GROWTH, SCLEROCHRONOLOGY AND DEVELOPMENT OF THE TRIDACNIDAE, WITH PARTICULAR REFERENCE TO *HIPPOPUS HIPPOPUS*

Thesis submitted by

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C. C. Shelley
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ABSTRACT

In recent years there has been growing interest in the mariculture of giant clams (F:Tridacnidae) for both re-stocking of reefs and commercial farming. A lack of information on giant clam growth rates and how they can vary, both between sites of close proximity and those geographically separated, initiated this thesis. Also the importance of understanding the reproduction of species of mariculture potential, led to the first detailed study of sexual development and reproductive periodicity in giant clams being undertaken. Shell material was collected throughout the Great Barrier Reef of Australia, with routine field sampling at Orpheus Island Research Station.

Growth of H.hippopus, Tridacna gigas, T.squamosa and T.maxima in the Palm Island Group, in the vicinity of Orpheus Island, was studied by tagging clams and measuring them repeatedly over a 2 year period. Also the growth and shell morphometry of H.hippopus, T.gigas, T.derasa and T.squamosa from numerous locations along the Great Barrier Reef were determined by sclerochronological examination of shells. Validation of the apparent seasonal growth bands seen in shell sections of giant clam was undertaken by studying shells of clams of known time of death, shell

microincrements and seasonal differences in both shell chemistry and fluorescent band formation.

To investigate the sexual development and reproductive cycle of H.hippopus, monthly organ indices, histology, biopsy sampling and anatomical development studies were utilised.

Calculation of parameters of the von Bertalanffy growth equation for tagged H.hippopus revealed considerable differences in L_{oo} and K between sites of close proximity, Iris Point Reef (L_{oo} = 347 mm, K = 0.204) and Pioneer Bay, Orpheus Island (L_{oo} = 414 mm, K = 0.159). Von Bertalanffy parameter values determined from tagged T.gigas (L_{oo} = 813-862 mm, K = 0.148-0.132), T.squamosa (L_{oo} = 384, K = 0.189) and T.maxima (L_{oo} = 271, K = 0.126) were similar to values elsewhere in their geographic range. Minimal seasonal growth of H.hippopus and T.gigas occured prior to the spawning season, during gametic development. Total mortality (Z) estimates were H.hippopus 0.05-017, T.gigas 0.20-0.22, T.squamosa 0.04 and T.maxima 0.28.

Validation of 2 seasonal growth bands to each year enabled shells to be aged. The validation work included the first record of fluorescent bands in giant clam shells and the first analysis of seasonal shell chemistry using an electronmicroprobe.

Morphometric analysis of H.hippopus revealed a trend of increasing flesh per unit shell length from south to north on the Great Barrier Reef. With regard to growth in shell

length, however, there was no similar latitudinal variation; with the exception that H.hippopus from close to their southern latitudinal limit at Hayman Island exhibited the lowest L_{oo} value. Comparison of growth rates using tag-remeasure and sclerochronological techniques from Orpheus Island were in close agreement for H.hippopus. This indicated the advantages of rapid sclerochronological investigation of shell death assemblages, which compared to the time consuming tag-remeasure method, did not result in less accurate growth estimates. Results indicated that site specific differences in growth rate within a locality, e.g. Torres Strait, Palm Islands, or between different parts of the Great Barrier Reef could be as large as any inter-country variation.

Gonad indices, gonad biopsy (including ooycte diameter), gross gonad anatomy and histological results were combined to demonstrate a mid-summer spawning season for H.hippopus. With maturation, the gonad and kidney, increased relative to other clam organs. The size at first male maturity was not ascertained, but was less than or equal to 77 mm, the smallest clam sampled. Male only gonads were found in clams of up to 140 mm in shell length. The shell length at first hermaphrodite maturity was approximately 146 mm, although one clam of 112 mm in length was found to be mature.

Combining growth and reproductive studies of H.hippopus, the age of male maturity (at a presumed shell length of 80

mm) was 2 years and hermaphrodite 4 years. The shell length at full hermaphrodite maturity of H.hippopus corresponded to the inflexion point in its growth curve, indicating there may be a causal relationship between the two events.

The wide variation in growth rates exhibited by all species both within and between sites, indicated that there was large scope to increase growth rates through careful site selection and selective breeding. Whilst the flesh yield of H.hippopus with age was less than that for T.gigas, further work to increase growth rate may see it join T.gigas and T.derasa as a commercially farmed species.

CONTENTS

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CHAPTER 1

GENERAL INTRODUCTION

1.1 AQUACULTURE OF THE TRIDACNIDAE

All species of giant clam have been drastically reduced in numbers throughout their ranges, some to extinction, both by traditional fishermen in South Pacific nations and by poachers from south-east Asia. For this reason alone, the culture of giant clams is a practical solution to assisting in the recovery of wild stocks, and in doing so providing another food source for many areas. Also the culture of giant clams appears to offer certain commercial advantages compared to other aquaculture ventures. Giant clams have a short larval life compared to most cultured bivalve species, and after settlement can grow successfully as long as they have seawater and sunlight. Most of their energy requirements appear to be provided by sunlight through the photosynthetic products of their symbiotic zooxanthellae. Giant clams are also amongst the fastest growing molluscs in the world.

The prospects for giant clam cultivation has been reviewed in recent years (Munro 1983, Munro & Heslinga 1983), a bibliography of giant clam literature produced (Munro & Nash 1985), and many papers have appeared over the last five years as a result of various collaborative

and individual studies at institutions in the Pacific region. Reference to most papers published in recent years can be found in the proceedings of a recent giant clam workshop (Copland & Lucas 1988).

When examining the commercial potential of a new species for aquaculture, several fundamental questions have to be examined. This thesis attempts to answer some of those questions concerning growth, reproduction and variation in growth rate and form relative to different geographical locations and environmental variables on Australia's Great Barrier Reef, with particular reference to Hippopus hippopus, the horseshoe clam.

1.2 THE GROWTH OF BIVALVES

Studies in modern times of the growth of bivalves have been comprehensively reviewed in 'Growth rhythms and the history of the Earth's rotation' (Rosenberg & Runcorn 1975) and 'Skeletal growth of aquatic organisms' (Rhoads & Lutz 1980).

This introductory chapter reviews many additional papers published since these two major reviews, together with earlier work, to set the theoretical background against which this study is undertaken. The amount of work in this field reflects the increasing effort by fisheries biologists to understand the factors affecting the growth of molluscs and similarly by paleoecologists attempting to extrapolate from the patterns of growth in modern molluscs to molluscs in the fossil record.

fluctuations of temperature.

For most species, growth rates are limited by temperatures, which might be either too high or too low. Within their normal temperature ranges, shell growth of bivalves usually increases logarithmically with increasing temperature, (e.g. in Mytilus edulis, Almada-Villela et al. 1982). However, decreased mean annual sea surface temperature resulted in increased growth rate in Spisula solidissima (Jones 1981) and similarly, the annual growth of three bivalve species were negatively correlated with spring and summer temperature anomalies (Menesguen & Dreves 1987). Too high a temperature can stress bivalves and this can result in decreased growth rates (Kennish & Olsen 1975). The effect of temperature can vary according to the age of the individual. This was noted by Jones et al. (1986) who found that in the post-inflexion growth phase in Tridacna maxima minimal calcification occurred in the warmer parts of the year, growth being limited to the cooler season.

Although changes in temperature have often been used to explain seasonal banding in bivalve shells, it is interesting to note that seasonal bands were still found in T. gigas when the annual variation in water temperature was less than 3°C, close to the equator (Bonham 1965). The prominence of growth lines in the shell of bivalves has been related to the air temperature during periods of emersion (Richardson 1980a), which may be related to the observations of Lutz & Clark (1984) that the percentage

of various shell microstructures may be correlated with mean annual sea temperature.

1.1.2 Food

Studies of the effects of food on the growth of bivalves have found that growth is related to the amount of food available (Winter & Langton 1975, Bricelj et al. 1987). The rate at which food is presented to a bivalve (i.e. the current speed of a given body of water) can control a bivalve's filtration rate (Walne 1972) and its growth (Wildish & Kritmanson 1985). Growth was found to be reduced at both low and high current speeds, whilst intermediate current speeds had no deleterious effect on growth.

For filter feeding bivalves it is not surprising their growth has been correlated to the levels of particulate organic and inorganic matter in the water column (Leighton 1979, Wallace & Reiness 1984). Most work on the effects of different types and amounts of food on bivalves has been undertaken on larvae and juveniles, whose growth under laboratory or hatchery conditions can be accurately monitored, whereas adults are usually reared under natural conditions. When bivalves are grown under natural conditions the synergistic effects of food supply with all other growth factors means that causal relationships between food and growth can only be hypothesised.

1.2.3 Depth and Emersion

Depth itself has little effect on the growth of bivalves, however variables which fluctuate with depth such as temperature, food availability and time of emersion certainly do. Growth of mussels decreases with increasing aerial exposure, resulting from increasing height on the shore (Bertness & Grosholz 1985, Griffiths 1981, Rodhouse et al. 1984). In the Icelandic scallop, Chlamys islandica, growth increased with depth to 12 m, but then decreased to 30 m. The increase in growth to 12 m was thought to be related to concentration of phytoplankton at the thermocline (depth 15 m). Similar work on the rock scallop, Hinnites multirugosus, showed increased growth at shallow depths, and this was related to both temperature and particulate organic matter concentrations (Leighton 1979). Radtke (in press) also found a decrease in growth with depth for Tridacna maxima. However, this is most likely a result of a decrease in light levels with depth, which is of great importance to the almost phototrophic clam.

1.2.4 Reproductive Cycle

Sexual maturation seems to have significant effects on the growth of bivalves. Kennish (1980b) found that a change in growth pattern accompanied sexual maturity, whilst Jones et al. (1986) described two separate growth phases for T. maxima, one for growth to sexual maturity and one after. However, Jones et al. did not assess the clams they worked on for stage of sexual maturity, but

based their hypothesis on the size at sexual maturity of T. maxima from a different geographic location (McMichael 1974).

Although it appears that sexual maturity affects growth, there does not appear to be a reciprocal relationship. Changes in growth rates do not seem to influence the age at sexual maturity (Vincent & Letourneau 1985).

The relative growth rates of gonadal and somatic tissue are such that somatic growth far exceeds gonadal growth, prior to sexual maturity. Therefore the relationship between genetic heterozygosity and improved growth can be most clearly examined in young bivalves prior to sexual maturity (Rodhouse et al. 1986). There appears to be competition between gonadic and somatic development in bivalves (Menesguen & Dreves 1987), which may be used to explain why there is often a poor correlation between shell and tissue growth, which can be exaggerated by spawning events (Seed 1980). Shell growth can nearly stop during reproductive periods, which can result in shell and somatic growth being out of synchronisation (Bricelj et al. 1987). In some bivalves the spawning period has been found to cause biochecks (Hall 1975) or spawning breaks (Kennish 1980b) (breaks in the normal deposition of growth lines) in shell growth.

1.2.5 Site and Latitude

The importance of site-specific differences in growth and growth form of bivalves was shown by Hickey (1987), who

found "maximal levels of intraspecific variation within each species occurred within different habitats". Hickey suggested that between site differences could be explained by genetic variation or by different ecophenotypic responses to their environment. Whilst examining growth in Mytilus edulis it was found that differences between sites accounted for most of the variation in tissue growth, stock related differences being much less significant (Mallett et al. 1987). The growth of the giant scallop, Placopecten magellanicus, was also found to vary between sites (MacDonald et al. 1987).

Changes in growth of species of bivalve with latitude have been described (Beukema & Meehen 1985, Harrington 1987) and reviewed (Beukema & Desprez 1986, Hall 1975). In addition to changes in growth rate with location, the numbers of microgrowth increments in seasonal bands have been found to vary with latitude (Hall 1975, Harrington 1987), as have relative shell dimensions and shell colour (Beukema & Meehen 1985). The main causal factors determining latitudinal differences in bivalve growth are considered to be temperature and food availability (Beukema & Desprez 1986).

1.2.6 Competition

Competition for space both in the wild and in cultivated bivalve systems has been shown to produce reduced growth rates in the mussel Geukensia demissa (Bertness &

Grasholz 1985), and the scallop, Pecten fumata (Cropp 1987). Similarly, Hamner (1978) found that competition for space amongst a population of the boring tridacnid, T.crocea inhibited their growth.

Adult bivalves compete for an adequate food supply, position to obtain optimal water currents and a site for attachment, in addition to space; however, little work appears to have related other such types of competition directly to growth.

1.2.7 Salinity

A combination of reduced salinity and high suspended matter has been suggested as the cause of a decrease in growth rate in Cerastoderma edule and M.edulis (Essink & Bos 1985). It is unclear how long-term exposure to low salinities affects the growth of bivalves. In short term trials on M.edulis, shell growth was not affected until the salinity fell below 50% seawater (Gruffydd et al. 1984). Davenport (1979) had already indicated that the reason for this was that the mussels isolate themselves from the surrounding medium by tightly closing their shells. To explain the possible long term affects of exposure to low salinity, Gruffydd et al. referred to Bohle's (1972) suggestion that any reductions in growth rate seen as a result of short term salinity fluctuations would be minimised as a result of acclimation.

At a microscopic level, the size of microstructures (daily, tidal growth lines) in shells of T.maxima were

found to be inversely proportional to salinity (Henocque 1977). How generally applicable to other shells and over what ranges this holds is unknown.

1.3 ASSESSING RELATIVE AND ABSOLUTE GROWTH OF BIVALVES

Condition indices have been used to assess relative growth in bivalves either between individuals or more commonly between populations or groups. The most commonly used indices are those which compare flesh mass to shell mass as shown in Lucas & Benninger (1985) who reviewed the use of condition indices for molluscs and recommended that the calculation of the Net Growth Efficiency Index, which measures the most important physiological parameters of a bivalve was the index which gives the best understanding of their growth. However, for ease of use in most routine investigations, they recommended that the comparison of shell to flesh weight was the best index to use for the study of juvenile and adult bivalves.

Using dry weights in condition indices removes the problem of amounts of water in tissue of varying through the year, which could lead to spurious estimates of true somatic growth. A further practical advantage to using Lucas & Benninger's dry weight index in fisheries research is that time is saved through decreased processing compared to indices involving ash free dry weights.

To obtain more detailed information than available from

condition indices alone, the differential growth rates of various body organs or tissues can be examined. Such allometric studies allow the determination of important fisheries parameters such as gonadal versus somatic growth, muscle growth with shell length and how these variables change with age, season and sexual maturity.

Morphometric studies can be undertaken which can further enhance the understanding of growth in bivalves. As "the shape of any shell depends ultimately on growth processes" (Seed 1980), the shape of several species have been studied (Berry 1978, Beukema & Meehan 1985, Griffiths 1981, Hickey 1987). Marked changes in morphometry have been related to latitude (Beukema & Meehen 1985), different sites (Hickey 1987, Mallet et al. 1987), and water depth (Wallace & Reiness 1984).

Allometric studies have revealed that shell growth and tissue growth are not necessarily simultaneous (Hilbish 1986) and that shell weight may increase faster than flesh weight (Richard 1977). To examine allometric and morphometric changes in bivalves growth, multivariate techniques such as discriminant analysis have been utilised (Hickey 1987).

Growth of bivalves have been found to be determinate and can be described using growth equations such as the von Bertalanffy growth curve (1938). Most studies of growth in bivalves have used shell length, rather than shell mass as the main variable. Assessing the growth of bivalves is undertaken either by measuring representative

samples on a regular basis or by remeasuring tagged individuals within a population.

1.4 SCLEROCHRONOLOGY, SHELL STRUCTURE AND FORM

1.4.1. Sclerochronology

Sclerochronology is the study of the relationship between the ontogenetic growth of organisms and the environment in which they live, where ontogenetic growth is defined as 'the life history of an individual as preserved in mineralized or otherwise refractory tissues' (Seed 1980). It is the zoological equivalent of the study of dendrochronology in botany, and is based on the study of periodically deposited increments in the calcified skeletons of organisms.

The first step in any sclerochronological study is to 'identify features in the growth record that have been deposited at regular intervals in time' (Dolman 1975). Having identified features, it is necessary to accurately define them, so that there can be no confusion. 'A growth record may be defined as a uniquely organised unit of shell constituents formed under a certain set of physiological conditions prevalent for a certain period of time between the mantle and shell surface' (Barker 1964). However well defined a growth increment is, currently it is a subjective decision by the reader as to what is an increment (Evans 1975). However, with advances to techniques which integrate video, digitising boards and computers, a fully automatic system should not be

many years away. The next crucial step is to validate the period over which increments form. If this is unknown or assumed, any growth determinations based on growth increments are of questionable value.

The different periodicities with which microgrowth increments are formed have been detailed by many workers including Barker (1964), Evans (1975) and Pannella and MacClintock (1968). Periods over which periodic increment patterns have been recorded include tidal, daily, fortnightly, monthly and seasonal. The patterns of increment formation have been determined by use of a variety of techniques which are detailed in section 1.4.5. Growth records must be carefully recorded as it is possible to count more than one type of period at once, e.g. fortnightly instead on monthly and so miscount the frequency of the increment (Dolman 1975).

1.4.2 Shell Shape

In addition to changes in growth as recorded in the shells of molluscs, changes in the shape of shells between geographical locations, as a response to habitat conditions have been recorded (O'Loughlin & Aldrich 1987). A dramatic example of the effect of environment on the shell shape of bivalves was demonstrated by Palmer (1980), who showed that regular handling of the scallop Argopecten irradians resulted in marginal thickening of valves, creating deformities in the shells. Differences in shell shape between intertidal and subtidal

populations of Perna perna were noted by Berry (1978). When examining shell shape parameters of 3 species of bivalve it was noted that there was a general decline in variability of parameters with age (Brown et al. 1976). Between populations of a bivalve from different locations there may be overlapping ranges of variation in shell shape, which may be largely explained by phenotypic responses (Eager et al. 1984). Differences in shell shape in molluscs may even be related to the sex of the specimen (Rao & Rao 1985).

Differences in shape between sites may give an insight into the way a mollusc is adapted to the environmental conditions found at that site and assist in explaining differences in growth found between sites, e.g. shells from exposed shores may be heavier and smoother than shells from a sheltered area, where shells maybe lighter and have more superficial growth on the shell such as scutes or flukes.

1.4.3. The Structure of Growth Increments

Although micro- and macro-growth increments have been measured, counted and utilised by scientists for years, there is still 'no satisfactory theory to account for growth-line formation' according to Deith (1985). The problem is that 'the bivalve shell is far more than a simple accretionary deposit' (Lutz & Clark 1984).

It is accepted by most researchers that the molluscan shell is usually composed of two basic components,

calcium carbonate and an organic matrix (referred to in earlier papers as conchiolin). Two papers in a symposium on the mechanisms of calcification in biological systems examined the role of the organic matrix in crystal nucleation. Greenfield et al. (1984) hypothesised that the sulfated fraction of the organic matrix appeared to be involved in crystal nucleation; whilst Wheeler and Sikes (1984) were only prepared to say that an organic matrix will affect the mechanical properties of a crystalline structure and that the role of the matrix in regulation of crystal growth appeared unclear. A result of the lack of certainty of the way in which the two components of shell interact in the deposition of shell can in part explain the many variations in theory as to the way in which growth increments are formed and of what they are composed.

Microgrowth increments are usually seen as series of dark and light bands. Many workers have referred to the darker lines as organic lines (Pannella 1976), lines being relatively rich in organics. However, Evans (1975) may have been the first to suggest that growth lines are not always a result of variations in the proportions of calcium carbonate and conchiolin (organic matrix), but can be built into the aragonite crystal itself. Deith (1985) demonstrated that some growth lines formed by periods of emersion, referred to as 'organic lines', have less, rather than more organic material than the lines formed during immersion. Also she revealed that the increments seen are related to the different relative

size of crystal units rather than their organic content. Such structural changes accounting for microgrowth increments agree with analysis of seasonal patterns in Geukensia demissa by Lutz & Rhoads (1978), who found distinctly different crystal structures in summer and winter growth bands.

A precise theoretical explanation of what a growth increment is composed of is irrelevant to the fisheries biologist wishing to age animals, because as long as the increments can be shown to be related to real events, e.g. tides, seasons, etc., age can be calculated.

1.4.4. The formation of growth increments

Seasonal growth bands, visible in sectioned shells, are most useful for the majority of fisheries studies. The most frequently seen pattern being two major bands, winter and summer, comprising one yearly increment, enabling aging of specimens to be undertaken.

Whilst it might be expected that seasonal differences in temperate regions lead to the most pronounced seasonal changes in growth and therefore seasonal growth band formation, marked seasonal bands have been found in molluscs from the sub-antarctic (Guzman & Rios 1987) to the tropics (Romanek et al. 1987, Radtke in press). Seasonal bands are often referred to as dark and light, or opaque and translucent. In Mercenaria mercenaria dark bands are usually associated with slower growth, and are formed in the summer and early fall. However, the time of

the growth band formation can vary with latitude (Fritz & Haven 1983). In some bivalves annual increments are not simply one light and one dark band. Bands can be interrupted by winter cessation lines (Fritz & Haven 1983) or spawning lines (Hall et al. 1974, Rhoads & Pannella 1970). In some molluscs spawning has been related to the deposition of the annual line (Muraurski et al. 1982) and in specimens which are not sexually mature, physiological mimicry of the reproductive cycle may produce the same result (Thompson et al. 1980). Spawning breaks have been distinguished from other events in the microgrowth record of a shell, the formation of 'daily' growth lines being abruptly disrupted, followed by a few days of slow growth (Rhoads & Pannella 1970). Another time at which the reproductive cycle of some molluscs has been implicated in depression of growth, is at the onset of gametogenesis (Ekaratne & Crisp 1982).

For many bivalves the period of maximum growth is during the summer months (Bachelet 1981, Utoh 1981, Wheeler & Wilbur 1977, Fritz & Lutz 1986). However, others form most of each annual increment during minimal temperatures (Zolotarev & Ignat'ev 1977). The season of slow growth can vary with latitude and periods of no growth at all are frequently recorded, especially with increasing age (Hall et al. 1974). In the period of slow growth, usually represented by the darker band, microgrowth increments are thin and closely spaced (Hall et al. 1974).

Kennish (1984), discussing the relative merits of reading daily or seasonal lines, commented that 'Although less accurate than counts of daily growth increments, counts of seasonal microgrowth patterns are especially practical when time constraints place limits on the use of daily growth increments'. One source of error which care should be taken to avoid is the confusion of lines caused by spawning or sickness with annual lines which can result in anomalous high ages (Tevesz 1972). Petersen & Ambrose (1985) found that the stability of the environment can affect the number of 'annual lines' formed. In muddy-sand Protothaca staminea formed one annual line. However, in clean sand, a more unstable environment, more than one line per year was formed, emphasising the need to check the assumption that 'annual lines' are just that. Some studies have relied on external rings deposited on shells to age molluscs, but it is now considered that patterns formed by microgrowth increments in the shell's internal structure give much better resolution (Rhoads & Pannella 1970).

As well as being used to age molluscs, seasonal bands can be used: 1. to assess good versus poor years of growth in any year class (Brown et al. 1976, Jones 1981), 2. establish the season of death (Rhoads & Pannella 1970, Lutz & Clark 1984), 3. establish the season of growth (Seed & Brown 1978, Ekaratne & Crisp 1984, Richardson et al 1980b) and even 4. examine the effects of major environmental fluctuations such as El Nino (Rollins & Sandweiss 1986). If one can determine the season of death

from seasonal bands, one can utilise all the shells of dead molluscs in an area (a death assemblage) in addition to those of live molluscs to examine life history traits. Kennish (1980b) considered that death assemblages give a more realistic picture of age structure of a population because they contain many generations of individuals.

Whereas earlier studies considered that most microgrowth lines in bivalves were deposited daily (Pannella & MacClintock 1968), there is now a wealth of evidence pointing to the influence of tides on the depostion of lines (Berry & Barker 1968, House & Farrow 1968, Pannella 1976, Richardson et al. 1979, 1980a, 1980b, 1981, Richardson 1987a, 1987b). The ways in which tide can directly or indirectly affect growth line formation depends on the tidal amplitude, the position of the mollusc with respect to low tide, the time of day of spring and neap tides (Pannella 1976), exposure to air (Muraurski et al. 1982, Rodhouse et al. 1984), the depth of water (Rhoads & Pannella 1970) and the period of valve closure during emersion (Kennish & Loveland 1980). Richardson's series of papers (see References) on growth line formation all mention that tidal lines are indistinct or absent in subtidal molluscs. Under conditions of continuous immersion with no tidal influence, however, one increment per day can be deposited by some species (Palmer 1980). This supports Richardson's (1987) hypothesis that molluscs possess an endogenous rhythm of deposition entrained by periods of

emersion.

If one microgrowth increment was formed each day, aging and interpretation of the growth record of the shell would be a simple task. It has been found however that not only do the number of lines deposited vary with the season (Wheeler & Wilbur 1977), but also with age. The number of increments per year decrease with age (Fritz & Haven 1983).

This section has outlined the reasons why growth line / band analysis should be analysed with caution, and indicated how important it is to validate the growth increments in the shell of the species under examination.

1.4.5. Techniques used in sclerochronology

As the usefulness of external shell growth patterns is very limited (Lutz & Rhoads 1980), examination of the internal structure of molluscan shells using thin sections, acetate or perspex (plexiglass) peels, scanning electron microscopy, and other techniques has become common practice. Thin sections of shell are produced so that one continuous series of growth increments can be viewed using transmitted light. Peels are used to make replica copies of growth lines emphasised by chemical etching. Peels can also be viewed by transmitted light; use often being made of interference microscopical techniques such as Nomarski, to enhance relief on the peels.

Shells are cut prior to examination, bivalve shells being commonly cut along the demarcation line (Seed 1980) between the umbo and growing edge in the region of maximum valve inflation, dividing the shell into anterior and posterior sections.

1.4.6 Ontogenetic changes in shell chemistry

Molluscan shell chemistry as summarised by Rosenberg (1975, 1980) is still poorly understood. He states that 'not one single bivalve species has been completely or adequately described'. However, relatively recent improvements in analytical tools and techniques available to sclerochronologists, such as the electron microprobe and the routine analysis of oxygen and carbon isotopes from powdered calcium carbonate, have provided valuable information on fluctuations in shell chemistry linked to structural changes. Earlier studies, often based on whole shell chemistry, do not necessarily reflect elemental concentration correctly. Elements are not usually uniformly distributed and studies based on parts of shells may similarly give atypical results (Rosenberg 1980). Both the electron microprobe and isotopic analysis allow sequential examination of the chemistry of shells. Therefore changes in shell structure and their relationship to shell chemistry can be examined.

As calcium (Ca) in the form of calcium carbonate is the major component of all molluscan shells, most studies of shell chemistry have examined its variation with respect to either structure, time or other elements. There seems

to be no fixed relationship between Ca and shell structure or time. Several investigators found Ca concentration to vary with season (Rosenberg 1975, Silina and Pozdnyakova 1982, Pozdnyakova and Silina 1985) and between sub-daily growth lines (Deith 1985). Others have found an increase in Ca concentration with age (Vinogradov 1953), Zolatarev 1974), whilst Rosenberg (1980) found no reliable patterns, there being large differences in Ca concentration between shells of the same species.

In the study of the chemical periodicity of calcified parts of fish using the electron microprobe, Ca, phosphorus (P), and strontium (Sr) have all been well related to growth increments (Radtke and Targett 1984, Radtke 1987, Cailliet and Radtke 1987). In molluscs, magnesium (Mg) (Dodd 1965, Silina and Pozdnyakova 1982, Pozdnyakova and Silina 1985), sulphur (S) (Rosenberg 1975a) and Sr (Eisma et al. 1976) have all been shown to fluctuate during the ontogeny of shells. Striking differences in oxygen and carbon isotope ratios (which are governed by water temperature) have been found between growth bands of the giant clam Tridacna maxima (Jones et al. 1986, Romanek et al. 1987).

If chemical periodicity in tridacnids is well defined, then chemical increments as well as structural units can be accessed for growth data. Should chemical periodicity be in phase with structural differences, this will provide further evidence to support the validation of the

seasonal growth increments used in this study. In Chapter 3 the chemistry of the seasonal bands of six species of tridacnid clam are investigated using an electron microprobe.

1.5 REPRODUCTION IN BIVALVES

Reproduction in bivalve molluscs has been reviewed recently by Sastry (1979) and Mackie (1984). This section merely seeks to summarise some of the important studies and approaches to the study of reproduction that have shaped the methodology applied in this study.

Studying the annual reproductive cycle in molluscs has often been undertaken using a gonad index (Sastry 1968, Griffiths 1981, Bricelj 1987, Garwood 1987) rather than examination of the gonad directly by histology or other means. This technique is based on the assumption that the mass of gonads, relative to other body organs changes during the course of the reproductive cycle. Changes in the gonad index of various molluscs has been related to food (Sastry 1968) and temperature (Lasiak 1987). In addition to changes in the gonad as development occurs, a decrease in the mass of somatic tissues was found by MacDonald and Thompson (1986) in the giant scallop, Placopecten magellanicus. Such changes in the wet mass of molluscan tissues may be a result of changes in water content (Castro & Mattio 1987). As the water content of tissues may mask changes in real tissue growth, examination of the gonad index of dried tissues has been

recommended (Grant & Tyler 1983a).

Grant & Tyler (1983a & b) give an excellent guide to the various techniques available for the analysis of data in studies of invertebrate reproduction. In addition to the use of indices, they recommend the measurement of oocyte diameters as a useful tool.

CHAPTER 2

MARK AND REMEASURE GROWTH TRIALS OF TRIDACNIDAE

2.1 INTRODUCTION

Most mark and recapture trials in fisheries science usually expect a small percentage of tagged fish returned; however, with sedentary benthic organisms such as giant clams almost all individuals tagged should be remeasured. Whereas most fisheries studies can examine thousands of individuals, giant clam populations of the larger species are numbered only in tens or hundreds on any one reef (Braley 1987). Therefore obtaining large samples of clams for comparative purposes in the wild has been something of a problem. The work of Pearson & Munro (in press) is the only growth study to work on a population of several hundred tagged giant clams.

Although fisheries science uses some moderately sophisticated statistics, it should be remembered that 'the degree of analytical sophistication should always be related to the kinds of questions being asked'(Seed 1980). In this chapter the basic questions being asked are simple; how fast do wild giant clams grow in the different habitats available in the Palm Island Group on the Australian GBR (Fig. 2.1), can the period of early exponential growth be indentified, and what are the best

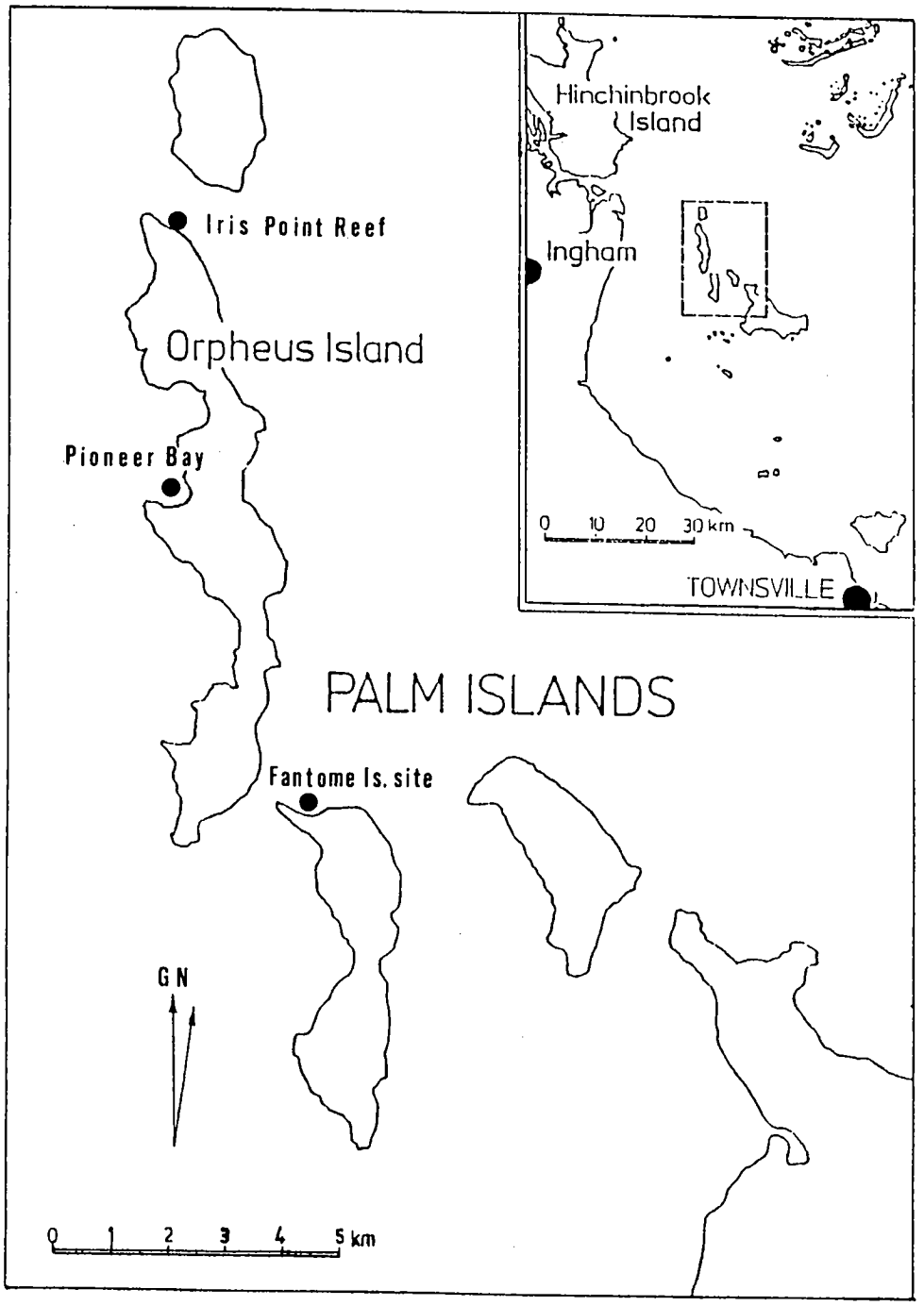
estimates of parameters describing the growth of the species studied?

2.2 SITE AND HABITAT DESCRIPTION

All tagging trials were carried out on tridacnid clams on the fringing reefs of Fantome Island or Orpheus Island, working from the Orpheus Island Research Station (Fig 2.1). These two islands are part of the Palm Island group (18° 37'S, 146° 30'E) situated within the Great Barrier Reef Marine Park. The islands are continental, high islands, about 13 km offshore. At this latitude the outer edge of the Great Barrier Reef (GBR) is around 130 km offshore, whilst mid-shelf reefs are found from 40 km offshore.

There is a pronounced wet and dry season in this region. At Lucinda on the mainland adjacent to the Palm Islands 73% of the rainfall occurs between December and March (Barnes 1984). The surface seawater temperature ranges from 31.2°C in January to 21.8°C in July in Cleveland Bay, some 60 km south of the Palm Islands (Archibald & Kenny 1980). Winds are predominantly from the south-east, with a north-easterly component during the summer months (Barnes 1984, Johnson & Risk 1987). During the wet season, freshwater run-off from the mainland can lower the salinity considerably. The edge of low salinity, freshwater plumes can extend seaward of the Palm Islands. Salinity on the GBR at the latitude of the Palm Islands can vary from under 32°/oo to over 35°/oo (Pickard 1977).

Figure 2.1 Location of the Palm Islands group (inset top right) and the study sites where tridacnid clams were tagged, Iris Point Reef, Pioneer Bay and Fantome Island. The Orpheus Island Research Station is located in Pioneer Bay.



(After Parnell (1987))

The tidal range and tides (semi-diurnal) at the Palm Islands are similar to that at Lucinda where the range is from MHWS 2.9 m to MLWS 0.46 m above chart datum (c.d.)(Parnell 1987).

Sites for tagging individual clams were determined following a survey of the main islands of the Palm Island group. The sites chosen were those reef areas which had the highest concentrations of giant clams and which were readily accessable by small boat from Orpheus Island Research Station.

Clams were tagged at three sites: Pioneer Bay and Iris Point on Orpheus Island, and the North-East Reef on Fantome Island (Fig.2.1). The species and number of Tridacnidae marked and repeatedly measured in their natural habitats are detailed in Table 2.1.

Table 2.1 Distribution of tagged and repeatedly measured giant clams

	Number of clams		
	Iris Point	Pioneer Bay	Fantome
Hippopus hippopus	50	56	1
Tridacna gigas	--	21	28
T.squamosa	--	3	38
T.maxima	--	--	21
T.derasa	--	--	2

The three sites are all distinctly different from one another.

