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Age, growth and population dynamics of tropical squid and sepioid populations in waters off Townsville, North Queensland, Australia

Thesis submitted by

George David JACKSON BSc(Hons) (JCU)

in February 1991

for the degree of Doctor of Philosophy in

the Department of Marine Biology

James Cook University of North Queensland

To Christine, who was a continual
source of encouragement and support
throughout this study.

Frontispiece: The near-shore loliginid squid
Loliolus noctiluca

"Very little is known about the longevity of squids...some of the smaller species might live for four years. It is almost certain that some of the large specimens of giant squids which have been taken were a good deal older than this. But how old nobody knows".

F.W. Lane *Kingdom of the Octopus*, 1974.

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ACKNOWLEDGMENTS

There are a number of people who have assisted with my research on this project. My supervisor, Professor Howard Choat provided much needed guidance and support throughout this project. His new-found interests in squids resulted in many interesting discussions and new ideas. His enthusiasm never waned (even through difficult times - such as the time I accidently embedded a squid jig in the skin of his back, during a research trip aboard the university vessel). My wife has provided unceasing help and support in many areas, throughout the years of this research. I would like to thank the crew of the 'James Kirby' who provided much needed assistance for the trawling trips. Many JCU students were also a great help during many of the vessel trips also. My associates in the Marine Biology lab (Frank Hoedt, Brett Molony and Lou) contributed to the interesting work environment of the lab. Many thanks to Frank who assisted with most of the trawling trips and a number of near-shore collecting trips. Natalie Moltschaniwskyj provided assistance with statistical analysis and provided some statoliths for examination. Leigh Winsor assisted with histological examination of the gonad material, Zolly Florian assisted with photography, Ann Sharp prepared the majority of tables and Bronwyn Scott provided specimen drawings. I would like to thank Dr. C.C. Lu who provided useful information. Dr. Lu and Mark Norman also assisted with research while I was in Melbourne. I would like to thank Roger Hanlon who sent statoliths and provided age information on cultured individuals of *Sepioteuthis lessoniana*. I would especially like to thank Dr. Ross Alford who assisted with theoretical aspects of curve-fitting and who offered editorial help on chapter seven. Last but not least I would like to thank my parents who have done all they could over the past years to encourage me in my studies.

ABSTRACT

Growth and ageing research was undertaken for squid and sepioid populations in waters off the Townsville region, North Queensland, Australia. The study species were the sepioid *Idiosepius pygmaeus*, and the loliginid squids *Loliolus noctiluca*, *Loligo chinensis*, *Loligo* sp. 1 and *Sepioteuthis lessoniana*. Age and growth parameters were determined by enumerating growth rings within the statolith microstructure of each of the species. Various mounting and grinding/polishing techniques were employed to reveal the growth rings. Daily periodicity in statolith growth rings was demonstrated to exist in 24 individuals in four of the study species; *I. pygmaeus* (6), *L. noctiluca* (9), *L. chinensis* (2) and *S. lessoniana* (7). Ring periodicity was determined by staining the statoliths *in situ* with tetracycline or calcein, and then maintaining the individuals in captivity to compare the rings laid down to the number of elapsed days. Double staining techniques were also employed to determine the rings laid down between stainings.

Field captured individuals of each of the study species were aged to construct growth curves. Growth was found to be rapid and maturity was found to be reached surprisingly fast. Tropical squid and sepioid growth is more correctly measured in days rather than months or years. The ageing studies with all five species did not reveal any individuals older than 200 days.

The ageing techniques developed were employed to discern any seasonality in squid and sepioid growth patterns. Regular sampling of *I. pygmaeus* and *L. chinensis* revealed different patterns of abundance, with *I. pygmaeus* more common during the winter period while *L. chinensis* was more abundant during the summer period.

Comparing growth based on statolith ageing to growth based on ELEFAN analysis on length frequency data revealed two diverging results. Length frequency analysis suggested that squid growth was asymptotic and that the life cycle was perennial. In contrast ageing research revealed that life span was short and that growth was rapid and non-asymptotic.

Seasonal size-at-age data for *I. pygmaeus* and *L. chinensis* revealed different patterns of growth during different seasonal periods. For *L. chinensis* growth was considerably slower during the winter period. For *I. pygmaeus* slower growth was also observed during the cooler period of the year. However, there was considerable scatter in the size-at-age data, probably as a result of greater variability in the near-shore environment. Differences were also detected in the relative statolith size in individuals of *L. chinensis* , with winter squids having larger statoliths for any given length than their summer counterparts. Alternatively, at comparable ages, winter squids had smaller statoliths than their summer counterparts, however, with increasing age, the longer-lived winter squids eventually obtained larger relative statoliths.

Determining individual size-at-age allowed for a more detailed understanding of age specific reproductive patterns. Male individuals of *Loligo chinensis* matured at a greater age in July (winter) than in January (summer) and no mature females were found in July. These age specific maturity patterns could not be discerned by an analysis of individual size. Development of the nidamental gland and hectocotylus was shown to follow a similar pattern to gonad development.

Mature individuals of *L. chinensis* were found throughout the year (except

July, 1989 for females). However, there was considerable fluctuations in the gonadosomatic index for both sexes with largest relative gonad sizes achieved during October for both years studied. This pattern appeared to be real and not biased by variation in the size of individuals captured during different months of the year.

