928	1.991	3.000	77.000	.122
	Hotelling's Trace	.078	1.991	3.000	77.000	.122
	Roy's Largest Root	.078	1.991	3.000	77.000	.122
SITE * DEPTH * YEAR	Pillai's Trace	.028	.729	3.000	77.000	.538
	Wilks' Lambda	.972	.729	3.000	77.000	.538
	Hotelling's Trace	.028	.729	3.000	77.000	.538
	Roy's Largest Root	.028	.729	3.000	77.000	.538
SITE * SIZE	Pillai's Trace	.028	.745	3.000	77.000	.528
	Wilks' Lambda	.972	.745	3.000	77.000	.528
	Hotelling's Trace	.029	.745	3.000	77.000	.528
	Roy's Largest Root	.029	.745	3.000	77.000	.528
DEPTH * SIZE	Pillai's Trace	.028	.730	3.000	77.000	.537
	Wilks' Lambda	.972	.730	3.000	77.000	.537
	Hotelling's Trace	.028	.730	3.000	77.000	.537
	Roy's Largest Root	.028	.730	3.000	77.000	.537
SITE * DEPTH * SIZE	Pillai's Trace	.023	.600	3.000	77.000	.617
	Wilks' Lambda	.977	.600	3.000	77.000	.617
	Hotelling's Trace	.023	.600	3.000	77.000	.617
	Roy's Largest Root	.023	.600	3.000	77.000	.617
YEAR * SIZE	Pillai's Trace	.090	2.548	3.000	77.000	.062
	Wilks' Lambda	.910	2.548	3.000	77.000	.062
	Hotelling's Trace	.099	2.548	3.000	77.000	.062
	Roy's Largest Root	.099	2.548	3.000	77.000	.062

SITE * YEAR * SIZE	Pillai's Trace	.045	1.208	3.000	77.000	.312
	Wilks' Lambda	.955	1.208	3.000	77.000	.312
	Hotelling's Trace	.047	1.208	3.000	77.000	.312
	Roy's Largest Root	.047	1.208	3.000	77.000	.312
DEPTH * YEAR * SIZE	Pillai's Trace	.045	1.203	3.000	77.000	.314
	Wilks' Lambda	.955	1.203	3.000	77.000	.314
	Hotelling's Trace	.047	1.203	3.000	77.000	.314
	Roy's Largest Root	.047	1.203	3.000	77.000	.314
SITE * DEPTH * YEAR * SIZE	Pillai's Trace	.020	.529	3.000	77.000	.664
	Wilks' Lambda	.980	.529	3.000	77.000	.664
	Hotelling's Trace	.021	.529	3.000	77.000	.664
	Roy's Largest Root	.021	.529	3.000	77.000	.664

a Exact statistic

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Inactive	3849.990	16	240.624	8.900	.000
	Male	3648.307	16	228.019	8.454	.000
	Female	3.015	16	.188	1.361	.184
Intercept	Inactive	8333.270	1	8333.270	308.206	.000
	Male	1410.771	1	1410.771	52.308	.000
	Female	.170	1	.170	1.224	.272
SITE	Inactive	114.844	1	114.844	4.248	.043
	Male	106.260	1	106.260	3.940	.051
	Female	.000	1	.000	.000	1.000
DEPTH	Inactive	189.844	1	189.844	7.021	.010
	Male	189.844	1	189.844	7.039	.010
	Female	.167	1	.167	1.203	.276
YEAR	Inactive	536.760	1	536.760	19.852	.000
	Male	481.510	1	481.510	17.853	.000
	Female	.667	1	.667	4.813	.031
SIZE	Inactive	2470.510	1	2470.510	91.372	.000
	Male	2370.094	1	2370.094	87.877	.000
	Female	.375	1	.375	2.707	.104
SAMPLING	Inactive	322.501	1	322.501	11.928	.001
	Male	293.151	1	293.151	10.869	.001
	Female	5.714E-02	1	5.714E-02	.413	.523
SITE * DEPTH	Inactive	15.844	1	15.844	.586	.446
	Male	21.094	1	21.094	.782	.379
	Female	4.167E-02	1	4.167E-02	.301	.585
SITE * YEAR	Inactive	.844	1	.844	.031	.860
	Male	1.260	1	1.260	.047	.829
	Female	4.167E-02	1	4.167E-02	.301	.585
DEPTH * YEAR	Inactive	19.260	1	19.260	.712	.401
	Male	21.094	1	21.094	.782	.379
	Female	.375	1	.375	2.707	.104
SITE * DEPTH * YEAR	Inactive	.510	1	.510	.019	.891
	Male	1.042E-02	1	1.042E-02	.000	.984
	Female	.167	1	.167	1.203	.276
SITE * SIZE	Inactive	.844	1	.844	.031	.860
	Male	.844	1	.844	.031	.860
	Female	.167	1	.167	1.203	.276
DEPTH * SIZE	Inactive	1.760	1	1.760	.065	.799
	Male	1.760	1	1.760	.065	.799
	Female	.167	1	.167	1.203	.276
SITE * DEPTH * SIZE	Inactive	3.760	1	3.760	.139	.710
	Male	3.010	1	3.010	.112	.739
	Female	4.167E-02	1	4.167E-02	.301	.585

YEAR * SIZE	Inactive	142.594	1	142.594	5.274	.024
	Male	133.010	1	133.010	4.932	.029
	Female	.000	1	.000	.000	1.000
SITE * YEAR * SIZE	Inactive	5.510	1	5.510	.204	.653
	Male	4.594	1	4.594	.170	.681
	Female	.375	1	.375	2.707	.104
DEPTH * YEAR * SIZE	Inactive	15.844	1	15.844	.586	.446
	Male	14.260	1	14.260	.529	.469
	Female	.375	1	.375	2.707	.104
SITE * DEPTH * YEAR * SIZE	Inactive	8.760	1	8.760	.324	.571
	Male	6.510	1	6.510	.241	.625
	Female	.000	1	.000	.000	1.000
Error	Inactive	2135.999	79	27.038		
	Male	2130.682	79	26.971		
	Female	10.943	79	.139		
Total	Inactive	35351.000	96			
	Male	20357.000	96			
	Female	16.000	96			
Corrected Total	Inactive	5985.990	95			
	Male	5778.990	95			
	Female	13.958	95			

a R Squared = .643 (Adjusted R Squared = .571)
b R Squared = .631 (Adjusted R Squared = .557)
c R Squared = .216 (Adjusted R Squared = .057)

8.3 Principal component analysis of length, height, thickness and weight total growth ($G_{T)}$

Total Variance Explained

	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total
1	1.834	45.841	45.841	1.834	45.841	45.841	1.802
2	1.123	28.064	73.905	1.123	28.064	73.905	1.202
3	.826	20.642	94.547				
4	.218	5.453	100.000				

Extraction Method: Principal Component Analysis.
a When components are correlated, sums of squared loadings cannot be added to obtain a total variance.

Correlation Matrix

		L	H	T	W
Sig. (1-tailed)	L		.000	.333	.213
	H	.000		.398	.324
	T	.333	.398		.289
	W	.213	.324	.289	

Structure Matrix

	Component	
	1	2
H	.937	.011
L	.924	.246
T	-.050	.821
W	.262	.683

Extraction Method: Principal Component Analysis. Rotation Method: Oblimin with Kaiser Normalization.

8.4 Stepwise regression analysis for the new variables LH and TW and dummy matrix

Dummy matrix

Site 1	Site 2	Depth 1	Depth 2	Year 1	Year 2	Size 1	Size 2	LH	TW
1	0	1	0	1	0	1	0	0.6527	1.0689
1	0	0	1	1	0	1	0	0.5479	1.012
1	0	1	0	1	0	0	1	1.2972	0.887
1	0	0	1	1	0	0	1	0.331	0.5132
1	0	1	0	0	1	1	0	-0.3521	0.6157
1	0	0	1	0	1	1	0	-1.2958	-1.8918
1	0	1	0	0	1	0	1	0.4455	-1.8764
1	0	0	1	0	1	0	1	-0.305	-0.8596
0	1	1	0	1	0	1	0	-0.3561	1.2362
0	1	0	1	1	0	1	0	-0.348	0.0364
0	1	1	0	1	0	0	1	1.613	-0.5211
0	1	0	1	1	0	0	1	1.7057	-0.1712
0	1	1	0	0	1	1	0	-1.4591	-0.118
0	1	0	1	0	1	1	0	-1.3126	1.0737
0	1	1	0	0	1	0	1	-0.3604	-0.6803
0	1	0	1	0	1	0	1	-0.804	-0.3247