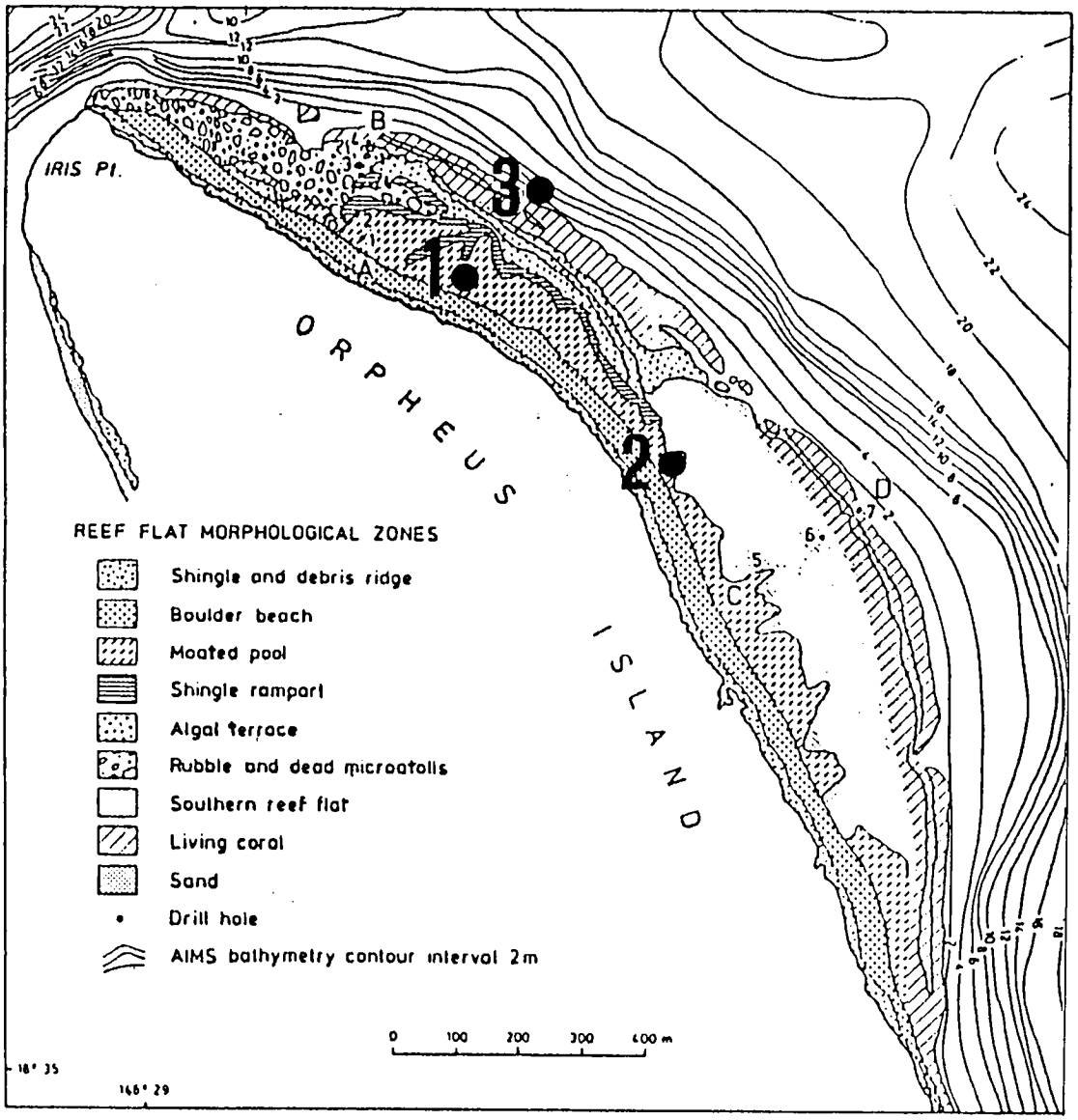
2.2.1 Iris Point

The fringing reef at Iris Point (Fig. 2.2) is the most exposed of the three sites, being open to wind and seas from the north-east to the south-east (Fig.2.2). The

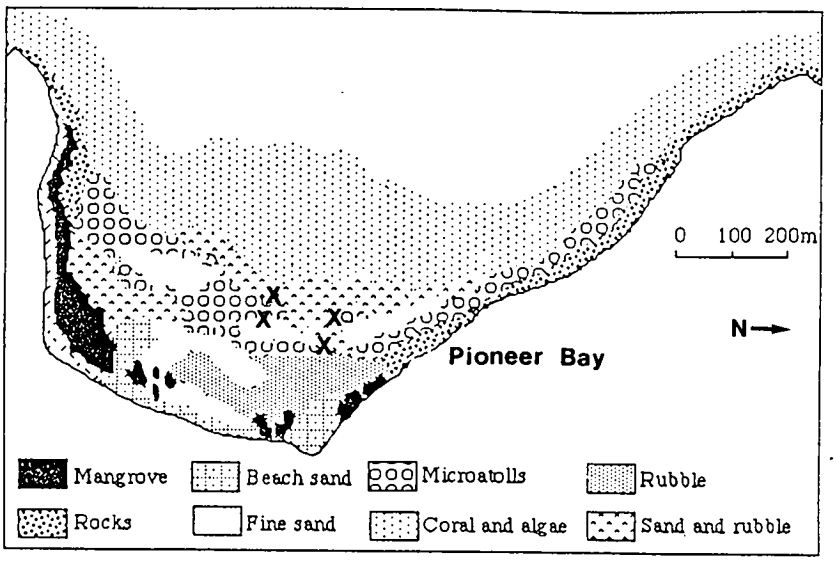
reef's structure and morphogenesis have been described by Barnes (1984) and Hopley & Barnes (1985). As a result of its exposed aspect, the reef crest and flat have a low coral species diversity. The reef has several prominent 'steps' of algal terraces coming up from just below extreme low water springs to a shingle rampart, inshore of which is a shallow inner moat (maximum depth 0.5 m, mean depth 0.3 m relative to algal ridge height) in which H.hippopus is found. At low water the depth of seawater in the moat is maintained at a level of 1.2-1.3 m above datum. This results in all but a few H.hippopus on raised substrates in the inner moat being almost constantly immersed, except during ELWS tides when drainage from the moat results in partial emersion of many clams. Two areas (1 & 2 in Fig. 2.2) on this reef had aggregations of H.hippopus. There was a distinct difference in size of individuals between the two locations (possibly a result of localised settlement within the lagoon). The site further east (site 2) consisted of mainly smaller clams (range 88-292 mm). The substrate on which H.hippopus is found in the inner moat consists of biogenic fragments and terrigenous gravels (Barnes 1984). A sub-littoral site to which some H.hippopus were translocated was situated at the base of the reef slope at 13 m depth, on sand (site 3, Fig. 2.2).

Figure 2.2 Iris Point Reef study sites. Numbers 1 & 2 mark the postions of the Hippopus hippopus sites located in the inner moat of the reef. Number 3 indicates the position of the sub-littoral site to which some H. hippopus were translocated.

Figure 2.3 Pioneer Bay study site. The four x's mark the extent of the Hippopus hippopus site. Tridacna gigas were found in the zones indicated as coral and algae, sand and rubble, and micro-atolls. T. maxima was only found in the zone coral and algae.



(After Barnes(1984))



After Parnell (1987)

2.2.2 Pioneer Bay

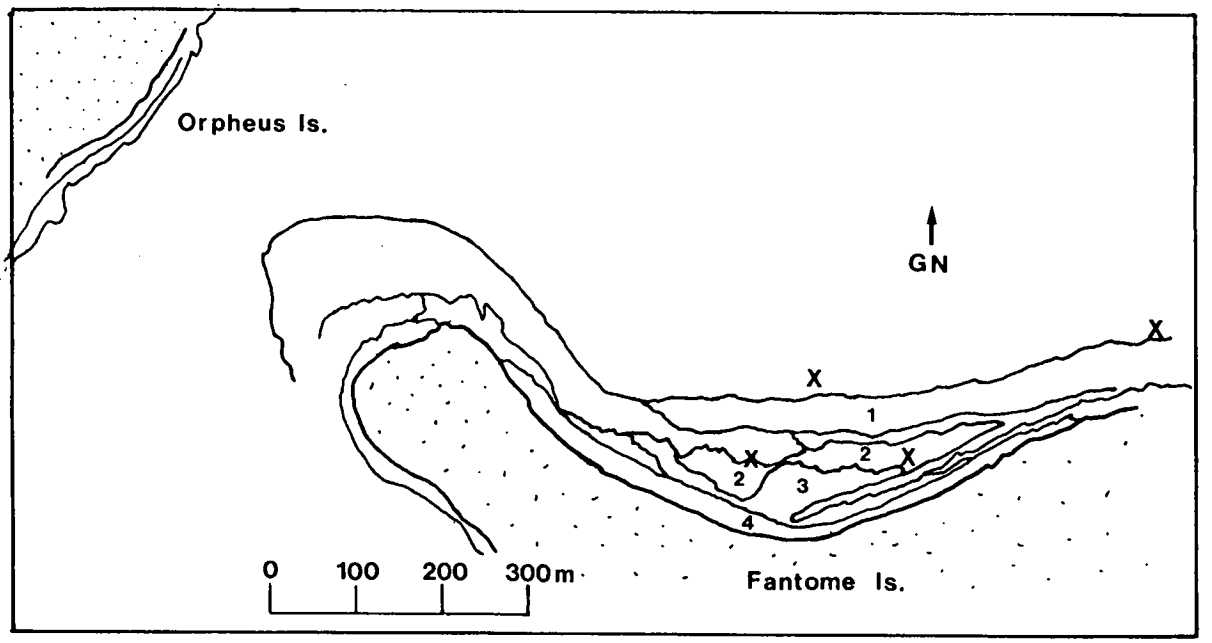
Pioneer Bay (Fig. 2.3) is the least exposed of the three sites, being sheltered from the predominantly easterly winds and is only exposed to infrequent westerly winds. Because of the short reach over the sea, winds from the west usually result in little wave action. The hydrodynamics of Pioneer Bay have been described by Parnell (1987). The reef flat (Fig. 2.3) is some 400 m in width and can be divided into 3 main zones: sand and coral rubble, transition zone and living reef (Slocombe 1981). Coral species diversity is moderate, with species tolerant of reasonably high sediment loads prospering. In Pioneer Bay H.hippopus is found in an area immediately inshore of the reef crest area, which at low water springs can be seen to be a slight depression in the reef flat, and is in a zone dominated by the coral Montipora ramosa. H.hippopus is found on sand or coral rubble. T.gigas is found from the reef flat down to the shallow sub-littoral, whilst T.squamosa is found only in the sub-littoral.

2.2.3 Fantome Island

The reef on the northern end of Fantome Island is moderately exposed compared to the other two sites, being sheltered from the prevailing south-easterlies, but open to the north-east (Fig. 2.4). There is little reef flat exposed even at low water springs, and related to this there is much branching staghorn coral (Acropora sp.) on the reef flat. Coral species diversity is the highest of the three sites, both on the reef flat and slope.

Figure 2.4 Fantome Island study site. The four x's mark the extent of the site within which clams were tagged.

Key: 1=sub-littoral coral zone, predominantly large branching Acropora sp. 2= reef flat coral zone 3= bedrock 4= beach


Orpheus Is.
GN
X
X
1
2
X
2
X
3
4
0
100
200
300m
Fantome Is.

T. gigas was found predominantly amongst branching coral or on top of rubble composed mainly of dead branching coral, or on a sandy bottom.

T. maxima was commonly embedded into dead coral on the reef flat of Fantome Island.

A few T. squamosa were found on the reef flat at Fantome; however, the reef flat at Fantome, excepting the inner most edge, is in the sub-littoral zone, which corresponds to the species distribution noted at Pioneer Bay. Most T. squamosa were situated on the reef slope. This species was commonly attached to coral rubble, amongst hard or soft corals, colonies of which were often found attached to the growing shell. It was also found on sandy bottoms.

Two T. derasa found sub-littoraly at Fantome Island, were the only specimens found at the three sites. This species of clam appears only to prosper in offshore reef areas. Such distribution could be related to a larval or adult intolerance to high sediment loads experienced inshore, or to an increased light requirement compared to the other species, requiring the clearer offshore water, or perhaps an intolerance to low salinities which can be experienced in inshore reefs from terrestrial run-off.

2.3 Methods

Clams were tagged with numbered aluminium tags. A small area of each shell was cleaned of algal growth using a wire brush, then a tag was pushed firmly to the surface

using aquatic adhesives. The two adhesives that worked best were Aquatepoxy and Epijoint, the latter being easier to manipulate underwater. The spatial positions of tagged clams were detailed on simple maps, which, whilst not drawn to scale or of great accuracy, were sufficient to relocate clams on a regular basis for remeasurement. Large floating buoys, stakes hammered into the reef and landmarks were used to relocate positions within sites from the surface. Underwater buoys floating 0.5 m above tagged clams and dominant underwater features assisted in sub-littoral relocation.

Clams were measured using calipers. Three sets of calipers were used, one pair was a set of commercial 30 cm stainless steel vernier calipers, another a large (length 1.5 m) pair made of solid aluminium, and a lightweight plastic pair (originally designed as a teaching aid) of 1 m in length. Shell length was measured to the nearest 1 mm. All calipers were fitted with extended 'jaws' to lessen the chance of damaging shell ends and to provide a larger surface area to hold against the shell end, so as to improve the accuracy of measurements. The large solid aluminium pair of calipers proved to be difficult to handle because of their rather large mass and their potential to cause shell damage, particularly when working in rough weather conditions, so the plastic calipers were used for all but the largest of shells of T.gigas.

Each clam was measured 3 times on every visit to the site and the mean of the three readings taken as the final

measurement. Clams were remeasured every 3 months where possible, from November 1984 to November 1986. Some additional clams were tagged to increase the sample sizes during the course of the trials, therefore there were not 2 years of data for all individuals. Clams on shallow reef flats were measured at low spring tides where possible, whilst scuba gear was used for sub-littoral work.

At the Iris Point site, 31 H.hippopus were transplanted from the littoral to the sub-littoral reef edge at a depth of 3.5 m on a sandy bottom (Fig. 2.2). This was to examine the effect that constant immersion had on the growth of the species by comparison with the tagged individuals on the nearby reef flats, which were regularly emersed. Ofcourse such trials results should be confirmed by laboratory trials under controlled conditions, which are being addressed by other members of the Giant Clam Project group at James Cook University.

The substrate and depth of each clam were recorded, together with any other observations considered of interest. In determining the size-frequency distribution of H.hippopus at both Iris Point and Pioneer Bay, clams in addition to those tagged were included in the measurements to get as large a sample as possible.

2.4 ANALYTICAL TECHNIQUES

The most commonly used growth function in fisheries science, that derived by von Bertalanffy (1938), has been rewritten in a number of ways for use in the analysis of

mark-remeasure data. Three such methods which can utilize variable time intervals between measurements are those of Gulland and Holt (1959), Fabens (1965) and Munro (1982). These methods have recently been adapted for microcomputers and are amalgamated in the programme MARCAP 3 packaged by John Munro of ICLARM.

The basic analytical approach used to determine the parameters of the von Bertalanffy growth function for clams closely follows that of Munro, Pearson & Gwyther (MS) and Pearson & Munro (in press), as follows.

(a) Estimate the asymptotic length (L_{oo}) and the Z/K ratio of the population from the size-frequency distributions of the clams using the Wetherall method (1986) as included in the ELEFAN 2B program of Brey and Pauly (1986). (see Note on Page 62.)

(b) The triplets of data, each consisting of the length when measured at time m, (L_m), the length when remeasured at time r, (L_r), and the time interval r-m (in days) are compiled, ignoring the fact that some clams may have been measured more times than others. For most clams more than one set of triplets was available, and the successive growth sectors were used as opposed to the cumulative growth from the initial size when first tagged.

(c) To estimate the length at the point of inflexion (between increasing and decreasing growth rate with increasing length) of the growth curve a plot of the average size increment (mm/yr) against the mean class size

is made. For each individual triplet within a class size, the average size between mark and remeasure ($(L_r + L_m)/2$) is taken as the size of that clam. Pearson & Munro (in press) note that "the plot is of the mean increment in successive size classes. Thus it is not a Gulland and Holt (1959) plot, which would be based on individual increments."

(d) It is assumed that the pre-inflexion phase of growth conforms to the Brody equation (Brody 1945)

$$L_t = Ae^{K't}$$

For animals of length L_m at time of marking, m, and length L_r at time of remeasurement, r,

$$A = L_m/e^{K'm} = L_r/e^{K'r}$$

so therefore

$$L_r/L_m = e^{K'r}/e^{K'm}$$

so that

$$\log_e (L_r/L_m) = K' (r - m)$$

so by plotting the value of $\log_e (L_r/L_m)$ against r-m a straight line of slope K' should be produced. The value of A in the Brody equation can only be calculated by reference to the length of clams of known age.

(e) Remove from the data set all triplets referring to small clams in their pre-inflexion phase of growth.

(f) This final data set is then processed using the Fabens (1965) method to obtain estimates of L_{oo} and K.

The specific growth rate (SGR) (Gruffydd et al. 1984) of the clams over different seasons was calculated to examine

seasonal differences in growth. The equation of the SGR used was:

$$SGR = \frac{(\log_e l_2 - \log_e l_1).}{t_2 - t_1}$$

2.5 RESULTS

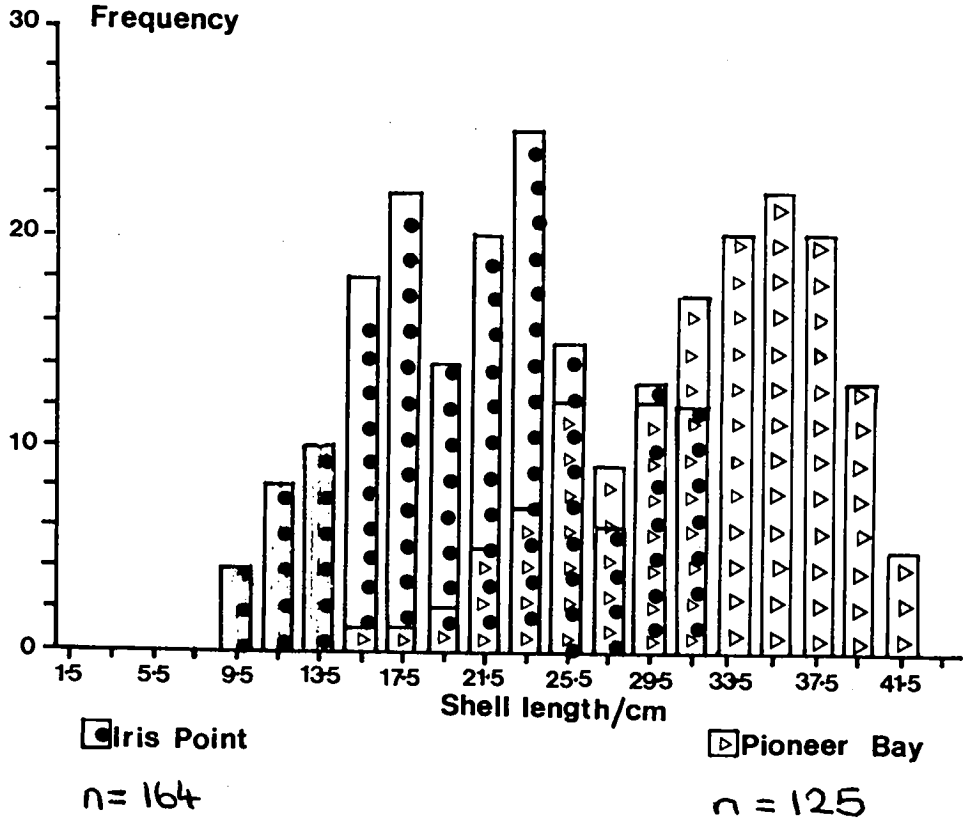
2.5.1 Hippopus hippopus

The size-frequency distributions of H.hippopus at Iris Point Reef and Pioneer Bay (Fig. 2.5) did not show a clear uni-modal pattern, typical of giant clam stocks elsewhere (Pearson & Munro in press). This is probably a reflection of the relatively small populations at both sites. The modal size-frequency at Iris Point Reef was 12 cm less than at Pioneer Bay. The probable cause of this difference is that the clams at Iris Point Reef are harvested occasionally by Palm Islanders, whereas those in Pioneer Bay directly in front of the research station are not. The Palm Islanders presumably select the larger clams. The lack of small individuals in Pioneer Bay presumably reflects poor recruitment in recent years.

The depth range for H.hippopus at Iris Point was 1-1.85 m (c.d.) and at Pioneer Bay they were found at approximately 0.5 m (c.d.).

Wetherall (1986) estimates of L_{oo} and Z/K are usually calculated using just those values of the descending, right arm of the size-frequency distribution plot. However, one can and should utilise all fully selected animals for a Wetherall plot (pers.com. J.Munro), and that

Figure 2.5 Size-frequency histogram for Hippopus hippopus at Iris Point Reef and Pioneer Bay.


30
Frequency
20
10
0
1·5
5·5
9·5
13·5
17·5
21·5
25·5
29·5
33·5
37·5
41·5
Shell length/cm
Iris Point
n= 164
Pioneer Bay
n = 125

is the procedure carried out in this analysis (Table 2.2)(Figs. 2.6 & 2.7).

Table 2.2 Values of Wetherall plot parameters for Hippopus hippopus

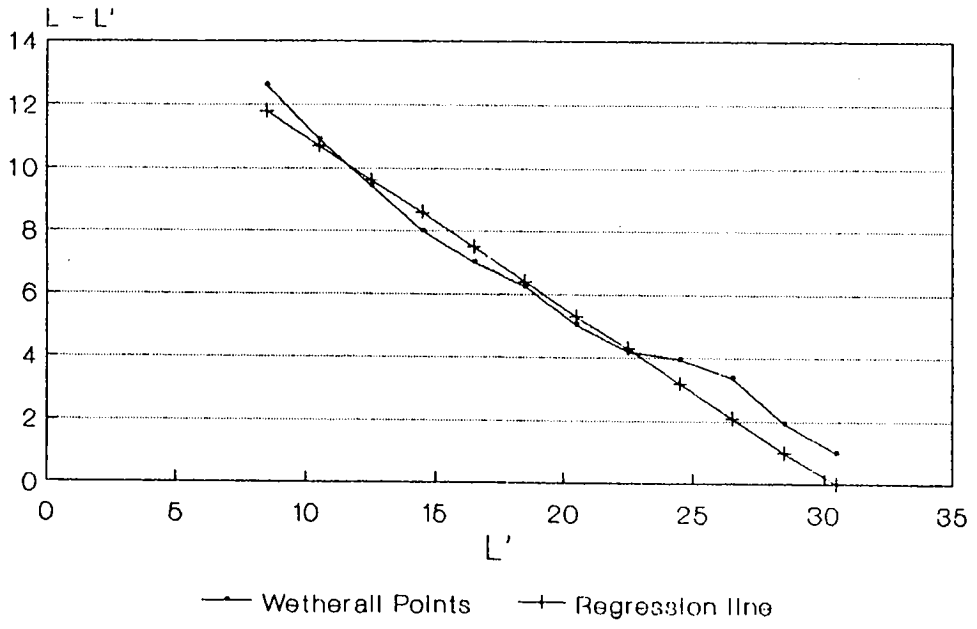
Site	Shell length (mm) L_{oo}	Z/K
Iris Point	305.4	0.86
Pioneer Bay	379.3	0.38

Plots of the mean annual length increment of successive length classes against mean length (Fig. 2.8) showed that at neither of the two intertidal sites were individuals still in their pre-inflexion growth phase. The decreasing growth rate of the post-inflexion growth curve of H.hippopus recorded in the mark-remeasure trials can therefore be described using the von Bertalanffy equation (1938). These graphs indicate that H.hippopus at Pioneer Bay had a higher growth rate for most given lengths of clam compared to clams on the reef flat at Iris Point Reef.

Clams transplanted to the sub-littoral off the edge of Iris Point Reef were found to have improved growth rates compared to clams from the reefs intertidal zone (Fig. 2.8). A few small clams were included in the sub-littoral sample and this resulted in finding a point of inflexion in the plot of mean increment length against mean length of length class (Fig. 2.8), which marks the change from an increasing to a decreasing growth rate with increasing length. The point of inflexion was in the length class 140-165 mm, the mean increment being 77.1 mm per year. The

Figure 2.6 Wetherall plot for Hippopus hippopus from the Iris Point Reef site. The mean lengths, L, of all clams above successive minimum lengths, L', are calculated and L-L' plotted against L'. Values in cm.

Figure 2.7 Wetherall plot of L-L' against L' for Hippopus hippopus from the Pioneer Bay site. Values in cm.


L - L'
14
12
10
8
6
4
2
0
0
5
10
15
20
25
30
35
L'
Wetherall Points
Regression line

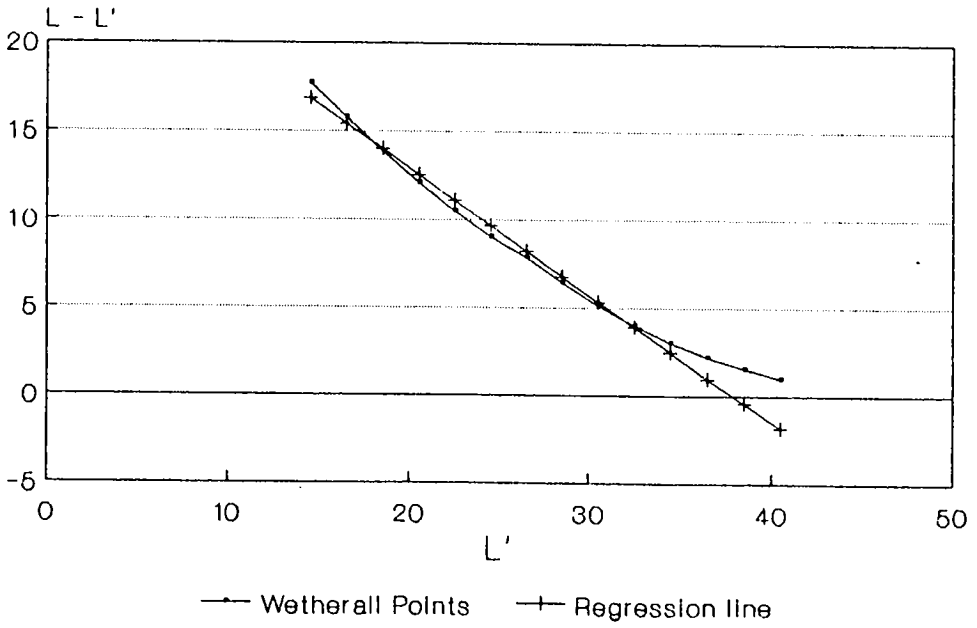
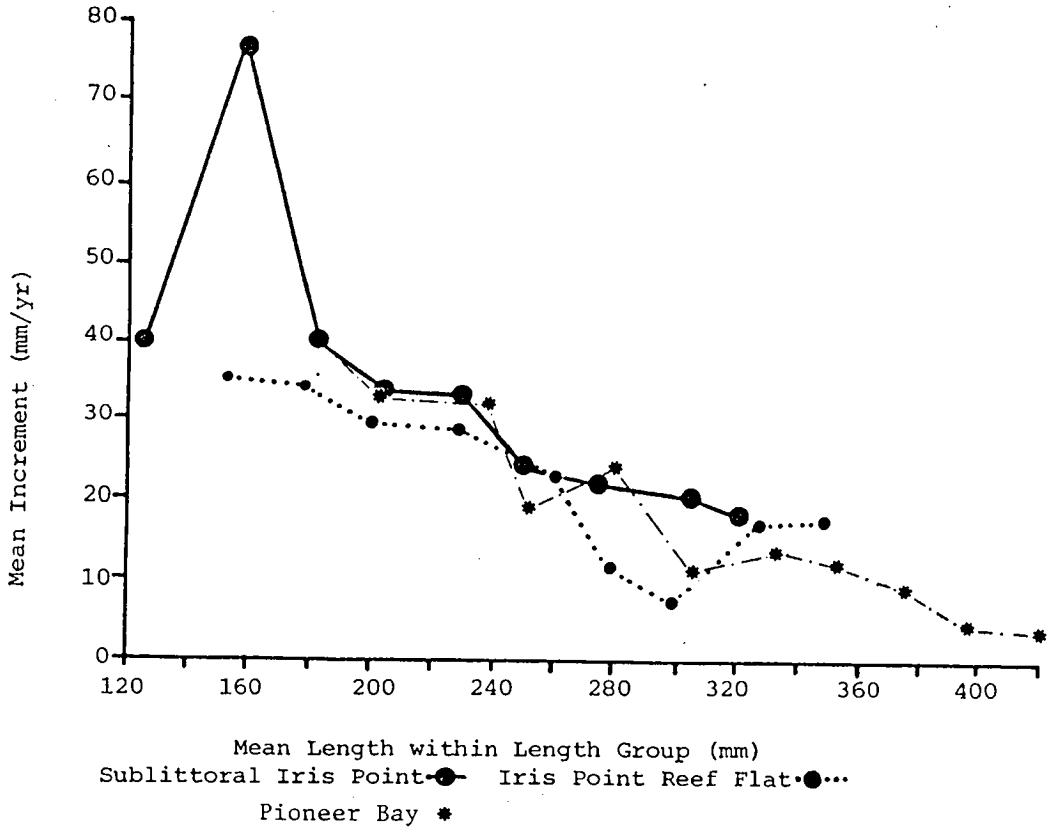

L - L'
20
15
10
5
0
-5
0
10
20
30
40
50
L'
Wetherall Points
Regression line

Figure 2.8 Plot of the mean increment in shell length of Hippopus hippopus for successive length groups against the mean length within each length group, for clams from the Iris Point reef flat site, the Iris Point sub-littoral site and the Pioneer Bay site.


80
70
60
50
40
30
20
10
0
Mean Increment (mm/yr)
120
160
200
240
280
320
360
400
Mean Length within Length Group (mm)
Sublittoral Iris Point
Iris Point Reef Flat
Pioneer Bay

slope of the graph K', of $\log_e(Lr/Lm)$ against r-m was 1.0834×10^{-3} (Fig. 2.9).

To estimate the parameters of the von Bertalanffy equation, data triplets below the point of inflexion for the sub-littoral clams were removed from the data set. The Fabens method was applied to the modified data set to produce initial estimates of L_{oo} and K (Table 2.3).

Table 2.3 Fabens estimates of parameters of the von Bertalanffy equation for Hippopus hippopus from mark-remeasure trials

Site	Data Set	Shell length (mm) L_{oo}	K	n
Iris Point Reef	All triplets	347.40	0.2047	248
Iris Point Reef, Sub-littoral	triplets less than inflexion removed	369.12	0.215	108
Pioneer Bay	All triplets	414.69	0.1549	388

Although the values of L_{oo} for Iris Point were less than those for Pioneer Bay, the values of K were higher (Table 2.3). The sub-littoral clams had a value of L_{oo} intermediate between those of the two main sites, and had the highest value of K.

Seasonal growth of H.hippopus examined by plotting SGR against time shows that growth is minimal at both Iris Point Reef and Pioneer Bay (Fig. 2.10) in the last quarter of each year, the quarter prior to the annual spawning season (see Chapter 5). The mean SGR rose to a maximum value during mid-winter of 1986 at Pioneer Bay, whereas in mid-winter 1985 it had been low, the best season for

Figure 2.9 Plot of ln (L_r / L_m) against r-m, the time in days between successive measurements of juvenile Hippopus hippopus from the sub-littoral site at Iris Point reef. The linear regression line on the plot has the slope equivalent to K', where the Brody growth equation is:

$$L_t = Ae^{K't}$$

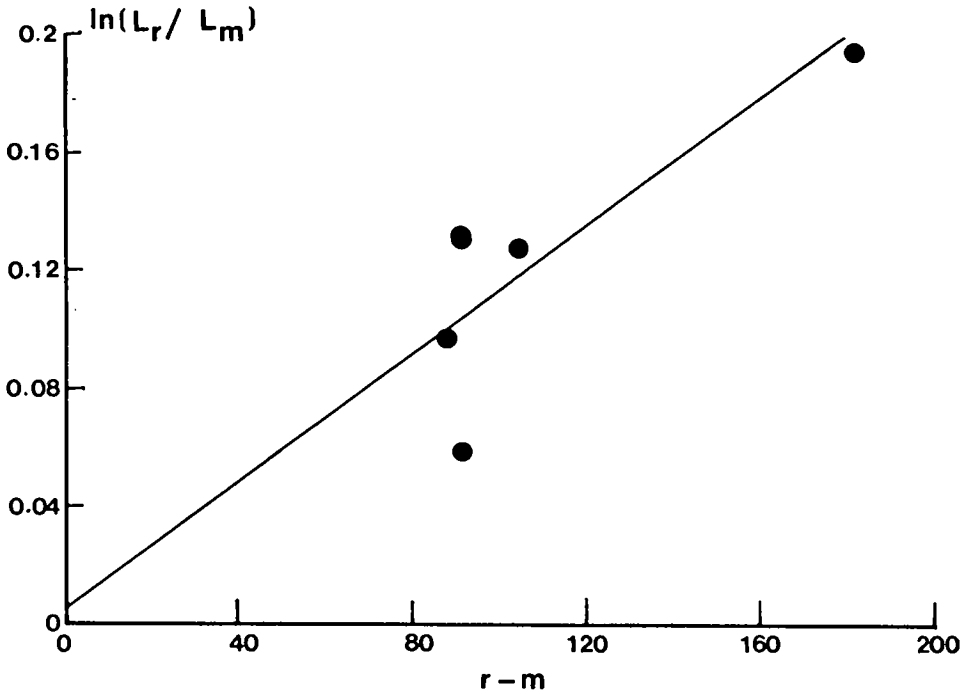
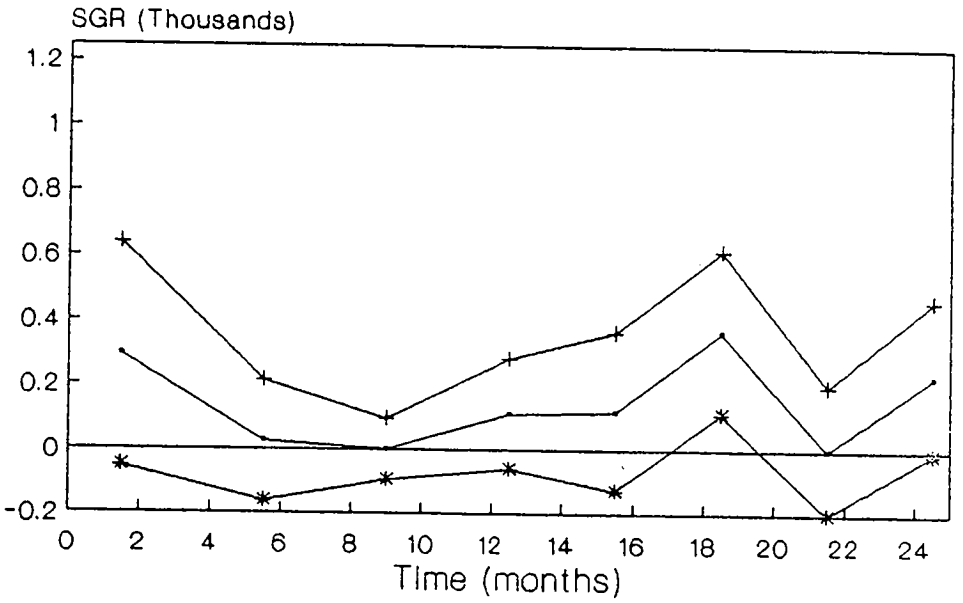
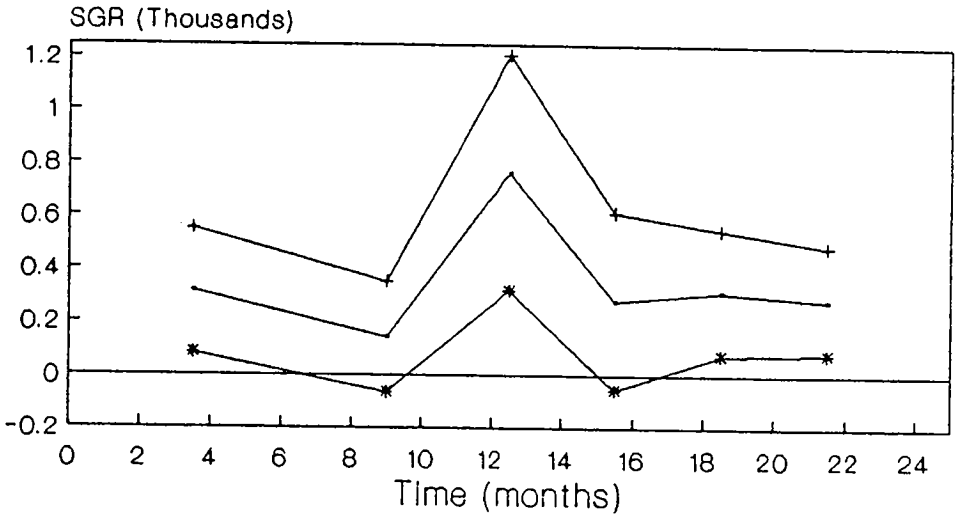

ln(L_r / L_m)
0.2
0.16
0.12
0.08
0.04
0
0
40
80
120
160
200
r – m

Figure 2.10 Seasonal growth of Hippopus hippopus at the Pioneer Bay and Iris Point Reef sites. SGR is plotted against time in months. The central line links mean values, whilst the upper and lower lines link points of + & - 1 standard deviation respectively.

Pioneer Bay



Iris Point Reef



growth appearing to differ between years. At Iris Point Reef SGR peaked during the summer 1985-6, following the spawning season. The maximum value of the SGR at Iris Point Reef was approximately twice that of the the peak value at Pioneer Bay. The differences between seasonal growth between the two sites and from 1985 to 1986 may well be a result of the wide range of the sizes of clams remeasured, which have a wide range of growth rates. A summary of these results has been presented in Shelley (1988)(Appendix 2.1). Seasonal growth would be best examined by following a single cohort over time.

2.5.2 Tridacna gigas

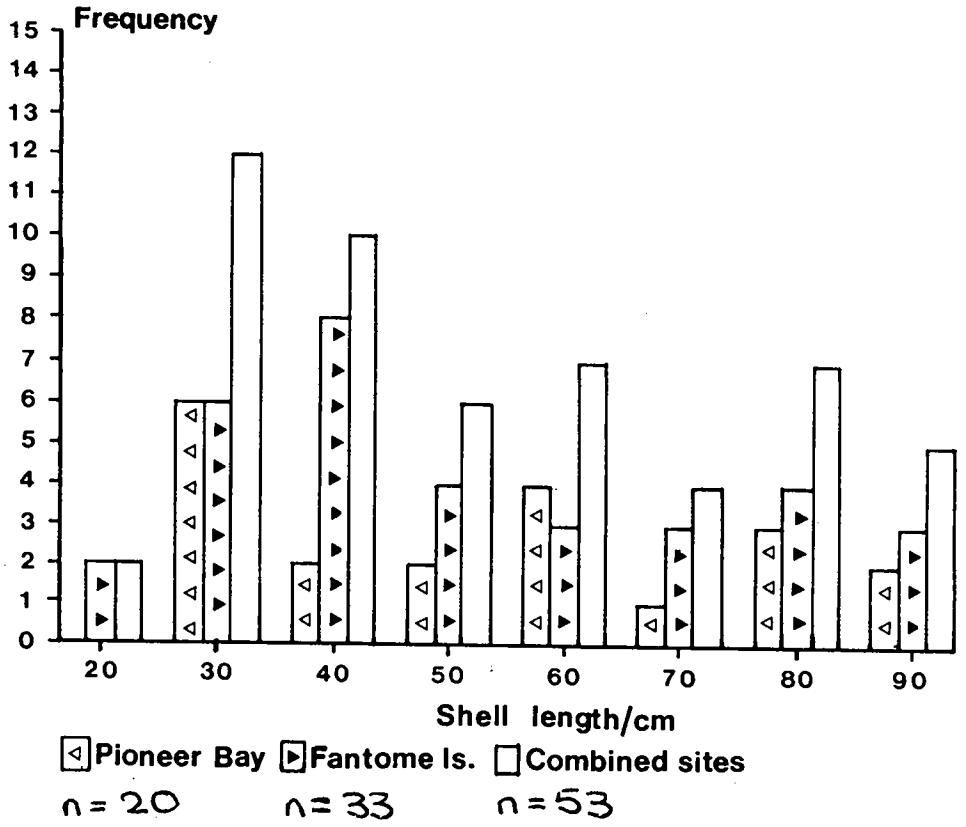
The depth range of T.gigas in Pioneer Bay was +0.2 to -1.5 m (c.d.) and at Fantome Island +0.3 to -2.6 m (c.d).

As there were only 21 T.gigas tagged and remeasured at Pioneer Bay and 28 at the Fantome Island site, it was not considered valid to estimate L_{oo} using the Wetherall method from their size-frequency distributions at the two sites, therefore data were combined. The size-frequency distributions of T.gigas at both sites and the for the combined sites are shown in Figure 2.11. The Wetherall method growth parameters obtained from using all points were L_{oo}=1031.57 mm and Z/K=1.54 (Fig 2.12).

A plot of the mean annual length increment of successive length classes against mean length for T.gigas at Pioneer Bay and Fantome Island (Figs. 2.13) showed that no individual clams in their pre-inflexion growth phase had

Figure 2.11 Size-frequency histogram of Tridacna gigas for clams from Pioneer Bay, Fantome Island and a third column representing the combined frequencies of both sites.

Figure 2.12 Wetherall plot of L-L' against L' for Tridacna gigas for the combined data set from Pioneer Bay and Fantome Island.