Idiosepius pygmaeus showed a different pattern in gonad growth with cooler season (spring) sepioids growing older and having larger gonads than warmer season (autumn) individuals, despite the fact that there was very little difference in individual size between the two seasons. Measurement of oocyte diameters within the ovaries of two *I. pygmaeus* females suggested that this species was a serial spawner. Maintenance experiments of captive *I. pygmaeus* females confirmed that this species lays its eggs in repeated batches, rather than all at once. Maximum egg output recorded was 922 eggs produced in 15 days. Moreover, histological examination of the oocyte size distribution of all five study species suggested that they were serial spawners, since a variety of oocyte sizes were present within the ovary.

More relevant tropical squid growth models could be developed as a result of obtaining individual size-at-age data. All the five study species displayed allometric growth, with *Loligo chinensis* and *Loligo* sp. 1 displaying significant differences in the length-weight relationship between males and females, while *Idiosepius pygmaeus* revealed sex-related differences in slope elevation of the L-W relationship.

Detailed growth models were developed for *L. chinensis* and *S. lessoniana*. *Loligo chinensis* were shown to exhibit exponential growth with a considerable

proportion of the lifespan spent as juveniles. There was no significant difference between the growth curves of male and female *L. chinensis* in either growth in length or weight. The growth of *S. lessoniana* could not be modelled with a single equation. Therefore, an exponential curve was fitted to the size-at-age data (for both mantle length and weight) for the first 70 days, while growth from 70 to 153 days could be described by a linear equation. These growth models were compared to growth curves developed for other loliginids raised in captivity. *Loligo chinensis* was found to grow faster than other temperate loliginids, while the growth of *S. lessoniana* revealed a remarkable similarity to the form of growth of the tropical *S. sepioidea.* Length-at-age data for *Loligo opalescens* was found to not differ greatly from results of growth of this species in captivity, although field individuals appeared to grow somewhat faster than captive individuals.

Very clear growth rings were also observed within the statolith microstructure of the giant squid *Architeuthis*, which suggested that ageing research and growth modelling based on size-at-age data could be expanded to a variety of other species of oceanic squids. Statolith growth ring analysis is thus one means to obtain important growth parameters for squid species in which we have little biological information for.

TABLE OF CONTENTS

CHAPTER ONE : GENERAL INTRODUCTION

CHAPTER TWO : SPECIES STUDIED, HABITATS AND GENERAL BIOLOGY

CHAPTER THREE : GENERAL METHODS

CHAPTER FOUR : STATOLITH ANALYSIS AND GROWTH RING VALIDATION

CHAPTER FIVE : THE PATTERN OF AGE AND SEASONAL SPECIFIC EVENTS

CHAPTER SIX : REPRODUCTIVE BIOLOGY

CHAPTER SEVEN : ALLOMETRY AND GROWTH MODELLING

CHAPTER EIGHT : GENERAL DISCUSSION AND CONCLUSIONS

LIST OF TABLES

CHAPTER FOUR

CHAPTER FIVE

CHAPTER SEVEN

LIST OF FIGURES

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

CHAPTER SIX

CHAPTER SEVEN

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

G. D. Jackson
22 February 1991

1.0 GENERAL INTRODUCTION

This thesis is focused on the validation of age estimates in nektonic cephalopods and the demographic application of size-at-age data. Research concentrated on the use of daily rings within the statolith microstructure to obtain age-specific growth and demographic parameters for tropical squids and sepioids. There was a considerable need to establish better growth parameters for nektonic cephalopods and especially tropical species. This research was particularly relevant due to the fact that cephalopods display a number of distinctive and intriguing aspects in their life histories, population demography and physiology.

The question of the form of growth in cephalopods is a particularly relevant one, especially in the light of opposing views which have been published with regards to cephalopod population dynamics. There has been considerable confusion and diversity of opinion with regard to the form of growth in squids (Hixon, 1980; Mangold, 1983; Boyle, 1990). Data presented in the literature has suggested conflicting views of both an extended lifespan and asymptotic growth (eg. Rao, 1954; Worms, 1983a; Pauly, 1985; Supongpan, 1988) as well as rapid, curvilinear, non-asymptotic growth (eg. Rosenberg *et al.*, 1981; Yang *et al.*, 1986; Forsythe & Van Heukelem, 1987; Forsythe & Hanlon, 1989). One factor contributing to this problem has been the use of inappropriate methodologies to describe squid growth. In many instances this has involved the unjustified application of teleost derived models to population statistics of pelagic cephalopods.

Although squids are molluscs, they do appear to function on an ecological level closer to fish than to other molluscs. Striking physical and ecological similarities have been shown to exist between squids and fish (Packard, 1972; Saville, 1987). However, unlike fish, squids are constrained by a number of physiological and metabolic factors (O'Dor & Webber, 1986; O'Dor, 1988). Some of these factors include a limited ability to store energy reserves and the inability to adequately digest and assimilate lipids (O'Dor & Wells, 1987).