Regression for LH

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.703	.494	.458	.73642
2	.866	.750	.712	.53672

a Predictors: (Constant), Age 1

b Predictors: (Constant), Age 1, Size 1

c Dependent Variable: LH

ANOVA

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	7.408	1	7.408	13.659	.002
	Residual	7.592	14	.542		
	Total	15.000	15			
2	Regression	11.255	2	5.628	19.536	.000
	Residual	3.745	13	.288		
	Total	15.000	15			

a Predictors: (Constant), Age 1

b Predictors: (Constant), Age 1, Size 1

c Dependent Variable: LH

Coefficients

		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
Model		B	Std. Error	Beta		
1	(Constant)	-.680	.260		-2.613	.020
	Age 1	1.361	.368	.703	3.696	.002
2	(Constant)	-.190	.232		-.818	.428
	Age 1	1.361	.268	.703	5.071	.000
	Size 1	-.981	.268	-.506	-3.655	.003

a Dependent Variable: LH

Regression for TW
Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.524	.275	.223	.88141

a Predictors: (Constant), Age 1
b Dependent Variable: WT

ANOVA

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	4.124	1	4.124	5.308	.037
	Residual	10.876	14	.777		
	Total	15.000	15			

a Predictors: (Constant), Age 1
b Dependent Variable: WT

Coefficients

		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
Model		B	Std. Error	Beta		
1	(Constant)	-.508	.312		-1.629	.126
	Age 1	1.015	.441	.524	2.304	.037

a Dependent Variable: WT

8.5 Principal component analysis of monthly instantaneous growth rate (G_{30}) of shell length, height, thickness and wet weight

Total Variance Explained

	Initial Eigenvalues			Extraction Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	3.145	78.635	78.635	3.145	78.635	78.635
2	.582	14.542	93.176			
3	.176	4.406	97.582			
4	9.673E-02	2.418	100.000			

Extraction Method: Principal Component Analysis.

Correlation Matrix[a]

		L MIG	H MIG	T MIG	W MIG
Correlation	L MIG	1.000	.893	.522	.863
	H MIG	.893	1.000	.578	.828
	T MIG	.522	.578	1.000	.548
	W MIG	.863	.828	.548	1.000
Sig. (1-tailed)	L MIG		.000	.000	.000
	H MIG	.000		.000	.000
	T MIG	.000	.000		.000
	W MIG	.000	.000	.000	

Determinant = 3.119E-02
Rotated Component Matrix
a Only one component was extracted. The solution cannot be rotated.

Component Matrix

	Component

	1
H MIG	.942
L MIG	.939
W MIG	.925
T MIG	.723

Extraction Method: Principal Component Analysis.
a 1 components extracted.

Communalities

	Initial	Extraction
L MIG	1.000	.882
H MIG	1.000	.887
T MIG	1.000	.522
W MIG	1.000	.856

Extraction Method: Principal Component Analysis.

8.6 Univariate ANOVA to test if sampling, site, depth, age and size has an effect on G_{30} factor scores

Tests of Between-Subjects Effects
Dependent Variable: G_{30} factor

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	78.455	75	1.046	7.684	.029
Intercept	.000	1	.000	.000	1.000
YEAR	10.744	1	10.744	78.924	.001
SIZE	6.498	1	6.498	47.732	.002
SITE	4.302E-02	1	4.302E-02	.316	.604
DEPTH	1.765E-02	1	1.765E-02	.130	.737
SAMPLING	29.841	4	7.460	54.803	.001
YEAR * SIZE	.693	1	.693	5.094	.087
YEAR * SITE	8.532E-03	1	8.532E-03	.063	.815
SIZE * SITE	.273	1	.273	2.003	.230
YEAR * SIZE * SITE	6.992E-02	1	6.992E-02	.514	.513
YEAR * DEPTH	1.313E-02	1	1.313E-02	.096	.772
SIZE * DEPTH	9.516E-02	1	9.516E-02	.699	.450
YEAR * SIZE * DEPTH	1.071E-02	1	1.071E-02	.079	.793
SITE * DEPTH	9.500E-03	1	9.500E-03	.070	.805
YEAR * SITE * DEPTH	1.745E-03	1	1.745E-03	.013	.915
SIZE * SITE * DEPTH	3.219E-03	1	3.219E-03	.024	.885
YEAR * SIZE * SITE * DEPTH	.390	1	.390	2.866	.166
YEAR * SAMPLING	15.144	4	3.786	27.811	.004
SIZE * SAMPLING	1.799	4	.450	3.304	.137
YEAR * SIZE * SAMPLING	.804	4	.201	1.476	.358
SITE * SAMPLING	1.511	4	.378	2.774	.173
YEAR * SITE * SAMPLING	1.645	4	.411	3.021	.155
SIZE * SITE * SAMPLING	1.315	4	.329	2.415	.207
YEAR * SIZE * SITE * SAMPLING	2.221	4	.555	4.079	.101
DEPTH * SAMPLING	1.518	4	.379	2.788	.172
YEAR * DEPTH * SAMPLING	.555	4	.139	1.019	.493
SIZE * DEPTH * SAMPLING	1.005	4	.251	1.846	.284
YEAR * SIZE * DEPTH * SAMPLING	1.034	4	.259	1.899	.275
SITE * DEPTH * SAMPLING	.527	4	.132	.968	.512
YEAR * SITE * DEPTH * SAMPLING	1.742E-02	4	4.355E-03	.032	.997
SIZE * SITE * DEPTH * SAMPLING	.649	4	.162	1.191	.435
Error	.545	4	.136		
Total	79.000	80			
Corrected Total	79.000	79			

[a] R Squared = .993 (Adjusted R Squared = .864)

Multiple Comparisons

Dependent Variable: G_{30} factor

Bonferroni

(I) Sampling	(J) Sampling	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Aug	Sep	-1.7045887	.13044596	.002	-2.4347688	-.9744085
	Oct	-.1666138	.13044596	1.000	-.8967940	.5635663
	Nov	-.4364495	.13044596	.287	-1.1666296	.2937307
	Dec	-.2758143	.13044596	1.000	-1.0059945	.4543659
Sep	Aug	1.7045887	.13044596	.002	.9744085	2.4347688
	Nov	1.5379748	.13044596	.003	.8077947	2.2681550
	Dec	1.2681392	.13044596	.006	.5379590	1.9983193
	Jan	1.4287744	.13044596	.004	.6985942	2.1589545
Nov	Aug	.1666138	.13044596	1.000	-.5635663	.8967940
	Sep	-1.5379748	.13044596	.003	-2.2681550	-.8077947
	Dec	-.2698356	.13044596	1.000	-1.0000158	.4603445
	Jan	-.1092005	.13044596	1.000	-.8393806	.6209797
Dec	Aug	.4364495	.13044596	.287	-.2937307	1.1666296
	Sep	-1.2681392	.13044596	.006	-1.9983193	-.5379590
	Nov	.2698356	.13044596	1.000	-.4603445	1.0000158
	Jan	.1606352	.13044596	1.000	-.5695450	.8908153
Jan	Aug	.2758143	.13044596	1.000	-.4543659	1.0059945
	Sep	-1.4287744	.13044596	.004	-2.1589545	-.6985942
	Nov	.1092005	.13044596	1.000	-.6209797	.8393806
	Dec	-.1606352	.13044596	1.000	-.8908153	.5695450

Based on observed means.

* The mean difference is significant at the .05 level.

8.7 Multiple linear regression analysis of $\log_{10}$ condition index and environmental variables

Correlations

		LOGCI	SPM	POM	pH	Salinity	Temperature	Rainfall	chloroa	chlorob	chloroc
Pearson Correlation	LOGCI	1.000	.097	.086	.058	.243	.204	-.005	.099	.128	.115
	SPM	.097	1.000	.405	-.553	-.242	-.322	-.198	.501	.526	.533
	POM	.086	.405	1.000	-.519	-.335	-.540	-.458	.569	.570	.586
	pH	.058	-.553	-.519	1.000	.570	.526	.091	-.619	-.588	-.627
	Salinity	.243	-.242	-.335	.570	1.000	.446	.258	-.302	-.280	-.310
	Temperature	.204	-.322	-.540	.526	.446	1.000	.618	-.218	-.191	-.223
	Rainfall	-.005	-.198	-.458	.091	.258	.618	1.000	-.224	-.250	-.238
	chloroa	.099	.501	.569	-.619	-.302	-.218	-.224	1.000	.988	.994
	chlorob	.128	.526	.570	-.588	-.280	-.191	-.250	.988	1.000	.996
	chloroc	.115	.533	.586	-.627	-.310	-.223	-.238	.994	.996	1.000
Sig. (1-tailed)	LOGCI	.	.174	.202	.288	.008	.023	.480	.168	.107	.131
	SPM	.174	.	.000	.000	.009	.001	.027	.000	.000	.000
	POM	.202	.000	.	.000	.000	.000	.000	.000	.000	.000
	pH	.288	.000	.000	.	.000	.000	.189	.000	.000	.000
	Salinity	.008	.009	.000	.000	.	.000	.006	.001	.003	.001
	Temperature	.023	.001	.000	.000	.000	.	.000	.016	.032	.014
	Rainfall	.480	.027	.000	.189	.006	.000	.	.014	.007	.010
	chloroa	.168	.000	.000	.000	.001	.016	.014	.	.000	.000
	chlorob	.107	.000	.000	.000	.003	.032	.007	.000	.	.000
	chloroc	.131	.000	.000	.000	.001	.014	.010	.000	.000	.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.433	.188	.103	.13561	.188	2.210	9	86	.029

a Predictors: (Constant), chloroc, Temperature, Salinity, SPM, Rainfall, POM, pH, chloroa, chlorob