Frequency
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
20
30
40
50
60
70
80
90
Shell length/cm
Pioneer Bay
Fantome Is.
Combined sites
n = 20
n = 33
n = 53

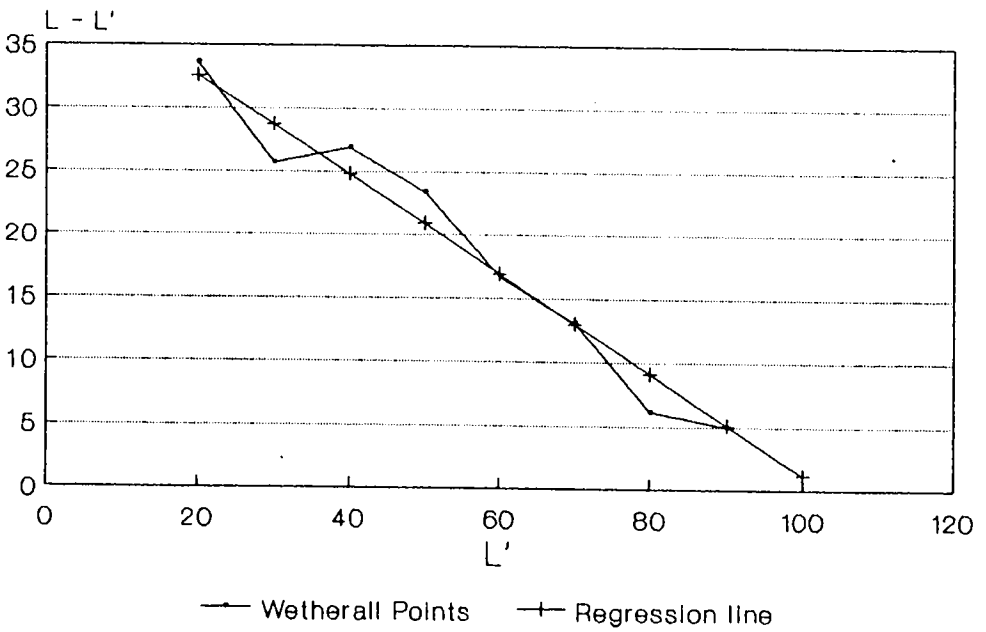
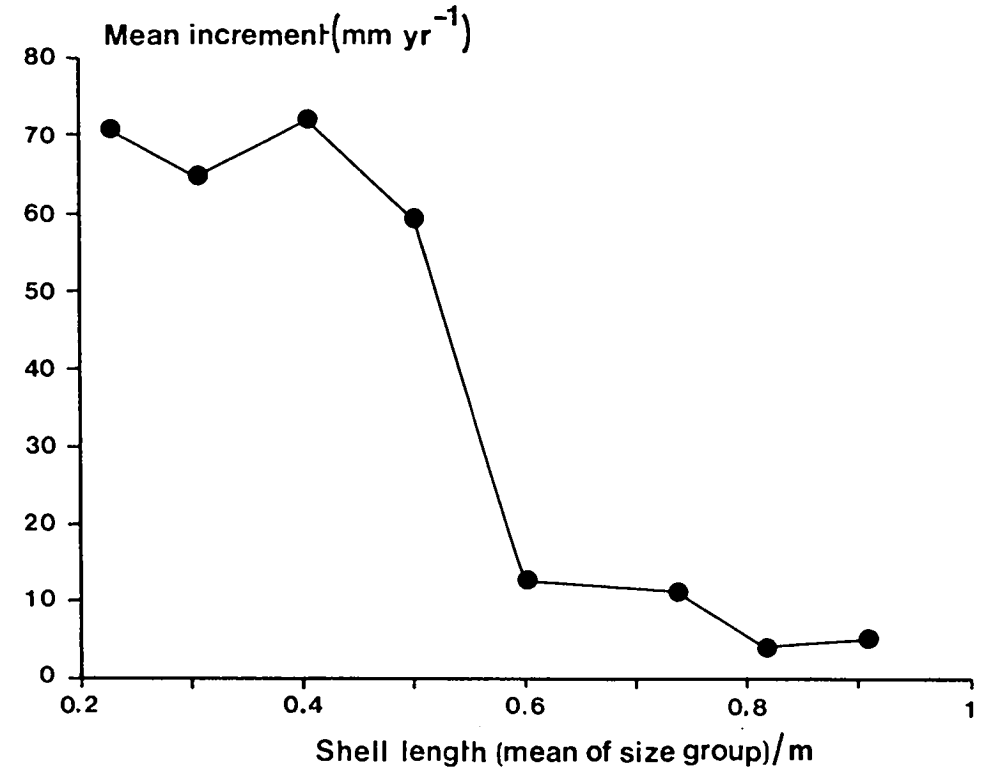
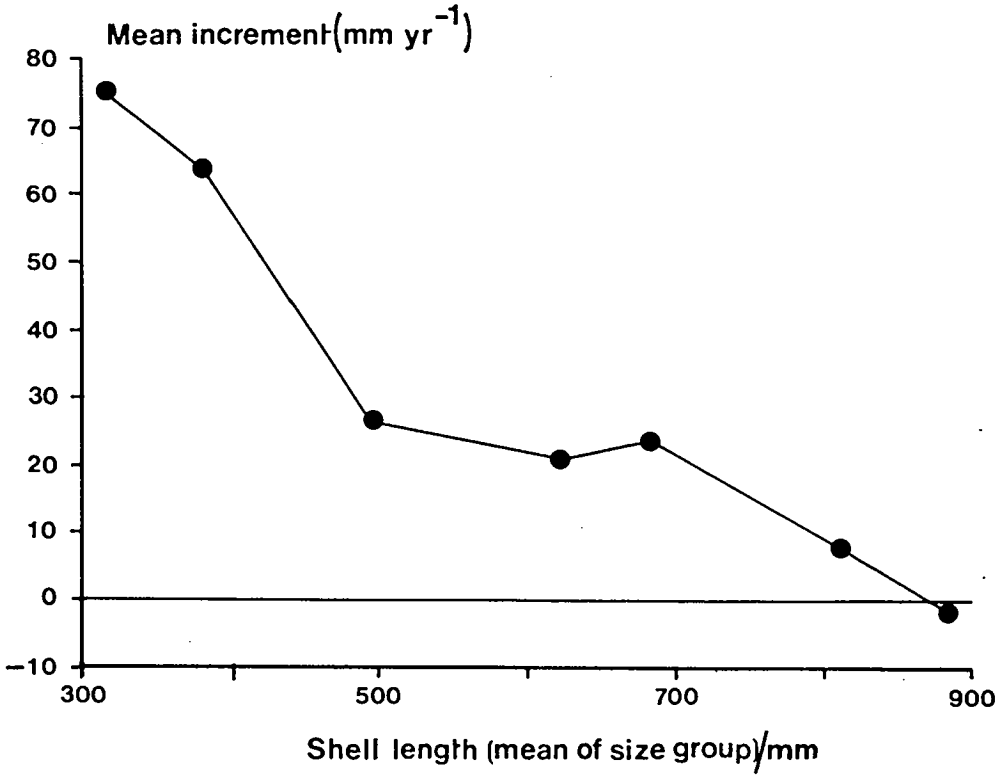

L - L'
35
30
25
20
15
10
5
0
0
20
40
60
80
100
120
L'
Wetherall Points
Regression line

Figure 2.13 Plot of the mean increment in shell length of Tridacna gigas for successive length groups against the mean length within each length group for clams from Fantome Island (upper figure) and Pioneer Bay (lower figure)


Mean increment (mm yr^{-1})
80
70
60
50
40
30
20
10
0
0.2
0.4
0.6
0.8
1
Shell length (mean of size group)/m


Mean increment (mm yr^{-1})
80
70
60
50
40
30
20
10
0
−10
300
500
700
900
Shell length (mean of size group)/mm

been included at Pioneer Bay. At Fantome a broad plateau from 200-400 mm, followed by a sharp decrease in the annual mean increment, suggests that the point of inflexion probably lies within this range.

As it was not strictly necessary to exclude any triplets from the pre-inflexion growth phase as it was not determined accurately, preliminary Fabens estimates were carried out on the complete data sets at both sites. The results are shown in Table 2.4.

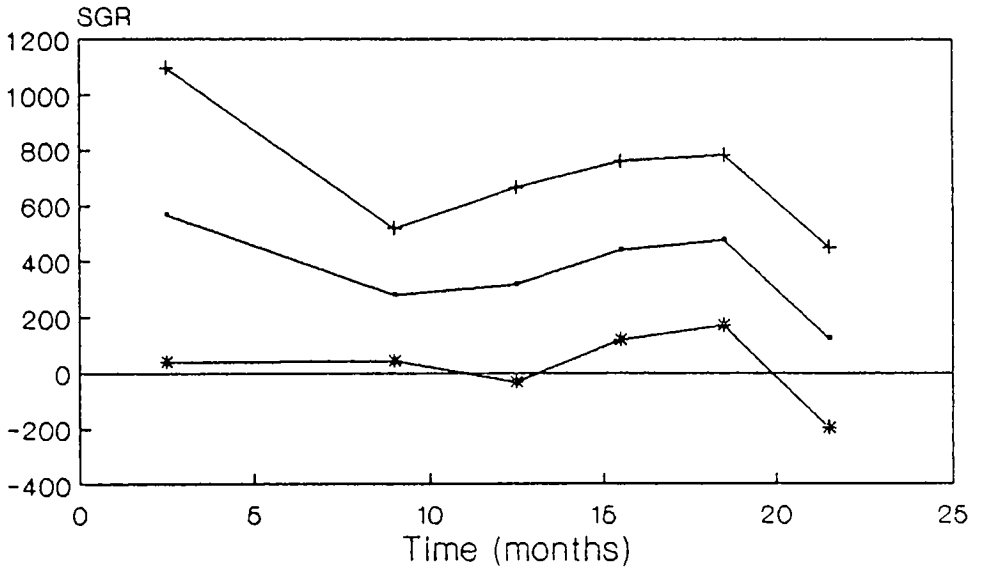
Table 2.4 Fabens method estimates of growth parameters for Tridacna gigas

Site	Shell length (mm) L_{oo}	K	n
Pioneer Bay	813.90	.148	73
Fantome Island	362.62	.132	97

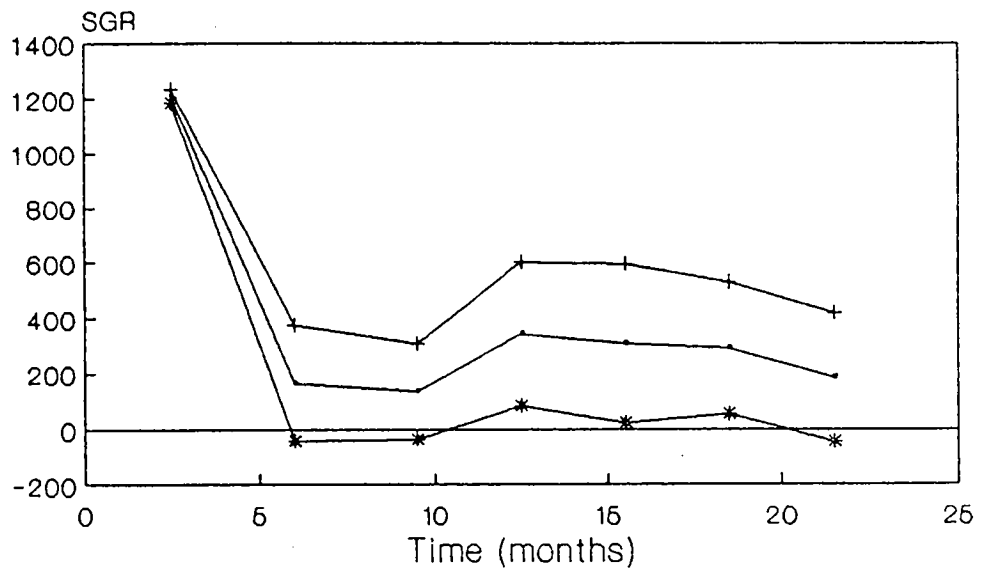
Seasonal growth of T.gigas (SGR) was minimal in the quarter August-November for the two years of data at Pioneer Bay and Fantome Island (Fig.2.14). As a result of bad weather clams at Fantome Island were only measured once in the first half of 1985, therefore it is only possible to comment on the maximum quarter for growth in 1986, which was February to May. At Pioneer Bay peak growth periods were observed from November 1984 to April 1985 and November to February 1986. Other than apparent minimal growth prior to the mid-summer spawning of T.gigas, as H.hippopus, no common seasonal growth pattern is evident between sites. As for H.hippopus, the wide size range of clams used, with their similarly wide range of growth rates. has probably obscured seasonal growth which

Figure 2.14 Seasonal growth of Tridacna gigas at Fantome Island (upper graph) and Pioneer Bay (lower graph). The SGR is plotted against time in months. The central line links points of the mean value, whilst upper and lower lines represent + & - 1 standard deviation respectively.

Fantome Island



Pioneer Bay



would be best examined by following a cohort over time.

2.5.3 Tridacna squamosa

The size-frequency distribution of shell length of T. squamosa from Fantome Island shows a typical pattern skewed to the right, with a modal length of approximately 32.0 cm (Fig. 2.15).

The depth range of T. squamosa at Fantome Island was +0.3 to -7.5 m (c.d.). A plot of frequency against depth for T. squamosa (Fig 2.16) shows that most clams were found between -3 to -5 m (c.d.).

Initial estimates of growth parameters using the Wetherall technique, (all points) were L_{oo} = 362.6 mm and Z/K = 0.25 (Fig. 2.17).

A plot of the mean annual length increment of successive length classes against mean length for T. squamosa (Fig. 2.18) showed that none of the tagged and remeasured clams were in their pre-inflexion growth phase.

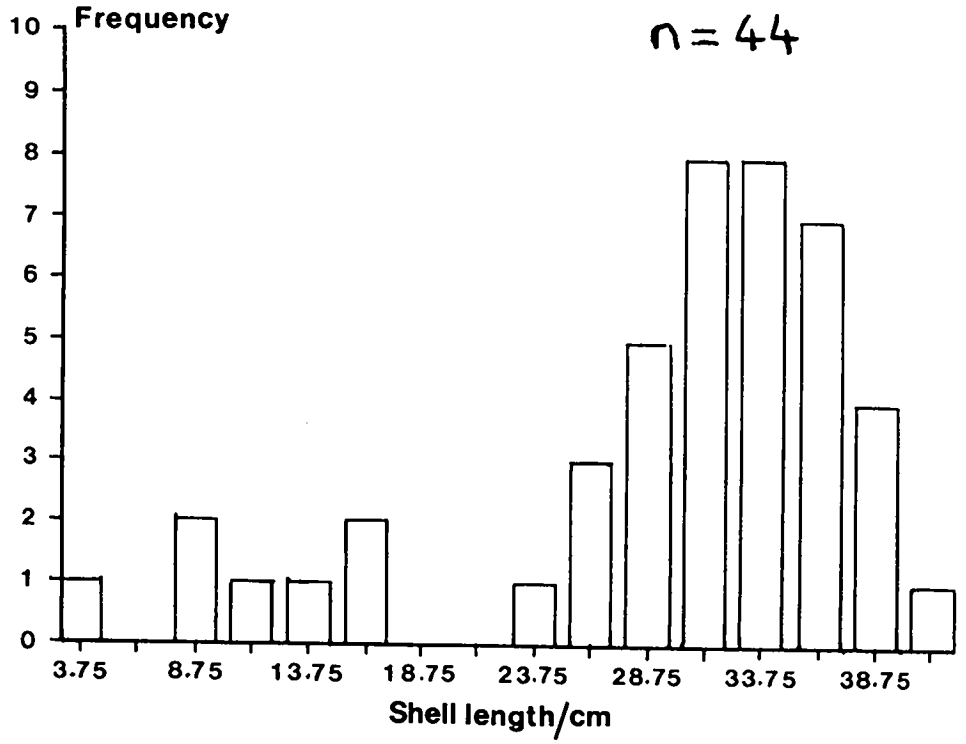
Growth parameters from the complete data set using the Fabens method are shown in Table 2.5. Seasonal growth of T. squamosa was only examined over an 18 month period. This proved to be too short a period to discern any pattern of seasonal growth.

Table 2.5 Fabens method estimate of growth parameters for Tridacna squamosa

Site	Shell length (mm) L_{oo}	K	n
Fantome	384.313	.189	115

Figure 2.15 Size-frequency distribution of Tridacna squamosa at Fantome Island.

Figure 2.16 Plot of frequency of Tridacna squamosa against depth at which they were found at Fantome Island.


Frequency
n = 44
10
9
8
7
6
5
4
3
2
1
0
3.75
8.75
13.75
18.75
23.75
28.75
33.75
38.75
Shell length/cm

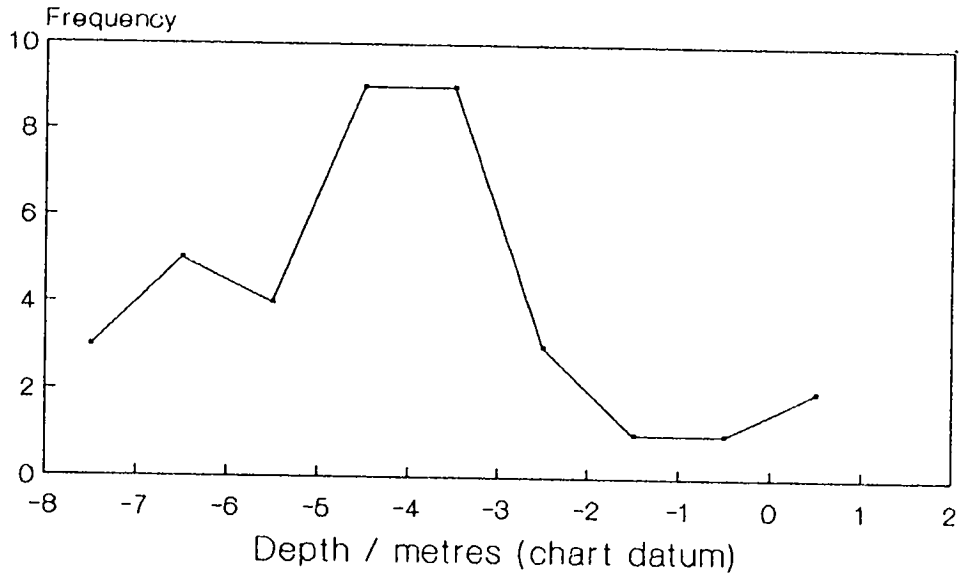
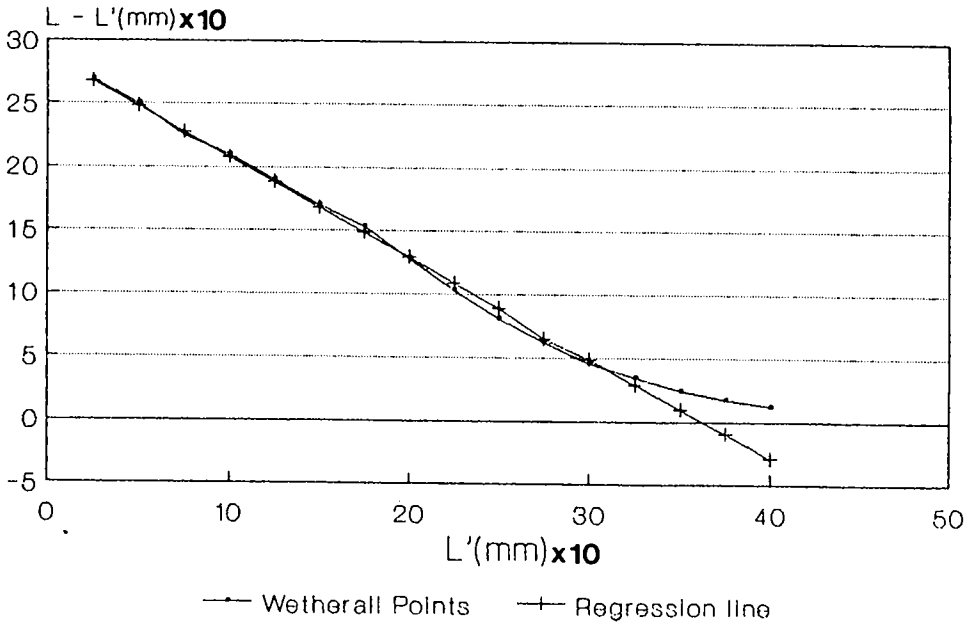
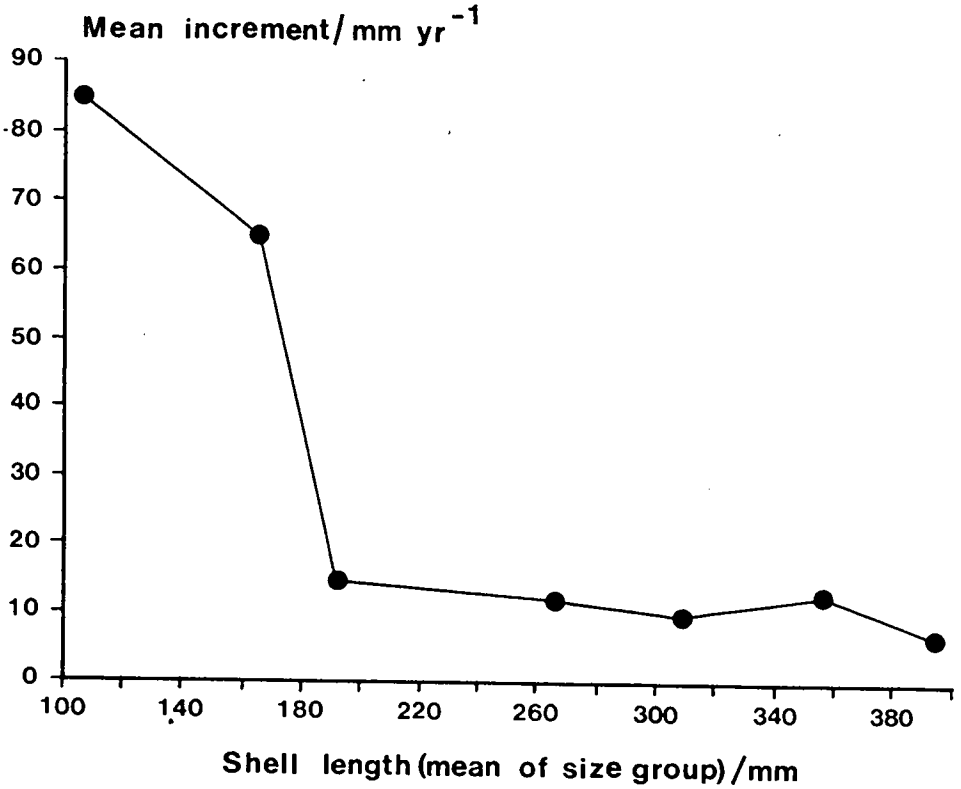

Frequency
10
8
6
4
2
0
-8
-7
-6
-5
-4
-3
-2
-1
0
1
2
Depth / metres (chart datum)

Figure 2.17 Wetherall plot for Tridacna squamosa from Fantome Island, of L-L' against L'. Values in cm.

Figure 2.18 Plot of the mean increment in shell length of Tridacna squamosa for successive length groups against the mean length within each length group for clams from Fantome Island.


L - L'(mm)x10
30
25
20
15
10
5
0
-5
0
10
20
30
40
50
L'(mm)x10
Wetherall Points
Regression line


Mean increment/mm yr-1
90
80
70
60
50
40
30
20
10
0
100
140
180
220
260
300
340
380
Shell length (mean of size group)/mm

2.5.4 Tridacna maxima

The size distribution of T. maxima at Fantome Island (Fig. 2.19) does not reflect the number of small individuals. There were many within the site, however many of them could not be accurately measured because they were sunken in the coral rock into which they bore.

The initial estimates using the Wetherall technique on all points gave the values of L_{oo} = 250.1 and Z/K = 0.27 (Fig. 20).

The depth range of T. maxima at Fantome Island was +0.3 to −1.3 m (c.d.).

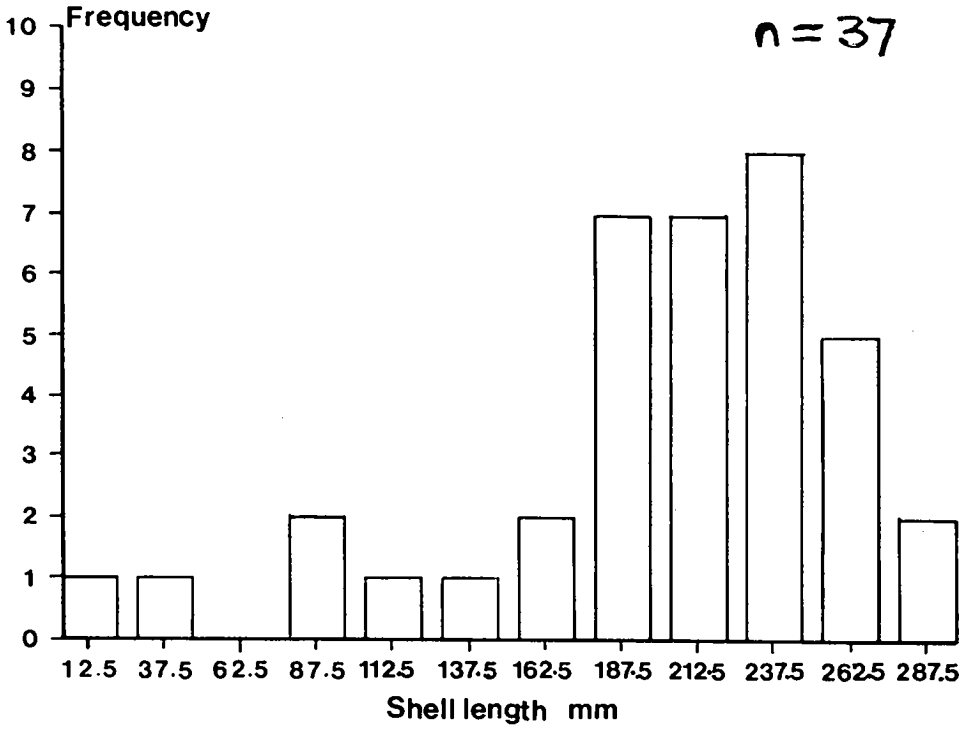
The plot of mean annual increment against mean length for T. maxima (Fig.2.21) showed a distinct inflexion at 140 mm. It is also interesting to note that there is a zero annual increment at just over 260 mm, suggesting that the L_{oo} = 250.1 estimate from the Wetherall plot is quite accurate, as one would expect L_{oo} to be close to the length at which the annual increment is zero.

To estimate the parameters of the juvenile (pre-inflexion point) growth a plot of $\log_e(L_r/L_m)$ against the time between measurements was produced (Fig. 2.22). Only five data points were available and the preliminary estimate of the mean slope of the regression line passing through the origin was $K' = 3.7 \times 10^{-4}$.

Triplets with values less than the point of inflexion were removed from the data set prior to the estimate of the

Figure 2.19 Size-frequency of Tridacna maxima at Fantome Island.

Figure 2.20 Wetherall plot for Tridacna maxima at Fantome Island of L-L' against L'. Values in mm.


Frequency
n = 37
10
9
8
7
6
5
4
3
2
1
0
12.5
37.5
62.5
87.5
112.5
137.5
162.5
187.5
212.5
237.5
262.5
287.5
Shell length mm

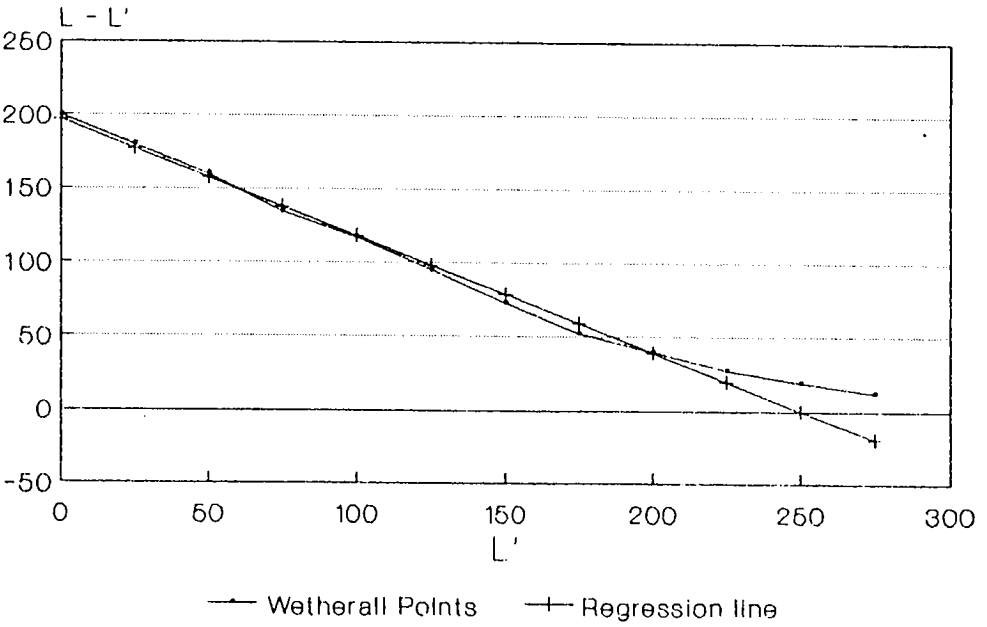
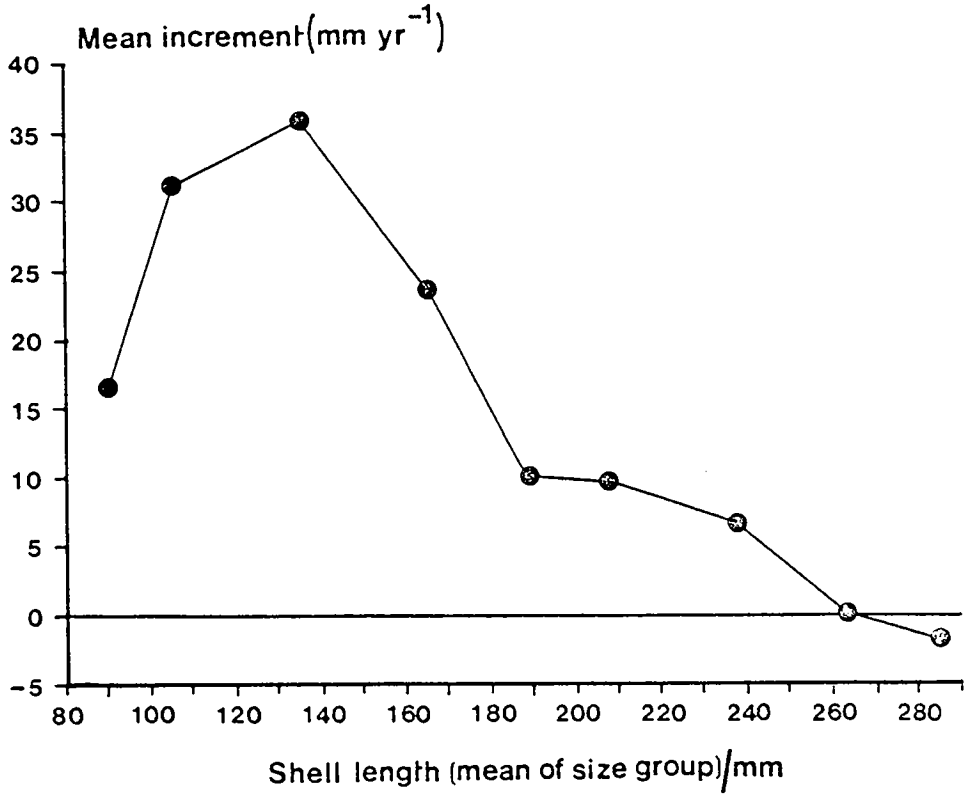
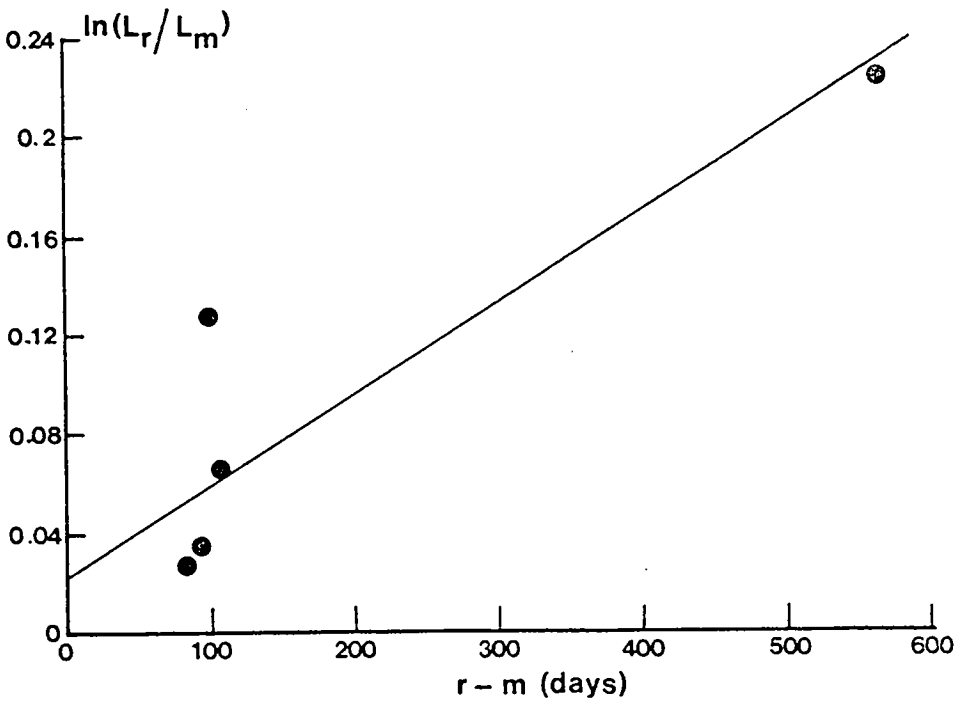

L - L'
250
200
150
100
50
0
-50
0
50
100
150
200
250
300
L'
Wetherall Points
Regression line

Figure 2.21 Plot of the mean increment in shell length of Tridacna maxima for successive length groups against the mean length within each length group for clams from Fantome Island.

Figure 2.22 Plot of $\ln(L_r / L_m)$ against r-m, the time in days between successive measurements of juvenile Tridacna maxima from Fantome Island. The linear regression line of the points has the slope K', where the Brody growth equation is:

$$Lt = Ae^{K't}.$$


Mean increment (mm yr^{-1})
40
35
30
25
20
15
10
5
0
−5
80
100
120
140
160
180
200
220
240
260
280
Shell length (mean of size group)/mm


ln(L$_r$/L$_m$)
0.24
0.2
0.16
0.12
0.08
0.04
0
0
100
200
300
400
500
600
r − m (days)

growth parameters using the Fabens method (Table 2.6).

Table 2.6 Fabens method estimate of growth parameters for *Tridacna maxima*

Site	L_{oo}	K	n
Fantome	271.52	.126	78

2.5.5 Environmental and observational results

During every remeasuring, sites were examined for new recruits. Additional efforts to find juvenile *H.hippopus* included transects across the algal terraces on the Iris Point Reef, examining 1 m x 1 m quadrats every 2 m across the outer reef flat and walks along the same reef at extreme low tide. Only two juveniles were found, both in depressions in upper levels of the algal terraces. Both individuals were kept in large saltwater tanks at the Orpheus Island Research Station. The two juveniles were 81 mm and 82 mm when found, and after one year had increased in length to 117 mm and 115 mm respectively, at which size their weights were 512 g and 545 g total wet weight.

Individual *H.hippopus* were found still bysally attached to 139 mm in length.

In Pioneer Bay several quadrats amongst the adult *H.hippopus* site were examined in great detail in attempts to find juveniles. The only one found was a 50 mm long *T.gigas* juvenile, attached to a small piece of dead coral.

The *H.hippopus* sites at Iris Point Reef and Pioneer Bay were quite homogeneous, therefore no attempt was made to

determine if there were intra-site specific differences in growth resulting from differing micro-environmental variables. Only inter-site differences were examined (see section 2.5).

In Pioneer Bay several T. gigas found on the reef slope showed signs of damage and some appeared to suffer a general malaise. Four clams showed damaged mantle edges which apparently were the result of bites from a predator. Several had mantles which had lost colour, turning white in patches, and occasionally mucous strands were noted floating upwards from the shell edge. Other features noticed were the retraction of the outer mantle layer from the growing shell edge revealing the inner mantle layer and insensitivity to touch (i.e. the clam did not close rapidly when the mantle was touched as is the normal response). These apparent signs of poor health persisted for over a year in one of the regularly measured clams (no.129).

New shell growth could sometimes be detected at the growing shell edge of clams. The shell there was clean as algal colonisation of the outer shell lagged behind the new shell growth.

All sites were affected by high winds and strong wave action resulting from Cyclone Winifred (2/2/86). At Fantome Island areas of the outer reef flat looked as if they had been scoured, whilst others were covered in newly formed piles of coral rubble. Some channels in the reef

edge were filled in by rubble, showing one of the ways in which fringing reefs can grow. Shell damage was particularly obvious in T.gigas, some of which were partially buried under broken branching coral. The shell damage often resulted from the valves closing on pieces of coral stuck between them. Several clams could not be found after the cyclone, whilst several others had moved position. Two T.squamosa were moved from their points of attachment on the reef slope and were found on sand at the base of the slope. At Iris Point Reef several H.hippopus were rolled several metres by the large waves associated with the cyclone.

A feature of H.hippopus at Iris Point Reef (11th August 1988) was that some had growing shell edges which noticeably overlapped, whereas usually they come together to a common point. This may be a result of tissue growth being outstripped by shell growth.

2.6 DISCUSSION

Length-frequency histograms of tridacnid clams in this study (Figs. 2.5, 2.11, 2.15. & 2.19) tended towards a uni-modal distribution, squewed to the right. According to Pearson & Munro (in press) this is probably the result of rapid juvenile growth, followed by the overlapping of year classes because of the variability of growth amongst individuals of any one year class. For species examined around the Palm Islands, it would appear that low or aperiodic recruitment also help to exaggerate the appearance of the length-frequency histograms.

Habitat differences, between sites of close geographic location can have noticeable affects on the growth of giant clams as demonstrated by H.hippopus from Pioneer Bay and Iris Point Reef. Also changes in the time of emersion of clams also affects growth. As was seen in this study, sub-littoral H.hippopus grew faster than clams which experienced occasional emersion and regular periods of heated water on the shallow reef flat. A similar decrease in growth with increasing tidal height and increased aerial exposure has been shown for many bivalves (Bertness & Grosholz 1985, Griffiths 1981, Rodhouse et al. 1984), whilst a similar comparison of an intertidal and subtidal site for Pinna bicolor, similarly showed slower intertidal growth (Butler 1987).

The effect of varying the amount of aerial exposure on the growth of H.hippopus was examined over a 7 month period (Appendix 2.2). Whilst results were not as clearcut as those of Nash (1988) who examined the effects of exposure on the growth of T.gigas using the same equipment, increasing the time of aerial exposure led to decreased growth in shell length, shell mass and flesh. Results were not as clear as those obtained by Nash (1988) who was able to utilise cohorts of T.gigas for his trial, whilst clams for the H.hippopus trial were of different ages and sizes (Appendix 2.2).