Research into the age and demography of cephalopod stocks is also relevant due to the importance these organisms play in the trophodynamics of the world's oceans. Squids serve as a major prey item for marine mammals (Fiscus, 1982; Clarke, 1983; Clarke & Pascoe, 1985; Pascoe, 1986; Pascoe *et al.*, 1990; Lipinski & David, 1990), marine birds (Rodhouse *et al.*, 1987; Rodhouse, 1989) and oceanic fishes (LeBrasseur, 1966; Voss, 1983; Okutani & Tsukada, 1988; Kobodera & Shimazaki, 1989). Alternatively, squids also are voracious carnivores (Nixon, 1987) and serve as important predators of fish stocks (Amaratunga, 1983; O'Sullivan & Cullen, 1983; Roper *et al*, 1983; Maurer & Bowman, 1985; Breiby & Jobling, 1985). Moreover, squids constitute important fisheries in many parts of the world (see reviews, Worms, 1983b; Voss, 1983; Rathjen & Voss, 1987). World cephalopod stocks are estimated to be enormous, exceeding 100 million tonnes. This compares to 70 million tonnes for the total annual catch of finfish and probably exceeds half of the biomass of the world's human population (Clarke, 1983).

Validation of the daily periodicity in statolith growth rings has been a major breakthrough and a key factor in the study of the growth dynamics of pelagic cephalopods. Without an ability to age individuals, it is difficult to obtain estimates of cohort age from population samples. Early attempts to age cephalopods have involved the enumeration of radular teeth (Nixon, 1969, 1973) as well as the detecting of laminae within the cuttlebone (Yagi, 1960; Choe, 1963; Packard, 1972; Richard, 1969), beak (Clarke, 1965) and gladius (LaRoe, 1971). However, none of these studies reached satisfactory conclusions with regards to using these structures to determine individual age.

All cephalopods excluding *Nautilus* possess paired calcium carbonate statoliths. Each statolith lies within a statocyst cavity situated at the base of the cephalic cartilage. The inside of the statocyst cavity is lined with a strip of sensory epithelium (the cristae) composed of hair cells and afferent neurones (Williamson, 1989a). This structure serves as part of the cephalopod equilibrium receptor system, contributing to detection of linear and angular acceleration and the providing of compensatory eye movements for the stabilisation of the retinal image (Budelmann, 1988; Young, 1989). The statolith cristae receptor system is considered analogous to the vertebrate semicircular canal system (Williamson, 1989b).

Statoliths are primarily calcium carbonate crystallised as aragonite, although they possess an organic protein matrix (Radtke, 1983). The distribution of protein within the statolith matrix has been found to be unevenly distributed. Concentrations of protein are in the statolith wing and dorsal dome (Lipinski, 1986), (see Clarke, 1978 and Rodhouse & Hatfield, 1990a for a description of the statolith microtopography).

Statolith growth rings were first documented by Clarke (1966). However, the majority of research has been carried out within the last decade. There were initially some reservations with regards to the presence of rings within the statolith microstructure. Dilly (1976) denied the existence of statolith growth rings and reported that there was no evidence of regular growth which might produce rings. Dilly (1976) attributed any changes in density within the statolith to changes in density due to thinner parts of the statolith passing more light than thicker parts. Furthermore, it was stated that any diurnal rhythm was probably too rapid to affect statoconia crystal growth.

Shortly after Dilly's (1976) report, the presence of statolith growth rings was verified with evidence provided from *Loligo opalescens* (Spratt, 1979) and *Illex illecebrosus* (Hurley & Beck, 1979; Lipinski, 1980). Subsequent to these initial findings there have been a number of studies which have documented the presence of statolith microstructural growth rings within a number of squid species and several sepioids (eg. Kristensen, 1980; Lipinski, 1981; Rosenberg *et al.*, 1981; Natsukari *et al.*, 1988; Kinoshita, 1989). Furthermore, several laboratory studies have also demonstrated that rings are produced daily (Hurley *et al.*, 1985; Dawe *et al.*, 1985; Lipinski, 1986; Nakamura & Sakurai, 1990). These rings bear a striking resemblance to daily rings in fish otoliths first documented by Pannella (1971).

This growing pool of background evidence regarding squid ageing suggested that accurate size-at-age information could be obtained for tropical species and used to construct age-specific growth and maturity schedules. The majority of cephalopod ageing research has been preliminary in scope and focused on temperate species. Therefore, there was a need to undertake a more detailed demographic analysis on the application of statolith age data for nektonic cephalopods a especially for tropical species. The tropical marine environments accessible to this study provided the means to analyse tropical squid and sepioid growth with a number of species. There was an order of magnitude difference in the size range of the study species (*Idiosepius pygmaeus, Loliolus noctiluca, Loligo chinensis, Loligo* sp. 1 and *Sepioteuthis lessoniana)* from the small *I. pygmaeus* less than 20 mm in mantle length to *S. lessoniana* which reached mantle lengths greater than 200 mm. The aims of this research was to not only obtain demographic and maturation information for these tropical cephalopods through statolith analysis, but to compare statolith ageing techniques to data obtained from population cohort analysis.

The research results are organised into four chapters (numbered 4-7) along with a chapter dealing with the general background biology and biogeography of each of the study species (Chapter 2). Chapter four deals with the establishment of techniques for the validation and enumeration of statolith growth rings, while the following three chapters deal with population biology and the application of ageing research to obtain demographic and maturity parameters for the study species (with *L. chinensis* and *I. pygmaeus* used as the focus for much of the research). The primary aims and topics addressed in each of the chapters are as follows:

Chapter 4 Do the respective species possess growth rings within the statolith microstructure and can techniques be employed to visualise and enumerate these growth rings? Can experimental methods be employed to determine the periodicity of these rings, and can the resulting information on size-at-age of field captured individuals be used to obtain an understanding of relevant demographic parameters?