ANOVA

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.366	9	.041	2.210	.029
	Residual	1.581	86	.018		
	Total	1.947	95			

a Predictors: (Constant), chloroc, Temperature, Salinity, SPM, Rainfall, POM, pH, chloroa, chlorob
b Dependent Variable: LOGCI

Coefficients

		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Correlations		
Model		B	Std. Error	Beta			Zero-order	Partial	Part
1	(Constant)	-3.749	1.608		-2.332	.022			
	SPM	2.887	5.794	.065	.498	.620	.097	.054	.048
	POM	7.607	6.311	.175	1.205	.231	.086	.129	.117
	pH	-.126	.150	-.169	-.839	.404	.058	-.090	-.082
	Salinity	8.432E-02	.035	.304	2.417	.018	.243	.252	.235
	Temperature	.136	.056	.457	2.421	.018	.204	.253	.235
	Rainfall	-3.445E-04	.000	-.251	-1.519	.132	-.005	-.162	-.148
	chloroa	-.550	.542	-1.037	-1.015	.313	.099	-.109	-.099
	chlorob	-.281	.513	-.732	-.548	.585	.128	-.059	-.053
	chloroc	.239	.252	1.769	.947	.346	.115	.102	.092

a Dependent Variable: LOGCI

8.8 Principal component analysis of environmental parameters

Total Variance Explained

	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	4.823	53.584	53.584	4.823	53.584	53.584	3.986	44.290	44.290
2	1.595	17.724	71.308	1.595	17.724	71.308	2.432	27.017	71.308
3	.960	10.664	81.972						
4	.669	7.433	89.405						
5	.474	5.265	94.670						
6	.342	3.804	98.474						
7	.127	1.408	99.883						
8	8.767E-03	9.741E-02	99.980						
9	1.802E-03	2.002E-02	100.000						

Extraction Method: Principal Component Analysis

Correlation Matrix

		SPM	POM	pH	Salinity	Temperature	Rainfall	Chloro a	Chloro b	Chloro c
Correlation	SPM	1.000	.405	-.553	-.242	-.322	-.198	.501	.526	.533
	POM	.405	1.000	-.519	-.335	-.540	-.458	.569	.570	.586
	pH	-.553	-.519	1.000	.570	.526	.091	-.619	-.588	-.627
	Salinity	-.242	-.335	.570	1.000	.446	.258	-.302	-.280	-.310
	Temperature	-.322	-.540	.526	.446	1.000	.618	-.218	-.191	-.223
	Rainfall	-.198	-.458	.091	.258	.618	1.000	-.224	-.250	-.238
	chloroa	.501	.569	-.619	-.302	-.218	-.224	1.000	.988	.994

	chlorob	.526	.570	-.588	-.280	-.191	-.250	.988	1.000	.996
	chloroc	.533	.586	-.627	-.310	-.223	-.238	.994	.996	1.000
Sig. (1-tailed)	SPM		.025	.003	.127	.063	.177	.006	.004	.004
	POM	.025		.005	.055	.003	.012	.002	.002	.001
	pH	.003	.005		.002	.004	.336	.001	.001	.001
	Salinity	.127	.055	.002		.014	.112	.075	.093	.070
	Temperature	.063	.003	.004	.014		.001	.153	.186	.147
	Rainfall	.177	.012	.336	.112	.001		.147	.119	.131
	chloroa	.006	.002	.001	.075	.153	.147		.000	.000
	chlorob	.004	.002	.001	.093	.186	.119	.000		.000
	chloroc	.004	.001	.001	.070	.147	.131	.000	.000	

a Determinant = 1.604E-06

Rotated Component Matrix

	Component	
	1	2
chloroc	.973	-.132
chlorob	.968	-.107
chloroa	.967	-.118
pH	-.659	.445
SPM	.604	-.282
Temperature	-.101	.914
Rainfall	-5.745E-02	.759
Salinity	-.260	.600
POM	.537	-.582

Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization.
a Rotation converged in 3 iterations

8.9 Multivariate analysis of covariance of component 1 (Food), component 2 (Physical) scores and pH

Levene's Test of Equality of Error Variances

	F	df1	df2	Sig.
Food	.635	3	20	.601
physical	.460	3	20	.713
PH	.769	3	20	.525

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.a Design: Intercept+TIME+SITE+DEPTH+SITE * DEPTH

Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.999	9162.612	3.000	17.000	.000
	Wilks' Lambda	.001	9162.612	3.000	17.000	.000
	Hotelling's Trace	1616.932	9162.612	3.000	17.000	.000
	Roy's Largest Root	1616.932	9162.612	3.000	17.000	.000
TIME	Pillai's Trace	.909	56.778	3.000	17.000	.000
	Wilks' Lambda	.091	56.778	3.000	17.000	.000
	Hotelling's Trace	10.020	56.778	3.000	17.000	.000
	Roy's Largest Root	10.020	56.778	3.000	17.000	.000
SITE	Pillai's Trace	.183	1.272	3.000	17.000	.316
	Wilks' Lambda	.817	1.272	3.000	17.000	.316
	Hotelling's Trace	.224	1.272	3.000	17.000	.316
	Roy's Largest Root	.224	1.272	3.000	17.000	.316
DEPTH	Pillai's Trace	.188	1.316	3.000	17.000	.302
	Wilks' Lambda	.812	1.316	3.000	17.000	.302
	Hotelling's Trace	.232	1.316	3.000	17.000	.302
	Roy's Largest Root	.232	1.316	3.000	17.000	.302
SITE * DEPTH	Pillai's Trace	.223	1.622	3.000	17.000	.221

	Wilks' Lambda	.777	1.622	3.000	17.000	.221
	Hotelling's Trace	.286	1.622	3.000	17.000	.221
	Roy's Largest Root	.286	1.622	3.000	17.000	.221

a Exact statistic
b Design: Intercept+TIME+SITE+DEPTH+SITE * DEPTH

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	food	15.077	4	3.769	9.039	.000
	physical	7.964	4	1.991	2.516	.076
	pH	.717	4	.179	21.529	.000
Intercept	food	10.788	1	10.788	25.870	.000
	physical	4.096	1	4.096	5.176	.035
	pH	254.278	1	254.278	30543.181	.000
TIME	food	13.356	1	13.356	32.030	.000
	physical	5.072	1	5.072	6.409	.020
	pH	.670	1	.670	80.517	.000
SITE	food	1.293	1	1.293	3.101	.094
	physical	1.501	1	1.501	1.896	.184
	pH	8.067E-03	1	8.067E-03	.969	.337
DEPTH	food	.421	1	.421	1.009	.328
	physical	1.625E-03	1	1.625E-03	.002	.964
	pH	1.927E-02	1	1.927E-02	2.314	.145
SITE * DEPTH	food	7.811E-03	1	7.811E-03	.019	.893
	physical	1.390	1	1.390	1.757	.201
	pH	1.927E-02	1	1.927E-02	2.314	.145
Error	food	7.923	19	.417		
	physical	15.036	19	.791		
	pH	.158	19	8.325E-03		
Total	food	23.000	24			
	physical	23.000	24			
	pH	1447.961	24			
Corrected Total	food	23.000	23			
	physical	23.000	23			
	pH	.875	23			

a R Squared = .656 (Adjusted R Squared = .583),b R Squared = .346 (Adjusted R Squared = .209),c R Squared = .819 (Adjusted R Squared = .781)

8.10 Discriminant analysis of sex, size (length, height, thickness) and weight

Eigenvalues

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	.241	99.4	99.4	.441
2	.002	.6	100.0	.039

a First 2 canonical discriminant functions were used in the analysis.

Wilks' Lambda

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 2	.804	625.178	6	.000
2	.998	4.484	2	.106

Standardized Canonical Discriminant Function Coefficients

	Function	
	1	2
LENGTH	.255	1.884

HEIGHT	.315	-.083
WEIGHT	.479	-1.736

Structure Matrix

	Function	
	1	2
WEIGHT	.967	-.210
HEIGHT	.948	.045
LENGTH	.933	.339
THICKNESS[a]	.714	-.084

a This variable not used in the analysis.