It was interesting to note that the size range which appeared to include the point of inflexion for the growth

curve of H.hippopus, 140-165 mm, also corresponded to the maximum size at which clams were still found to be byssally attached (139 mm). Only further work could determine if there was any causal relationship between these events.

The finding that minimal seasonal growth was found prior to the spawning season, during gametic development, is presumably the result of energy reserves being directed preferentialy to the gonad, thereby limiting both somatic and shell growth.

The observation that at some times of the year that shell and tissue production may not be synchronous, corresponds to similar findings in other bivalve species (Hilbish 1986, Borrero & Hilbish 1988). Such information can be particularly useful when considering the best time of year to maximise flesh yield in a mariculture situation.

One can combine values of K obtained using the Fabens technique, which has been shown to give the most accurate estimates of the von Bertalanffy equation (Sundberg 1984), with the values of Z/K from Wetherall's technique to obtain estimates of Z (Table 2.7).

The higher value of Z for H.hippopus at Iris Point Reef compared to Pioneer Bay, reflects the fact that the population there was occassionally harvested, so that the fishing mortality (F) had a direct impact on the total mortality Z, where Z = F+M, M being the natural mortality. These estimates of mortality rates are very approximate. Pearson & Munro (in press) showed that mortality rate

varies with size for T. gigas, therefore at best the mortality estimates presented here are estimates of the average mortality for all clams in the samples.

Table 2.7 Estimates of Z (total mortality) for giant clams of the Palm Island Group

Species	Wetherall estimates of Z/K, using all data points	Fabens estimate of K	Z
H. hippopus			
(Iris Point Reef)	0.86	0.204	0.17
(Pioneer Bay)	0.38	0.154	0.05
T. gigas			
(Pioneer Bay)	1.54	0.148	0.22
(Fantome Is.)	1.54	0.132	0.20
T. squamosa			
(Fantome Is.)	0.25	0.189	0.04
T. maxima	2.27	0.126	0.28

Note: The Wetherall method is primarily used to obtain preliminary estimates of the Z/K ratio and that the estimate of L_{oo} is of secondary interest in the context of this thesis, because it is independently derived by the Fabens method and from the results of the sclerochronological studies.

CHAPTER 3

SCLEROCHRONOLOGY 1 - GROWTH INCREMENT VALIDATION AND DESCRIPTION

3.1 INTRODUCTION

The validation of seasonal or annual bands in the shell of a mollusc requires that they should be directly related to real time. Validation allows the use of the bands in growth studies, in the same way that annual bands are used to age trees.

There are a number of options for assessing the time of formation of growth bands. One is to examine the development of bands with time in shells of known age or time of death. A second is to mark points in a shell's microgrowth record by either chemical dyes or stress, from which time periods can be accurately measured. A third is the analysis of chemical and structural changes in the growth record to see if these changes reflect apparent banding patterns and can themselves be related to time. Finally, it was found in this study that giant clams, like some corals (Isdale 1984), have fluorescent areas in their shells. By checking the correspondence of fluorescent lines to other patterns in the shell, these areas of fluorescence may themselves indicate seasonal differences.

A number of these techniques have been used in this study to examine growth band formation in giant clams.

3.2. METHODS

3.2.1 Examination of shells of known time of death

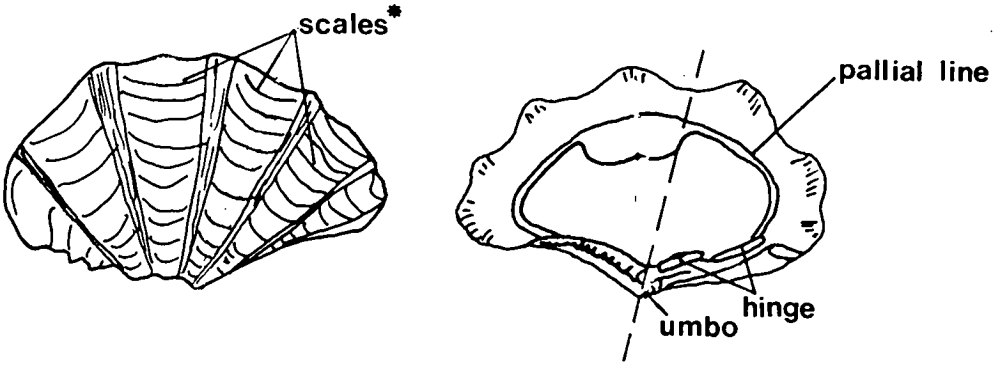
Three or four adult clams were sacrificed each month over the course of a year during the course of the study of the reproductive development and cycle of H.hippopus. In addition, small H.hippopus were killed in an attempt to determine the time of first sexual maturity and the allometric growth of different organs in relation to shell growth. These shells of known time of death were initially sectioned along the demarcation line using a diamond tipped blade, and then sectioned again to produce a section of shell 1-2 cm thick (Fig. 3.1). As the 'seasonal' growth bands are well defined in the inner shell layer (Fig. 3.2), and erosion can affect resolution of growth bands in the outer shell layer, the outer (most recently formed) band of the inner shell layer was examined at the time of death to determine which part of a growth band was being formed.

3.2.2 Shell microincrement examinations

Sections of H.hippopus and T.gigas were prepared as detailed in 3.2.1, and then etched for 30 seconds in 5% hydrochloric acid. They were then gently rinsed with distilled water and air dried. The sections were sputter coated with a 200 angstrom layer of gold to prepare them for Scanning Electron Microscope (SEM) examination. Obvious 'seasonal' bands were examined using the SEM (Fig. 3.3) to determine the structural differences between them

Figure 3.1 Diagram of two valves showing pallial line, umbo, hinge and scales. (* obvious scales are only found on *Tridacna maxima* & *T. squamosa*) Other species of tridacnids have ridges on the outer shell surface, which are refered to as external growth lines in this study). The dashed line across the shell is the demarcation line, where shells were cut.

Figure 3.2 Diagram of a section of *Hippopus hippopus* showing the pallial lines (P), the outer shell layer (O), the inner shell layer (I) and the umbonal shell layer (U). The open circles mark the surface of shell accretion. The appearance of internal seasonal growth bands can be seen in both the inner and umbonal shell layer.



(After Rosewater (1965))

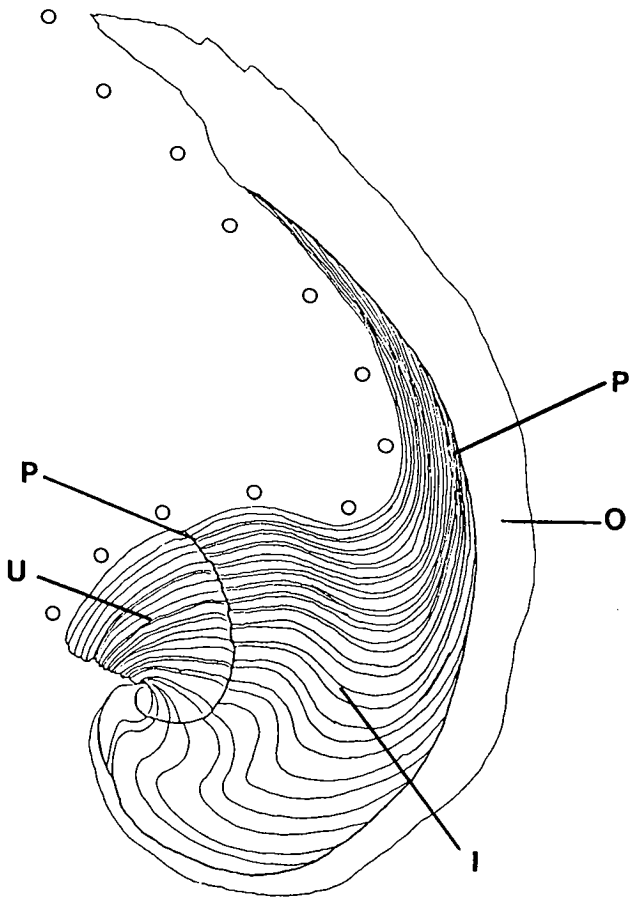
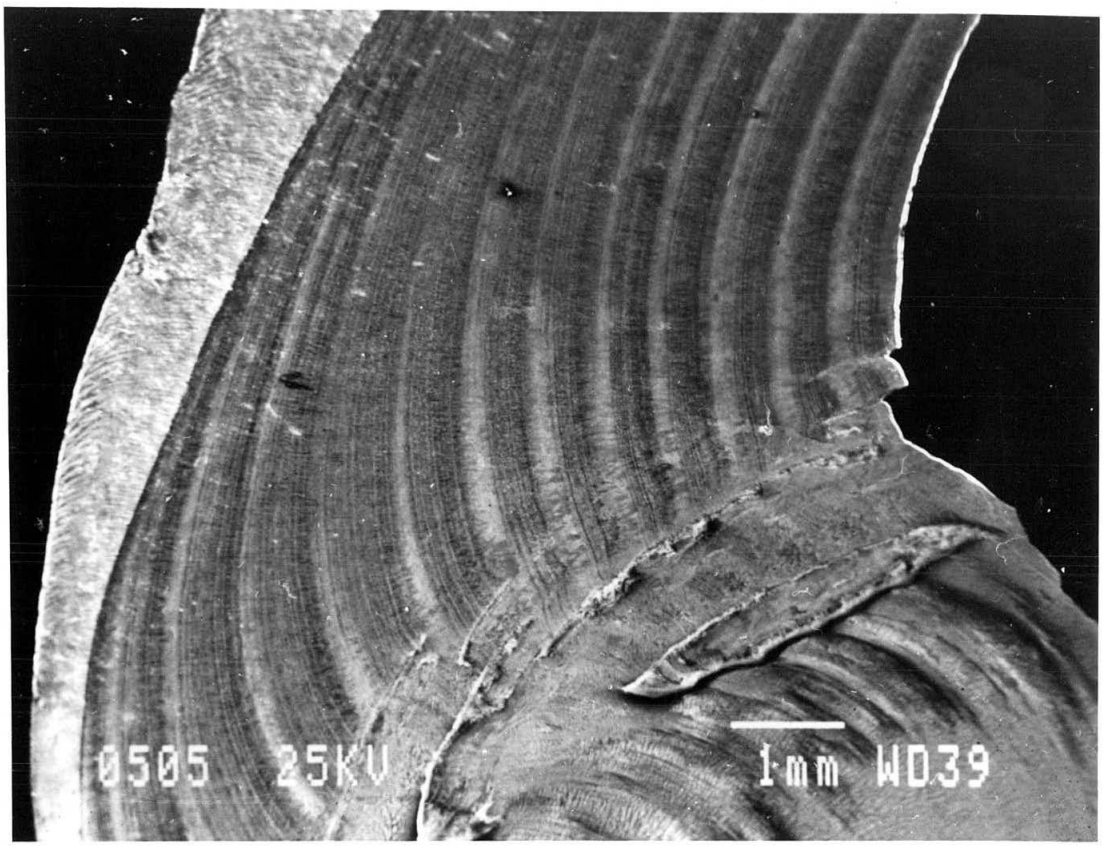


Figure 3.3 Scanning electron microscope photograph showing the differential etching of major seasonal growth bands across a shell section of Tridacna crocea.


0505 25KV
1mm WD39

and to examine microincrements and their periodicity.

Juvenile shells of H.hippopus and T.gigas were fixed to SEM stubs with glue and similarly sputter coated with gold. External growth lines on the clam shells were examined to determine whether or not they corresponded to periodical environmental fluctuations.

3.2.3 Seasonal differences in shell chemistry

Sections of a few centimetres thickness were cut dorso-ventrally through the centre of the umbo using diamond tipped blades. One cut was made as close as possible to a line which would pass through the prodissoconch. The cut was made to run parallel to the dorso-ventral line of the shell through the umbo, the demarcation line (Fig. 3.1). Sections were produced from the shells of H.hippopus, T.gigas, T.derasa, T.squamosa, T.maxima and T.crocea.

From these sections, rectangular blocks were cut from the inner shell layer of a size to fit on a standard microscope slide using a diamond tipped blade. These blocks were air dried (drying on a hot plate resulted in shell fractures) and attached to slides which had been roughened to improve adhesion using 815 epoxy resin.

Thick sections of approximately 2 mm were then produced from the blocks using a low speed microtome fitted with a diamond tipped wafering blade and a chuck into which the slide fitted. The thickness of the section was controlled by a micrometer mechanism.

These thick sections were then polished through a series of powders and grits using both manual lapidary wheels and automatic polishing machines. The size series of grits were 400, 600, and 1000, followed by 5 and 1 um alumina powder.

The highly polished shells were then sputter coated with a 200 A layer of carbon to ensure adequate conductivity of electrons. As the distinct seasonal opaque and translucent bands were not visible on the scanner of the microanalyser, the centres of bands were marked with indian ink under a low power dissecting microscope to distinguish them from one another.

The shells were then examined using wavelength dispersive spectrometry on a Jeol JXA-840A Electronprobe Microanalyser. Elements probed for were barium, calcium, iron, manganese, magnesium, phosphorus, strontium, and sulphur. All sections were probed from the oldest to the most recently deposited growth band.

Standards used for calibrating the spectrometers were: barite ($BaSO_4$), wollastonite ($CaSiO_3$), haematite (Fe_2O_3), rhodonite ($MnSO_3$), periclase (MgO), apatite ($Ca_5(PO_4)_3(OH,F,Cl)$), celestite ($SrSO_4$), and barite ($BaSO_4$). A beam current of 10.0 +/- 0.03 nA was used and probe times were from 5-40 seconds, depending on the element. The probe was positioned close to the end of the indian ink marks to ensure the readings from the different bands were taken accurately.

The weight % of each minerals oxide was measured and the weight % of each element calculated by direct stoiciometry.

3.2.4 Fluorescent line examination

Examination of fluorescent lines in shell sections of Hippopus hippopus, Tridacna gigas, T.derasa, and T.squamosa (prepared as 3.2.3) was undertaken using both a low and high frequency ultra-violet light source. Shell sections, cut for growth line analysis, were examined to determine:-

1) how the distribution of fluorescent lines was related to obvious structural growth bands

2) if the amount of fluorescence is related to the location of the clam across the continental shelf

3) if fluorescent lines differ between species of giant clam.

Shells were examined from as many different reefs as possible. Reef names and locations are listed in the Results section (Appendices 3.1 - 3.4). One shell of each species from each reef was examined.

3.3 RESULTS

3.3.1 Examination of shells of known time of death

Examination of shell sections of H.hippopus killed for reproductive cycle analysis showed that the translucent band, of the presumed annual couplet (one translucent and one opaque band) was only deposited between the months of

October 1986 and January 1987. An opaque band, was found to be deposited on the outer growing edge of the inner shell layer for all other months of the reproductive study.

A batch of H.hippopus killed in December 1986, in an attempt to determine age at first sexual maturity were all found to be in the process of depositing a translucent band at the outer edge of the inner shell layer.
These results point to the synchronous deposition of opaque and translucent 'seasonal' bands within a given population, all experiencing similar conditions, and that two bands are deposited each year forming one annual couplet.

Examination of some shells of T.gigas, known to be 2.6 years at time of death, revealed that examination of sections may underestimate their age. The clams should have had two opaque, winter bands and a third being laid down. Only one opaque band and one in the process of being laid down were apparent. This is a reflection of the relatively small bands laid down in the first year of growth, being overshadowed by the relatively large band of the next year. Although examination of shells using acetate peel or scanning electron microscope, would have distinguished the true number of bands, both these techniques are time consuming and relatively expensive. Therefore, when aging a large number of shells, as described in chapter 4, it was advisable to add one year to the age estimated from simply counting the number of

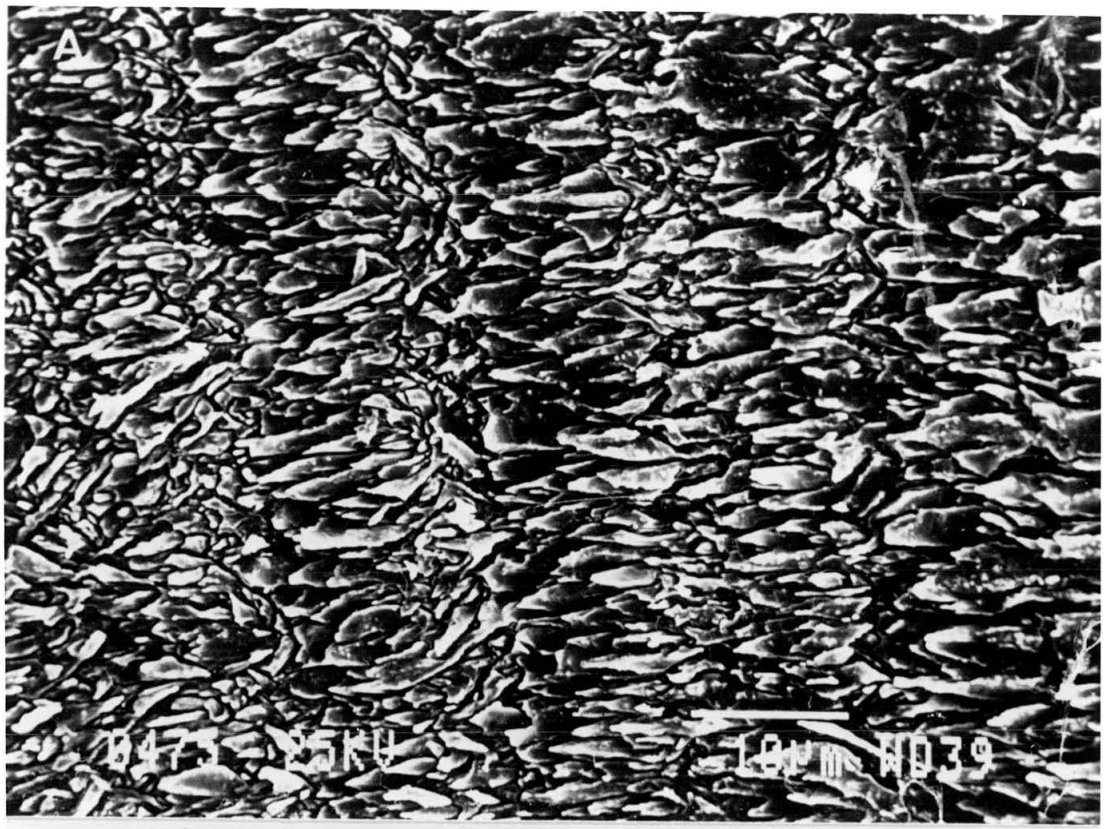
annual couplets of growth bands to find the true age of giant clam shells.

3.3.2 Shell microincrement examination

Comparison of the two photographs in Figure 3.4 shows the differences in crystal size between translucent and opaque growth bands of etched H.hippopus sections. Also it was evident that microincrements, whilst clearly visible by the alternation of orientation of the etched crystals in the translucent growth band, were not visible in the opaque band. Crystals of the translucent band were smaller and more irregular in both shape and orientation than those of the opaque band which were elongated in the c-crystallographic axis (horizontal axis in Figs. 3.4) and quite regular in size, shape and orientation.

The series of 5 plates in Figure 3.5 shows growth bands of H.hippopus at 5 different magnifications. As magnification is increased on the translucent band of the two seasonal bands (Fig. 3.5 (a)) groupings of microincrements are seen (Fig. 3.5 (b)). Then an increment with a double sized low etched area is noted (Fig 3.5 (c)), which is then highlighted (Fig. 3.5 (d)), until at 3000x magnification, just two complete microincrements are seen (Fig. 3.5 (e)). Examination of comparative microincrements of T.gigas at the same magnification (as Fig. 3.5 (f)) revealed crystals which were narrower and sharper than those of H.hippopus Examining the outer surface of a shell of H.hippopus (Fig 3.6), shell length 57 mm, shell mass 0.012 g, which was known to be 135 days old, only 120 microincrement growth

Figure 3.4 Scanning electron microscope photograph of etched crystals of the translucent (upper) and opaque (lower) growth bands of Hippopus hippopus


A
0473 25KV
10µm WD39

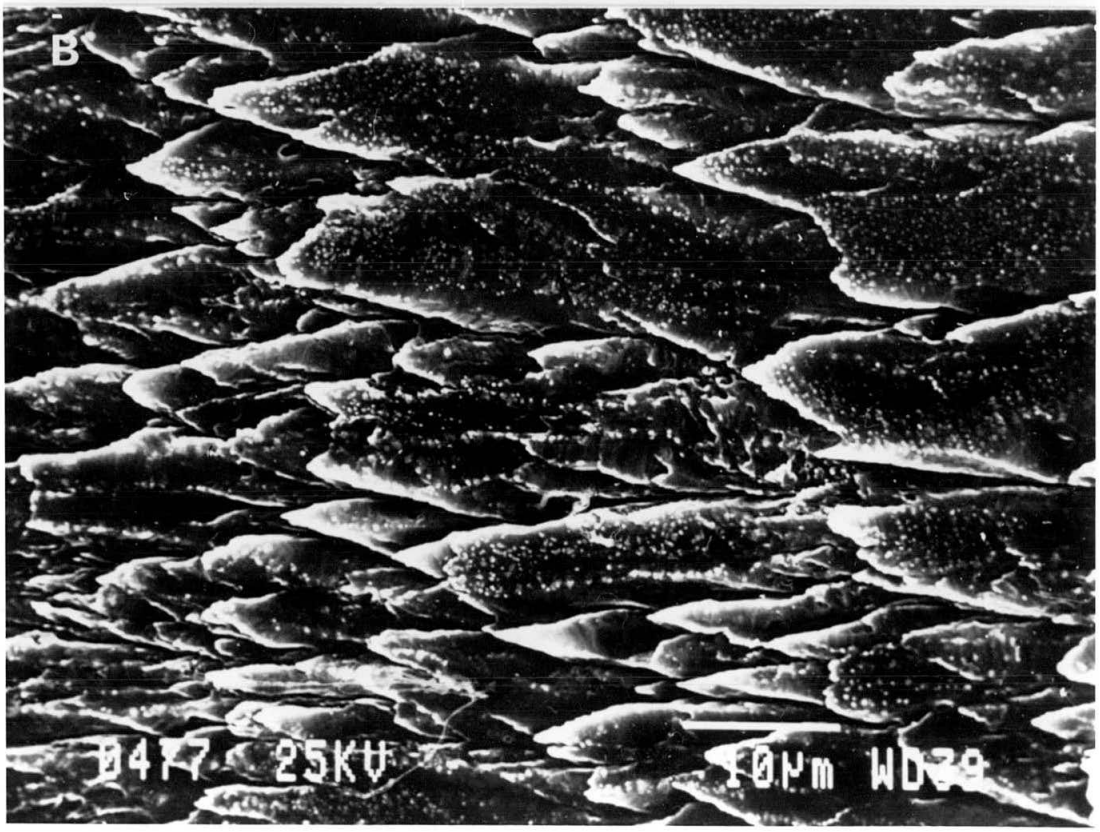

B
0477 25KV
10µm WD39

Figure 3.5 Scanning electron microscope photographs of etched Hippopus hippopus sections. Note b-e are all from the translucent band of the clam shell, seen centrally in a.

a. Two seasonal bands are shown.

b. Approximately 40 microincrements (growth lines).

c. A double sized increment is seen amongst other microincrements.

d. The double sized, deeply etched microincrement is highlighted.

e. Two microincrements. The difference in crystal size and orientation can be seen between the two different zones which make up each microincrement.

f. Two microincrements of Tridacna gigas at the same magnification as e. above. It is noticeable that the crystals appear sharper and narrower than those of Hippopus hippopus.

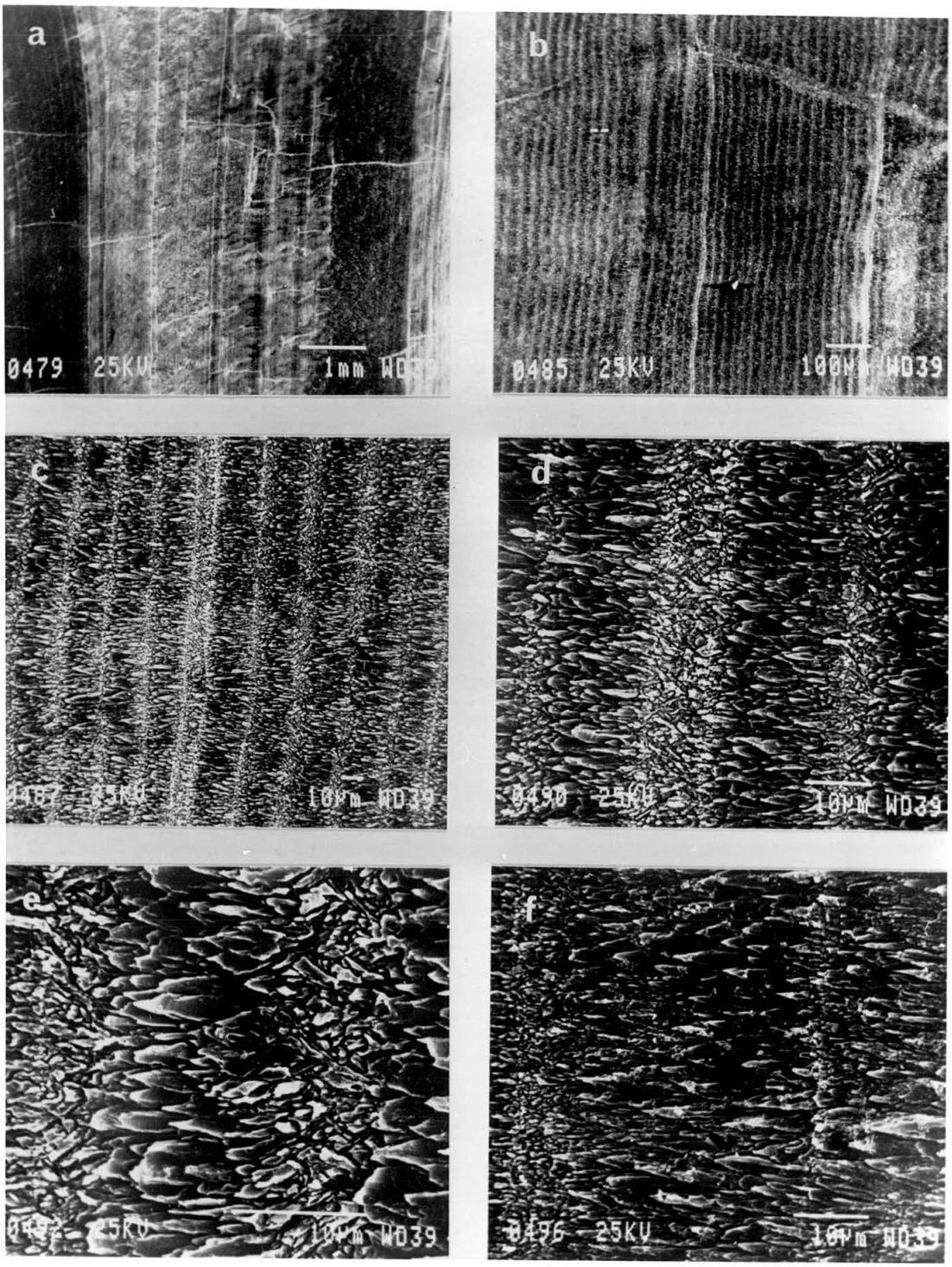
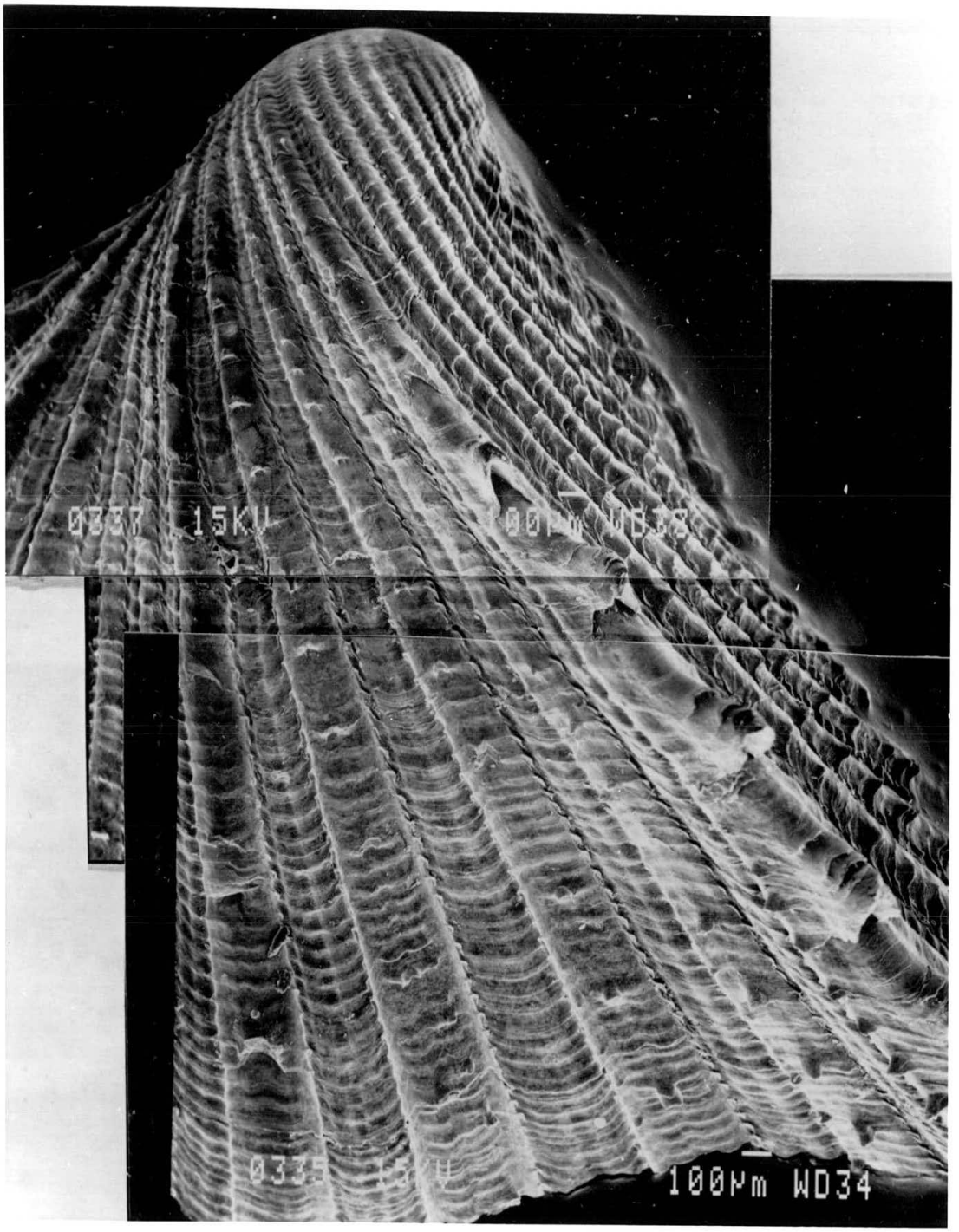

a
0479 25KV 1mm WD
b
0485 25KV 100µm WD39
c
10µm WD39
d
0490 25KV 10µm WD39
e
25KV 10µm WD39
f
0496 25KV 10µm WD39

Figure 3.6 A composite photograph (from the scanning electron microscope) of the external growth lines of a 135 day old juvenile Hippopus hippopus.


0337 15KV
100µm WD34

lines were counted. However the prodissoconch of the shell exhibited no obvious growth lines, which would account for the differences. Similar examination of a 190 day old shell of T.gigas (shell length 55 mm, shell mass 0.0145 g) revealed 163 microincrement growth lines. Therefore after discounting the slight discrepancy resulting from the absence of growth lines in the prodissoconch, it appears that in juvenile clams at least there is approximately one external microincrement laid down per day. These juveniles were kept in on-shore seawater tanks and experienced no direct tidal emersion / immersion pattern. The daily growth lines therefore most likely reflect the day / night cycle. The significance of daylight relates to the importance of the clam's symbiotic zooxanthellae (through photosynthesis), and the probable role these symbionts play in the control of calcification in clams, as they do in corals. Observations of giant clam's behaviour suggest that they respond little to mechanical stimulus at night.

3.3.3 Seasonal differences in shell chemistry

3.3.3.1 Hippopus hippopus

Ten pairs of 'seasonal' growth bands, which should correspond to 10 years of growth were probed (using the electron microprobe of the SEM) from a section of H.hippopus (Appendix 3.5). Only 3 of the elements probed for, Ca, Mg and Mn showed regular variation in concentration corresponding to the banding pattern (Figs. 3.7, 3.8, 3.9). In the first 7 pairs of bands probed for Ca, the opaque band had a lower wt% oxide than the

Figure 3.7 Graph of the elecronmicroprobe analysis for calcium (Ca) measured in Wt% oxide, from a shell section of Hippopus hippopus. Points marked'o' are from opaque bands and those marked 't' from the translucent band (bands appearance when viewed using transmitted light).

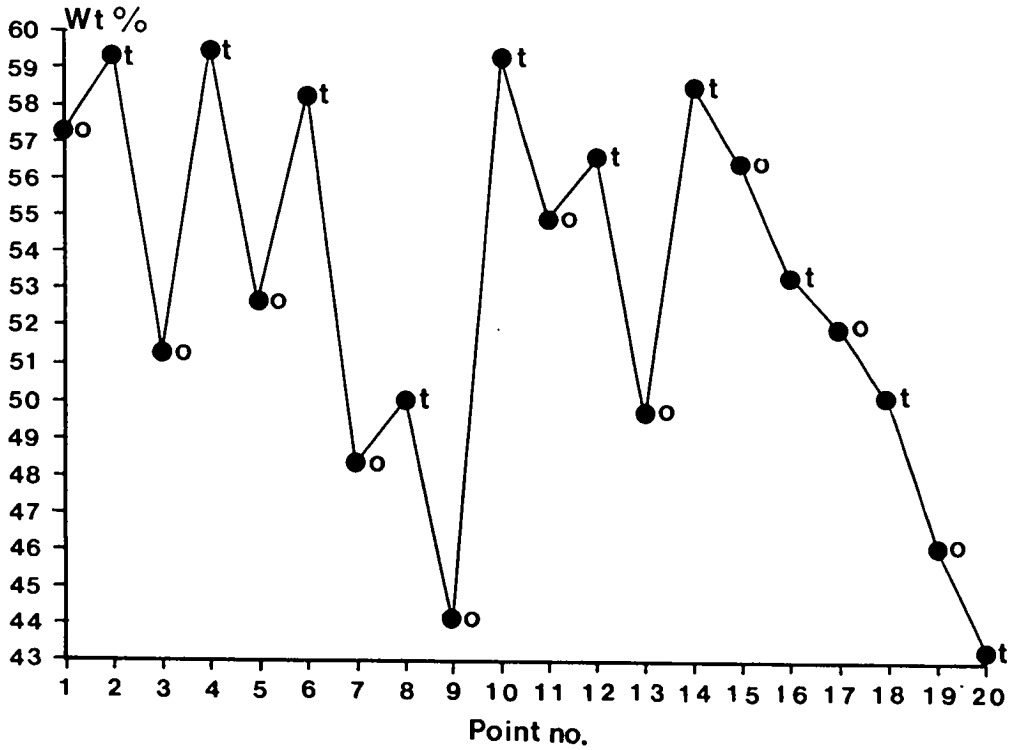
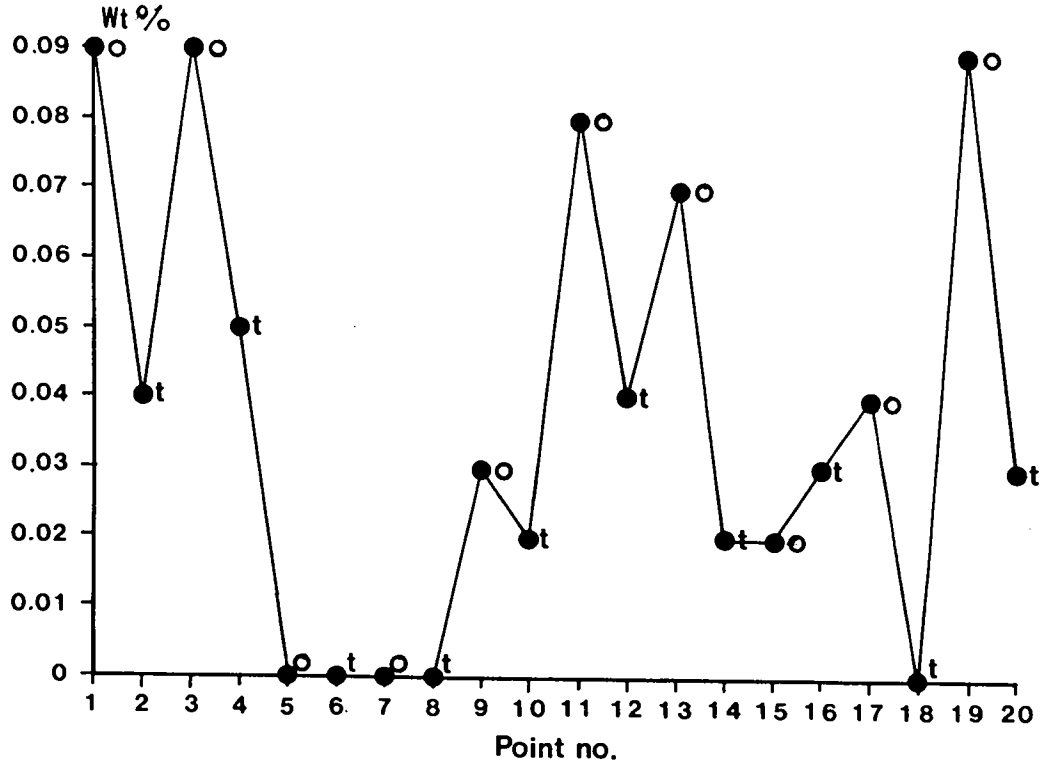

Wt %
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Point no.
o t o t o t o t o t o t o t o t o t o t o t

Figure 3.8 Graph of electronmicroprobe analysis for magnesium (Mg) from a shell section of Hippopus hippopus

Figure 3.9 Graph of electronmicroprobe analysis for manganese (Mn) from a shell section of Hippopus hippopus.


Wt %
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Point no.