Chapter 5 What is the seasonal abundance of *I. pygmaeus* and *L. chinensis* and can an understanding of age parameters be related to trends in population abundance? By obtaining regular samples of these two species it was possible to carry out more traditional length frequency analysis to obtain estimates of growth based on cohort modal progressions, and to compare this technique with statolith ageing. The results obtained were considered in the context of current methodologies of establishing growth parameters for squids, and current theories of squid growth. Seasonal differences in growth of these two species were also investigated. Finally, the growth of the statolith in relation to growth of the mantle was explored for *L. chinensis* and compared to current findings in fish soma otolith size relationships.

Chapter 6 What can individual age analysis reveal about the timing of maturity processes in cephalopods? Gonad analysis was therefore carried out to determine age-specific schedules of maturity during different seasonal periods. Temporal variability in gonad growth and growth of associated reproductive structures was also considered.

Chapter 7 Growth and allometry of the study species were considered and discussed in relation to conclusions reached for other species. Although size-at-age data could provide information about the form of growth in squids, more detailed growth modelling was carried out to determine if size-at-age information could be used as a basis to mathematically describe growth in *L. chinensis* and *S. lessoniana.* Cephalopod growth modelling is reviewed and avenues for further research are considered.

Finally, a summary of the factors influencing cephalopod growth, and the fundamental differences between cephalopods and fish are considered in the general discussion. The appendices provide summary publications of the validation and application of the statolith ageing techniques carried out in this study.

2.0 SPECIES STUDIED, HABITATS AND GENERAL BIOLOGY

The continental shelf waters off Townsville, as well as the local near-shore environments are well situated for the study of tropical Cephalopoda. The variety of marine habitats provide a diversity of different areas inhabited by cephalopods. Furthermore, the obtaining of live specimens for experimental work was promoted by the accessibility of these diverse marine habitats. Although this research focused primarily on loliginid squids and one sepioid, there are also a variety of other sepiolids and cuttlefishes which can be obtained in local waters.

2.1 Species studied

This study focused on one sepioid, *Idiosepius pygmaeus* and four loliginid squids, *Sepioteuthis lessoniana*, *Loliolus noctiluca*, *Loligo chinensis* and *Loligo* sp.1 (a new species currently being described). *Loligo chinensis, Loligo* sp. 1 and *I. pygmaeus* are predominantly restricted to tropical and subtropical waters while *S. lessoniana* and *L. noctiluca* also have a tropical distribution, but extend into temperate waters.

As mentioned previously, the scope of this study enabled the analysis of growth in cephalopods, covering an order of magnitude in size from the small *I. pygmaeus* which matures at less than 20 mm mantle length to the large *S. lessoniana* which reaches lengths greater than 200 mm in mantle length (Fig. 2.1).

2.1.1 *Idiosepius pygmaeus*

Idiosepius is the only genus now recognised as belonging to the family

Idiosepiidae, which was originally described by Steenstrup (1881). The family consists of six species distributed throughout the Indo-West Pacific; *I. biserialis* and *I. macrocheir* in South Africa (Voss, 1962); *I. pygmaeus* in the Philippines (Voss, 1963), Indonesia (Berry, 1921), South China Sea, Palau Is. and northern and northeastern Australia (Nesis, 1987); *I. picteti* in eastern Indonesia (Berry, 1921); *I. paradoxus* in Japan and South Korea (Nesis, 1987) and *I. notoides* an endemic Australian species recorded from South Australia (Berry, 1921), Victoria (Burn, 1957, 1959), north to Sydney (English, 1981) and west to Cockburn Sound (Wells & Bryce, 1986).

The genus has a number of distinguishing features such as: small body size, with all recorded individuals less than 20 mm in mantle length; the hectocotylisation of both ventral arms in the male; the presence of a unique dorsal adhering organ which is used for attachment to objects (Sasaki, 1923); and the fact that the newly hatched paralarvae lack tentacles (these are formed during post-embryonic development) (Boletzky, 1977).

Preliminary work with *I. pygmaeus* in the Townsville region (Jackson, 1986, 1989) indicated that this species was exceptionally abundant during certain periods of the year in near-shore mangrove and breakwater habitats. The large numbers and the accessibility of this species provided a basis for a more detailed population and ecological study.

2.1.1.1 Habitat and biology of *I. pygmaeus*

Idiosepius pygmaeus was readily observed along mangrove mud banks or along rocky breakwaters in relatively shallow water. In many situations (especially in the mangrove tributaries) they occurred in the vicinity of flotsam, and often attached to floating dead mangrove leaves with their attachment organ. Although most reports of temperate *Idiosepius* species indicate a benthic or seagrass habitat preference (Sasaki, 1929; Burn 1959), *I. pygmaeus* appeared to be predominantly free swimming although littoral in distribution. The distribution of *I. pygmaeus* in shallow water along the Townsville breakwaters bears a close resemblance to the habitat description given by Moynihan (1983) for Koror, Palau, in which individuals were captured in shallow water (less than 1 m) "over hard and rather bare surfaces, natural rock and coral or artificial concrete and iron" and "floating or swimming high in the shallows near the shoreline on bright sunny days".

Individuals were most commonly observed singly or in pairs, although it was not uncommon to find four or five individuals closely associated together. On several occasions schools up to six individuals were seen. *Idiosepius pygmaeus* most commonly was observed at the surface, and even after considerable disturbance (eg. missing them with a dip-net) they usually returned back to the surface after several minutes.