Functions at Group Centroids

	Function	
SEX	1	2
.00	-.414	-1.284E-03
1.00	.578	8.458E-03
2.00	.850	-.561

Unstandardized canonical discriminant functions evaluated at group means

Classification Function Coefficients

	SEX		
	.00	1.00	2.00
LENGTH	.523	.537	.484
HEIGHT	.771	.788	.796
WEIGHT	-.169	-.165	-.154
(Constant)	-59.130	-64.693	-66.408

Fisher's linear discriminant functions

Classification Results

			Predicted Group Membership			Total
		SEX	.00	1.00	2.00	
Original	Count	.00	1349	330	0	1679
		1.00	505	678	0	1183
		2.00	5	9	0	14
	%	.00	80.3	19.7	.0	100.0
		1.00	42.7	57.3	.0	100.0
		2.00	35.7	64.3	.0	100.0

a 70.5% of original grouped cases correctly classified.

8.11 Pearson's correlation between inactive, male and female oysters and environmental parameters

Correlations

		Inactive	Male	Female	SPM	POM	pH	Salinity	Temperature	Rainfall	Chloro a	Chloro b	Chloro c
Inactive	Pearson	1	-.998	-.359	.114	.053	-.209	-.062	-.261	-.217	.073	.083	.090

	Correlation												
	Sig. (2-tailed)	.	.000	.000	.268	.606	.041	.545	.010	.034	.481	.421	.386
	N	96	96	96	96	96	96	96	96	96	96	96	96
Male	Pearson Correlation	-.998	1	.319	-.113	-.048	.204	.055	.261	.217	-.071	-.081	-.087
	Sig. (2-tailed)	.000	.	.002	.271	.642	.046	.594	.010	.034	.494	.432	.397
	N	96	96	96	96	96	96	96	96	96	96	96	96
Female	Pearson Correlation	-.359	.319	1	.084	-.029	.052	.091	.070	.164	.050	.051	.042
	Sig. (2-tailed)	.000	.002	.	.418	.780	.613	.380	.499	.110	.629	.625	.687
	N	96	96	96	96	96	96	96	96	96	96	96	96
SPM	Pearson Correlation	.114	-.113	.084	1	.405	-.553	-.242	-.322	-.198	.501	.526	.533
	Sig. (2-tailed)	.268	.271	.418	.	.000	.000	.017	.001	.053	.000	.000	.000
	N	96	96	96	96	96	96	96	96	96	96	96	96
POM	Pearson Correlation	.053	-.048	-.029	.405	1	-.519	-.335	-.540	-.458	.569	.570	.586
	Sig. (2-tailed)	.606	.642	.780	.000	.	.000	.001	.000	.000	.000	.000	.000
	N	96	96	96	96	96	96	96	96	96	96	96	96
pH	Pearson Correlation	-.209	.204	.052	-.553	-.519	1	.570	.526	.091	-.619	-.588	-.627
	Sig. (2-tailed)	.041	.046	.613	.000	.000	.	.000	.000	.377	.000	.000	.000
	N	96	96	96	96	96	96	96	96	96	96	96	96
Salinity	Pearson Correlation	-.062	.055	.091	-.242	-.335	.570	1	.446	.258	-.302	-.280	-.310
	Sig. (2-tailed)	.545	.594	.380	.017	.001	.000	.	.000	.011	.003	.006	.002
	N	96	96	96	96	96	96	96	96	96	96	96	96
Temperature	Pearson Correlation	-.261	.261	.070	-.322	-.540	.526	.446	1	.618	-.218	-.191	-.223
	Sig. (2-tailed)	.010	.010	.499	.001	.000	.000	.000	.	.000	.033	.063	.029
	N	96	96	96	96	96	96	96	96	96	96	96	96
Rainfall	Pearson Correlation	-.217	.217	.164	-.198	-.458	.091	.258	.618	1	-.224	-.250	-.238
	Sig. (2-tailed)	.034	.034	.110	.053	.000	.377	.011	.000	.	.028	.014	.019
	N	96	96	96	96	96	96	96	96	96	96	96	96
Chloroa	Pearson Correlation	.073	-.071	.050	.501	.569	-.619	-.302	-.218	-.224	1	.988	.994
	Sig. (2-tailed)	.481	.494	.629	.000	.000	.000	.003	.033	.028	.	.000	.000
	N	96	96	96	96	96	96	96	96	96	96	96	96
Chlorob	Pearson Correlation	.083	-.081	.051	.526	.570	-.588	-.280	-.191	-.250	.988	1	.996
	Sig. (2-tailed)	.421	.432	.625	.000	.000	.000	.006	.063	.014	.000	.	.000
	N	96	96	96	96	96	96	96	96	96	96	96	96
Chloroc	Pearson Correlation	.090	-.087	.042	.533	.586	-.627	-.310	-.223	-.238	.994	.996	1
	Sig. (2-tailed)	.386	.397	.687	.000	.000	.000	.002	.029	.019	.000	.000	.
	N	96	96	96	96	96	96	96	96	96	96	96	96

** Correlation is significant at the 0.01 level (2-tailed).

Correlation is significant at the 0.05 level (2-tailed).

APPENDIX B

CHEMICALS AND FIXATIVES

Chapter 3

90 % Acetone (v/v)

Acetone	90 ml
Add to 10 ml distilled water	

Chapter 8

FAACC gonad fixative

40% Formaldehyde solution	10 ml
Glacial acetic acid	5 ml
Calcium chloride dihydrate	1.3 g
Add to 85 ml of distilled water.	

APPENDIX C

HISTOLOGY

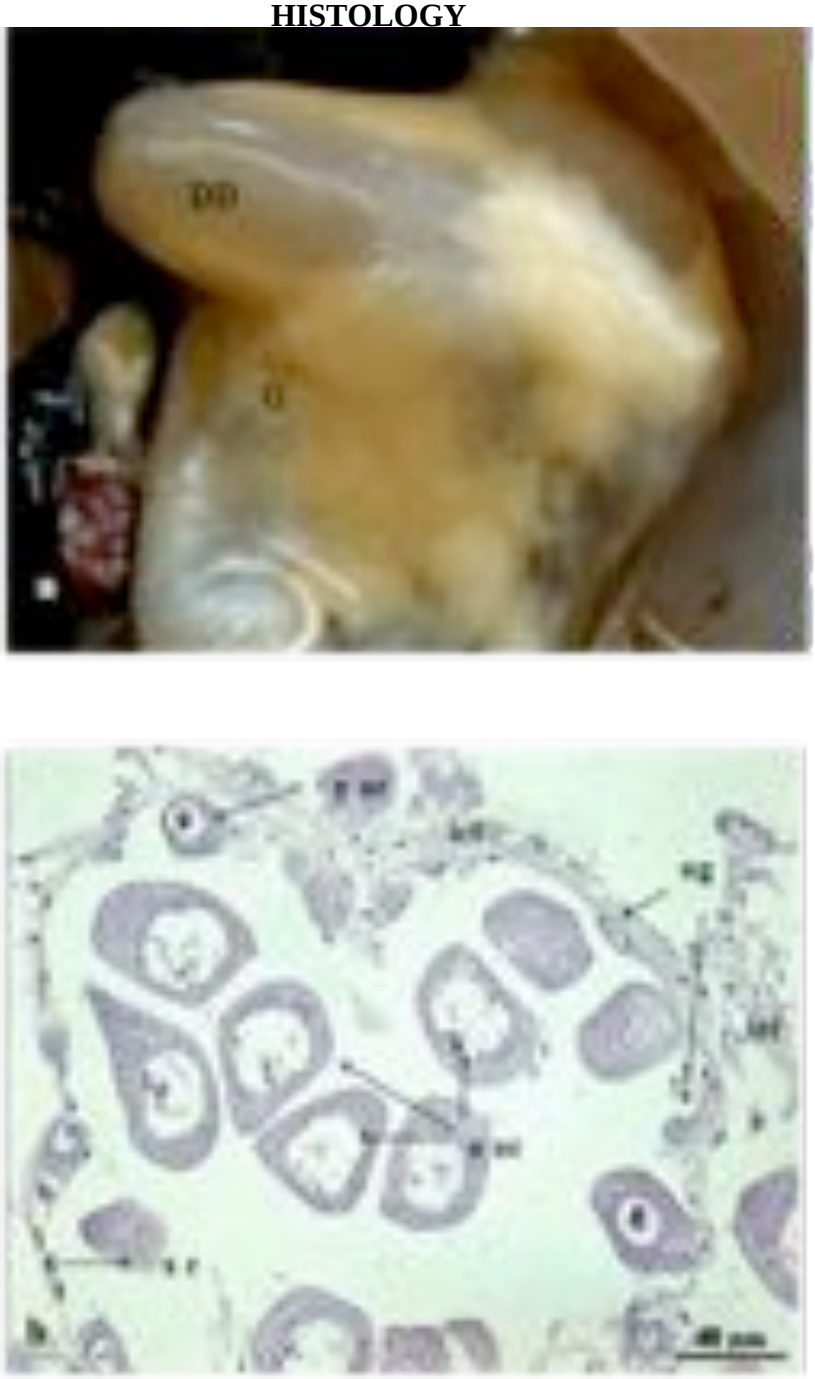
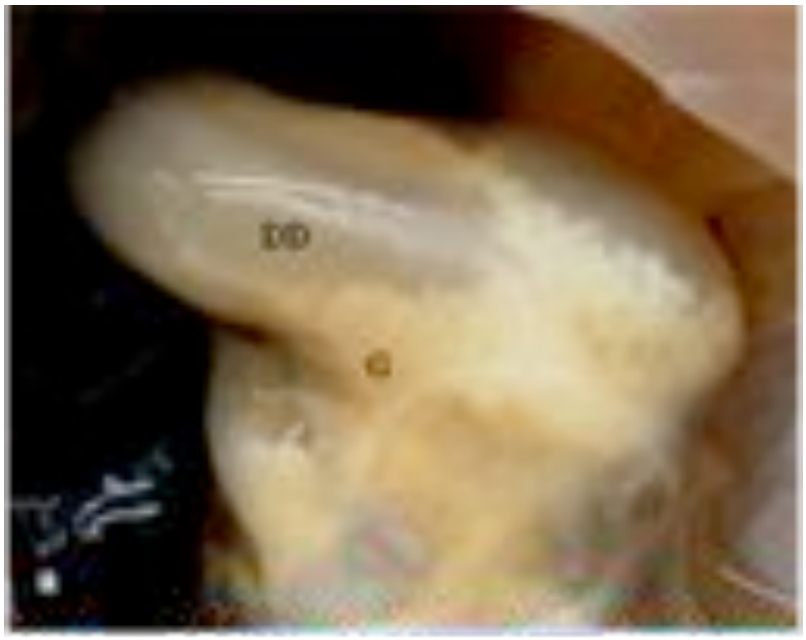