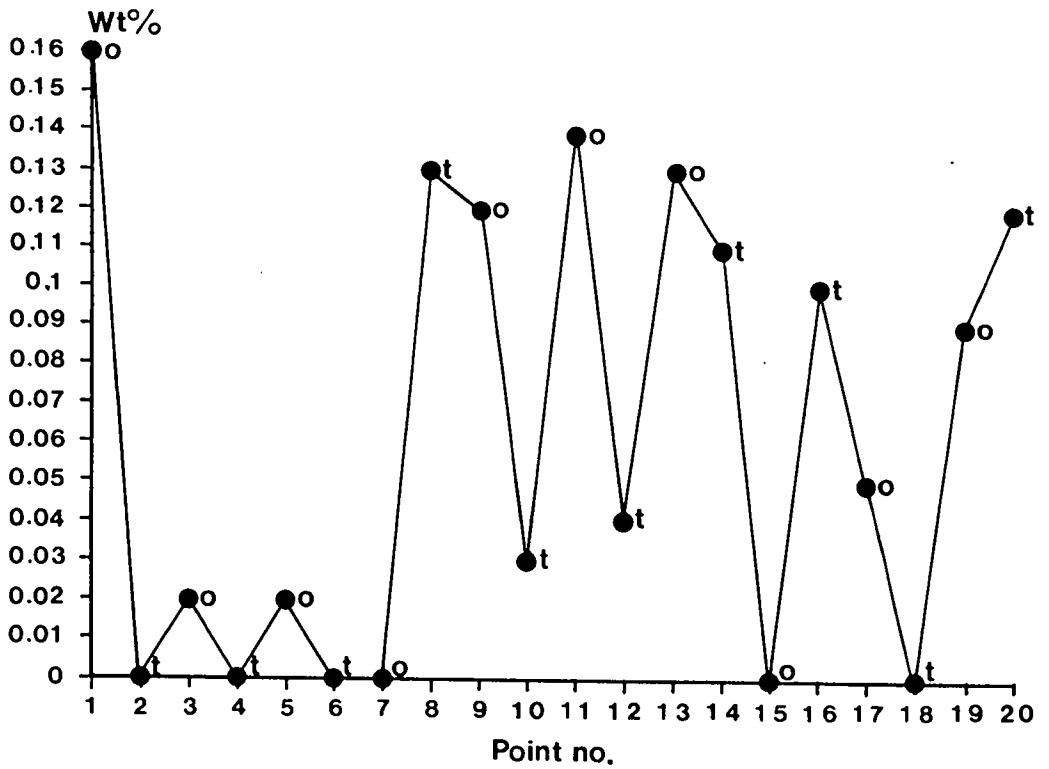
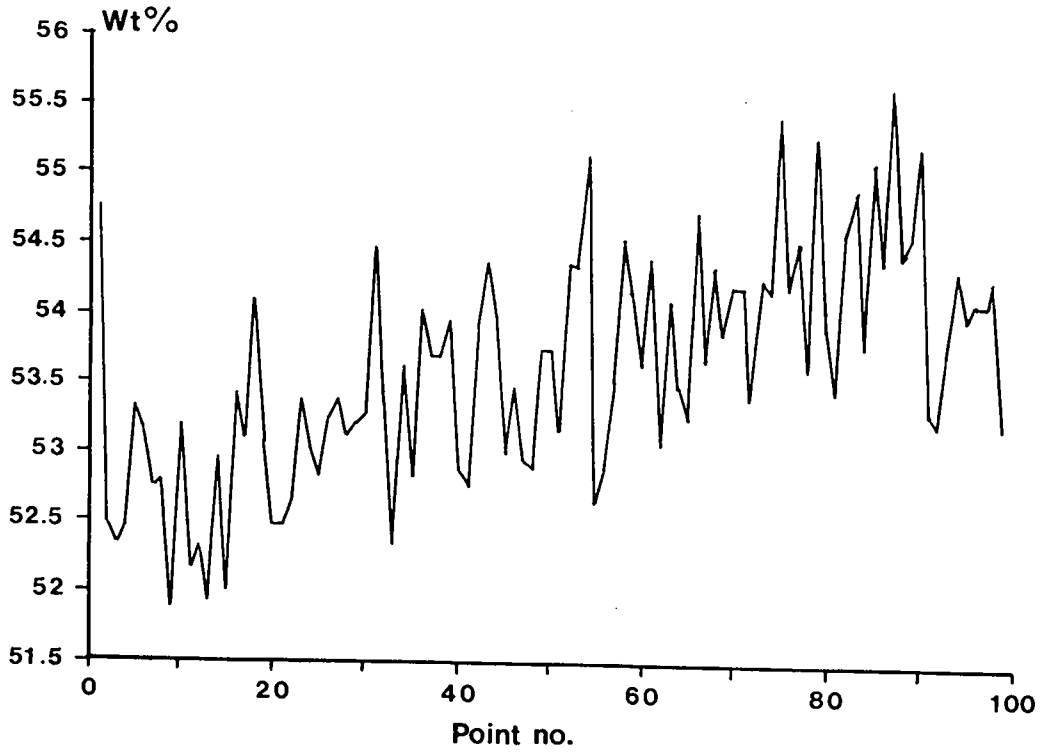
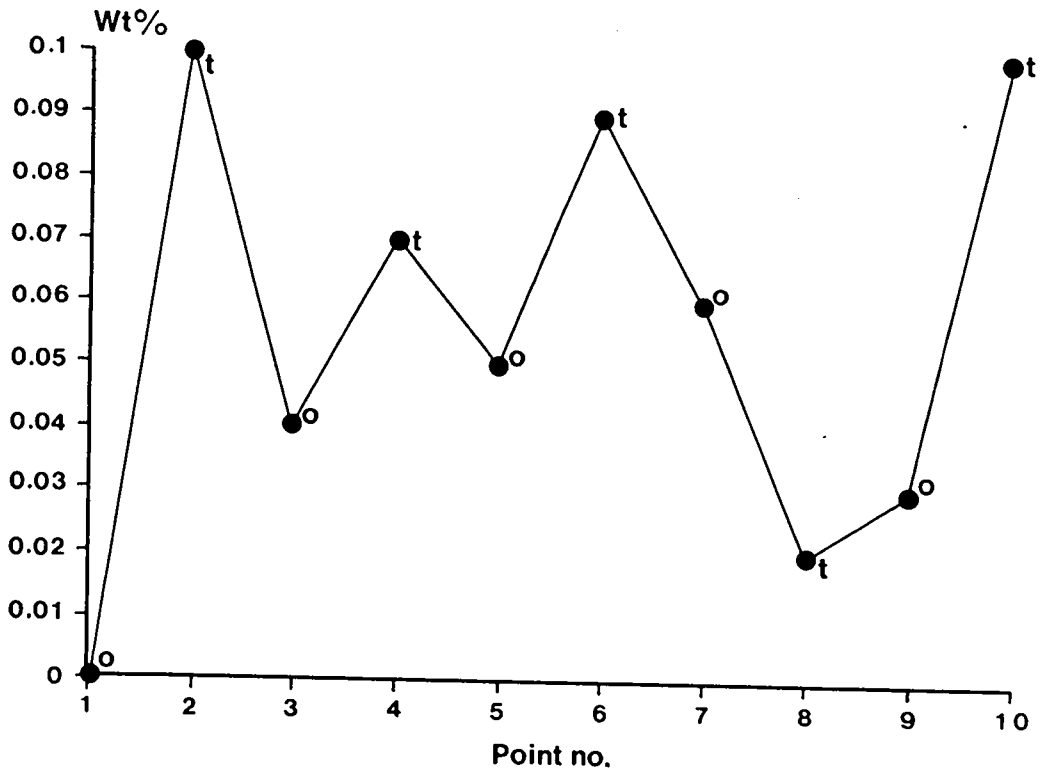

Wt%
0.16
0.15
0.14
0.13
0.12
0.11
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Point no.

Figure 3.10 Graph of a wave scan using the electron microprobe of 100 points across 3 opaque and 2 translucent bands of a shell section of Hippopus hippopus. The different bands are not shown clearly by variations in calcium concentration.

Figure 3.11 Graph of electronmicroprobe analysis for manganese (Mn) in a shell section of Tridacna derasa.


Wt%
56
55.5
55
54.5
54
53.5
53
52.5
52
51.5
0
20
40
60
80
100
Point no.


Wt%
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
1
2
3
4
5
6
7
8
9
10
Point no.

preceding transparent band. After these, the concentration of Ca decreased with each band, and with increasing clam age (Fig. 3.7). For 7 pairs of seasonal bands, both Mg and Mn showed a lower concentration in the transparent band compared to the preceding opaque band.

All other elements probed for in sections of H.hippopus showed variation in concentrations; however, fluctuations were not in synchrony with the two'seasonal' bands.

In an attempt to get greater resolution of the variation of the concentration of Ca with band type, a wave scan of 100 points was undertaken across just 5 seasonal bands (3 opaque, 2 translucent) (Fig. 3.10). The results show considerable variation in elemental composition within each seasonal band, indicating that chemical composition and its variation may have to be examined at a still higher resolution. Marked chemical variations may occur with periodicities of a month, a day or even between tides.

3.3.3.2 Tridacna gigas

As the size of the seasonal growth bands of the fast growing T.gigas are the largest of all, only 2 pairs of growth lines (2 years) could be probed at once (Appendix 3.6). Therefore variation in chemical composition of the different types of seasonal band may only be guessed at from such a small sample.

3.3.3.3 Tridacna derasa

Five pairs of seasonal bands of the shell of Tridacna

derasa were probed (Appendix 3.7). The best correspondence in differences in concentration between bands was shown for Mn, where for 4 of the 5 pairs the concentration of Mn was higher in the translucent than the opaque band (Fig. 3.11).

3.3.3.4 Tridacna squamosa

Three pairs of 'seasonal' growth bands (3 years) of T. squamosa were probed (Appendix 3.8). The only element that seemed to vary in concentration with the seasonal bands was Ba, which was lower in the translucent than the opaque layers. There was an apparent decrease in the concentration of Ca with age and an increase in Mn.

3.3.3.5 Tridacna maxima

Of the seven pairs of 'seasonal' bands of T. maxima probed (Appendix 3.9), six of the pairs showed a lower concentration of Ca in the translucent than the opaque bands (Fig. 3.12). There were no other discernible relationships between band type and chemical make-up.

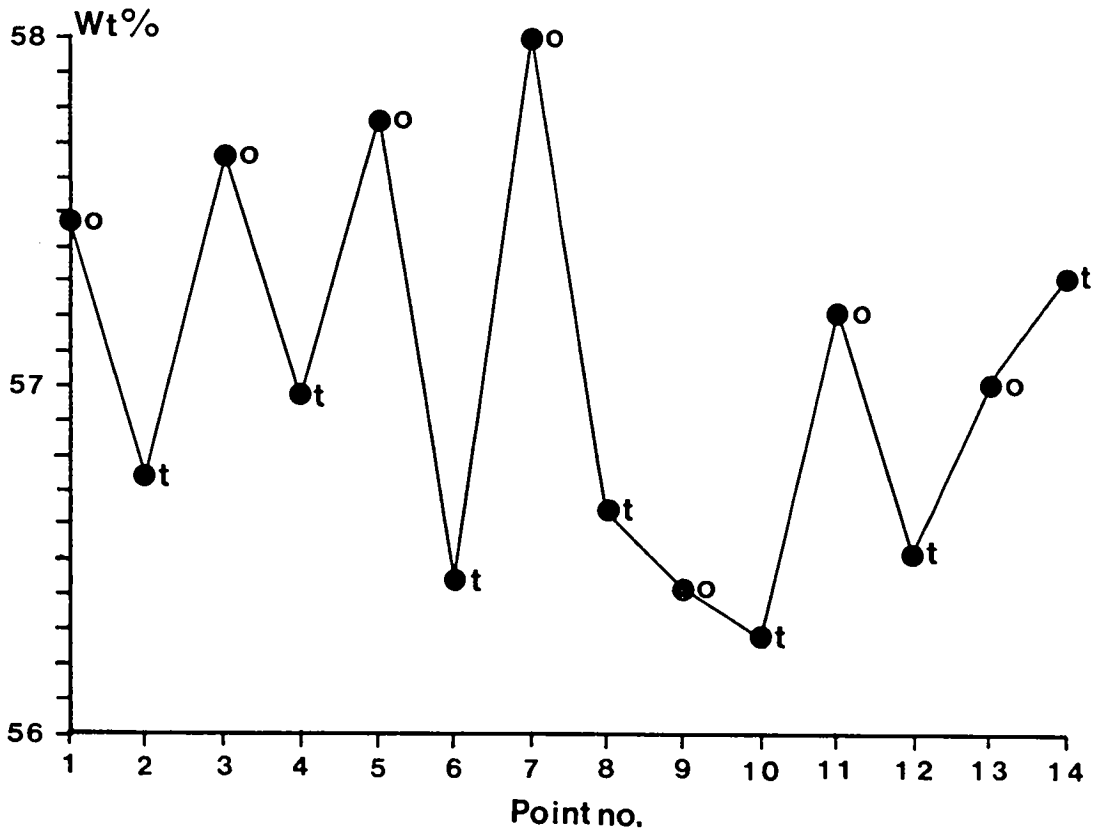
3.3.3.6 Tridacna crocea

Six pairs of 'seasonal' bands of T. maxima were examined and no relationship between fluctuations in chemical composition and seasonal bands was found (Appendix 3.10).

3.3.3.7 Chemical composition of growth bands

The chemical composition of the opaque and translucent bands from all species of clam probed are summarised in

Figure 3.12 Graph of the electronmicroprobe analysis for calcium (Ca) in a shell section of *Tridacna maxima*.


Wt%
58
57
56
o
t
o
o
t
o
t
o
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o
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11
12
13
14
Point no.

Tables 3.1 & 3.2. This is the first time that giant clam shells have been examined chemically using this technique.

3.3.4 Fluorescent line examination

The translucent band contained the fluorescence which was usually light blue in colour. Occasionally some yellow fluorescence was noted. Observations on individual shell sections are detailed in appendices 3.1 - 3.4. In most shells the pallial lines show the brightest fluorescence. There were species differences in fluorescence intensity, with T. squamosa the brightest, followed by T. derasa, T. gigas and H. hippopus in descending order of intensity. Although no measurement was made of the amount of fluorescence, it appeared that shells of H. hippopus, T. gigas and T. squamosa from reefs on the outer edge of the Great Barrier Reef, such as Myrmidon Reef, exhibit lower intensity fluorescence, compared with inshore reefs.

In addition to differences in intensity between species, other variations were noted. Shell sections of H. hippopus, T. gigas and T. squamosa had fluorescent lines which showed distinctly different intensities in different growth bands. The relative size of the translucent bands were seen to increase with age, compared to opaque bands in all species except T. derasa, where translucent bands were always relatively larger than opaque bands. Only in T. derasa was it noticeable on some shells that the fluorescent bands apparently extended to a slightly greater width than the corresponding translucent band. This may have been an anomaly related to technique (angle

Table 3.1 * Summary of mean chemical content of seasonal opaque (o) and translucent (t) growth lines measured in wt. % of oxide

	Ho	Ht	Go	Gt	Do	Dt
Ba	0.057	0.052	0.070	0.025	0.062	0.032
Ca	51.280	54.850	61.050	62.550	60.570	60.270
Fe	0.086	0.065	0.135	0.020	0.108	0.086
Mg	0.051	0.023	0.000	0.015	0.022	0.036
Mn	0.073	0.053	0.055	0.025	0.036	0.076
P	0.014	0.026	0.010	0.000	0.024	0.022
S	0.040	0.039	0.045	0.030	0.038	0.026
Sr	0.152	0.144	0.150	0.150	0.152	0.190
n =	10	10	2	2	5	5

	So	St	Mo	Mt	Co	Ct
Ba	0.046	0.086	0.067	0.025	0.051	0.078
Ca	47.430	51.490	57.360	56.700	54.740	54.840
Fe	0.033	0.000	0.025	0.072	0.055	0.033
Mg	0.030	0.023	0.010	0.017	0.023	0.020
Mn	0.070	0.026	0.068	0.052	0.085	0.071
P	0.033	0.003	0.012	0.012	0.023	0.020
S	0.023	0.043	0.020	0.008	0.026	0.028
Sr	0.183	0.146	0.190	0.174	0.196	0.243
n =	3	3	7	7	6	6

Table 3.2 * Summary of mean chemical content of seasonal opaque (o) and translucent (t) growth lines measured in wt. % of element

	Ho	Ht	Go	Gt	Do	Dt
Ba	0.051	0.047	0.063	0.022	0.056	0.029
Ca	36.650	39.201	43.632	44.704	43.289	43.075
Fe	0.067	0.051	0.105	0.016	0.084	0.067
Mg	0.031	0.014	0.000	0.009	0.013	0.022
Mn	0.056	0.041	0.043	0.019	0.028	0.059
P	0.006	0.011	0.004	0.000	0.010	0.010
S	0.016	0.016	0.018	0.012	0.015	0.010
Sr	0.129	0.122	0.127	0.127	0.129	0.161
n =	10	10	2	2	5	5

	So	St	Mo	Mt	Co	Ct
Ba	0.041	0.077	0.060	0.022	0.046	0.070
Ca	33.898	36.800	40.995	40.523	39.122	39.194
Fe	0.026	0.000	0.019	0.056	0.043	0.026
Mg	0.018	0.014	0.006	0.010	0.014	0.012
Mn	0.054	0.020	0.053	0.040	0.066	0.055
P	0.014	0.001	0.005	0.005	0.010	0.009
S	0.009	0.017	0.008	0.003	0.010	0.011
Sr	0.155	0.123	0.161	0.147	0.166	0.205
n =	3	3	7	7	6	6

Key*: H=Hippopus, G=T.gigas, D=T.derasa, S=T.squamosa, M=T.maxima, C=T.crocea

of cut of shell, section thickness), rather than to the bands themselves.

3.4 DISCUSSION

The production of two annual growth bands to form one annual couplet in giant clams, noted by examination of shells of known time of death, is in agreement with results commonly found in molluscs (Ropes & Jearld 1987). This result confirms the work of Jones et al. (1986) who found that apparent annual banding in shells of a giant clam, T. maxima, corresponded well to changes in the stable isotopic composition of the bands, which was related to seasonal changes in the seawater temperature.

As microincrements were not apparent in the opaque zone of H. hippopus , counts of the number of microincrements per year could not be undertaken using the techniques described in 3.2.2. However, patterns seen from the translucent bands were very similar to those described by Pannella (1976) for T. squamosa. Cross-over areas which reflect the change over from spring to neap tidal regimes are seen in Figures 3.5 (a-e), just as shown by Pannella (1976). Approximately one microincrement laid down per day was demonstrated for both T. maxima and T. crocea by Ohno (1985). External microincrements, whilst not as reliable as internal lines (Ropes & Jearld 1987), were also shown to approximate to one per day for H. hippopus and T. gigas juveniles in this study. Microincrement formation resulting from the relative size of crystal units, as seen in Figure 3.4, and different crystal structures in this

study agrees with the findings of Deith (1985).

Although only a few elements showed any periodic variation between different types of band (opaque or translucent) for the species of giant clam examined, larger sample sizes and perhaps examination of other elements may reveal chemical periodicity between band type. The variability in fluorescence between species and between growth bands in a particular clam point to differences in chemical composition outside the scope of this study. However, results which suggested differences in intensity between inshore and offshore reefs are similar to results found for corals (Isdale 1984, Boto & Isdale 1985), where fluorescence was found to be limited to inshore reefs.

As was noted by Ohno (1985), the importance of photosynthesis to the zooxanthellae of giant clams is reflected in the clams growth form, where microincrement formation appears dominated by the day / night cycle; whereas in most littoral molluscs microincrement formation is governed mainly by tidal cycles (Richardson 1987, Richardson et al. 1980b, Lonne & Gray 1988). Having determined this, it is not surprising that a couplet of seasonal bands are laid down forming annual bands very reminiscent of growth bands in trees, where photosynthesis similarly is the major energy source. The validation of the annual nature of the growth bands permits giant clam shells from death assemblages to be aged with a degree of confidence.

CHAPTER 4

SCLEROCHRONOLOGY II - MORPHOMETRY AND GROWTH

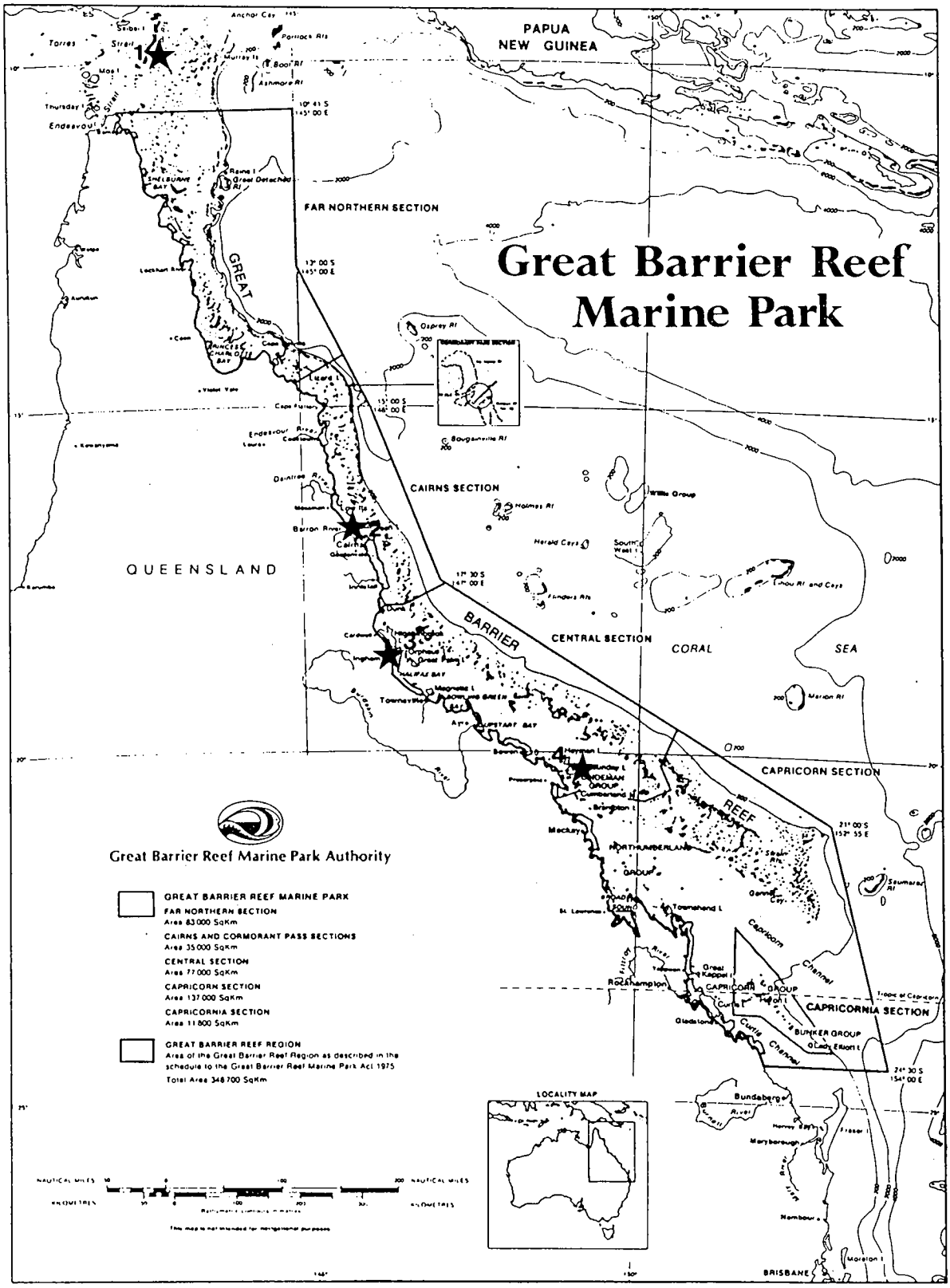
4.1 INTRODUCTION

The validation of the annual periodicity of each couplet of seasonal bands in the inner shell of giant clams (Chapter 3) enables shells to be aged. Shells collected from death assemblages from different locations and shells from clams killed to obtain reproductive material were aged so that growth rates and patterns from different sites could be examined. Measuring growth of shell length alone may not fully reflect the possible differences in growth rate and shell form within or between sites. Therefore in this chapter a number of shell parameters were examined in an attempt to describe growth more accurately. This information can also help identify optimal sites for giant clam growth, and determine if generalisations can be made about variation in growth rate and form, relative to latitudinal, cross-shelf and small scale environmental variation.

4.2 SITES

Shells of dead clams were collected from the southern end of the Great Barrier Reef to the most northerly reefs in the Torres Strait (Fig. 4.1). Cross-shelf variation was

Figure 4.1 Location of main centres of shell collection along the Great Barrier Reef. Sites are marked with a star and a number. The numbers refer to:
1 = Torres Straits, 2 = Cairns, northern site, 3 = Palm Islands group and 4 = Hayman Island (Whitsunday Islands group.



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SEPTEMBER 1984

examined in the Central Section of the Great Barrier Reef, most easily accessible from Townsville, whilst shells for examination of small scale environmental effect on growth were collected from the northern Torres Straits, and the Palm Island Group (Fig. 4.1).

Hippopus hippopus provided the largest number of shells in good condition to examine from the greatest variety of sites.

Although death assemblages of shells provide an excellent resource for the sclerochronologist to work with, the fact that the shells are from dead individuals means that in most circumstances only very general environmental descriptions applicable to a given reef are reliable. As shown in Chapter 2 shells can be moved considerable distances during tropical cyclones or storms. This means that the clam may have grown under different micro-environmental conditions to those of the site where the dead shell was located. This generalisation is more applicable to the smaller giant clam species, H. hippopus, T. squamosa and T. maxima, than for the more massive T. gigas and T. derasa.

4.3 METHODS

Shells were marked with permanent ink for later identification. Superficial fouling growths such as corals, bivalves, sponges, algae and other sedentary marine organisms found attached to the shells were removed where possible. All shells were sun dried. For each valve examined the following information was recorded: species,

location, shell side, shell length, shell height, hinge length, unhinge length, external growth anomalies, and dry mass, and, for those species with a byssal gape, byssal gape length and width (Fig. 4.2).

Following the external measurements, the shells were processed to obtain shell sections (see 3.2.1). For the largest shell specimens of T.gigas and T.derasa a stonemason's gantry saw with a diamond tipped blade capable of a 25.4 cm deep cut was used. The very largest shells were cut from one side, turned over and then cut from the other side, to completely cut through a valve. Shell sections were often cut several cm thick because shells were found to be prone to fracture, especially along lines between seasonal bands. Smaller shells were cut using diamond tipped saws in the Engineering and Geology Departments of James Cook University.

Further measurements of some H.hippopus shell sections were made using a Bioquant Digitising Tablet and software with an Apple 2e computer. Each section had 13 further morphometric attributes measured (Fig. 4.3).

Each shell section was aged to the nearest whole year using the annual couplets of seasonal growth bands present in the inner shell layer. If couplets were particularly distorted or difficult to read the specimen was not aged. One problem found with some of the larger T.gigas and T.derasa was that erosion of the umbonal region had removed some of the earlier growth couplets from the inner shell layer making

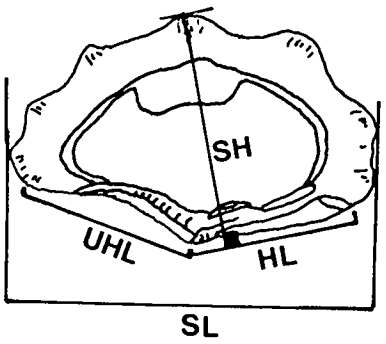
Figure 4.2 Shell with standard measurements indicated. SL = shell length, SH = shell height (measured at right angles to the hinge line), HL = hinge length and UHL = unhinge length.

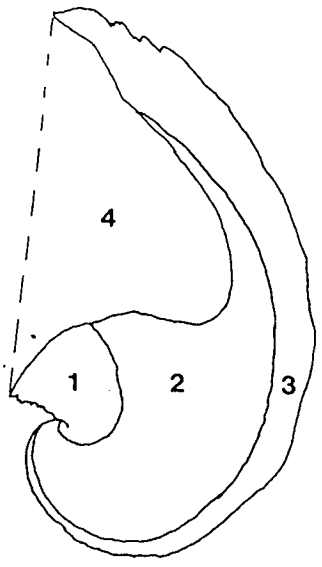
Figure 4.3 Shell sections of Hippopus hippopus showing parameters measured using the Bioquant Digitising Tablet.

Areas: 1 = umbonal, 2 = inner shell, 3 = outer shell, 4 = internal shell

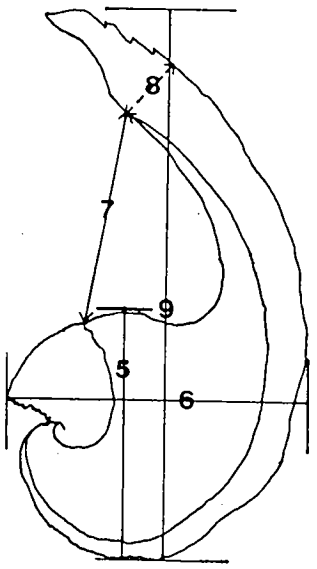
Lengths 1: 5 = umbonal height, 6 = section width, 7 = pallial to pallial length, 8 = shell thickness at end of pallial, 9 = section height

Lengths 2: 10 = pallial to pallial distance, 11 = pallial to outer end, 12 = maximum growth line (this line links asymptotic points of growth lines in the inner shell layer (see Fig. 3.2)), 13 = umbonal distance

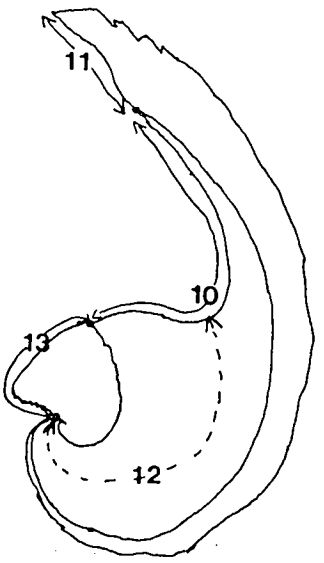




Areas



Lengths 1.



Lengths 2.

accurate ageing difficult.

4.4 ANALYTICAL METHODS

An important consideration for any shellfish candidate for commercial aquaculture is to assess the ratio of useful flesh to shell, and to see if this varies with location. Without killing a lot of giant clams, one way to examine this was to examine the relative proportions of the cross-sectional area of shell sections which would have been taken up by flesh, compared to that of shell, had the clam been alive. In this study cross-sectional areas of the inner shell layer were compared to the area which would have been occupied by the living animal (called internal shell area) (Fig 4.3).

To see what major differences occurred in the shell morphometry of H.hippopus with latitude, the ratios of shell length to shell height and internal area to inner shell layer area of sections were calculated. The allometric relationships of the form $y = ax^b$ between the maximum growth line (see Fig 4.3 for definition) and shell mass with shell length were examined by a linear correlation of logarithmic values. This allows values of the transformed allometric equation, $\log y = \log a + b \log x$, to be found.

To determine with what accuracy clam age could be determined by counting external growth lines, age (determined by examination of shell sections) was correlated against counts of external growth lines.

To describe the growth curves of the clams, age and length data were processed using the program Fishparm to estimate the von Bertalanffy parameters and to make comparisons of growth between different locations for one species and also between species. For H.hippopus, values of the growth index $\phi' = \log K + 2 \log_{10} L_{oo}$, which has been shown to be best index for several fish species (Moreau et al. 1986) were also calculated as a further aid to examining differences in growth beween sites.

4.5 RESULTS

4.5.1 Morphometry of Hippopus hippopus

There was little difference in the ratio of shell length to shell height for shells of H.hippopus from different locations (Table 4.1). The maximum difference in shell height between sites for a 300 mm long shell was less than 10 mm. The ratio of shell length to height for H.hippopus in the GBR region is therefore relatively constant at approximately 1.4 : 1.

Table 4.1 Mean values of the ratios shell length to shell height (sl/sh) and internal section area to inner shell area (int/inn) for Hippopus hippopus

Site	Hayman Is.	Central GBR	North GBR	Torres Straits
sl/sh				
x	1.40	1.43	1.47	1.41
sd	0.086	0.098	0.091	0.094
n	53	582	27	91
int/inn				
x	1.13	1.66	1.80	1.78
sd	0.312	1.014	0.353	0.660
n	26	107	8	48

The internal shell area and the inner shell area (Fig. 4.3) can be taken to represent the relative amount of flesh and shell respectively in a giant clam and used to examine changes in relative flesh yield between sites. The larger the value of the ratio, the more flesh for any given size of clam.

There was an apparent trend towards more flesh per given size of clam from south to north, with the Hayman Island sample having the lowest value and the top values in the Northern and Torres Strait samples (Table 4.1).

Table 4.2 Parameters for the relationship $y=ax^b$ for shell mass (y) against shell length (x) for Hippopus hippopus

Site	a	b	c.c	n
Hayman Is	5.14955	3.14056	0.954233	53
Central G.B.R.	3.59	2.78244	0.96226	581
North G.B.R.	1.29223	2.92129	0.961282	19
Torres Straits	2.41496	3.23623	0.979098	95

The relationship between shell length and shell mass closely followed an isometric growth curve with values of b (from the equation $y=ax^b$) approximately 3 (Table 4.2). There was a high correlation coefficient for the relationships from all four latitudinal areas.

Table 4.3 Values for the relationship between maximum growth line and shell length for the equation $y=ax^b$, where y=maxgl and x=sle of *Hippopus hippopus*

Site	a	b	c.c	n
Hayman Is.	7.081495	0.493302	0.26	32
Central G.B.R.	17.96883	1.31052	0.78	105
Torres Straits	83.34508	1.584	0.74	55

The line of maximum growth (Fig. 4.3) had an unexpectedly poor correlation with shell length of the form $y = a + bx$, but a slightly better correlation for the equation $y = ax^b$. The very poor correlation coefficient for the Hayman Island shells can be partially explained by the restricted range of the shells collected there. The lack of a good relationship between the line of maximum growth and shell length may be a result of the former's curvilinear direction in the inner shell layer.

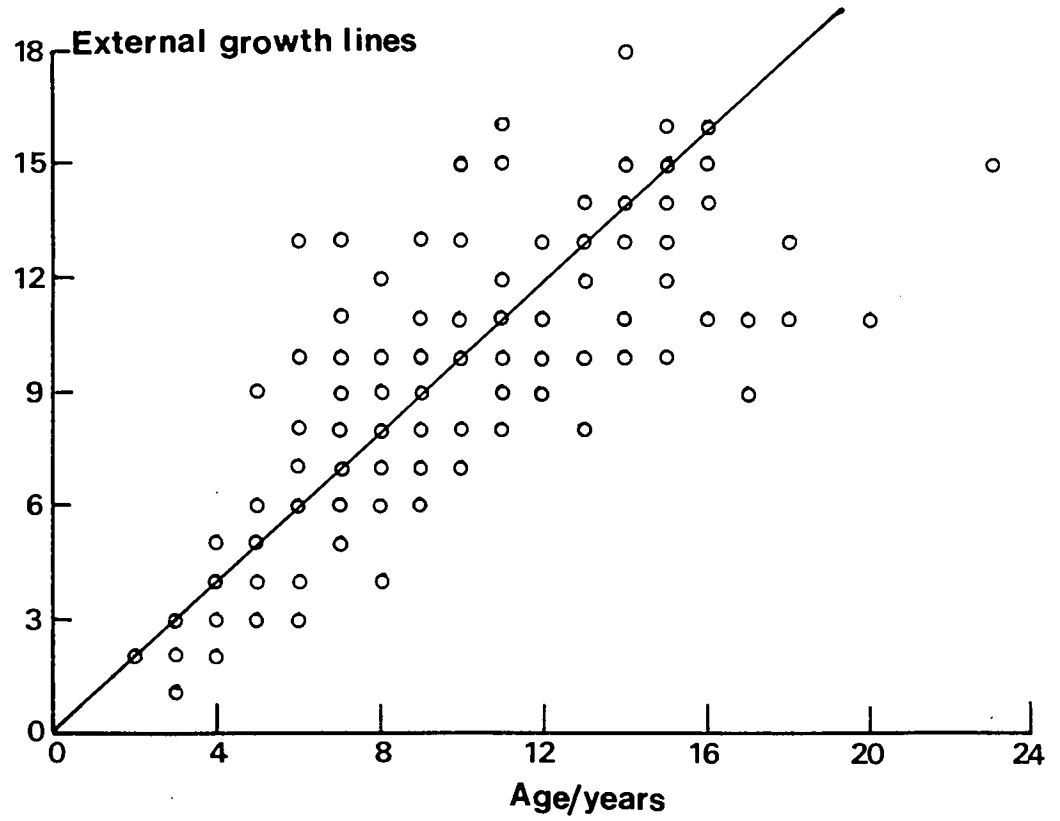
Table 4.4 Parameters for shell mass (y) to shell length (x) of the form $y= ax^b$, for *Tridacna* spp.

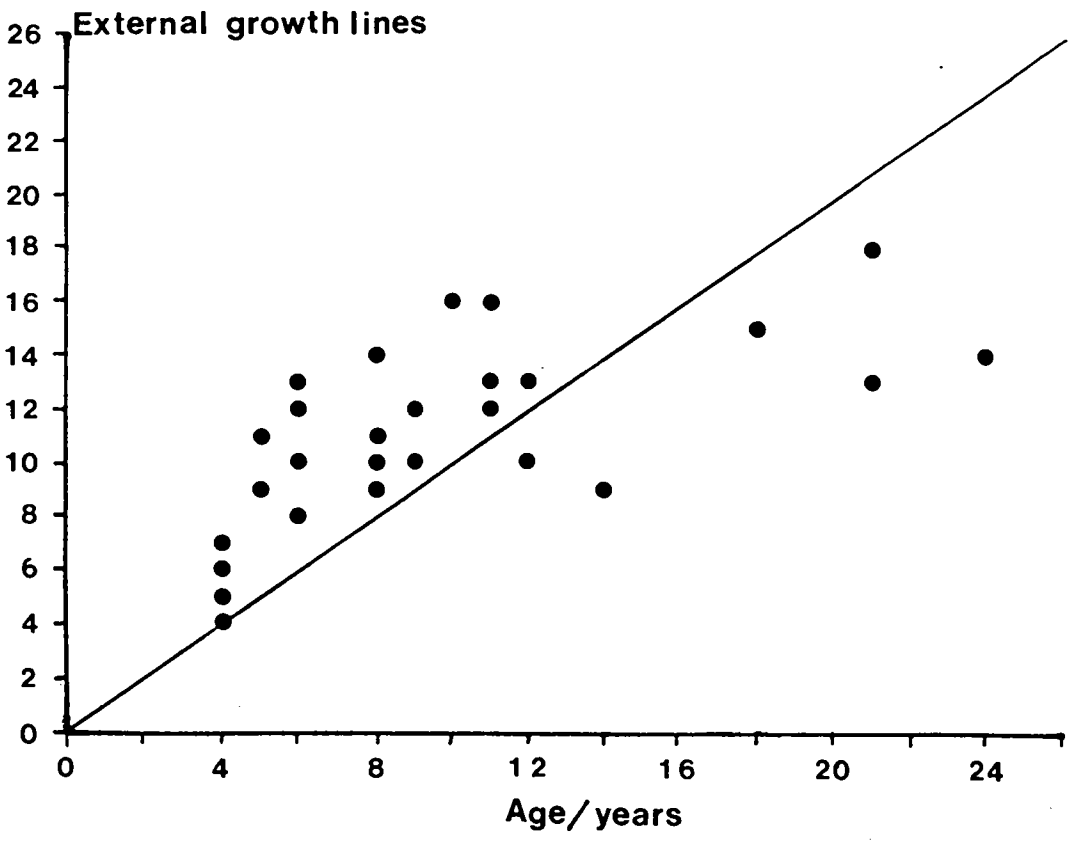
Species	a	b	c.c	n
T.gigas	1.43139	3.27923	0.979235	29
T.derasa	6.36253	2.71878	0.679056	54
T.squamosa	1.45941	3.74062	0.978805	33

Comparison of external growth lines to internal annual growth band couplets revealed that carefully read external growth bands can provide a reasonable estimate of the age of *H.hippopus* at certain sites (Fig. 4.4). The usefulness of the external growth lines is dependent on their clarity which appeared to differ between sites. Factors such as

Figure 4.4 External growth lines of Hippopus hippopus from Pioneer Bay, plotted against the age of the clam in years as determined by examination of the inner shell layer growth bands. The line on the graph marks a 1:1 correspondence.