Both field and laboratory observations have revealed that *I. pygmaeus* preys predominantly on *Acetes sibogae australis, a* small sergested shrimp which periodically swarms in large numbers in near-shore waters. Only on one occasion was an individual of *I. pygmaeus* observed eating a fish (*Gobiopterus*) in the field. At this time very few *Acetes* were observed, however, there were exceptional numbers of juvenile *Gobiopterus* schooling along the breakwater where individuals of *I. pygmaeus* were captured.

Due to its small size and robustness *I. pygmaeus* was easy to collect and maintain in captivity in relatively small aquaria. Copulatory activity was often observed in the field and in captivity. Females also readily laid eggs in captivity.

The fact that this species was often found up estuarine tributaries in very shallow water suggests that this species is probably considerably tolerant of variable water conditions and salinities. *Idiosepius pygmaeus* has also been captured in sizeable numbers in light traps in shallow, reef waters around Lizard Is. in the northern Great Barrier Reef (McCormick & Jackson, unpublished data).

2.1.2 *Sepioteuthis lessoniana*

The genus *Sepioteuthis* is a very distinctive genus with large ovate marginal fins and a very robust thickset body. This is in contrast to most other loliginid squids which tend toward a longer, streamlined body form. *Sepioteuthis* therefore has a somewhat intermediate body form between the other loliginids and the cuttlefishes (Boycott, 1965). The long marginal fins of *S. lessoniana* also promote greater manoeuvrability and the ability to hover in one position, unlike the other loliginids (Boycott, 1965; Hanlon, 1990).

The genus has three species; *S. sepioides* a common, reef species found in the tropical western Atlantic from Bermuda and Florida to southern Brazil; *S. lessoniana* in the Indo-West Pacific from the Red Sea and southern Africa to Hokkaido, Hawaii and Australia; and *S. australis* in southern Australia, north to Dampier in the west and up the east coast to Brisbane (Nesis, 1987).

The range of *S. lessoniana* in Australia extends across the top and down both the east and west coasts into sub-tropical waters, to south of Fraser Is. 27°12'S on the east coast and to south of Geraldon on the west coast 28°12'S.

In the west the distribution of *S. lessoniana* overlaps with the distribution of *S. australis*. *Sepioteuthis lessoniana* is common in inshore waters and extends in depth to at least 110 m (Lu & Tait, 1983).

Within the Townsville sampling area, juvenile individuals of *S. lessoniana* (usually less than 10 cm in length) were regularly observed and dip-netted off the breakwaters during the warmer time of the year. On several occasions adults were also observed along the breakwater. Larger individuals were commonly observed around boats at night and off lighted jetties in the local Townsville region as well as around boats in the Great Barrier Reef Lagoon and on reefs. *Sepioteuthis lessoniana* appears to be predominantly reef associated in the Townsville area, as on only several occasions were juveniles captured while trawling.

2.1.3 *Loliolus noctiluca*

Loliolus is a genus of small loliginid squids less than 100 mm in length (Lu *et al*, 1985). There are three species; *L. hardwicki* ranging from the Persian Gulf throughout Indonesia and off the north Chinese coast opposite Taiwan, in shallow near-shore and estuarine waters to a minimum of 30 m; *L. affinis* found from India to Indonesia; and *L. noctiluca* which occurs on the east coast of Australia from New Guinea to Tasmania, predominantly in bays and estuaries, although individuals have been captured as deep as 46 m. *Loliolus noctiluca* has also been reported from estuaries with bottom salinities as low as 24 ppt and surface salinities as low as 17.5 ppt (Lu *et al.*, 1985).

In the Townsville study area, individuals of *L. noctiluca* were captured in near-shore habitats, often within metres of the beach. Individuals were also captured in trawls taken close to the shore (eg. near Long Beach on Cape Cleveland) and many juveniles were captured with beach seines, especially during the winter. *Loliolus noctiluca* was also found to be relatively hardy and easy to trasport and maintain in captivity.

2.1.4 *Loligo chinensis*

The genus *Loligo*, unlike the other study genera discussed, is very speciose, and has representative species found throughout much of the continental shelf and slope waters in tropical and temperate seas. *Loligo chinensis* is common in the south Pacific and occurs in the western Pacific, in the south and east China Seas to Japan. It is fished throughout much of its range and is an important commercial species (Roper *et al.*, 1984). Within Australian waters, *L. chinensis* occurs in the Arafura Sea and south to at least New South Wales and is jigged in waters as deep as 300 m (Winstanley *et al.*, 1983). Currently it is not known how far this species extends south along New South Wales (Dunning, personal commmunication). Nesis (1987) however, has reported the distribution of *L. chinensis* to include Tasmania.

In the Townsville region, this species was regularly captured with bottom trawls and individuals were jigged around boat and jetty lights. *Loligo chinensis* formed a small part of the trawl catch in Cleveland Bay, with numbers usually low in the winter. However, on several occasions during the summer period, very large numbers of squids were captured, with squids forming the dominant segment of the trawl.