Figure 1 Photo and micrograph of Stage 1 female *P. maxima*.

(a) Sex can be determined as female based on colour (yellowish-orange) but development is slight and patchy. The gonad (G) is still flaccid and the colour somewhat dull and development is only along the base of the digestive diverticulum (DD).

(b) Early oogenesis. Acinus show some stem cells (sc), oogonia (og) and young oocytes (y oc) developing along the inner wall. Larger and older oocytes (o oc) are found further within the acinar lumen. Interconnective tissue (ict) support area between acini.



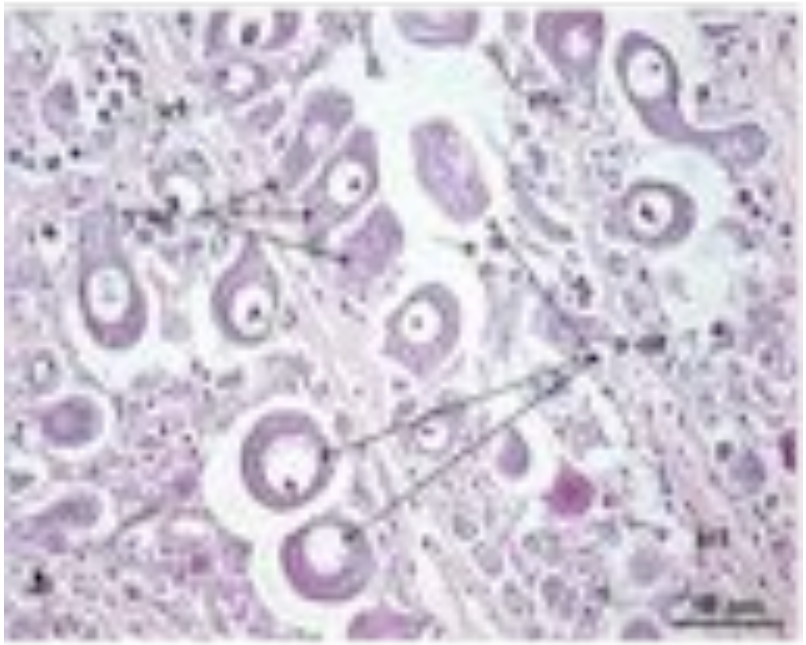


Figure 2 Photo and micrograph of Stage 2 female *P. maxima*.

(a) Development has progressed, with the gamete patches (G) becoming larger and more closely associated. Larger area of the digestive diverticulum (DD) is being filled with cloudy gametes.

(b) Mid oogenesis in stage 2 female. Young oocytes (y oc) are actively proliferating along inner acinar wall and older oocytes (o oc) are beginning to fill lumen of acini.

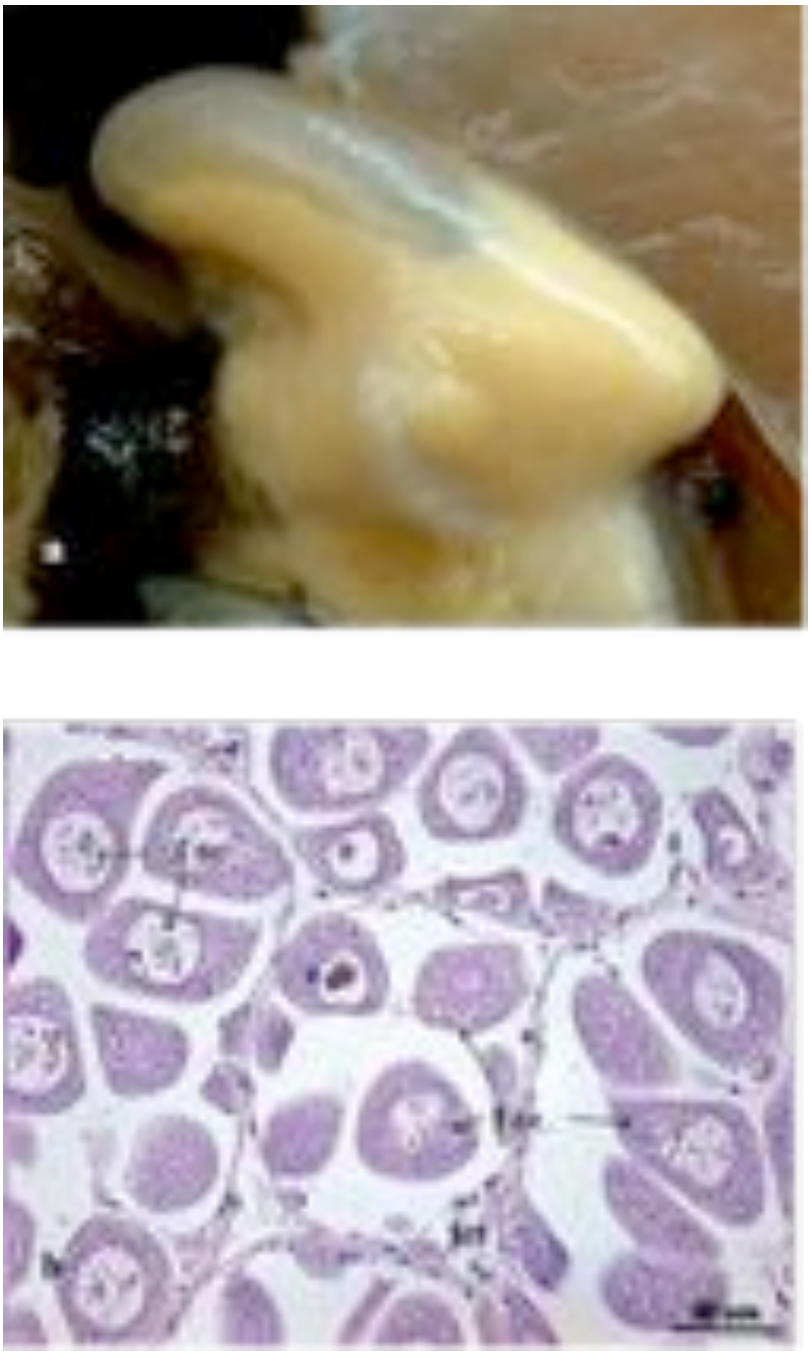


Figure 3 Photo and micrograph of Stage 3 female *P. maxima*.

(a) Patches of gametes are more confluent and have taken on a more solid appearance. Gametes (G) are visible along length of digestive diverticulum.

(b) Mid-developmental phase of stage 3 female. Acini are still visible and lumens of acini are filled with increasingly larger free oocytes (f oc) and acinar-connected oocytes (c oc). The proportion of interconnective tissue (ict) between acini is decreasing.