Figure 4.5 External growth lines of Tridacna gigas from Pioneer Bay and Fantome Island, plotted against the age of the clam in years as determined by examination of the inner shell layer growth bands. The line on the graph marks a 1:1 correspondence.


External growth lines
18
15
12
9
6
3
0
0
4
8
12
16
20
24
Age/years


External growth lines
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
4
8
12
16
20
24
Age/years

algal, coral and other growths on shells outer surface are influential in field reading of external growth lines. One obvious use of ageing using external growth lines is that by comparison of age and size to local growth curves, animals of superior growth rate can be picked for use as broodstock to enhance maricultural operations.

Other parameters of shells measured (Fig. 4.3) had very poor correlations with shell length or mass, and showed no geographic trends.

4.5.2 Morphometry of *Tridacna* species

The relationship between shell length and mass was also examined for *T. gigas*, *T. derasa* and *T. squamosa*, treating all clams collected from the G.B.R. as members of one population for the purpose of this calculation. Whilst length to mass calculations for *T. gigas* and *T. squamosa* showed good correlation coefficients, that of *T. derasa* was poor (Table 4.4).

Most *T. derasa* shells collected had no obvious external growth bands. One reason for this is that, of all the shells collected, *T. derasa* seemed the most prone to extensive boring action in the outer shell layer. External growth lines and scute formation on the outer surface of *T. squamosa* were difficult to differentiate between, and plotting external growth bands against age gave no recognisable relationship. However *T. gigas* generally had quite clear external growth lines. There appeared to be a tendency to overestimate the younger and to underestimate

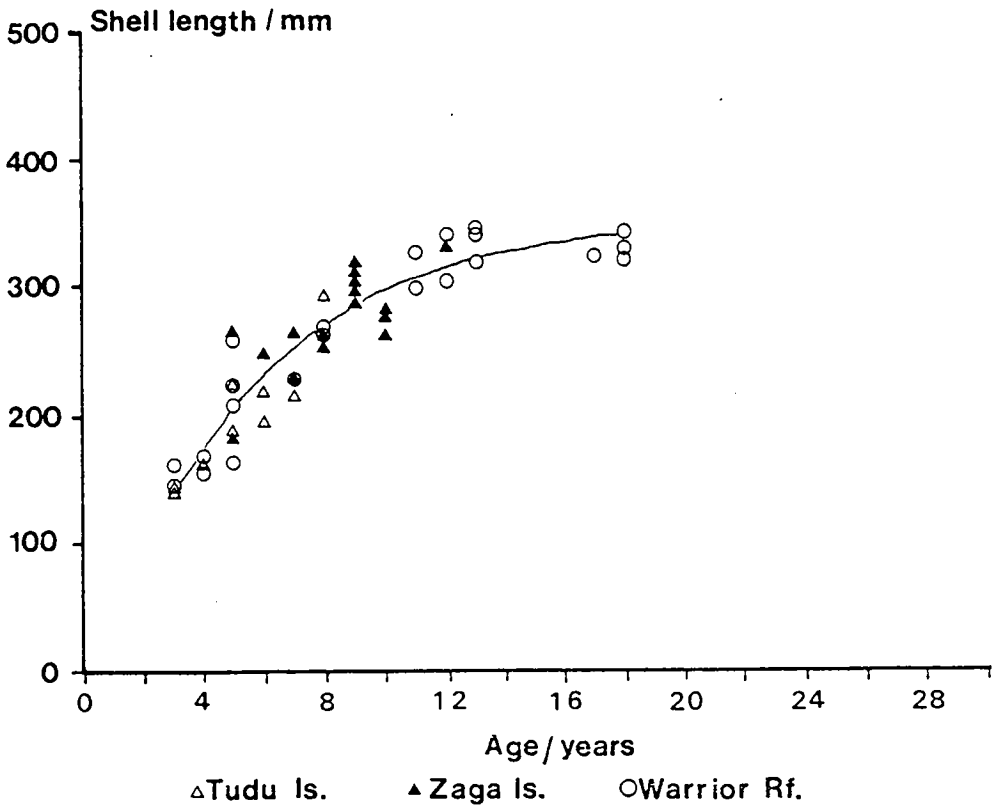
the age of older clams by examination of external growth lines (Fig. 4.5 (p.94)).

4.5.3 Growth of Hippopus hippopus

Examination of changes in growth (determined by examination of internal seasonal bands), based on the von Bertalanffy parameters, of H.hippopus with latitude revealed no general north - south trends (Table 4.5). Whilst shells from Hayman Island (the most southerly site at which H.hippopus was located in this study) had the lowest asymptotic length (L_{oo}) of 279.1 mm, the next most northerly area (Central G.B.R.) had the highest value of 422.3 mm. Similarly values of the growth coefficient, K, varied considerably between sites, but without a latitudinal trend. High values of $K > 0.3$, from shells from Hayman Island and the Northern G.B.R. may be a result of the predomiantly old shells in the case of Hayman Island; or the low sample size in the death assemblage for the Northern G.B.R.; the low sample size, and for both sites a restricted size range. A plot of shell length against age (Fig. 4.7) illustrates the noticeable difference between Hayman Island and other sites where H.hippopus was collected, and may well reflect the species growth at its southern most limit of distribution. Examination of mid-shelf reefs from south of the Whitsunday Island group to the southern end of the G.B.R. found no specimens of H.hippopus.

Figure 4.6 Plot of shell length against age for Hippopus hippopus from the Torres Straits. The von Bertalanaffy curve for the combined data set is shown.

Figure 4.7 Plot of shell length against age for Hippopus hippopus from Northern, Outer Reef and Hayman Island sites. Results for a combined Central section data set are plotted for comparison.


Shell length / mm
500
400
300
200
100
0
0
4
8
12
16
20
24
28
Age / years
△Tudu Is.
▲Zaga Is.
○Warrior Rf.

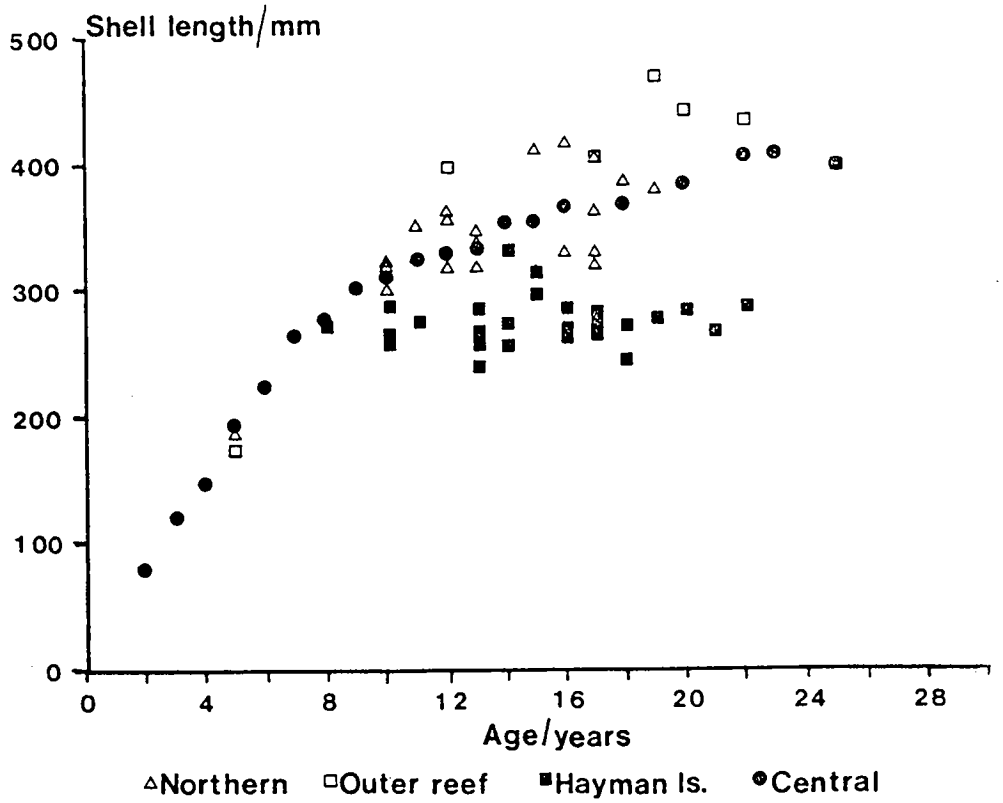

Shell length/mm
500
400
300
200
100
0
0
4
8
12
16
20
24
28
Age/years
△Northern
□Outer reef
■Hayman Is.
●Central

Table 4.5 Parameters of the von Bertalanffy equation and the growth index ϕ' for Hippopus hippopus. Table is arranged so that the most northerly site is found at the top of the table.

Location	L_{oo}	K	to	ϕ'	n
Warrior Reef	350.7	.19	.38	2.39	23
Tudu Island *	1108	.02	-1.76	2.54	8
Zagai Island *	624	.03	-6.29	2.18	18
Combined Torres Straits sample	355.6	.19	.39	2.38	59
Northern GBR (Cairns)	373.7	.24	2.13	2.54	20
Iris Point Rf.	347.8	.22	1.08	2.43	201
Pioneer Bay	412.3	.13	-.71	2.37	52
Outer reefs (Central)*	433.4	.29	3.28	2.74	7
Combined Central Section GBR sample	422.3	.13	.53	2.39	279
Hayman Island *	279.1	.37	-.02	2.46	30

* indicates that either sample size very small or size range of shells age restricted. The implications for von Bertalanffy calculations for either of these cases is discussed in the text.

Differences in growth of H. hippopus between sites of close proximity, were examined in the Torres Strait and Central G.B.R..

Values of Loo and K from the three Torres Straits sites, Warrior Reef, Tudu Island and Zagai Island varied considerably (Table 4.5). The extraordinary high values of Loo from Zagai and Tudu Islands reflect the restricted size range of shells found at these sites, which can badly bias von Bertalanffy calculations, making comparisons of parameter values between such sites of doubtful value. Combining data from all three Torres Straits sites (Fig. 4.6(p.97)), it appears that the clams from different sites are at different points along the same growth curve which

is estimated in Table 4.5 (Torres Straits combined), showing that there was little difference in growth between the sites. Although the sites were apparently quite different, the Warrior Reef shells being collected from the westerly back-reef area, Tudu Island shells collected from the islands large reef flat and Zagai Island shells collected from its unusually raised lagoon, they all experienced a relatively sheltered environment.

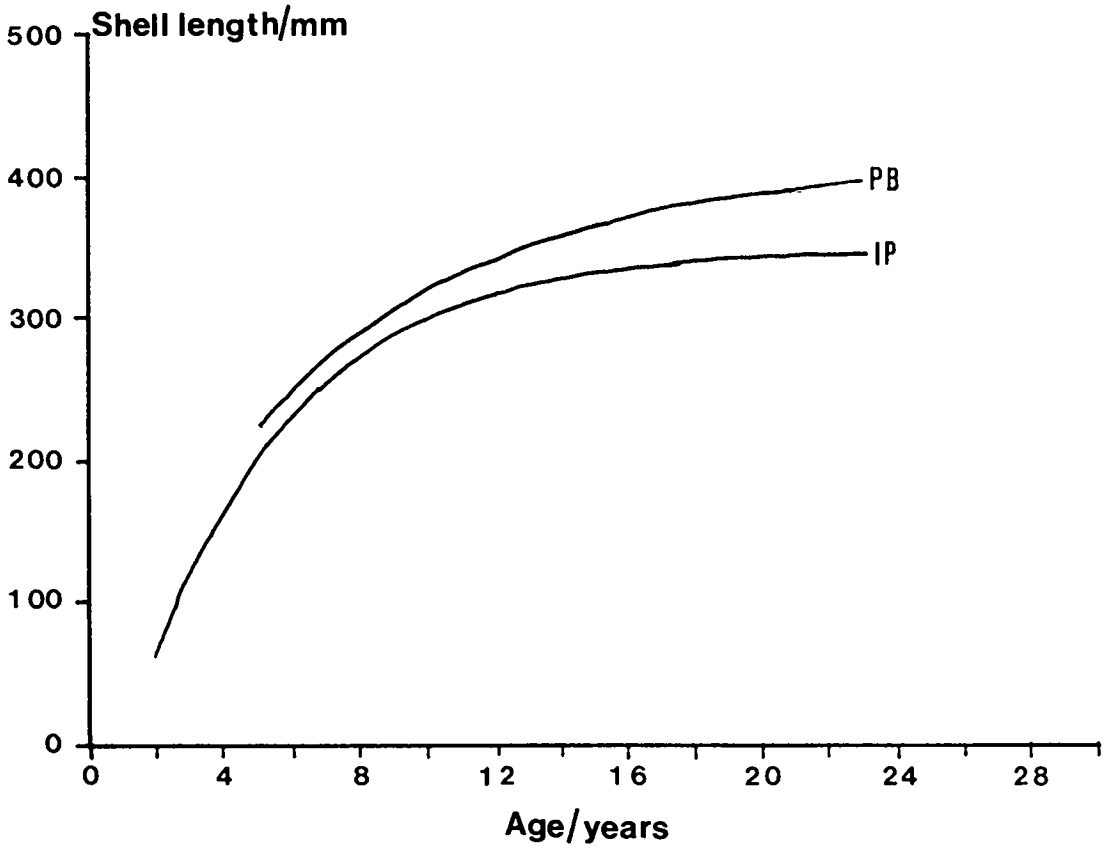
There was a difference of 65 mm in the L_{oo} of H.hippopus at the two Opheus Island reef sites, Iris Point Reef and Pioneer Bay (Table 4.5). The von Bertalanffy growth curves for H.hippopus from Iris Point and Pioneer Bay calculated from 201 and 52 shells, respectively, are shown in Figure 4.8. Differences between these curves may reflect that where physical and environmental variation is high between sites of close proximity, as was the case for the two Orpheus Island sites, this can be reflected in real differences in growth rate.

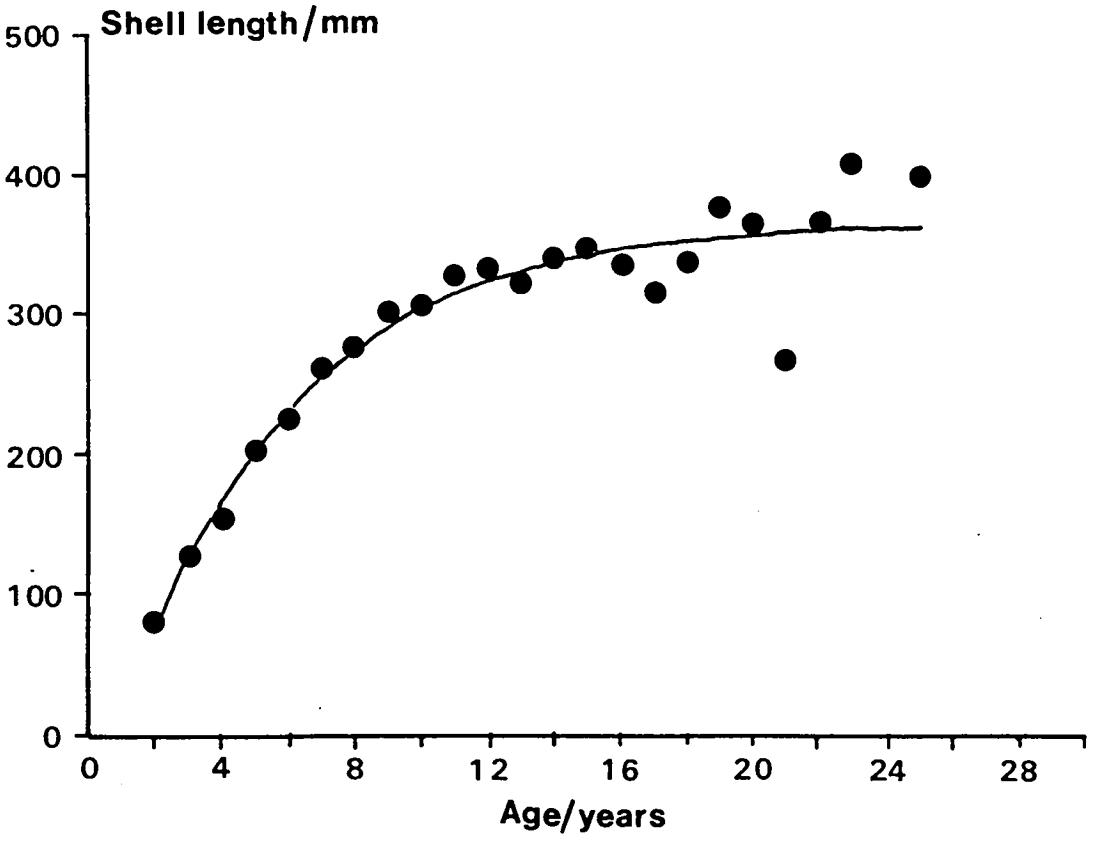
For comparative purposes with overseas studies, all age-length data for the G.B.R. were combined as if the shells were all from one population (Fig. 4.9).

Examination of the growth index ϕ' as a further guide to differences in growth performance of H.hippopus between sites (Table 4.5) did not show any clear trends with latitude.

Figure 4.8 Plots of the von Bertalanaffy curves for Hippopus hippopus from Pioneer Bay and Iris Point reef.

Figure 4.9 Plot of shell length against age for all Hippopus hippopus aged from the Great Barrier reef, including the the von Bertalanaffy curve for the data set.


Shell length/mm
500
400
300
200
100
0
PB
IP
0
4
8
12
16
20
24
28
Age/years


Shell length/mm
500
400
300
200
100
0
0
4
8
12
16
20
24
28
Age/years

4.5.4 Growth of *Tridacna gigas*

The large value of L_{oo} found for *T. gigas* in the Central G.B.R. (Table 4.6) is in part a result of one of the largest giant clams on record being included in the sample. One valve collected from Myrmidon Reef (using first a lifting bag and then a winch onto the research vessel), weighed 215 kg, was 1056 mm in length and was estimated to be 63 years old. The implications of the mass of one valve of a giant clam being 215 kg are that the total shell weight, combined with the flesh weight of the clam put the potential total (shell + tissue) weight of a large giant clam at approximately half a tonne. Estimates of the von Bertalanaffy curve for the same data set excluding the extremely large clam are also included in Table 4.6 and depicted in Figure 4.10.

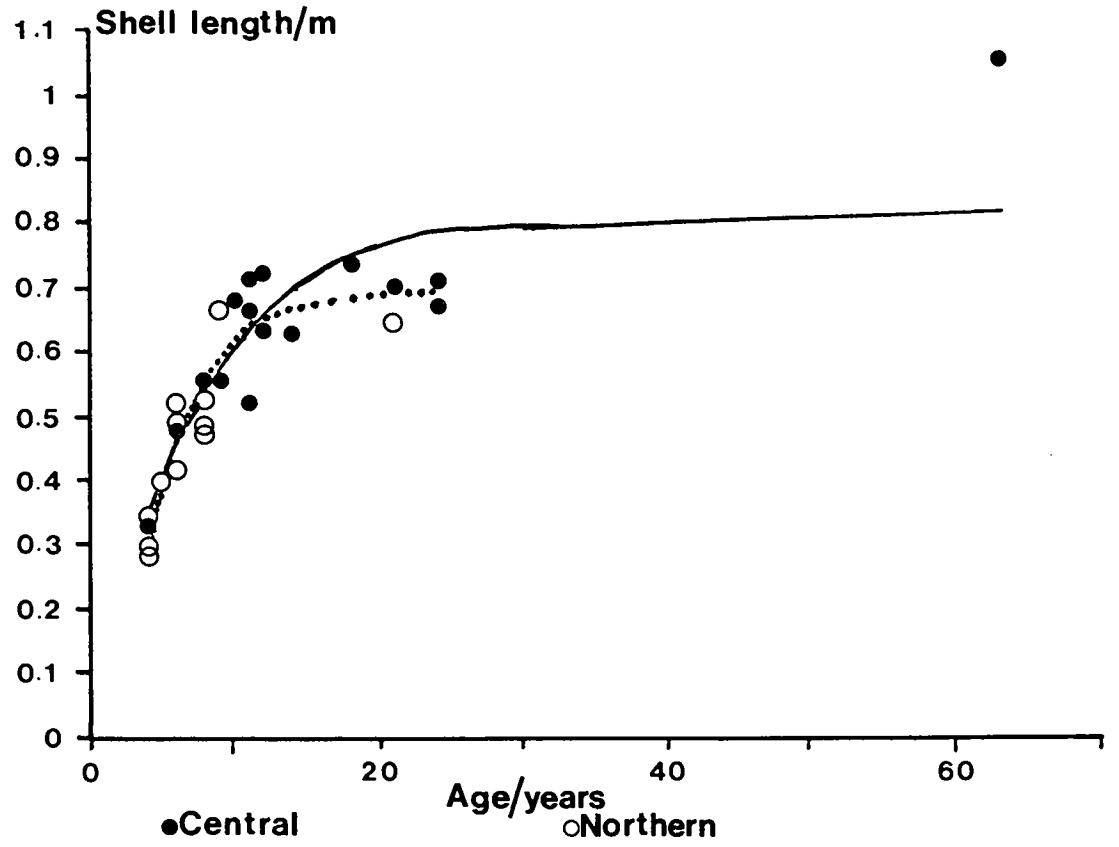
Table 4.6 Parameters for the von Bertalanffy growth equation for *Tridacna gigas*.

Location	Loo	K	to	n
Central GBR	1109	.33	-.12	16
Northern GBR	661.8	.29	1.80	19
Combined GBR sample	819.7	.12	-.29	35
Combined GBR sample, excluding 63 yr old	699.2	.25	1.6	34

The shells of the Northern G.B.R. were collected predominantly from the Michaelmas Cay reef, and as such results can be compared directly to those of Munro (1988) and Pearson and Munro (in press), who measured clams at this site repeatedly over several years. The large difference in parameters of the von Bertalanffy curves (Table 4.6) calculated for the 2 sites were in part a

Figure 4.10 Plot of shell length against age for Tridacna gigas for clams from the central and northern (Cairns) sections of the Great Barrier Reef. The solid line marks the von Bertalanaffy curve for the combined data set, whilst the dotted line marks the curve for the same data set minus the exceptionally large 63 year old clam.

Figure 4.11 Plot of shell length against age for Tridacna derasa for clams from the central and northern (Cairns) section of the Great Barrier Reef. The solid line is the von Bertalanaffy curve for the combined data set.


Shell length/m
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
Age/years
●Central
○Northern

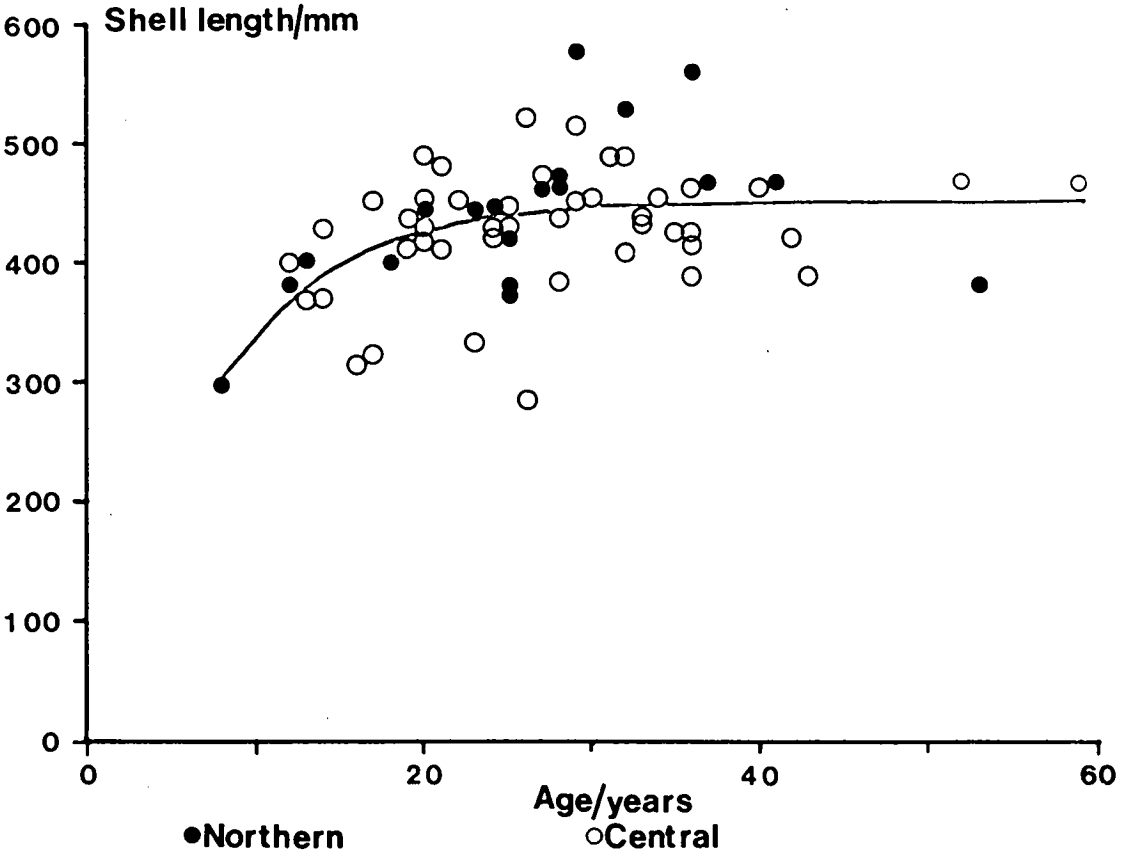
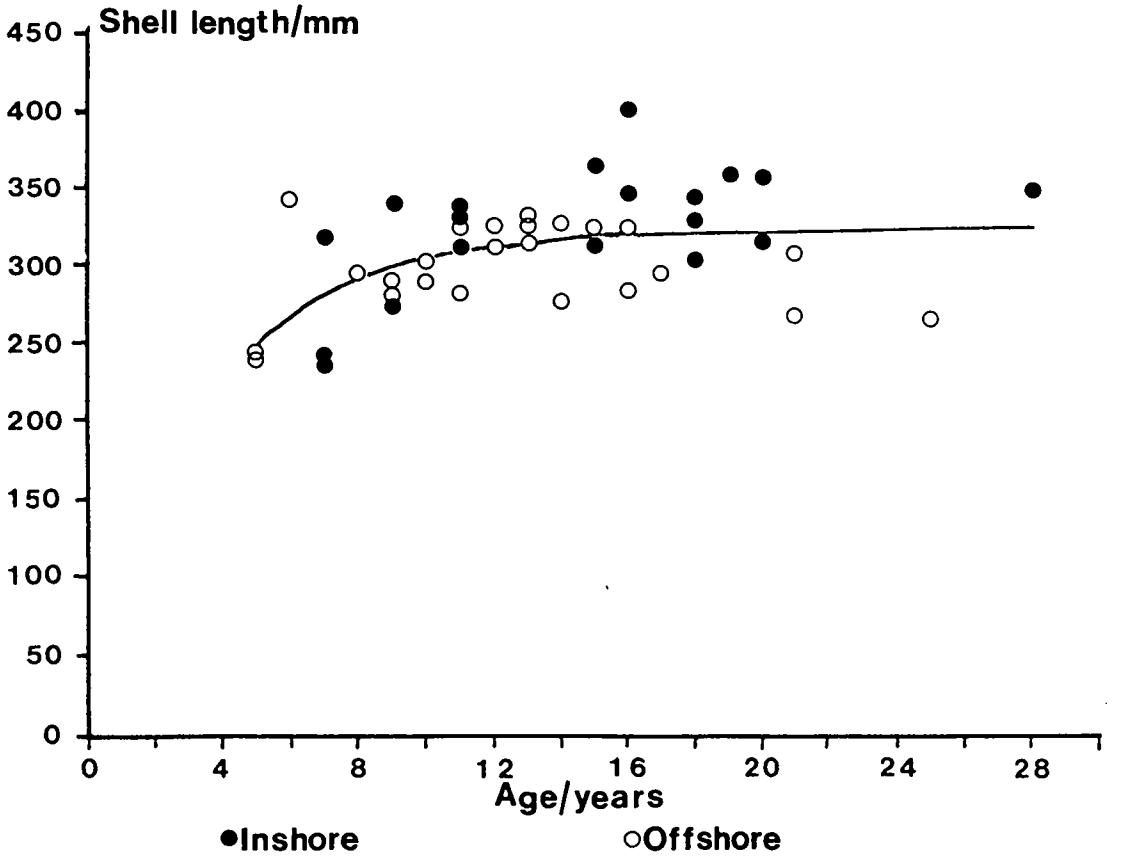

Shell length/mm
600
500
400
300
200
100
0
0
20
40
60
Age/years
●Northern
○Central

Fig. 4.12??


Shell length/mm
450
400
350
300
250
200
150
100
50
0
0
4
8
12
16
20
24
28
Age/years
●Inshore
○Offshore

result of the small sample sizes. Combining the results of the clams from the two sites, an intermediate value of Loo is obtained (Table 4.6)(Fig. 4.10) which may be more representative of average growth values for T.gigas from the Great Barrier Reef.

4.5.5 Growth of Tridacna derasa

The von Bertalanffy parameters for Tridacna derasa (Table 4.7) show there to be little difference in growth between the two groups of clams. On the GBR T.derasa only occurs in numbers on mid- or outer- shelf reefs, where environmental conditions such as water turbidity are very similar. Therefore, closely comparable growth rates were expected for the two groups of clams examined. Combining the data for the two groups of clams, yielded a von Bertalanffy curve (Fig 4.11), the parameters of which are given in Table 4.7. This represents an estimate of the average growth rate of T.derasa for the G.B.R..

Table 4.7 Parameters for the von Bertalanffy growth equation for Tridacna derasa

Location	Loo	K	to	n
Central GBR	448.9	.14	-.65	48
Northern GBR	480.1	.11	-.89	20
Combined GBR sample	456.8	.14	.4	68

4.5.6 Growth of Tridacna squamosa

Comparison of the growth of T.squamosa on inshore reefs, with those on offshore reefs suggests that conditions may be more favourable in inshore reefs as von Bertalanffy curves had higher K and L_{oo} values (Table 4.8). The von

Bertalanffy growth curve from a combined data set for T. squamosa are shown in Figure 4.12. As for some H. hippopus sites, the size range of T. squamosa was limited and therefore the accuracy of the von Bertalanffy estimates are questionable, especially as sample sizes were also small.

4.5.7 Growth of Tridacna maxima

Shells of T. maxima collected for growth analyses were in general riddled with marine borers or badly eroded. The shells were therefore of no practical value for aging studies, as growth lines were generally not well defined.

4.6 DISCUSSION

The values of the von Bertalanffy equation for Pioneer Bay and Iris Point Reef (North Orpheus) from sclerochronological age determination and from mark - remeasure results (Chapter 2) are very similar, especially when one examines the results of the Fabens (1965) estimates for all data triplets (Table 2.3). For values of L_{oo} there is only a 2 mm difference between the two techniques for Pioneer Bay, whilst the L_{oo} values for Iris Point Reef were identical (to the nearest mm). The estimated values of K showed slightly larger differences, with the Pioneer Bay mark - remeasure value of 0.155 compared to sclerochronological estimate of 0.139. A similar comparison of K values for Iris Point shows K values of .205 and 0.221, respectively. This agreement between the two techniques provides further support to the

Table 4.8 Parameters for the von Bertalanffy growth equation for *Tridacna squamosa*

Location	L_{oo}	K	to	n
Inshore Reefs	344.6	.39	3.2	19
Offshore Reefs	302	.11	4.8	24
Combined sample	324.1	.28	-.15	43

MISSING. - see page before No 103.

Figure 4.12 Plot of shell length against age for Tridacna squamosa from inshore and offshore sites on the Great Barrier Reef. The solid line indicates the von Bertalanaffy curve for the combined data set.

usefulness of sclerochronology which can reveal growth performance without time consuming remeasuring of shells over a protracted time period, as long as there is a reasonable number of shells in the death assemblage at a site covering the full size range of the species.

Results of growth analysis presented here, together with the recent work of Pearson and Munro (in press) provide baseline data of natural growth rates for tridacnid clams from the GBR, against which aquaculture operations can assess their growth rates. These growth data, combined with the relationships of shell length to shell mass, are useful for projecting potential flesh yields when utilised together with ratios of flesh mass to shell length or shell mass.

The attempt to measure the flesh to shell ratio of clams from different locations by examining the internal area / inner shell area of a shell section (Table 4.1), showed a trend of more flesh to shell, the further north the site was. This would be significant to aquaculture operations looking for new farm sites especially if growth rates in terms of shell length were constant throughout the G.B.R..

The usefulness of external growth lines for ageing of both T.gigas and H.hippopus, and so being able to identify rapidly growing individuals should not be underestimated in its potential importance to maricultural operations. If clam farms had to wait 4 - 5 years, the time to sexual hermaphroditic maturity, before selection of rapid growers as broodstock in order to improve growth rates,

they would be sacrificing several years of increased growth which would presumably result from deliberately crossing rapid growers. Munro (1988) elegantly calculated the advantages of culling slow growing individuals in giant clam aquaculture. Combine this technique together with selective breeding for growth and increases in potential yields of clam farms should be greatly increased.

Growth estimates of T.gigas for all the shells examined gave a value of L_{oo} of 819 mm. This value is similar to L_{oo} estimates of 813 mm for clams from Pioneer Bay, and 862 mm for those from Fantome Island, based on mark-remeasure data (Chapter 2), and are close to Munro's (1988) estimate of 800 mm from Michaelmas Cay. The importance of obtaining as large a sample as possible for growth estimates is highlighted by the differences in L_{oo} and K values found from a limited number of shells (19) of T.gigas from Michaelmas Cay (Table 4.6, Northern G.B.R.), compared to the values from Pearson and Munro's (in press) large sample from the same location. The estimate of K for T.gigas from the total shell sample (K = 0.129) was slightly lower than estimates obtained from mark-remeasure trials (range of K 0.132 - 0.174), and that of the best individuals at Michaelmas Cay (Munro 1988)(K = 0.15).

The estimates on the growth of T.squamosa detailed in this study show differences between von Bertalanffy parameters based on sclerochronology and on mark - remeasure data. However the values of Loo and K for the inshore sample

(L_{oo} = 344.6, K =0.3957) are not that different to those obtained for the inshore Fantome Island (L_{oo} = 354.4, K = 0.250) using the mark-remeasure technique.

Results of the sclerochronological examination of T.derasa from the northern G.B.R. (L_{oo} = 480.1, K = 0.1129) are very close to values obtained from Michaelmas Cay (a reef in the northern G.B.R.)(Munro & Pearson in press)(L_{oo} = 469.0, K = 0.108), adding more weight to the value of sclerochronological work.

One area in which sclerochronological results were not as useful as had been hoped, was in the comparisons of shell length with the line of maximum growth. It had been hoped to use measurements along the maximum growth line between seasonal growth bands, as scales or otoliths are used for fish. However it became apparent that not only was there a poor relationship between the two parameters amongst the population (Table 4.3), but also that the values across the maximum growth line could vary with the exact angle of cut of a shell section, which was very difficult to control. Therefore no attempts were made at using back - calculation techniques.

Table 4.9 is an expansion of Munro & Heslinga's (1983) summary, the results of Adams et al. (1988), Munro (1988), Ratke (MS), Villanoy et al. (1988) and this study being the new additions.

Table 4.9 Summary of published growth estimates for adult tridacnid clams from different locations, including largest sizes L_{max} (cm shell length), recorded in various areas and estimates of the von Bertalanffy growth function. $L_{(oo)}$ is an estimate of asymptotic size, L_{oo}, based upon maximum size data. This table is an expanded version of Table 3 from Munro & Heslinga's (1983) work. See the key at the end of the table for reference numbers

Tridacna gigas

L_{max}	= 137 cm	1 Sumatra
	= 101-110 cm	2 Helen Reef
	= 111-120 cm	2 Palau
	= 94 cm	3 PNG
$L_{(oo)}$	= 100 cm K = 0.087 $\pm$ 0.007	3 PNG
	= 100 cm K = 0.136	4 Palau
L_{oo}	= 93 cm K = 0.13	9 Philippines
	= 80.0 cm K = 0.145	5 Michaelmas Reef
	= 81.3 cm K = 0.148	6 Pioneer Bay GBR
	= 86.2 cm K = 0.132	6 Fantome Is. GBR
	= 69.9 cm K = 0.25	6 Combined GBR

Tridacna derasa

L_{max}	= 51.4 cm	1 ?
	= 52.5 cm	7 Tonga
$L_{(oo)}$	= 50 cm K = 0.167	4 Palau
	= 50 cm K = 0.136	4 Tonga
L_{oo}	= 46.9 cm K = 0.108	5 Michaelmas Reef
	= 47.3 cm K = 0.134	8 Fiji
	= 44.8 cm K = 0.14	6 Central GBR
	= 48.0 cm K = 0.11	6 Northern GBR
	= 45.6 cm K = 0.14	6 Combined GBR

Table 4.9 (continued)

Tridacna squamosa

L_{max}	= 40.8 cm	1 Thailand
	= 38.5 cm	3 PNG
	= 37.2 cm	5 Tonga
$L_{(oo)}$	= 40 cm K = 0.091	4 Palau
	= 40 cm K = 0.187	4 Tonga
	= 38.5 cm K = 0.140 $\pm$ 0.021	3 PNG
L_{oo}	= 30.0 cm K = 0.23	9 Philippines
	= 32.4 cm K = 0.28	6 GBR
	= 38.4 cm K = 0.189	6 Fantome Is. GBR

Tridacna maxima

L_{max}	= 35.3 cm	1 Philippines
	= 25.0 cm	10 Queensland
	= 30.5 cm	3 PNG
	= 30.1 cm	4 Tonga
	= 23.5 cm	11 American Samoa
	= 17.0 cm	12 French Polynesia
$L_{(oo)}$	= 30.5 cm K = 0.082	4 Tonga
	= 30.5 cm K = 0.112 $\pm$ 0.032	3 PNG
	= 27.5 cm K = 0.074	4 Queensland
L_{oo}	= 12.4 cm K = 0.26	12 French Polynesia
	= 29.4 cm K = 0.065	13 American Samoa
	= 27.1 cm K = 0.126	6 Fantome Is.