2.1.5 *Loligo* sp. 1

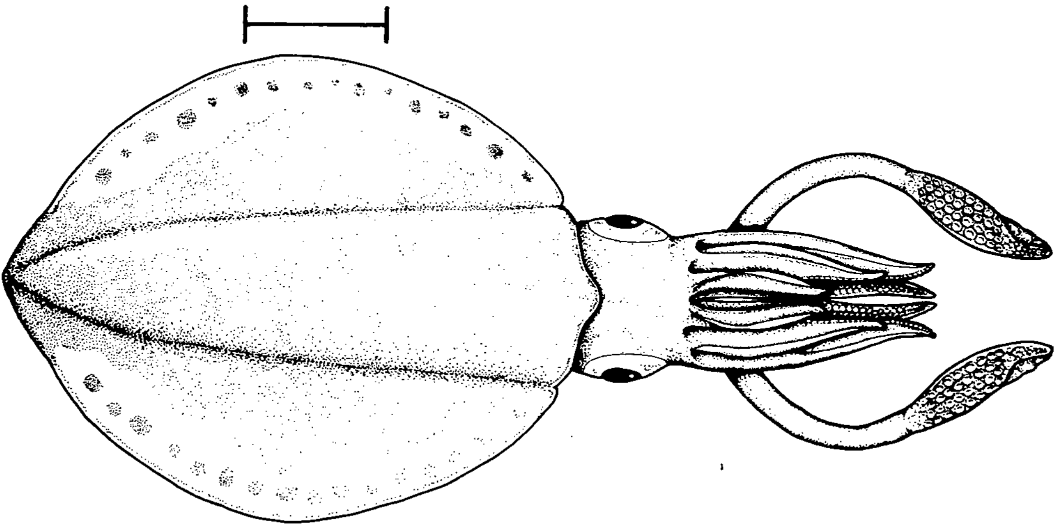
This species is currently being described by C.C. Lu, and very little is known of its biology. In Townsville waters it occurred concurrently with *L. chinensis,* although it was captured only sporadically throughout the year. As with *L. chinensis,* on several occasions during the summer, very large numbers of predominantly mature *Loligo* sp. 1 were captured in association with *L. chinensis* off Florence Bay, Magnetic Island which suggests that this species may move inshore into specific areas for spawning.

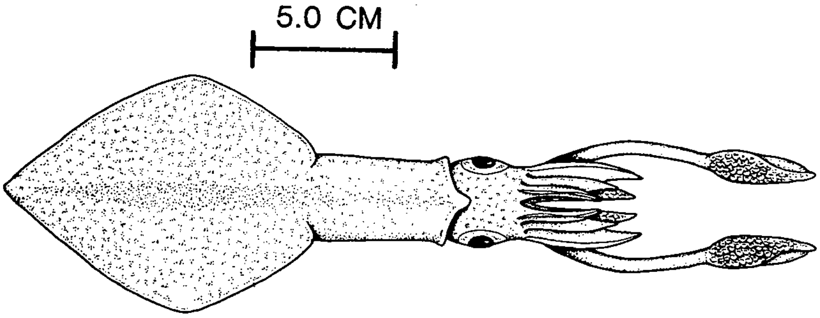
The capture of *Loligo* sp. 1 during this study represents the furthest southern collection of this species along the Australian East Coast. Although the distributional data of this species is incomplete, it extends from at least the Townsville region on the east coast, and north across the top of Australia and down the west coast to at least Shark Bay (C.C. Lu, personal communication).

Figure 2.1

Relative sizes of four of the study species, from left to right; *Sepioteuthis lessoniana, Loligo chinensis, Loliolus noctiluca, Idiosepius pygmaeus.*

Loligo sp.1 is similar in size to *Loligo chinensis*

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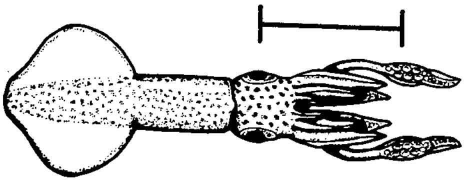

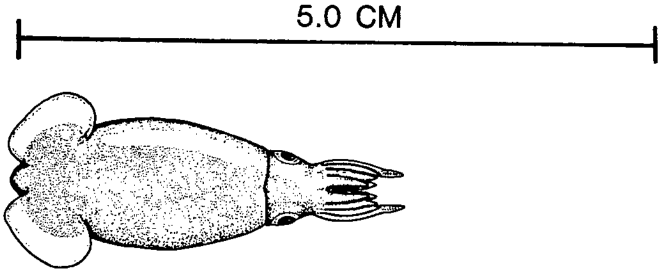
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3.0 GENERAL METHODS

This chapter provides background information with regards to the localities and the marine environments sampled during the research program. General collection and preservation techniques are also described. More detailed specific methods are given in each of the respective chapters.

3.1 Study area

All sampling was undertaken in local inshore waters in the vicinity of the Townsville harbour/breakwater complex, and within the waters of Cleveland Bay (Fig. 3.1). Cleveland Bay lies within the Central Great Barrier Reef province. Although the environment is tropical (the locality of Townsville Harbour is 19°15'S, 146°50'S), there is a marked seasonal fluctuation in temperature, with temperatures ranging from 19.3°C to 30.9°C (Walker, 1981). Sampling was carried out in the near-shore localities in less than 1 m water depth, while trawling in Cleveland Bay was carried out in water between 2 and 20 m depth.

3.2 The near-shore environment

The local harbour/breakwater environment provided a convenient collecting venue for littoral squids and sepioids. Although *I. pygmaeus* was predominantly collected along the breakwaters, juvenile *S. lessoniana* were often observed and collected as well.