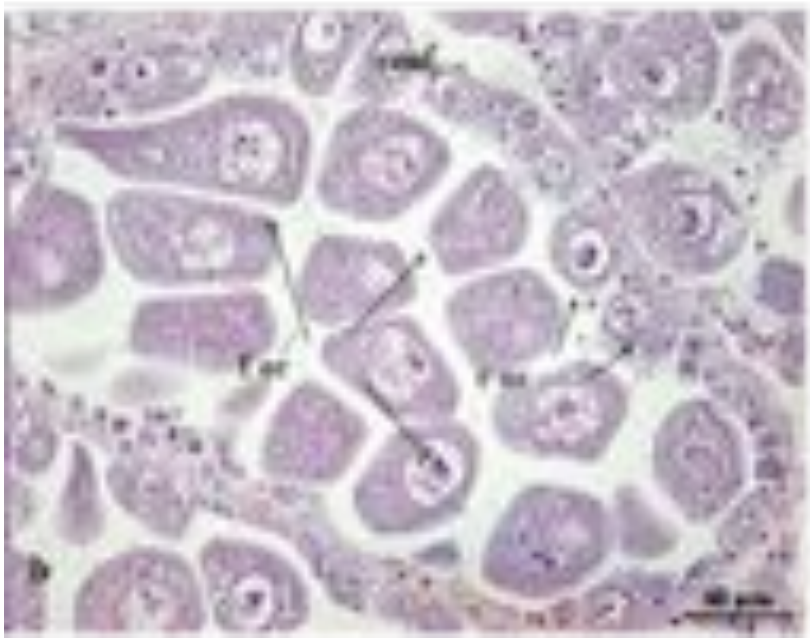
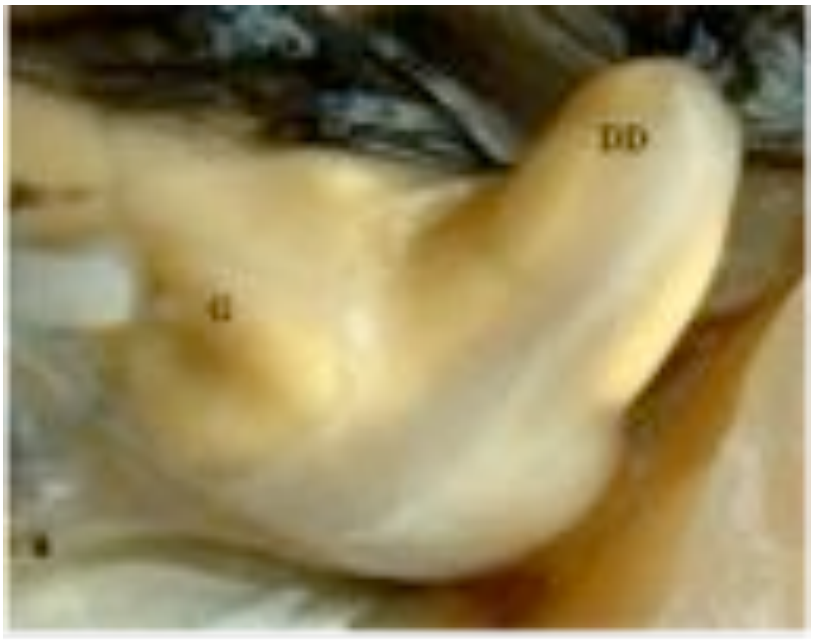


Figure 4 Photo and micrograph of Stage 4 female *P. maxima.*

(a) Gonad (G) is turgid, however, only one side of the gonad is well developed.

(b) Actively developing ovary. Acini start to become confluent (conf) and are filled with free oocytes (f oc) and connected oocytes (c oc).



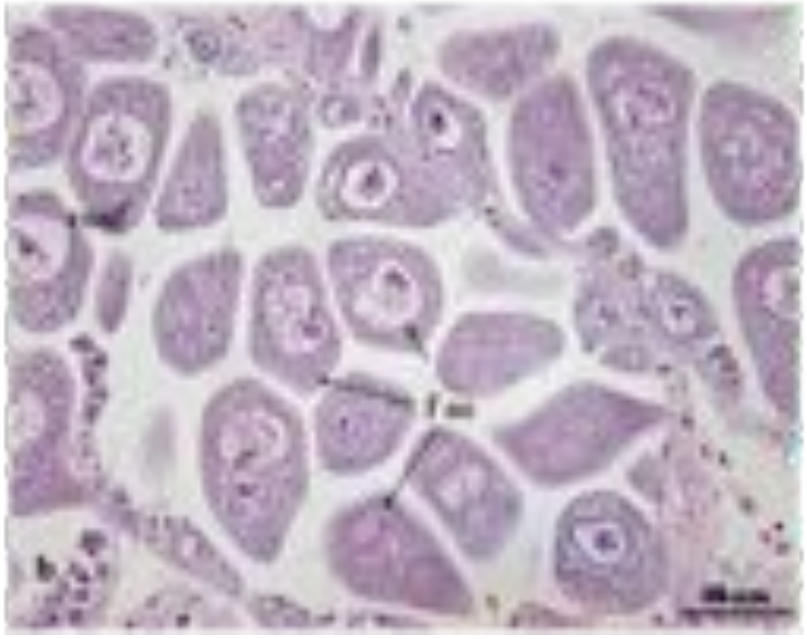


Figure 5 Photo and micrograph of Stage 5 female *P. maxima.*

(a) Female gonads (G) now appear as bands of colour with both sides of the gonad well developed and visible along the length of the digestive diverticulum (DD).

(b) Near ripe ovary. More acini become confluent and lumen starts to be filled with greater number of free oocytes, although connected oocytes are still observed.

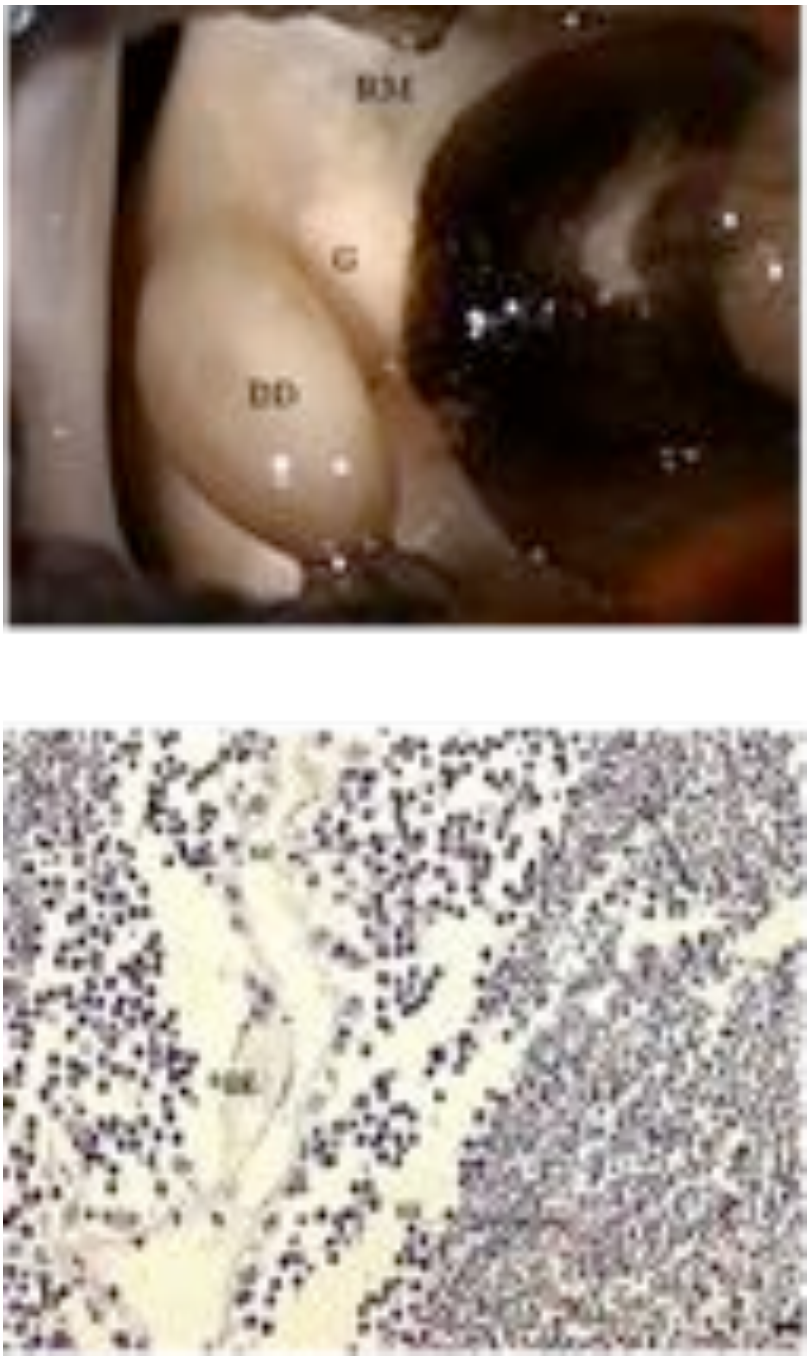
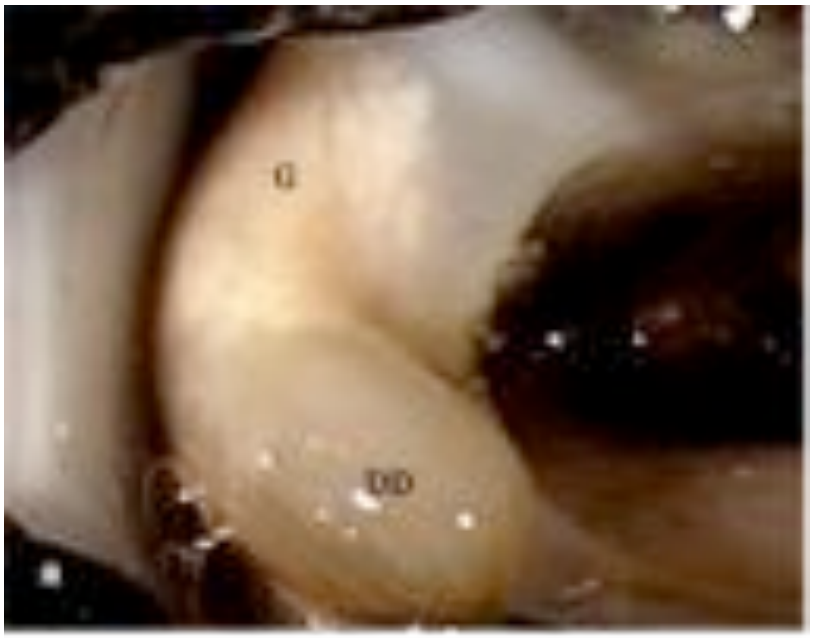