Hippopus hippopus

L_{max}	= 33.7 cm	1 Philippines
	= 39.7 cm	1 Queensland
	= 33.0 cm	14 Queensland
	= 40.0 cm	3 Milne Bay, PNG
	= 33.0 cm	3 Motupore Is., PNG
	= 47.9 cm	7 Tonga
$L_{(oo)}$	= 40 cm K = 0.213	3 Motupore Is., PNG
	= 40 cm K = 0.1 $\pm$ 0.013	4 Palau

Table 4.9 (continued)

H. hippopus (contd)		
L_{oo} = 43.7 cm K = 0.13		9 Philippines
= 34.7 cm K = 0.20		6 Iris Point Reef(a)
= 34.7 cm K = 0.22		6 Iris Point Reef(b)
= 41.4 cm K = 0.15		6 Pioneer Bay(a)
= 41.2 cm K = 0.13		6 Pioneer Bay(b)
= 35.5 cm K = 0.19		6 Torres Straits
= 37.3 cm K = 0.24		6 Cairns Region, GBR
= 42.2 cm K = 0.13		6 Central, GBR
= 27.9 cm K = 0.37		6 Hayman Island
Hippopus porcellanus		
L_{oo} = 43.6 cm K = 0.13		9 Philippines

Key to Table 4.9
Numbers correspond to following references:
1 Rosewater (1965), 2 Motoda (1938), 3 Munro & Gwyther (MS), 4 Munro & Heslinga (1983), 5 Munro (1988), 6 This thesis, 7 McKoy (1980), 8 Adams et al. (1988), 9 Villanoy et al. (1988), 10 McMichael (1975), 11 Wass (n.d.), 12 Richard (1978), 13 Ratke (MS), 14 Stephenson (1934)
For *Hippopus hippopus*:
(a) Values derived from marked and remeasure tagged clams
(b) Values derived from shells from death assemblage

PNG = Papua New Guinea

The wide variation in individual growth rates that can be found in *Tridacna gigas* within a particular region is evident when one compares values of L_{oo} from the GBR (69.9-86.2 cm) (Table 4.9) with the value of L_{max} from the GBR (105 cm). Values of K for *T. gigas* also ranged from 0.087 to 0.25. These results again highlight the potential to improve growth rates of cultured *T. gigas* by careful site selection and selective breeding.

Comparing values of L_{oo}, and K in Table 4.9 differences within the GBR are often larger than differences between countries, suggesting locality rather than gross geographic differences in growth can be more important.

CHAPTER 5

THE REPRODUCTIVE CYCLE OF *HIPPOPUS HIPPOPUS*

5.1. INTRODUCTION

The only previous study of the reproductive cycle of *Hippopus hippopus* was undertaken by Stephenson (1934) on the Low Isles of the Great Barrier Reef. Her study indicated that the spawning season was between January and March, the latter half of the Austral summer.

Braley (1984,1986) used a biopsy technique to sample gonad condition in *Tridacna gigas* and *T.derasa* throughout the year and found that peak spawning activity in the G.B.R. region was in the Austral summer. The results of these studies have been confirmed in part by the first histological study of the reproductive season in *T.gigas* (Nash et al. 1988). All of the recent studies of aspects of the reproduction of giant clams, including an electron microscopical study of the gonad of *T.crocea* (Kawaguti et al. 1982), have been a direct consequence of the interest in their commercial production.

Interactions between growth of the shell and somatic tissues of clams, and the processes of maturation and cyclic reproduction need to be examined for the process of growth in giant clams to be more fully understood. This chapter details several different methods used to determine the annual reproductive cycle of *H.hippopus* at

Orpheus Island, the sizes at which male and hermaphroditic sexual maturity occurs, fluctuations in organ morphometry related to the sexual cycle, and the first anatomical and histological description of the gonad of this species.

5.2. METHODS

5.2.1. Constraints on sampling

Small sample sizes were used in the histological and gonad index studies because the natural populations of large mature H. hippopus were limited at the study site. The use of a variety of techniques to maximise the value of the available resources was the approach adopted.

5.2.2 Morphometry

Three or four clams were killed each month. Each clam was dissected into its components (mantle, gonad, kidney, digestive gland, adductor muscle, heart, pericardium, and renal sphincter) and their wet mass recorded. Shell length and weight were also recorded. Each organ was then dried at 60°C to a constant mass . Dried samples were then ashed in a muffle furnace at 500°C for 6 hrs to determine the ash free dry mass (AFDM) of the organs. Indices which reflect the mean monthly contribution of an organ to the animal's total mass (either wet, dry or AFDM) were calculated from these measurements. The indices were calculated as follows:

$$\text{Index} = \frac{\text{mass of organ}}{\text{total mass of clam tissue}} \times 100$$

where the mass of organ and total tissue measured were either wet, dry or ash free.

Notes were recorded on the gross anatomy and condition of the gonad during dissection of the clams.

5.2.3 Histology

From all dissected clams, a subsample of approximately half of each gonad was taken after the wet mass of the total organ had been recorded. This piece of gonad was fixed in 10% (V/V) formal saline solution. Each gonad was sliced regularly at right angles to its greatest length and injected with the fixative to ensure adequate absorption of the fixative into this large organ. Six tissue blocks were cut from each half gonad approximately equidistantly along its length. Each block was stored in 70% alcohol. Tissue blocks were processed to paraffin wax, sectioned at 6 um, and stained by haematoxylin and eosin (Winsor 1984). The slides produced were examined to determine the reproductive status of each gonad and to determine whether gametogenesis was synchronous in different parts of the gonad.

When examining the gonad sections the following notes were recorded for each section:-

1. For male portions of the gonad:

(a) The relative abundance of the different stages of sperm development. Three stages were distinguished: stage 1. round cell, chromatin not very condensed

(spermatogonium); stage 2. smaller round cell, chromatin condensed (spermatid); stage 3. flagellated spermatozoa.

(b) Description of the lumen structure.

2. For female portions of the gonad:

(a) The colour of eggs as stained by haematoxylin and eosin.

(b) The size, shape and structure of the eggs.

(c) Description of the ascinii and the follicle structure.

3. General observations on:

(a) The amount of inter-follicular, inter-ascinii material

(b) The size of channels between ascinii.

(c) The relative cross-sectional area of the male and female components.

Gonad development has frequently been recorded by bivalve researchers by assigning a stage of development to a histological section. Nash et al. (1988) assigned stages to Tridacna gigas gonad sections, and for comparative purposes the same stages and gonad index score were used in this study (Appendix 5.1).

5.2.4 Biopsy

The biopsy technique used to sample H.hippopus is described in Shelley and Reid (1988), a copy of which forms Appendix 5.2. The gonad of each clam dissected for histological study was also biopsied so that a histological sample and a biopsy sample of the same clam could be compared. Smears were prepared from these biopsy samples as described in Winsor (1984). These smears were

stained using Geimsa stain (Winsor 1984), so that nuclei, eosinophils, basiphils, neutrophils and cytoplasm could be easily distinguished. In addition, a group of clams (initially 39 in number) was biopsied monthly over a 15 month period (March 1986 - June 1987) to follow the sexual development of some individual clams. The clams that were regularly biopsied were kept in a group on the fringing reef in Pioneer Bay (Fig. 2.3). The diameters of 30 oocytes were recorded from each sample (or the diameters of all oocytes present, if less than 30) using an eyepiece micrometer, and the mean value recorded.

5.2.5 Size at sexual maturity

Small, presumably young, clams were collected from Iris Point Reef (Fig. 2.2) to determine the sizes at sexual maturity of both the male and hermaphroditic phase of Hippopus hippopus. The size range of clams collected was from 77 to over 200 mm in shell length. Each clam was dissected, its organs weighed and one histological sample prepared from each gonad as detailed in 5.2.3. Clams were collected in December 1986 and March 1987 for this study. In addition to examining the development of the gonad with size, photocopies of longitudinal sections through the entire visceral mass were made, from which drawings of the anatomical developmental stages of the gonad in relation to the digestive gland and foot were made.

5.3 RESULTS

5.3.1 Seasonal changes in organ indices of Hippopus hippopus

5.3.1.1 Gonad Indices

Changes in the gonad index (dry mass) of H.hippopus have been described in Shelley and Southgate (1988) (Appendix 5.3). Whilst both the wet and dry gonad index (Fig. 5.1) peaked in September 1986, the AFDM gonad index (Fig. 5.2) peaked in October 1986. All three gonad indices fell to minimum values in the first quarter of 1987.

5.3.1.2. Adductor Indices

No clear pattern was seen in the adductor indices (Figs. 5.1 & 5.2)

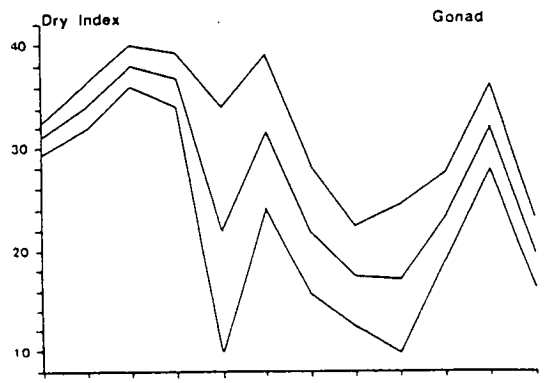
5.3.1.3. Digestive Gland Indices

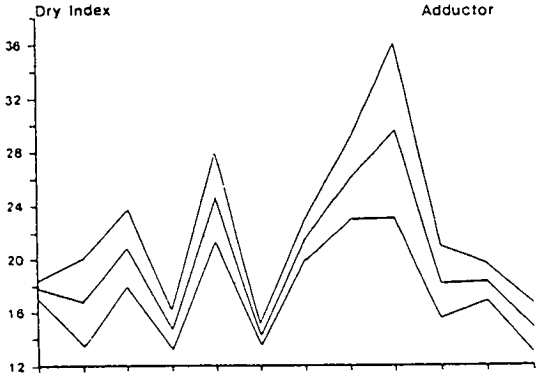
No periodicity of an annual nature is shown by either the wet, dry or AFDM digestive gland indices (Figs. 5.1 & 5.2).

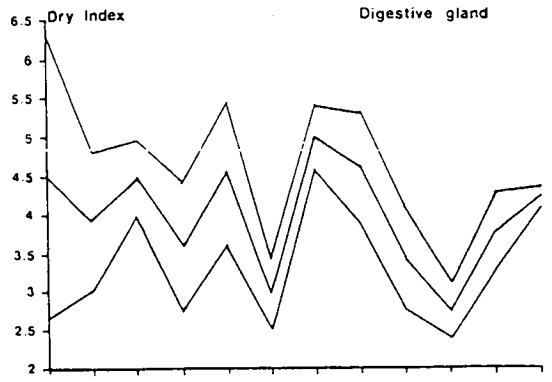
5.3.1.4. Kidney Indices

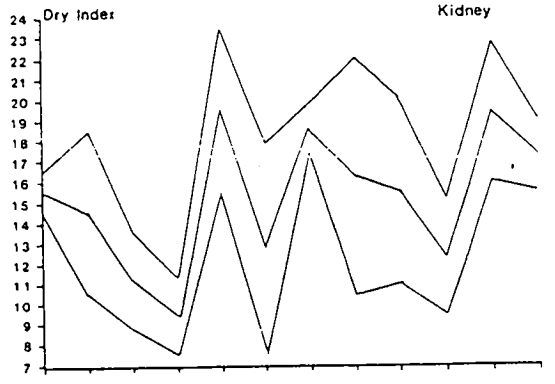
Whilst no gradual annual periodic cycles are detectable in any kidney indices, all show a doubling; from October to November 1986 for the dry index (Fig. 5.1), from September to November for the AFDM index (Fig. 5.2). This suggests an increase in the mass of the kidney may be linked to the onset of the spawning season.

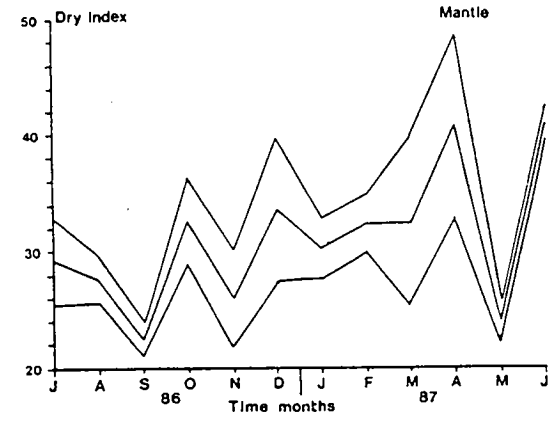
Figure 5.1 Dry weight organ indices of Hippopus hippopus. Indices for gonad, adductor, digestive gland, kidney, mantle and ctenidia are shown. The central line of the three lines on each graph link mean values whilst the upper and lower lines link points of + & - 1 standard deviation.


Dry Index
Gonad
40
30
20
10


Dry Index
Adductor
36
32
28
24
20
16
12


Dry Index
Digestive gland
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2


Dry Index
Kidney
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7


Dry Index
Mantle
50
40
30
20
J A S O N D J F M A M J
86
87
Time months

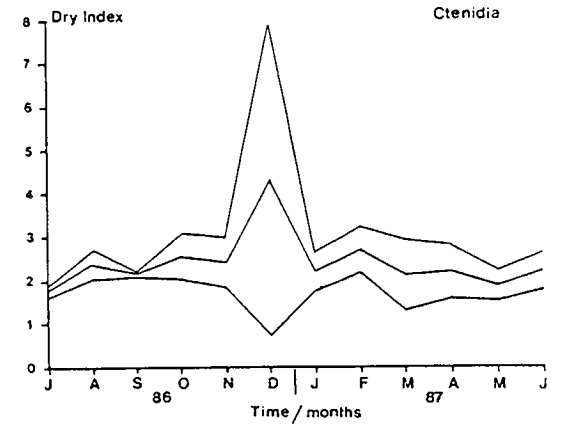
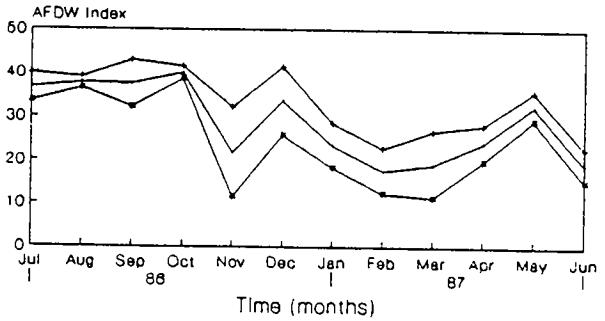
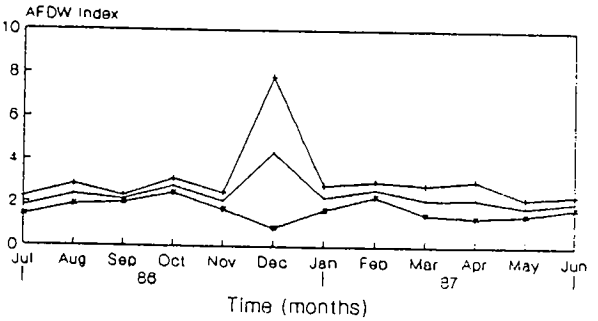

Dry Index
Ctenidia
8
7
6
5
4
3
2
1
0
J A S O N D J F M A M J
86
87
Time / months

Figure 5.2 AFDW indices of Hippopus hippopus. Indices for gonad, adductor, digestive gland, kidney, mantle and ctenidia are shown. The central line of the three lines on each graph link mean values whilst the upper and lower lines link points of + & - 1 standard deviation.

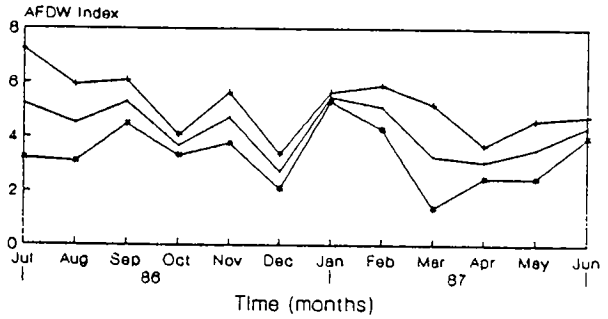
Gonad



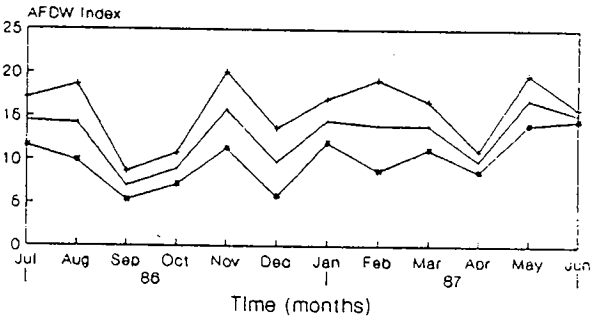
Ctenidia



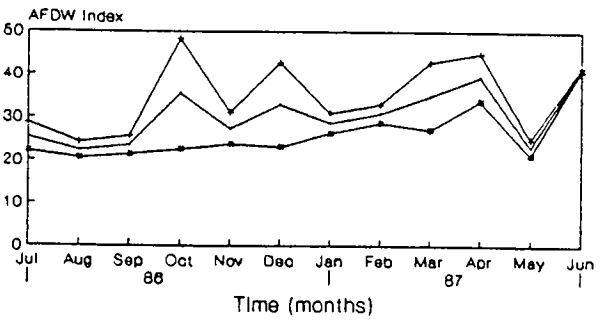
Digestive gland



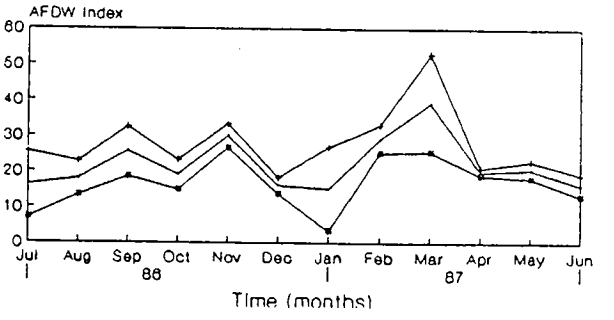
Kidney



Mantle



Adductor



5.3.1.5. Mantle and Ctenidia Indices

No seasonal patterns were observable in mantle or ctenidial indices (Figs. 5.1 & 5.2). The extremely large standard deviation in the December 1986 ctenidial indices is most likely an artifact of the small sample size.

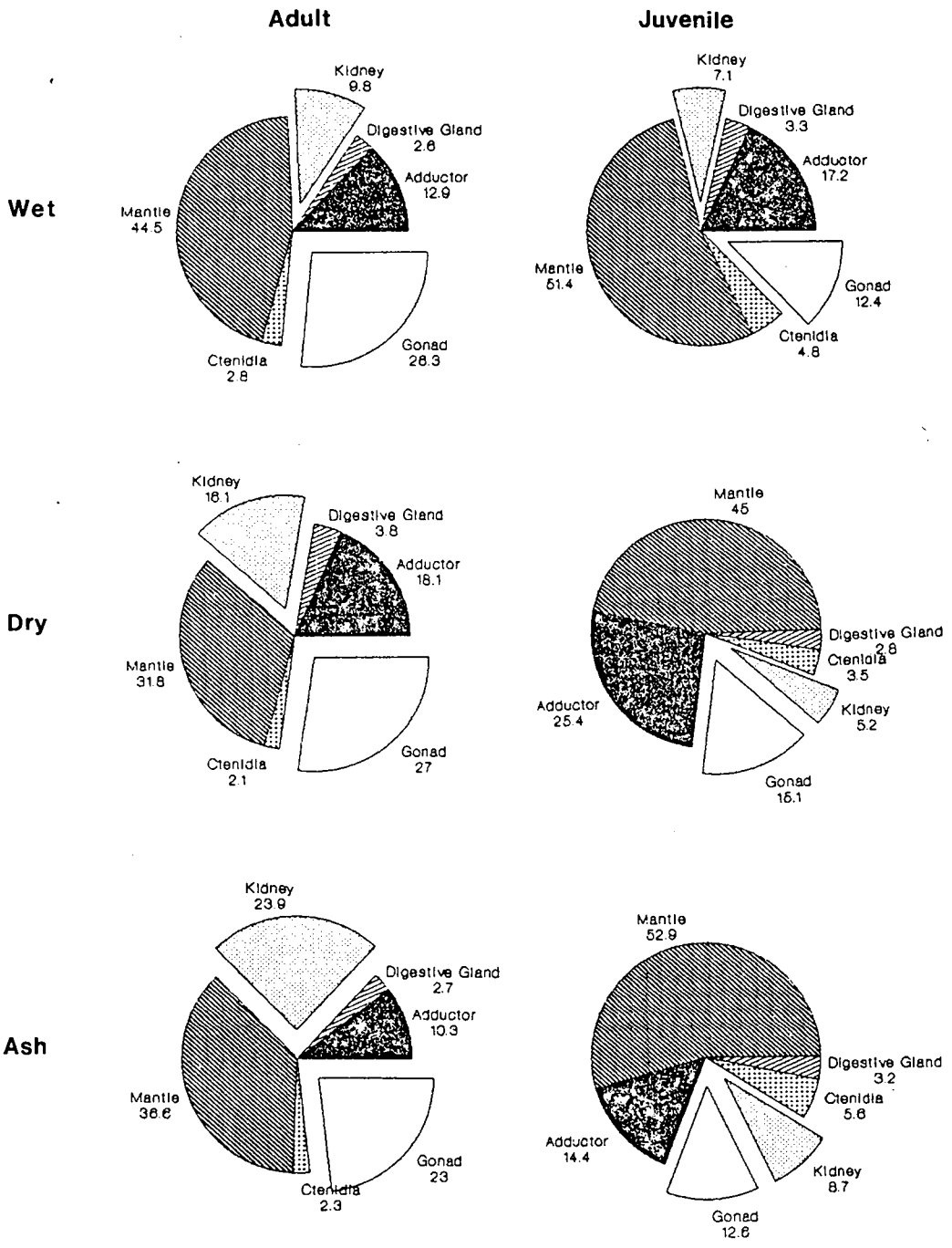
5.3.2. Morphometry of organs of Hippopus hippopus

Measurements of shell length and shell mass were combined with organ wet, dry and AFDM data to examine the morphometry of H. hippopus. These data were collected from clams sampled monthly to examine their reproductive periodicity, and from smaller clams sampled to determine the size and, hence, age at male and female sexual maturity.

As expected whilst relationships between organ masses and shell length are of the form (1), $y = ax^b$, shell mass was found to be linearly related to organ masses i.e. (2) $y = a + bx$, where y = organ mass and x = shell length in (1) and shell mass in (2). Relationships between organ masses and shell length are detailed in Table 5.1, together with the relationship between shell length and shell mass.

Comparison of pie charts of the average composition of the small clams collected for study of the size at sexual maturity and large clams collected for reproductive periodicity (Fig 5.3) show several major differences in relative organ size. The lower relative percentage composition of the mantle and adductor muscle in the larger clams, is countered by increases in the relative

Figure 5.3 Pie charts showing the relative organ composition mature and juvenile Hippopus hippopus, representing wet, dry and ashed samples.


Adult
Juvenile
Wet
Dry
Ash
Kidney 9.8
Digestive Gland 2.6
Adductor 12.9
Mantle 44.5
Ctenidia 2.8
Gonad 26.3
Kidney 7.1
Digestive Gland 3.3
Adductor 17.2
Mantle 51.4
Gonad 12.4
Ctenidia 4.8
Kidney 18.1
Digestive Gland 3.8
Adductor 18.1
Mantle 31.8
Ctenidia 2.1
Gonad 27
Mantle 45
Digestive Gland 2.8
Ctenidia 3.5
Kidney 5.2
Adductor 25.4
Gonad 15.1
Kidney 23.9
Digestive Gland 2.7
Adductor 10.3
Mantle 36.6
Ctenidia 2.3
Gonad 23
Mantle 52.9
Digestive Gland 3.2
Ctenidia 5.6
Kidney 8.7
Adductor 14.4
Gonad 12.6

sizes of both the kidney and gonad. The dry and ashed masses of the heart, pericardium and renal sphincter were not measured for the smaller clams.

Table 5.1 Morphometric relationships of the form $y = ax^b$, where x = shell length (mm) and y = dry mass of organ (g), for the 4 major organs of Hippopus hippopus

Organ	a	b	Correlation Coefficient
Adductor	1.5×10^{-6}	2.85	0.98
Mantle	5.8×10^{-6}	2.71	0.97
Gonad	2.6×10^{-10}	4.39	0.94
Kidney	2.1×10^{-10}	4.33	0.98
Shell	2.2×10^{-4}	2.87	0.99

5.3.3. Gross anatomy of monthly gonad samples

Gross changes in the appearance of the gonads corresponded with changes in the gonad index. From the months April to May, gonads were mostly flaccid. Gonads were moderately turgid in June. Prominent tubules which appeared as channels just under the surface of the gonads, were conspicuous in both June and July. The gonads from August to October appeared full and turgid. In November and December both turgid and flacid gonads were present in the samples, indicating that some animals had spawned during this period. Although the gonads examined from January to March were a mixture of moderately turgid to flaccid, when cut open the gonads were loosely packed with material, compared to the turgid gonads which were full and firm.

Full turgid gonads were white to orange-white in colour,

whilst flaccid gonads were generally off-white with darker markings on the anterior end which could be brown / yellow / orange or purple. These observations on gross anatomy were presented in Shelley and Southgate (1988)(Appendix 5.3.).

5.3.4 Histology of gonads from monthly samples

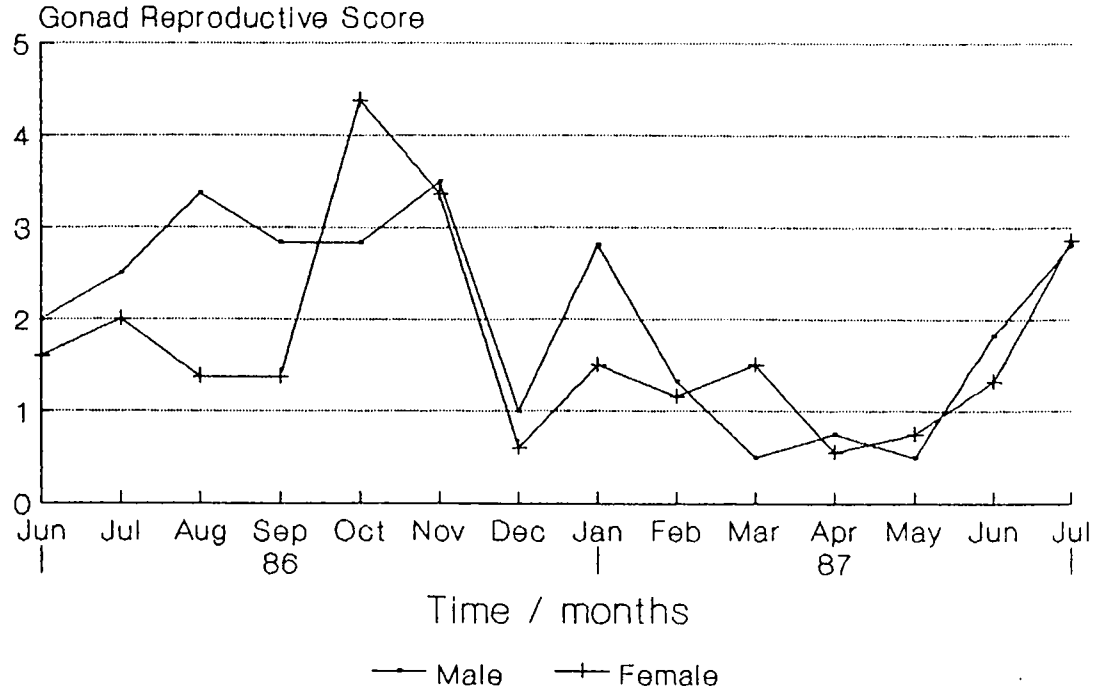
5.3.4.1. Female

It was found to be difficult to assign a single stage as defined by Nash et al. (1988)(Appendix 5.1) to describe the histological structure of H.hippopus gonads. In addition to overlapping stages; in a number of cases the characteristics of 3 stages could be found in one gonad. For the calculation of the gonad reproductive score (GRS)(Fig. 5.4), average scores were recorded where more than one stage was noted on a gonad section.

The GRS (Fig. 5.4) decreased from a maximum value in November to a minimum in April, indicating spawning probably occurred over that period. The onset of marked oogenesis appeared in May, and gradual increases in G.R.S. were seen through June and July. Mid-development to late-development / ripe G.R.S. values were found from August to November.

In addition to scoring the gonads using the same GRS criteria as Nash et al. (1988), several other features of the female portions of the gonad were noted. The distance between adjacent follicles and the relative density of

Figure 5.4 The mean gonad reproductive score plotted against time for both male and female portions of the gonad of Hippopus hippopus.


Gonad Reproductive Score
5
4
3
2
1
0
Jun
Jul
Aug
Sep
86
Oct
Nov
Dec
Jan
Feb
Mar
Apr
87
May
Jun
Jul
Time / months
Male
Female

inter-follicular hemocytes (IFH) in that space were found to vary between clams. The distance between follicles was noticeably larger over the period November to March, and the relative density of IFH was correspondingly high over the same period. The highest levels of IFH were found in November.

Small, purple stained (with haematoxylin and eosin) primary oocytes were seen in female follicles throughout the year. These oocytes were commonly found at the junction of the gel coats of several oocytes that were in the process of breaking down. This suggests that the purple staining oocytes may be involved with the resorption of unspawned eggs, in addition to presumably being the precursors for the next generation of oocytes. In some gonads where ova were undergoing cytolysis (Fig. 5.5 (e)), the purple staining oocytes were very irregular in shape.

Only one parasite was found in the 258 gonad sections examined. It has been tentatively identified as a digenean trematode, possibly a Protoces sp. (Fig. 5.5 (f)). By comparison, in Tridacna crocea approximately 12% of gonads had a parasitic infection of a bucephalid trematode (Shelley et al. 1988)(Appendix 5.4).

5.3.4.2. Male

In addition to the features of male follicles mentioned by Nash et al. (1988), it was observed that the inner walls of mature follicles had a lining of cells with

Figure 5.5 Microscopic photographs of the gonad of Hippopus hippopus.

a. Stage 1 female portion of gonad. Female follicle containing only primary oocytes (po). Inter-follicular hemocyte (ifh) concentration high. Scale bar = 5 um

b. Stage 2 gonad. Stalked ooyctes noticeable and follicles only partially filled. Scale bar = 5 um

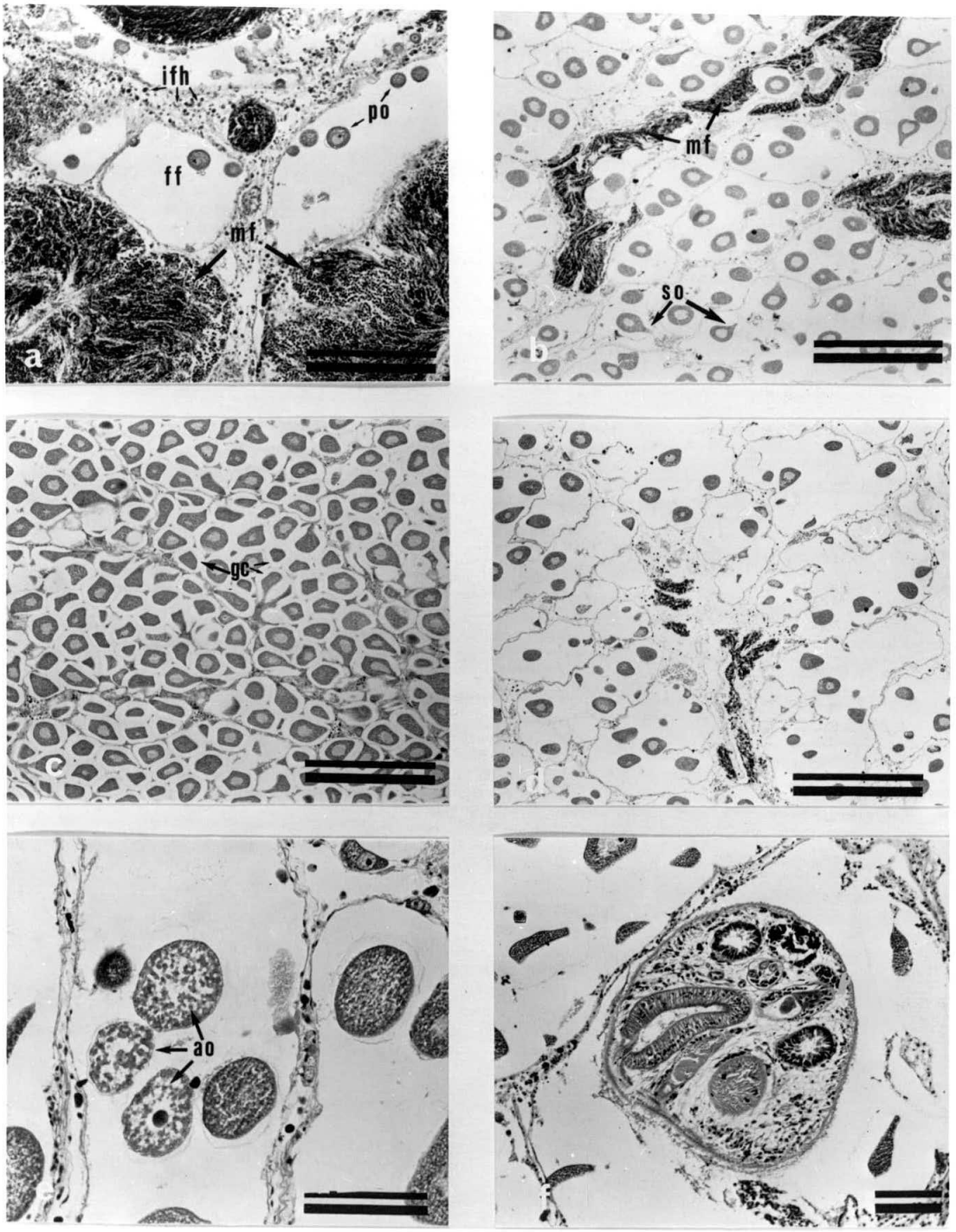
c. Stage 3 female portion of gonad. Ripe gonad showing polygonal oocytes, gel coats (gc) compressed and follicles full. Scale bar = 5 x 10 um

d. Stage 4/5 gonad. Follicles appear empty, walls of female follicles crenulated, and some oocytes undergoing cytolysis (see e.). Scale bar = 5 um

e. Attritic oocytes (ao), undergoing cytolysis. Scale bar = 1 um

f. A parasitic digenian trematode in a female follicle of Hippopus hippopus. Scale = 1 um

Key: ff = female follicles, gc = gel coat (chorion layer), ifh = inter follicular hemocytes, mf = male follicles, po = primary oocytes, so = stalked oocytes.

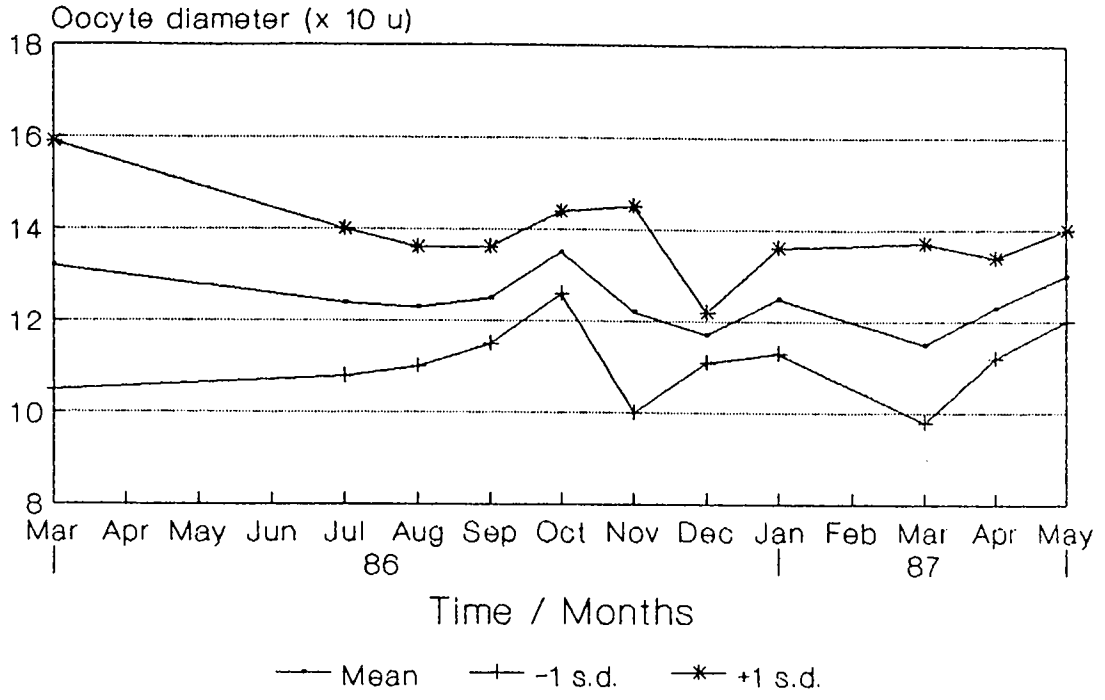

ifh
po
ff
mf
a
mf
so
gc
ao

'cilia like' structures extending to the periphery of the inner follicle structure, inside which sperm at different developmental stages were found (Fig. 5.6 (e) & (f)). These cells lining the male follicles are similar in structure to stereocilia found in vertebrates. Stereocilia, whilst superficially resembling cilia, are in fact non-motile, long flexuous microvilli. " Their (stereocilia) function is not well established, but the epididymal epithelium (where they occur) is absorptive and it is assumed that they promote this function by amplifying the cell surface " (Bloom & Fawcett 1975).

5.3.4.3 Zooxanthellae in the gonad

Zooxanthellae were found just inside the gonad wall of H.hippopus, and there appeared to be no barrier between them and hemocytes which freely move throughout the gonad (Fig. 5.6 (g). It is possible that these zooxanthellae are merely moving through the gonad from the mantle to another organ; however, if there exists a direct interaction between zooxanthellae, hemocytes and gonad production it would make the giant clam - zooxanthellae symbiosis more understandable. As hemocytes are the basic 'unit of circulation' for molluscs, the transfer of photosynthetic products by a physical process directly to hemocytes and then to other body organs would seem logical and may be a useful hypothesis for workers in the field to examine.

Figure 5.6 Oocyte diameter from biopsy samples plotted against time for Hippopus hippopus.


Oocyte diameter (x 10 u)
18
16
14
12
10
8
Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr May
86
87
Time / Months
Mean
-1 s.d.
+1 s.d.

5.3.5 Synchronisity of development

Each of the six sections from each gonad was found to be similarly staged. Therefore each gonad of H.hippopus exhibited synchronisity of development along their length. Although male and female ascinii in one gonad may be at different stages, according to the stage criteria of Nash et al.(1988) (Appendix 5.1), the plot of GRS against time indicated that annual cycles of gametogenesis were synchronous between the sexes, as would be expected for successful fertilisation.

The cross-sectional area of male to female ascinii was found to vary between sections within a gonad; however, these variations are small compared to differences between different gonads sampled at the same time. Whilst the cross-sectional area of male ascinii in a gonad section averaged less than 50% of the total, some individuals had up to 95%, whilst one clam appeared to be 100% female.

5.3.6 Geisma stained biopsy smears

Geisma stained smears of biopsy samples were found to provide similar results to that found from histological preparation. Oocytes stained dark purple-blue, whilst the chorion layers were violet. Sperm nuclei at all stages of development stained light blue-dark blue, whilst mature sperm had eosinophilic tails. The most common type of hemocyte found was the eosinophilic (acidophilic) granulocyte (Fig. 5.7 (f)) (mean diameter approx. 7um), which had a central, slightly irregular nucleus (mean

diameter approx. 3um). The slightly larger granulocytes (mean diameter approx. 10u), which stained purple with haematoxylin and eosin (in standard histological preparation)(Fig. 5.7 (f)), with an eccentric round nucleus, were not evident in the biopsy samples.