The inshore sampling localities were concentrated predominantly on two breakwater systems (Fig. 3.2, 3.3). The western breakwater marina complex was used for visual census of *I. pygmaeus.* The habitat was left undisturbed, except for the occasional specimens collected for experimental work. The distance covered for each sampling period was approximately 3.21 km. For visual sampling, the position of each sepioid was recorded on a map of the breakwater, and later enumerated. The eastern breakwater complex served as a regular collecting locality for individuals of *I. pygmaeus* for length frequency and ageing analysis. This breakwater is only temporary and the area it encloses will be later filled for land reclamation (B. Holden, Townsville Harbour Board, personal communication) The area covered for each collecting trip along this breakwater was approximately 1.72 km. Samples taken consisted of two collecting trips on different days, usually within one 48 hour period. Regular collecting of juvenile squids and sepioids was carried out with a hand dip-net. The dip-net was triangular in shape (approximately 35 cm in width) and was constructed of 500 μm plankton mesh.

Other near-shore sampling was also undertaken for juvenile individuals of *L. noctiluca* off the Strand Beach, Townsville (Fig. 3.1) with 1 mm and 8 mm beach seine nets.

3.3 Offshore sampling and jigging

Collection of the larger species (predominantly *L. chinensis* and *Loligo* sp. 1) was carried out with dual otter trawls from the James Cook University Research Vessel 'James Kirby' (19.5 m length, 4.72 m breadth, 2.15 m maximum draft, steel hull vessel, equipped with a 300 HP engine). Each trawl net had an 11 m gape and 3.8 cm mesh. Trawls were generally 20 minutes in duration. Samples were taken predominantly between Florence Bay, Magnetic Island and Long Beach, Cape Cleveland (Fig. 3.1).

Adult individuals of *S. lessoniana* were never captured by trawling, however, they were often observed around reef areas and around jetty lights at night. Therefore individuals were collected at night using squid jigs off the jetty at the Australian Institute of Marine Science (AIMS) Cape Ferguson, and the Picnic Bay Jetty, Magnetic Island (Fig. 3.1). The jigs which were an imitation of a prawn (eg. the Japanese made YO-ZURI jig) were particularly effective in catching squids. No success was achieved with the more traditional plastic, 'barrel shaped' squid jig.

3.4 Preservation of samples

All squids and sepioids were immediately preserved upon collection. Ten percent seawater-formalin buffered with borax was used as the primary fixative for the larger squid species. The smaller species (*I. pygmaeus, L. noctiluca* and juvenile *S. lessoniana*) were fixed directly in 70% ethanol.

3.5 Terminology

When general reference is made to the Cephalopoda as a whole in this study, especially in regards to growth dynamics and demography, this is in reference to the subclass Coleoidea (Teuthoids, Sepioids and Octopods). Due to the very different characteristics of growth and demography of *Nautilus*, the subclass Nautiloidea is considered separately in the general discussion.

Standard scientific abbreviations are used throughout this thesis; μm=micrometre, mm=millimetre, cm=centimetre, m=metre, km=kilometre, °C=degrees celsius, h=hours, mg=milligrams, g=grams, kg=kilograms, millilitres=ml, l=litres, UV=ultraviolet and rate per day is given as d^{-1}. Cephalopod length in this thesis refers to dorsal mantle length (DML). The periodic laminae (daily rings) are referred to in this thesis as either 'increments' or 'growth rings', terms which are both used in the literature.

3.5.1 Larvae/paralarvae/juveniles/subadults

There has been some confusion in the past with regards to the terminology used for young cephalopods. The problem has been due to the fact that the young of cephalopods are generally miniatures of the adults and do not go through metamorphosis. Boletzky (1974) first dealt with this problem and suggested that cephalopods do not possess true larvae since they do not undergo metamorphic changes as outlined by Geigy & Portmann (cited in Boletzky, 1974), namely (a) elimination of the larval parts of the animal, (b) development of some adult features from rudiments that remained in the embryonic stage and (c) continuation of the formation of structures not affected by metamorphosis. Boyle (1983c) emphasised that the second process in the above outline is generally lacking in the development of cephalopods. He suggested that the term 'larva' could only be used as a convenient abbreviation rather than a strict concept.

Although most cephalopods are simply miniatures of the adults, some species, such as the ommastrephid squids do possess a hatchling which is very different from the adult (eg. fused tentacles forming a proboscis in the ommastrephid rhynchoteuthion) (O'Dor *et al.*, 1985). Boyle (1983c) suggested that for these forms, the use of the term 'larva' is justified due to the distinct morphological differences between the rhynchoteuthion and the adult. The problem is further complicated due to the wide range in both hatchling size and the mode of life of young cephalopods. Many benthic forms such as octopods and sepioids have hatchlings which are very similar to the adults and inhabit the same benthic environment as the adults. In contrast, many pelagic squids and some benthic octopods produce very small planktonic hatchlings.