Figure 6 Photo and micrograph of Stage 1 male *P. maxima.*

(a) Male gonad (G) is clearly visible as creamy white patches. Patches are visible at the base of the digestive diverticulum (DD) around the dorsal edge of the retractor muscle (RM).

(b) Early stages of gametogenesis. Large spermatogonia (sg) lining inner wall of acinus and sparse spermatocytes (sc) and greater number of spermatids (st) visible in the inner lumen. Tails of spermatozoa (sz) can be seen within the pockets of spermatids.



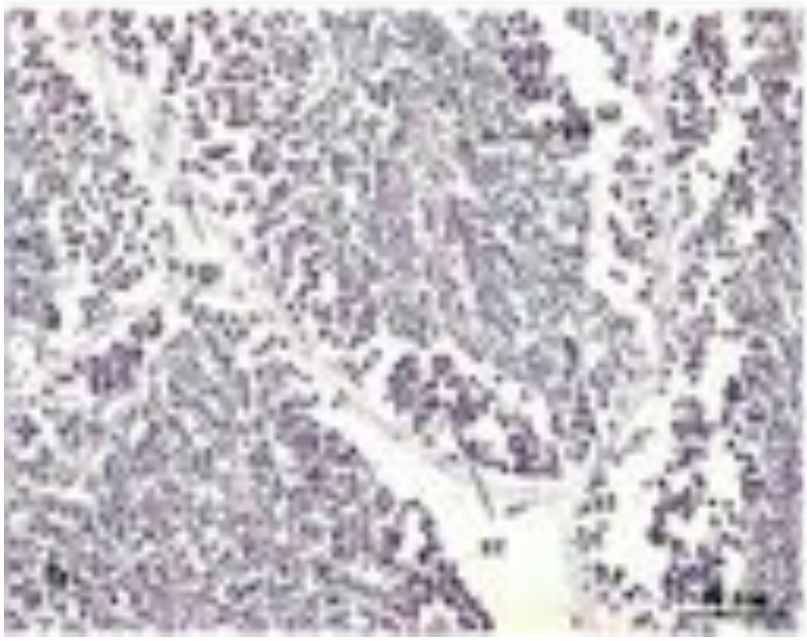


Figure 7 Photo and micrograph of Stage 2 male *P. maxima.*

(a) Gametes (G) have moved further along the base of the digestive diverticulum (DD). Development is still patchy and cloudy.

(b) Actively developing testis. Spermatogonia (sg) can be seen lining the inner wall of acini, large spermatocytes (sc) and small spermatids (st) are scattered at the periphery while spermatozoa (sz) with visible tails are in the centre.

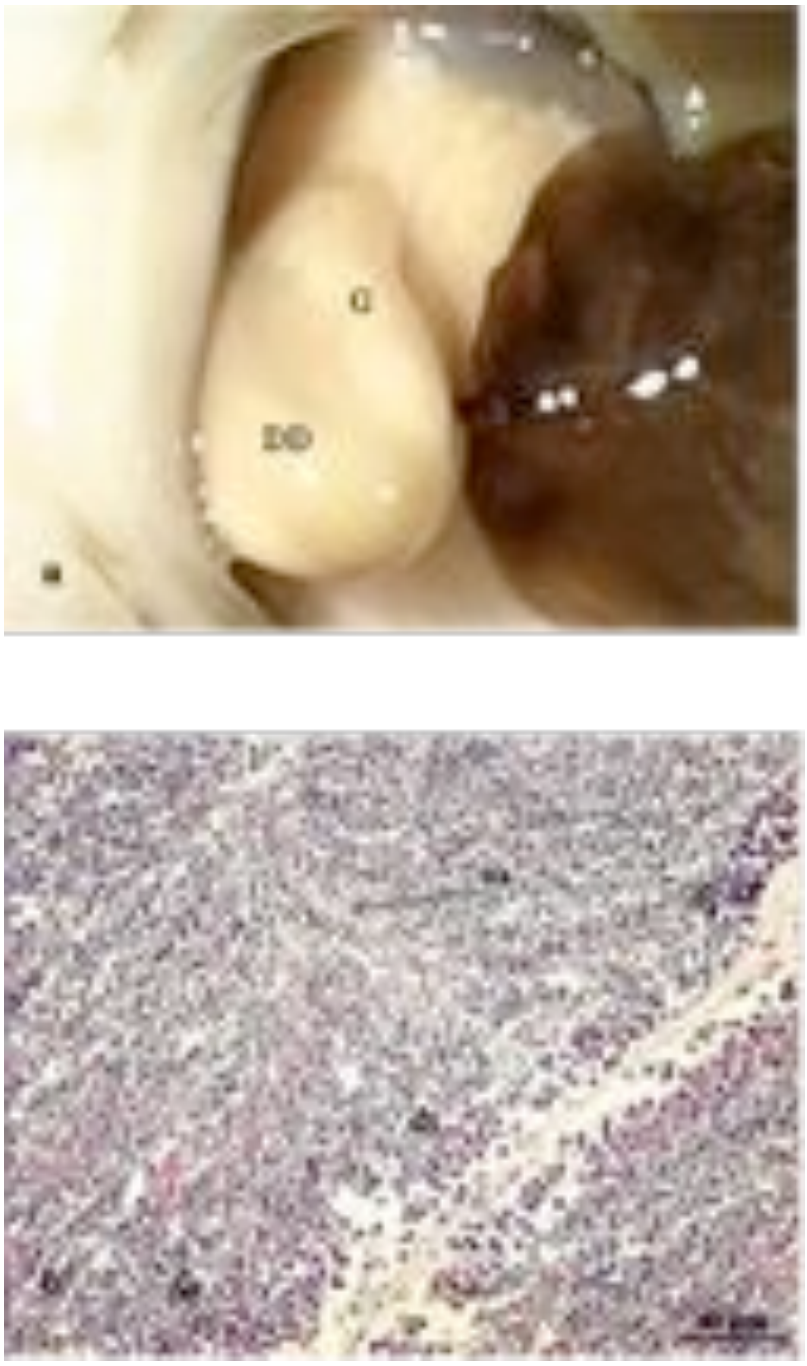
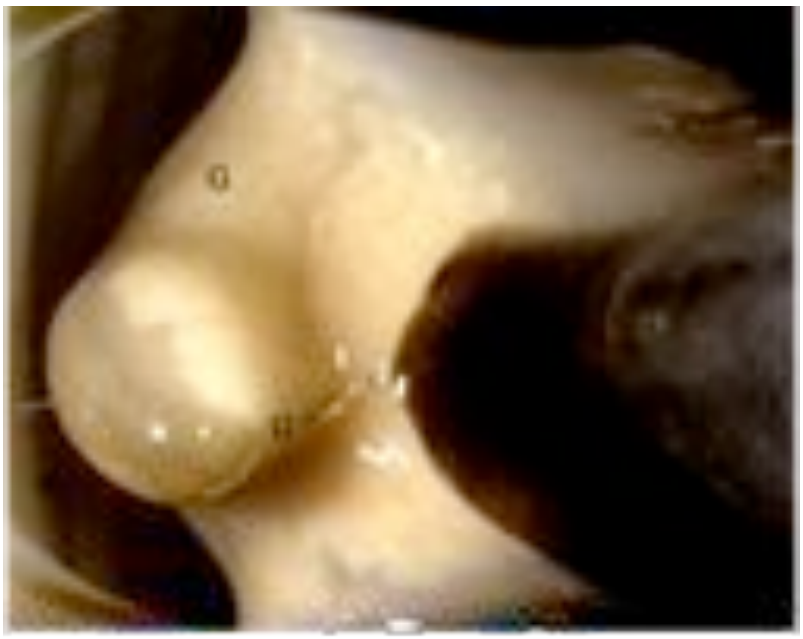


Figure 8 Photo and micrograph of Stage 3 male *P. maxima.*

(a) Gametes (G) patches have become larger and progressed further along the digestive diverticulum (DD). Development is observed on only one side of the digestive diverticulum.