Biopsy samples containing regular, oval oocytes, with some oocytes still contained inside follicles were characteristic of the ripening to ripe gonads described in the histology section. Some oocytes retained a slightly polygonal shape, even when excised from a follicle by the biopsy technique. Spent and partially spent gonads were seen to have a lot of loose fine particulate material in the smear, with oocytes in various stages of degeneration. As the oocytes breakdown, their structure becomes more open and attritic (Fig. 5.5 (e)). It was noted that the material remaining inside a follicle in a smear was much more lightly stained than loose material, indicating that the follicle walls were slightly impervious to the stain. Clumps of male follicles in spent gonads had concentrations of the eosinophilic granulocytes amongst them.

Whilst the information obtained from the smear technique is limited compared to that from histology, similar staging of the ripeness of gonads using a smear technique could be undertaken. The ease of processing and speed at which results are obtained, compared to full histological processing, are features in favour of the biopsy techniques commonly used in giant clam mariculture.

5.3.7 Repetitive monthly biopsy samples

A plot of the mean oocyte diameter against time (Fig. 5.6) showed that the largest value occurred in October 1986. The lowest values were found in November 1986 and March 1987. In March 1986 a large mean oocyte diameter may have indicated that the peak oocyte size was later, or that perhaps the maximum size of oocyte obtained was larger than in the 1986-87 spawning season.

Clam mortalities during the course of the biopsy sampling programme, as discussed in Shelley and Reid (1988)(Appendix 5.2) indicated that biopsy sampling of H. hippopus should not be undertaken as a routine procedure, unless a large number of broodstock were available. The missing values in the data set (Fig. 5.6) resulted from the loss of a box containing biopsy samples taken over several months.

5.3.8 Size at hermaphroditic sexual maturity

Of the 50 clams collected from Iris Point Reef to assess size at sexual maturity (shell length range 77-206 mm), none were small enough to show a complete absence of sexual development, i.e. no clams of indeterminate sex were found. This indicates that the size at first sexual maturity is less than 77 mm, the smallest clam collected for this study.

Small ova without a recognisable female follicular structure were found in some clams. As all samples were

Figure 5.7 Microscopic photographs of stages of the development of the gonad of Hippopus hippopus.

a. Gonad showing aggregations of granulocytes (ag) which appear to be the precursors of follicle formation. Scale bar = 2 um

b. Three primordial male follicles (mp). Scale bar = 2 um

c. Aggregations of granulocytes (ag), and primordial male (mp) and female follicles containing primary oocytes. Scale bar = 2 um

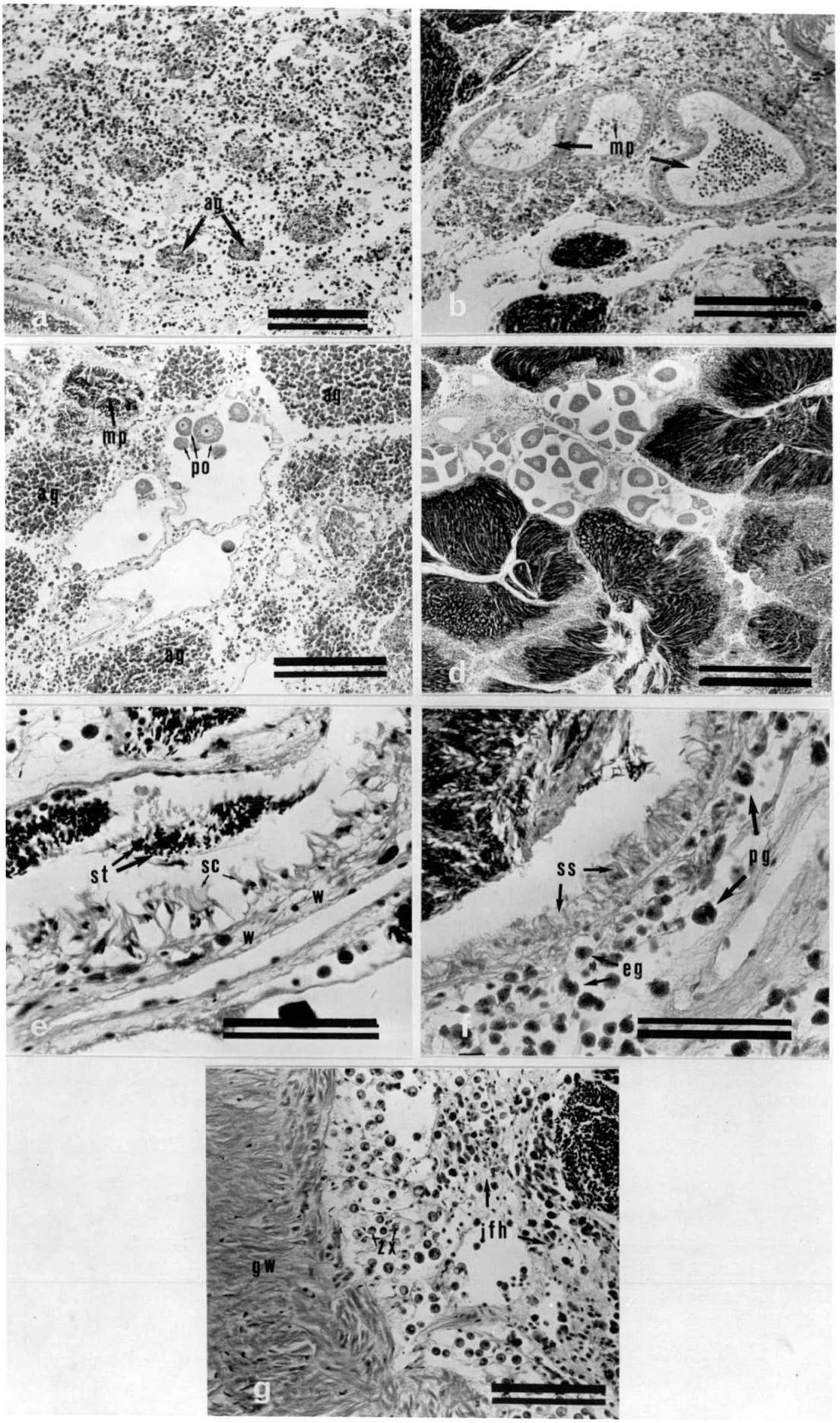
d. A mature (male and female) gonad. The high cross-sectional area of male compared to female gonad indicates the gonad is from a small clam which has only recently obtained female maturity. Scale bar = 5 um

e. Sterocilia (sc) lining the wall (w) of a male follicle containing predominantly spermatids (st). Scale bar = 1 um

f. Sterocilia (ss) lining the wall of a male follicle. Eosinophillic granulocytes (eg) and purple staining larger granulocytes (pg) seen in the inter-follicular channels. Scale bar = 1 um

g. The co-occurrence of zooxanthellae (zx) and inter-follicular hemocytes (ifh) is shown at the outer edge of the gonad, just inside the gonads muscular wall (gw). Scale bar = 1 um

Key ag = aggregations of hemocytes, eg = eosinophilic granulocytes, gw = muscular gonad wall, ifh = inter follicular hemocytes, mp = male primordial follicles, pg = large purple stained (with H & E) granulocytes, po = primary oocytes, sc & ss = sterocilia, st = spermatids, w = wall of male follicle.


ag
mp
ag
mp
po
ag
ag
st
sc
w
w
ss
pg
eg
gw
zx
jfh

collected during, or at the end of the spawning season, a clam was only classified as having mature female characteristics if some of its follicles had ripe, degenerating or bimodal oocytes present. An example of a gonad with a unimodal oocyte distribution is seen in Figure 5.7 (c). An obvious bimodal oocyte size distribution usually shows that the clam has been through at least one previous spawning season, and new oocytes are beginning to form. The largest clam which had a solely male gonad was 140 mm in shell length. The smallest clam showing any female structures (immature), was 77mm in length, the smallest clam collected. However, the shell length of the smallest mature hermaphrodite was 112 mm. Clams which showed signs of female oogenesis without maturation ranged from 77-146 mm in shell length.

5.3.9 Histological development of hermaphroditism in Hippopus hippopus

No H. hippopus gonads were found without male follicles. Gonads containing only male follicles contained dense concentrations of hemocytes (Fig. 5.7 (a)), which could account for up to 25% of the cross-sectional area of a gonad section. Primordial male follicles (Fig. 5.7 (b)&(c)) were found commonly in gonads already containing some mature male follicles. It was rare to see follicle primordia in gonads of fully mature clams, suggesting that the number of new follicles produced may decrease with the age of the clam. Therefore, it is probable that increases in gonad size of the largest clams may be a result of an

increase in size of individual follicles of both sexes, probably combined with subdivision of existing follicles to create more follicles.

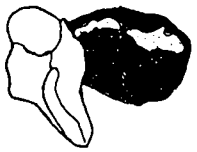
5.3.10 Anatomical gonad development

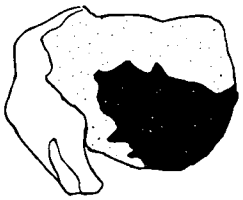
Figure 5.8 details the progressive anatomical development of the gonad of H.hippopus, from clams of shell length 89 to 206 mm. The longitudinal section through the visceral mass from a clam of 89 mm shell length shows that the gonad at this stage is composed of a thin band of tissue across the upper surface of the digestive gland. Gradually the band of gonad increases its coverage of the digestive gland, also becoming thicker, until the digestive gland is completely surrounded by gonad. This progression is shown in Figure 5.8 from clams of shell length 89,112,140,171 and 209 mm.

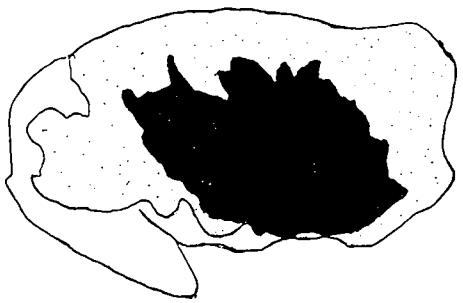
5.4. DISCUSSION

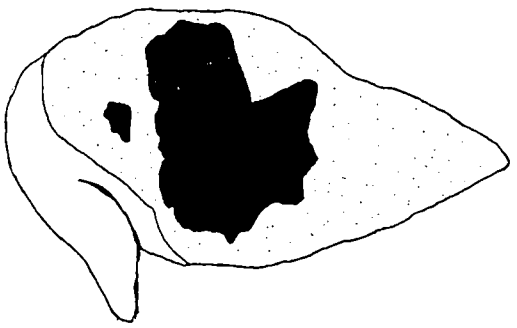
Histological examination, monthly biopsy samples, gonad index and field observations of Hippopus hippopus(see Shelley and Southgate 1988, Appendix 5.3) all indicate that gonad condition peaks at the start of the Austral summer (September/October), and is followed by a spawning season which may extend to March, at which time the gonad condition is at its lowest, confirming Stephenson's (1934) earlier work. Similar annual reproductive cycles, peaking during the Austral summer, have been reported for T.gigas (Braley 1986, Nash et al. 1988) and T.crocea (Shelley & Southgate 1988) from the Great Barrier Reef and for

Figure 5.8 Anatomical development of the gonad of Hippopus hippopus, shown by examination of longitudinal sections of the visceral mass. Scale is real size. Stippled area = gonad, black area = digestive gland, and area without shading = foot. From top to bottom the gonads are from clams of shell length 89, 112, 140, 171, & 209 mm respectively.









T. squamosa from Papua New Guinea (Baria et al. 1987). However, Gwyther & Munro (1981) reviewed spawning of giant clams to 1980 and showed no well defined reproductive season. Their own study showed that H. hippopus could be induced to spawn sperm throughout the year, and T. maxima could be induced to spawn eggs over a 6 month period. One major factor causing the apparent contradiction between results of spawning trials with results of examination of reproductive cycles is that most giant clams can be induced to at least release sperm, at almost any time of the year. The availability of ripe eggs, capable of producing larvae that can progress through settlement to production of juveniles, is the acid test of a clam's maturity. Therefore, accounts of induced spawnings, which do not result in larval or juvenile production, do not validate reproductive periodicity or the lack of it, at any particular site. Reports of the lack of a well defined reproductive cycle from sites close to the equator (Solomon Islands - pers comm John Munro, Palau - pers comm Gerald Heslinga), are similar to reports for other invertebrates which show increasingly well defined reproductive cycles the further they are from the equator (Pearse 1968).

Examination of organ indices revealed no obvious relationships between adductor, mantle, kidney and gonad. Interactions between these organs would be best studied at a biochemical and physiological level. Much of the recent work in this area has been reviewed by Sastry (1979). It

is interesting to note that peak index values for adductor muscle following the spawning season, as found in this study (Fig. 5.1 & 5.2), have been reported for other bivalves (Taylor & Venn 1979, Taylor & Vahl 1981). The increase in the relative size of the gonads of juvenile to adult clams, is an expected consequence of sexual maturation. However the relative increase in size of the kidney is not so easily explained (Fig. 5.3). A possible explanation is that the mobilisation and reorganisation of biochemicals involved in storage and energy reserves at different times of the year are reflected in the size of the kidney (pers. comm. P.Southgate).

Comparing the body components of adult H.hippopus to that of a representative adult T.squamosa (Baria et al. 1987), it was found that whilst the mantle and foot represent higher percentages in T.squamosa, the kidney of H.hippopus was relatively larger. The greater percentage of mantle in T.squamosa is a result of the usual hypertrophied Tridacna mantle, compared to the comparatively restricted mantle of H.hippopus, which does not extend beyond the outer shell limits. The relatively large foot of T.squamosa is a reflection of its lifestyle. Whilst T.squamosa maintains its foot, and a large byssal opening throughout its life, H.hippopus loses its byssal opening with growth, and its foot appears of little value in its adult state. That the kidney of H.hippopus is almost twice the relative size of that of T.squamosa may indicate a slightly different relationship between the zooxanthellae and energy assimilation, or a greater dependence of filtered food in

H. hippopus compared to T. squamosa.

The processes of breakdown and resorption of unused gametes and the formation of new gametes seems a continuous process, in that there seems to be no resting stage present in the histological development of mature H. hippopus. This was also found for T. gigas (Nash et al. 1988). Empty follicles of either sex are rarely seen in fully mature clams. The developmental stages of follicles of both sexes are best observed in juvenile, newly mature clams, where ascinii structure is less confused than in fully mature clams. The number and percentage cover of sections of female follicles increases in larger clams until they are more numerous and take up a larger percentage of the gonad, than the male follicles, as was also found in T. crocea (Kawaguti et al. 1982).

The 'stereocilia like' lining of male follicles (Figs. 5.7 (e)&(f)) and the primary, purple staining oocytes of female follicles (Figs. 5.5 (a)& 5.7 (c)) appear to be involved in the resorption of material from their respective follicles. Similarly, in Mytilus edulis the released oocyte contents (following oocyte degeneration) were reabsorbed by the epithelial cells of the gonoducts (Pipe 1987). Whether or not a similar mechanism is occurring in H. hippopus, and whether or not some small purple staining primary oocytes identified in this study are in fact follicle cells requires a more detailed histological and cytological investigation.

Whilst the concentrations of the two main types of hemocytes varied in the hemocoelic spaces between follicles, they were rarely found inside a follicle. This leads one to the conclusion that their role in transport of material throughout the clam is their major function, contrary to the conclusions of Braley's (1986) study of Tridacna gigas. Other evidence pointing to this includes Thompson's (1977) study on Placopecten magellanicus, which showed that the amount of glycogen in circulating hemocytes was highest during gametogenesis. Photographs depicting phagocytic amoebocytes amongst biopsy samples of T.gigas gonads (Braley 1986) are far from convincing. Use of a staining technique to distinguish different cell types in the present study, showed that much of the material classified in Braley's study as being regressive was probably cellular debris resulting from the biopsy technique used. In addition, histological examination of H.hippopus gonads gave no support to Braley's suggestion that spermatogenesis occurs at the site of regressing ova. Male and female follicles are quite independent, with all evidence pointing to spermatogenesis occurring at the periphery of male follicles. The spermatogonia lying close to the hemocoelic space that surrounds follicles, as noted in a mytilid (Bernard et al. 1988). It is likely that there are independent male and female ducts to the gonadal pores, a mechanism which would allow clams to spawn first male and then female gametes.

Synchronisity of development throughout the large gonad of

H.hippopus, parallels the findings of Nash et al.(1988) for T.gigas.

The peak mean oocyte diameter of 135 um, in monthly biopsy samples, is very similar to the size at which Jameson (1976) observed H.hippopus oocytes to develop, but slightly smaller than the value of 143 um given as the size of mature eggs in H.hippopus from the Philippines (Alcala et al. 1986).

The only records of the size or age at sexual maturity in giant clams are those of Murukoshi & Kawaguti (1986a & b), who found that T.crocea less than 55 mm in shell length were all males; Heslinga & Perron (1983) who stated T.derasa became mature males after 3 years; and Radtke (pers. comm.) who found that all T.maxima greater than 37 mm were hermaphrodites. This study showed that there is some overlap between the size of the largest solely male H.hippopus and the smallest mature hermaphrodite. If male and female gametes are required a clam of shell length greater than 140 mm is required.

A factor not determined in this study is whether or not a clam that histologically appears mature is capable of spawning. A combined spawning induction and histological study is required to determine if this is so.

CHAPTER 6

SYNTHESIS AND CONCLUSIONS

6.1 GROWTH AND SEXUAL MATURITY

In Chapter 5 the sizes at male and full hermaphrodite maturity for H.hippopus were determined as being less than 77 mm and 146 mm respectively. Chapter 4 detailed the age: shell length relationship for the same species, from the same location, Iris Point Reef, Orpheus Island. The average shell length at age 2 was 80 mm; at age 4, 146.8 mm; therefore establishing the age of male maturity at approximately 2 years and full hermaphrodicity at 4 years of age.

In Chapter 2 the inflexion point in the growth curve of H.hippopus at Iris Point Reef (sub-littoral sample) was found to be in the range of 140-165 mm. Therefore it is apparent that the size at full hermaphroditic maturity corresponds well to the inflexion point in the growth curve of H.hippopus. This marked decrease in growth rate with sexual maturity is in agreement with speculation in a paper on the growth of T.maxima (Jones et al. 1986), who suggested that the growth rate changed at the size that McMichael (1975) had given as the size of sexual maturity for that species. Evidence of marked physiological changes in another clam, T.crocea (Banaszak, Shelley and

Kenny in prep), at an age and size which probably corresponds to that species' onset of full hermaphroditic maturity, according to Murakoshi & Kawaguti (1986a & b), provides further evidence for the hypothesis that quite major changes occur in tridacnid clams at the time of becoming viable females.

6.2 AGING AND SITE ASSESSMENT USING SCLEROCHRONOLOGY

Confirmation that the translucent seasonal band was formed during Austral summer and the opaque band during winter by examination of internal growth band formation (chapter 3) confirms the results of Aharon & Chappell (1983) who examined oxygen and carbon isotope concentrations across giant clam shells relating changes in composition to seasonal temperature changes. Validation of seasonal growth bands, best seen in the inner shell layer of shell sections, allowed counting of annual couplets of seasonal bands (summer & winter) to age giant clams. The potential usefulness of sclerochronological aging, demonstrated in Chapter 4, makes it possible to visit a site, collect as many dead clam shells as possible, section them and within a very short period of time provide growth rates for that site. Death assemblages also enable estimates of mortality rates with size to be made. One of the few problems with this technique is that smaller clams are probably not proportionally represented, as they are more prone to being smashed or washed ashore than larger shells, and hence not sampled. If information on juveniles in the population can be obtained, it then enables life tables to

be produced which can be particularly useful for assessing sites for clam farm operation. Kennish (1980a) showed that life tables for 4 natural sites of Mercenaria mercenaria differed so much between sites that the number of specimens surviving after 7 years, per 1000 initially there, varied from 2 to 227, so the importance of site selection should not be underestimated.

6.3 MORPHOMETRY AND FLESH YIELD

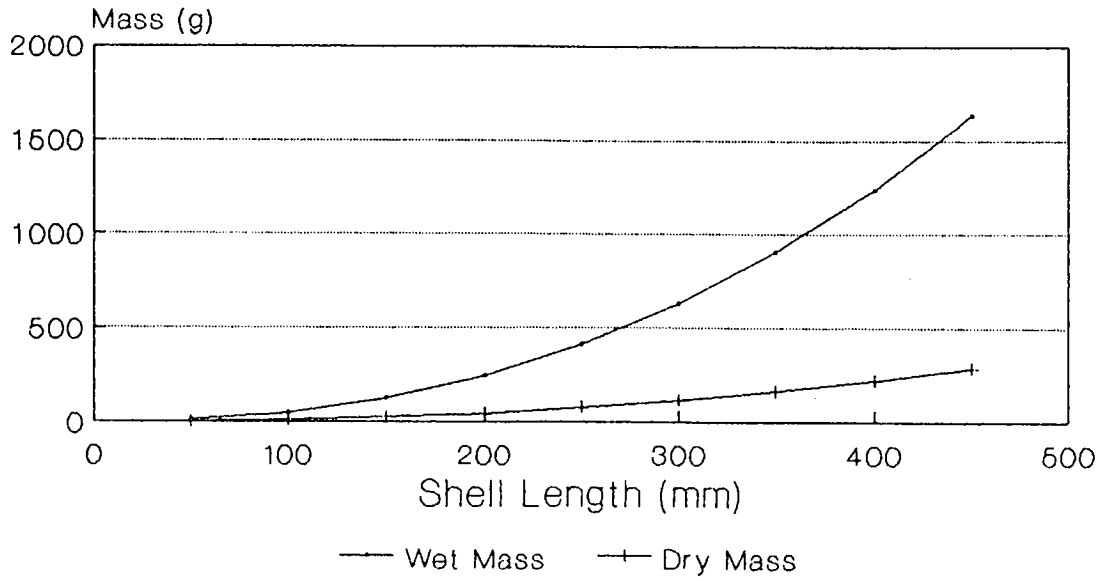
Using the morphometric relationships between wet mass (flesh) and dry mass (flesh) with shell length from Shelley & Southgate (1988), flesh yield data with length of H.hippopus from Pioneer Bay are shown in Figure 6.1. Comparing these yields to those of T.gigas grown at Orpheus Island (Barker et al. 1988)(Appendix 6.1), a 3 year old T.gigas produces approximately the same flesh yield as a 5 year old H.hippopus. In Chapter 4 it was suggested that the flesh to shell ratio may increase northerly along the G.B.R., and that growth rates vary between sites, therefore a comparison of the yields of T.gigas to H.hippopus could vary considerably with location of the clams. Data from this study are just part of the data base required to define production parameters throughout the geographic range of giant clams (Munro 1986)

6.4 GROWTH AND REPRODUCTION

The growth rates obtained for H.hippopus (as well as other tridacnid clams) could be improved by selective breeding

Fig. 6.1 Plot of wet and dry mass flesh yields of Hippopus hippopus with shell length for clams obtained from Pioneer Bay, Orpheus Island.

Flesh yield of Hippopus hippopus



*Masses exclusive of shell mass. Yields obtained from clams, Pioneer Bay Orpheus Island.

programs, and quite probably by the use of triploid induction to neuter clams, so that flesh production is confined to somatic rather than gametic tissue. Polyploidy of bivalves, particularly of the genus Crassostrea, has been induced using both chemical (Stanley et al. 1981) and high pressure techniques (Chaiton & Allen 1985), and its favourable impact on growth rates described (Stanley et al. 1984). The effect of gametogenesis on the growth rate of both H.hippopus and T.gigas was demonstrated in Chapter 2, where both species showed minimal growth the quarter year preceding their spawning seasons.

6.5 DETERMINATION OF REPRODUCTIVE STATE AND CYCLE

An improved biopsy technique (Shelley & Reid 1988) and a better way of examining biopsy samples using a staining technique (Chapter 5), both developed during this study now make examination of reproductive condition in giant clams safer and more accurate respectively. The usefulness of histology for examination of reproductive cycles and screening for disease is unquestionable, but as a rapid procedure for identifying reproductive condition, biopsy sampling is useful.

The identification of the reproductive cycle of H.hippopus, together with an examination of its gonad development and ages at sexual maturity has helped to fill in information on the details of the reproductive cycles of giant clams which have been lacking (Munro & Heslinga 1982). An annual reproductive cycle supports the findings

of Crawford et al. of T.gigas (1987) who considered that cycles were restricted to summer in higher latitudes, with year round spawning close to the equator.

6.6 GROWTH FACTORS AND GROWTH RATES

The most important factors determining an organisms growth rate are best examined under controlled experimental conditions. Initial attempts adopting this approach have been used by Mingoa (1988) and Mingoa & Shelley (1988) to examine the growth of juvenile T.gigas. In this study large scale environmental differences such as different latitudes, inshore versus offshore position, and exposed versus sheltered were examined. The period of emersion which giant clams experience (Appendix 2.2) and the degree of exposure to wave action (Pioneer Bay compared to Iris Point Reef) were identified as important growth factors for H.hippopus. Low growth rates and a smaller Loo value for H.hippopus from Hayman Island, at the southern limit of its distribution confirm the observation of Munro & Heslinga (1982), that decreased growth rate occurs towards the limits of a giant clams distribution. Obtaining large sample sizes of some of the larger clams (T.gigas & T.derasa) proved a problem both of logistics, cost, and sheer unavailability of material on some reefs. Giant Clams are protected under the C.I.T.E.S. treaty and their population numbers on any one reef are limited (Braley 1987), therefore there was no attempt to use live material (except in the case of Hippopus on Orpheus Island). Values for von Bertalanffy growth curves for T.gigas, T.derasa,

T. squamosa and T. maxima in this study join the growing database on the growth of giant clams from both wild and maricultured clams, and form baseline values against which the growth of maricultured giant clams in the G.B.R. region can be compared.

The von Bertalanaffy growth function (VBGF) has been used extensively to describe the growth of giant clams in this study, however Munro (1986) suggested that it " might not be entirely adequate to describe the detailed features of growth ". This study has shown the VGBF to be a useful tool to compare growth rates, and age - length data has shown a reasonable conformity to the form of the VGBF.

6.7 GIANT CLAM SHELL CHEMISTRY AND FLUORESCENCE

With the relatively short lifespan of most giant clams, it would seem that some coral species with colony lifespans of several 100 years, will remain more important indicators of past environmental conditions by examination of features such as seasonal growth and levels of fluorescence in their calcium based skeletal material (Isdale 1984, Boto & Isdale 1985). The shell chemistry of the tridacnids, briefly examined in this study (Chapter 3), may yet prove useful both in detection of pollutants incorporated into the shell and in the further understanding of calcification in bivalves, especially interesting in tridacnids because of their symbiotic zooxanthellae. The relatively large growth bands in the tridacnids means that examination by the electron microprobe can be made at a far higher resolution than in

smaller bivalves where electron beam width can prove a limiting factor.

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APPENDICES CHAPTER 2

Appendix 2.1

Growth Rates of *Hippopus hippopus* from Orpheus Island, Great Barrier Reef

C.C. Shelley*

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Appendix 2.2 Growth of Hippopus hippopus in relation to tidal emersion.

Methods

In order to study the effects of varying degrees of emersion on the growth of H.hippopus, clams were positioned on a staircase structure, constructed on a reef flat, in Pioneer Bay, Orpheus Island. The staircase provided the following heights above chart datum: 0, 50, 80, 105 and 120 cm. The site and structure are fully described in Nash (1988), who similarly examined the affect of emersion on the growth of Tridacna gigas. The H.hippopus used in the trial were of different sizes even though the clams used were the smallest which could be found. At each level 8 clams were placed forming one group; the total weight and lengths of the 5 groups of clams were arranged to be as close as possible. The total masses of the groups of clams ranged from 1353-1591 g, whilst the combined lengths ranged from 1214-1265 mm. Over a seven month (6/11/86 - 8/6/87) period each month the shell length, and total wet mass of each clam was recorded.

Results

It was found that generally increasing emersion led to a decrease in growth of shell and total wet mass. The exception to this rule was that the growth rate at the 50 cm level was better than at the 0 cm level, for both shell length and total wet mass (Table A1).

Table A1. Mean daily growth of H.hippopus at different levels of emersion over 7 month trial period

	n=8	n=8	n=8	n=8	n=8
Ht. above chart datum (cm)	0	50	80	105	120
Daily increase length (mm)	.099	.150	.135	.089	.063
Daily increase mass (g)	3.14	3.67	2.82	2.45	1.81

Discussion

When examining the affect of emersion on T.gigas, Nash (1988) found there to be a marked difference in growth between clams growing at lower 3 and the top 2 levels; growth at the top 2 levels being much slower. In H.hippopus it appears that there is a gradual decline with exposure, rather than a sudden decrease. This may be because the shells of T.gigas cannot close tightly for extended periods, whereas H.hippopus can. Therefore H.hippopus presumably loses less water when emersed, suffering less stress, than T.gigas under the same conditions. As it was not possible to work with cohorts of H.hippopus as Nash (1988) had with T.gigas, or with as large a sample size, these results should be considered preliminary.

APPENDICES CHAPTER 3

NOTE: In appendices 3.1 - 3.4, FLS = fluorescent lines, GLS = growth bands and FS = fluorescence.

Appendix 3.1 Examination of the fluorescent lines of *Hippopus hippopus* on the GBR

Location	Description of fluorescence in GLS
1. Warrior Rf.	The intensity of FLS vary, some indistinct
2. Tudu Is.	Pallial lines distinctly fluoresce, GLS in umbo and outer shell brighter FS than lines in inner shell layer.
3. Orpheus Is.	Pallial (1) brightly fluoresces, GLS in inner and umbo fluoresce distinctly, outer shell layer GLS indistinct, pallial (2) also fluoresces distinctly.
4. Fantome Is.	Pallial (1) distinctly fluoresces, otherwise low intensity FS, GLS in outer shell most discernible
5. Thetford Rf.	Slight FS in Pallial (1), FLS discernible, but very weak
6. Brewer Rf.	FLS distinct although weak, Pallial (1) weakly FS. Variation in intensity between growth bands.
7. Myrmidon Rf.	Pallial lines distinct. Could not discern boundaries between very weak FLS.
8. Michaelmas Cay	Pallial and growth bands distinctly FS. in umbo and inner shell. No FLS seen in outer shell.
9. Hayman Is.	Pallial (1) & (2) FS distinctly, and FLS discernible. FLS appear relatively larger than in other *H.hippopus* shells.

Appendix 3.2 Examination of the fluorescent lines of *Tridacna gigas*

Location	Description of Fluorescent lines (FLS)
1. Orpheus Is.	Pallial and GLS in inner & outer shell FS. Umbo very weakly FS.
2. Michaelmas Cay.	Pallial distinct. GLS FS in all shell layers.
3. Myrmidon Rf.	Very weak FS. Whilst pallial barely FS, growth bands just discernible FS.
4. Fantome Is.	FS distinct in all layers. Little noticeable difference in intensity between GLS.

Appendix 3.3 Examination of the fluorescent lines of *Tridacna derasa*

Location	Description of Fluorescent Lines (FLS)
1. Leopard Rf.	Pallial line strongly FS, whilst GLS FS lighter blue, umbo area strongest FS, where borers have entered shell yellow FS seen.
2. Myrmidon Rf.	Pallial line bright blue, whilst GLS FS clear, inner shell layer stronger FS than outer.
3. Brewer Rf.	Pallial line (1) very bright, GLS very distinct, as is pallial (2), umbo very bright FS.
4. Rib Rf.	Pallial (1) & (2), GLS & umbo brightly FS.
5. Dip Rf.	Pallial lines, & GLS FS light blue.
6. Michaelmas Cay	Pallial (2) very bright FS, in umbo some yellow FS in addition to light blue.

Appendix 3.4 Examination of the fluorescent lines of *Tridacna squamosa*

Location	Description of Fluorescent Lines (FLS)
1. Stanley Reef	Pallial lines very distinct FS. Marked differences in intensity between GLS
2. Tiger Reef	Marked differences in intensity FS between GLS, umbo very bright FS
3. Palm Is.	Differences in intensity in GLS are echoed in umbo & outer shell layers.
4. Dip Rf.	Brighter FS than 1.- 3. above. Some yellowness seen in some FLS. Some bands FS very strong
5. Rib Rf.	Little FS in umbo region. Variation in brightness FS between GLS
6. Brewer Rf.	Yellowness noted in FLS, FS relatively low density.
7. Fantome Is.	Very bright FLS, some yellow FS.
8. Michaelmas Cay	Less bright FLS, FL relatively larger than Fantome Is.
9. Myrmidon Rf.	FLS indistinct, pallial line weakly FS.

Appendix 3.5 Results of electron microprobe analysis of a *Hippopus hippopus* shell section

Point No.	P	S	Fe	Ba	Ca	Mg	Mn	Sr
1	57.24	0.02	0.04	0.09	0.16	0	0.22	0.06
2	59.34	0.02	0.12	0.04	0	0	0.21	0.06
3	51.26	0.04	0.04	0.09	0.02	0.09	0.2	0.08
4	59.47	0	0.07	0.05	0	0	0.1	0.06
5	52.61	0	0.05	0	0.02	0.17	0.15	0
6	58.22	0.05	0.07	0	0	0.01	0.16	0.03
7	48.36	0.02	0	0	0	0	0.06	0.11
8	50.02	0.02	0	0	0.13	0.12	0.1	0.17
9	44.17	0	0.07	0.03	0.12	0.14	0.11	0.06
10	59.34	0.06	0.06	0.02	0.03	0.06	0.17	0.09
11	54.85	0.04	0.1	0.08	0.14	0	0.2	0.06
12	56.59	0	0	0.04	0.04	0.08	0.22	0
13	49.73	0	0.03	0.07	0.13	0.19	0.16	0.11
14	58.58	0	0.07	0.02	0.11	0	0.16	0
15	56.49	0.01	0.07	0.02	0	0.18	0.17	0
16	53.35	0.05	0	0.03	0.1	0.08	0.16	0
17	51.98	0.01	0	0.04	0.05	0.03	0.15	0
18	50.14	0.04	0	0	0	0.13	0.15	0.11
19	46.15	0	0	0.09	0.09	0.06	0.1	0.09
20	43.3	0.02	0	0.03	0.12	0.17	0.01	0

Appendix 3.6 Results of electron microprobe analysis of a *Tridacna gigas* shell section

Point No.	P	S	Fe	Ba	Ca	Mg	Mn	Sr
1	59	0	0.06	0	0.05	0	0.17	0
2	58.14	0	0.07	0	0	0.11	0.28	0.14
3	66.11	0	0	0.03	0	0.04	0.13	0.05
4	63.97	0.02	0.02	0	0.11	0.16	0.02	0

Appendix 3.7 Results of electron microprobe analysis of a *Tridacna derasa* shell section

Point No.	P	S	Fe	Ba	Ca	Mg	Mn	Sr
1	0	0.04	0.1	0.07	59.37	0.02	0	0.07
2	0.03	0.13	0.17	0.03	64.14	0.03	0.1	0.19
3	0.04	0	0.04	0.09	63.27	0.02	0.04	0.16
4	0.04	0	0.08	0	62.89	0.04	0.07	0.2
5	0.02	0.04	0.07	0	65.72	0	0.05	0.17
6	0	0	0.11	0.1	61.08	0.05	0.09	0.24
7	0.03	0.04	0.15	0.15	57.63	0.03	0.06	0.19
8	0.04	0	0.04	0	57.21	0.03	0.02	0.18
9	0.03	0.07	0.18	0	56.87	0.04	0.03	0.17
10	0	0	0.03	0.03	56.01	0.03	0.1	0.14

Appendix 3.8 Results of electron microprobe analysis of a *Tridacna squamosa* shell section

Point No.	P	S	Fe	Ba	Ca	Mg	Mn	Sr
1	51.46	0.05	0.07	0.05	0.06	0.1	0.25	0.05
2	55.34	0	0.07	0.03	0.02	0	0.16	0.17
3	48.25	0.05	0	0	0.05	0	0.12	0.03
4	53.8	0	0.06	0	0	0	0.13	0
5	42.59	0	0	0.04	0.1	0	0.18	0.06
6	45.32	0.01	0	0.04	0.06	0	0.15	0.09

Appendix 3.9 Results of electron microprobe analysis of a *Tridacna maxima* shell section

Point No.	P	S	Fe	Ba	Ca	Mg	Mn	Sr
1	0.03	0.03	0.04	0.12	57.47	0	0.09	0.23
2	0	0	0.16	0	56.74	0	0.02	0.16
3	0	0	0	0.1	57.66	0	0	0.17
4	0	0.04	0	0.02	56.96	0.02	0.1	0.18
5	0	0	0.05	0.03	57.76	0.02	0	0.18
6	0.03	0	0.11	0.03	56.44	0.03	0.08	0.2
7	0	0.04	0	0	58	0.02	0.11	0.19
8	0	0	0.05	0	56.64	0	0.09	0.19
9	0.01	0.03	0	0.17	56.42	0	0.1	0.17
10	0	0	0.05	0	56.28	0.02	0.02	0.08
11	0.02	0.04	0.09	0.02	57.21	0.03	0.09	0.16
12	0.03	0	0	0.06	56.51	0.02	0.06	0.25
13	0.03	0	0	0.03	57.01	0	0.09	0.23
14	0.03	0.02	0.14	0.07	57.31	0.03	0	0.16

Appendix 3.10 Results of electron microprobe analysis of a *Tridacna crocea* shell section

Point No.	P	S	Fe	Ba	Ca	Mg	Mn	Sr
1	0	0.04	0.21	0.1	54.14	0.03	0.06	0.14
2	0.04	0	0.05	0	54.92	0.05	0.03	0.23
3	0.07	0	0.05	0.07	53.1	0	0.11	0.18
4	0.05	0.04	0.02	0.09	53.33	0.03	0.12	0.21
5	0	0.04	0.07	0.07	49.09	0.03	0.1	0.16
6	0.03	0	0.1	0	49.72	0.02	0.12	0.24
7	0.06	0.04	0	0.03	58.82	0.05	0.08	0.23
8	0	0.08	0	0.1	57.94	0	0	0.22
9	0.01	0	0	0	57.04	0.03	0.07	0.21
10	0	0.01	0.03	0.06	56.36	0	0.08	0.33
11	0	0.04	0	0.04	56.27	0	0.09	0.26
12	0	0.04	0	0.22	56.78	0.02	0.08	0.23

APPENDICES CHAPTER 5

Appendix 5.1 From Nash et al. (1988)

Appendix 5.2

An Improved Gonad Biopsy Technique for *Hippopus hippopus*

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Appendix 5.3

Reproductive Periodicity and Morphometry of *Hippopus hippopus* and *Tridacna crocea*

C.C. Shelley* and P.C. Southgate**

Appendix 5.4

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Trematode (Digenea: Bucephalidae) infection in the burrowing clam *Tridacna crocea* from the Great Barrier Reef

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APPENDIX CHAPTER 6

Appendix 6.1

Ocean-Nursery Technology and Production Data for the Giant Clam *Tridacna gigas*

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