(b) Near ripe testis. Within the acinus, the outer darker bands of cells at the border are developing sperm (ds) while the more mature spermatozoa (sz) fill the middle with tails visible.



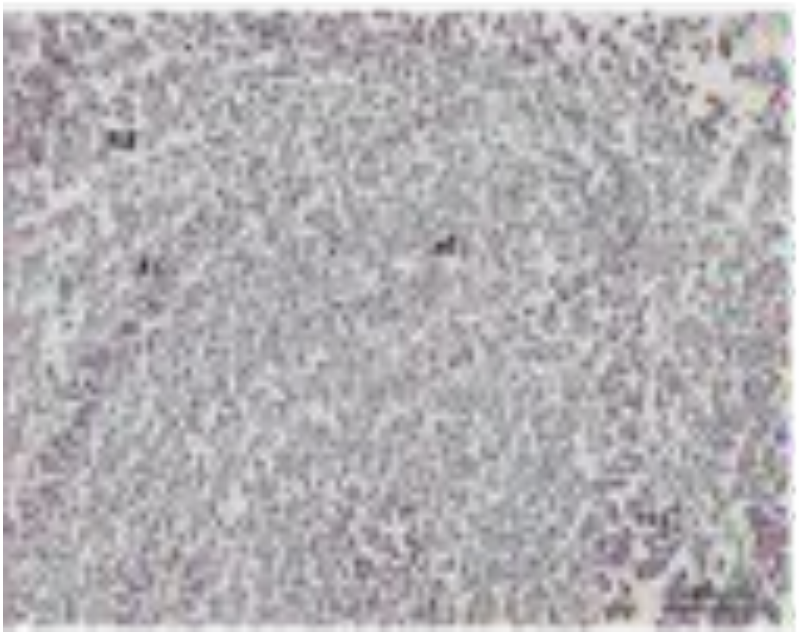


Figure 9 Photo and micrograph of Stage 4 male *P. maxima.*

(a) Gonad is turgid. Patches are large and more confluent and only one side of the digestive diverticulum has well developed gametes.

(b) Near ripe testis, with confluent acini filled predominantly with sperm in the later stages of development i.e spermatids (st) and spermatozoa with tails (sz). Small bands of spermatocytes (sc) can also be seen.

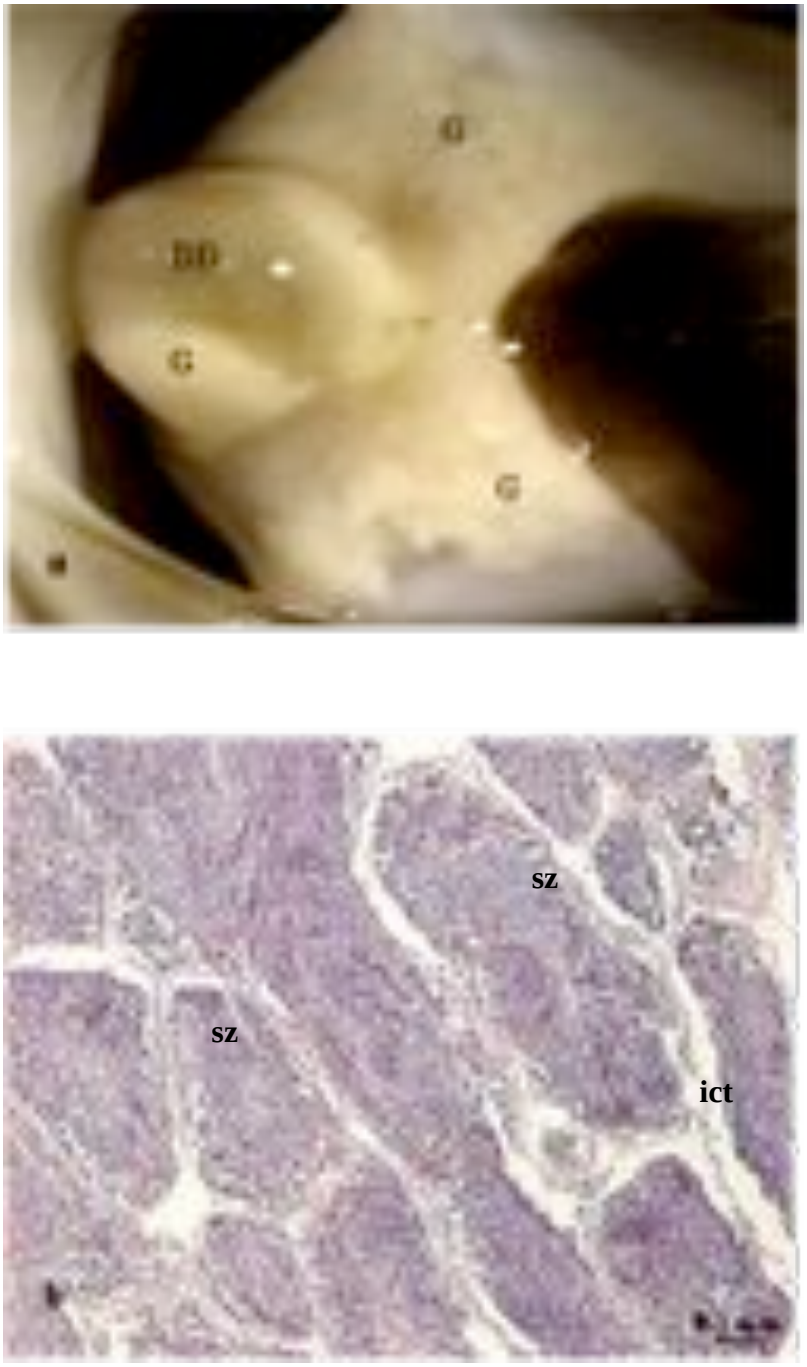


Figure 10 Photo and micrograph of Stage 5 male *P. maxima.*

(a) Gonad patches are found along both sides of the digestive diverticulum and are well developed.

(b) Near ripe testis. Acini are packed tightly with sperm cells, with spermatozoa (sz) in the centre of each lumen. Interconnective tissue (ict) between acini start to decrease.

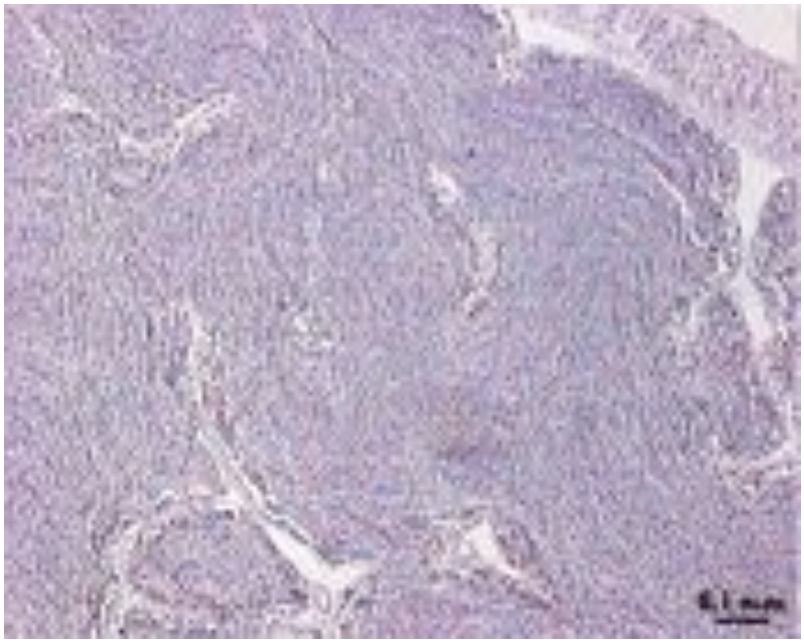


Figure 11 Photo and micrograph of Stage 6 male *P. maxima.*

(a) The digestive diverticulum is turgid and filled with gametes. Gonad is full.

(b) Boundaries between acini are difficult to distinguish. Acini are fully filled with mature sperm cells.

APPENDIX D

Publication

CSIRO PUBLISHING

Marine and Freshwater Research, 2008, **59**, 22–31 www.publish.csiro.au/journals/mfr

Modelling and comparison of growth of the silver-lip pearl oyster *Pinctada maxima* (Jameson) (Mollusca : Pteriidae) cultured in West Papua, Indonesia

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 10.1071/MF07112 1323-1650/08/010022