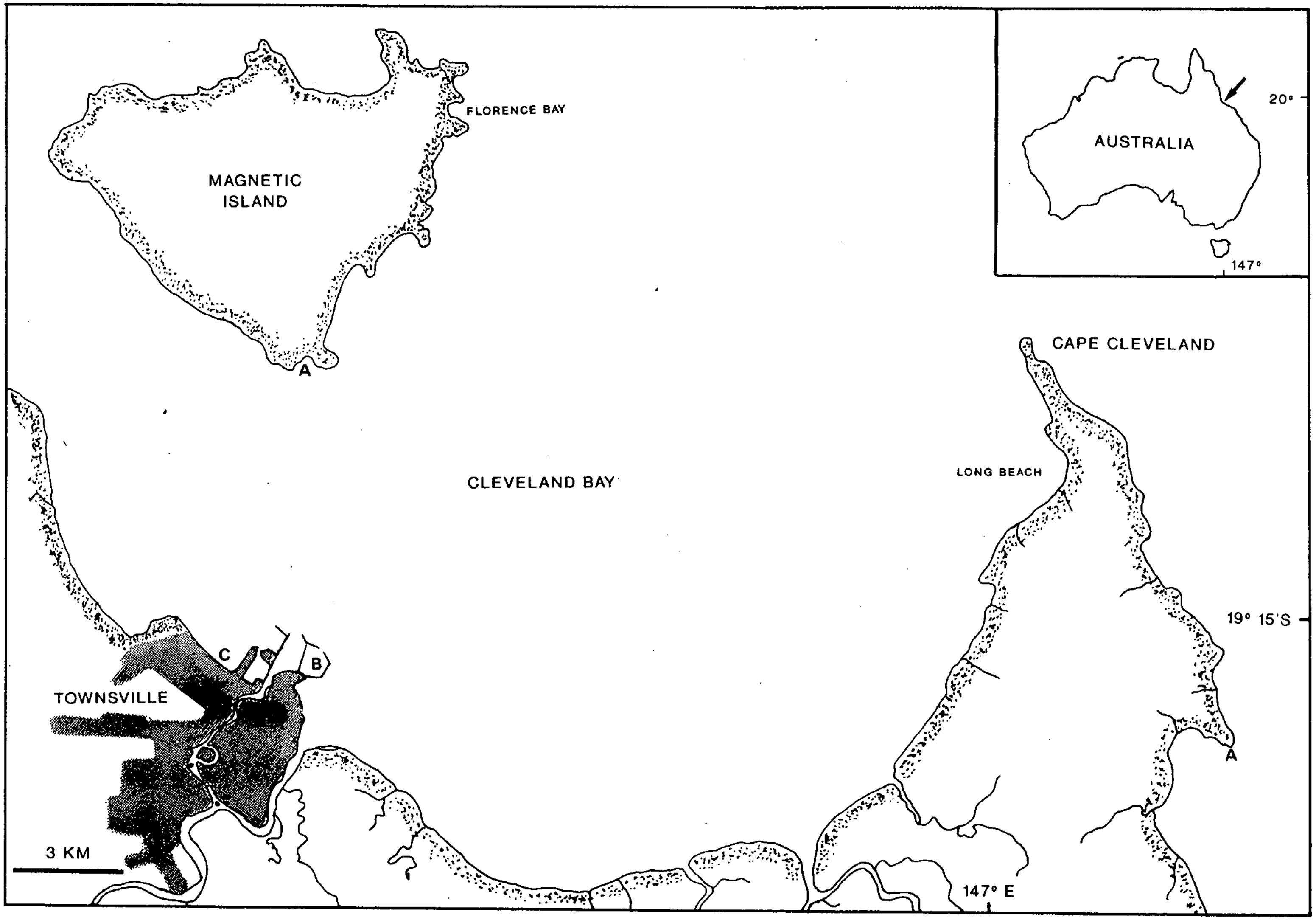
This dilemma in terminology has been somewhat clarified by Young & Harmon (1988). They attempted to provide a more generally used, universal means to describe young cephalopods. They suggested that some terms such as 'juvenile', as especially applied to the pelagic squids is so broad that it has little use. Young & Harmon (1988) thus proposed an alternative term 'paralarva' to define the young of species which have post-hatchlings with a very different mode of life than the older conspecifics. According to this criteria, only teuthoids, and octopods which produce planktonic young would possess paralarvae.

Although Young & Harmon (1988) provide both ecological and morphological criteria for the delineation of the term 'paralarva', they also suggest a simple criterion to use if morphological or ecological data is not available. According to this simple rule, a young cephalopod can be considered a 'paralarva' if it can be sampled quantitatively in near-surface waters during the day. The post-'paralarval' stage is then considered to be the subadult stage, which is characterised by the ability to distinguish all diagnostic morphological characteristics recognised to define the species.

The use of the term 'paralarvae' provides a means to clear up some of the past discrepancies that have existed in the literature. This study concurs with the use of the term 'paralarva' in reference to very small individuals which can be sampled with plankton nets. The use of the term 'juvenile' in this study is used in a more general sense in reference to young squids and encompasses the term 'paralarva'.

Figure 3.1

Squid and sepioid collecting sites in the Townsville region. (A) Collecting localities for *Sepioteuthis lessoniana* at the Picnic Bay jetty, Magnetic Island and the Australian Institute of Marine Science jetty, Cape Ferguson. (B) Collecting site for *Idiosepius pygmaeus*, Townsville eastern breakwater complex. (C) Collecting site for juvenile *Loliolus noctiluca*, Strand Beach, Townsville. Individuals of *Loligo chinensis, Loligo* sp. 1 and adult *Loliolus noctiluca* were trawled predominantly between Florence Bay, Magnetic Island and Long Beach, Cape Cleveland.



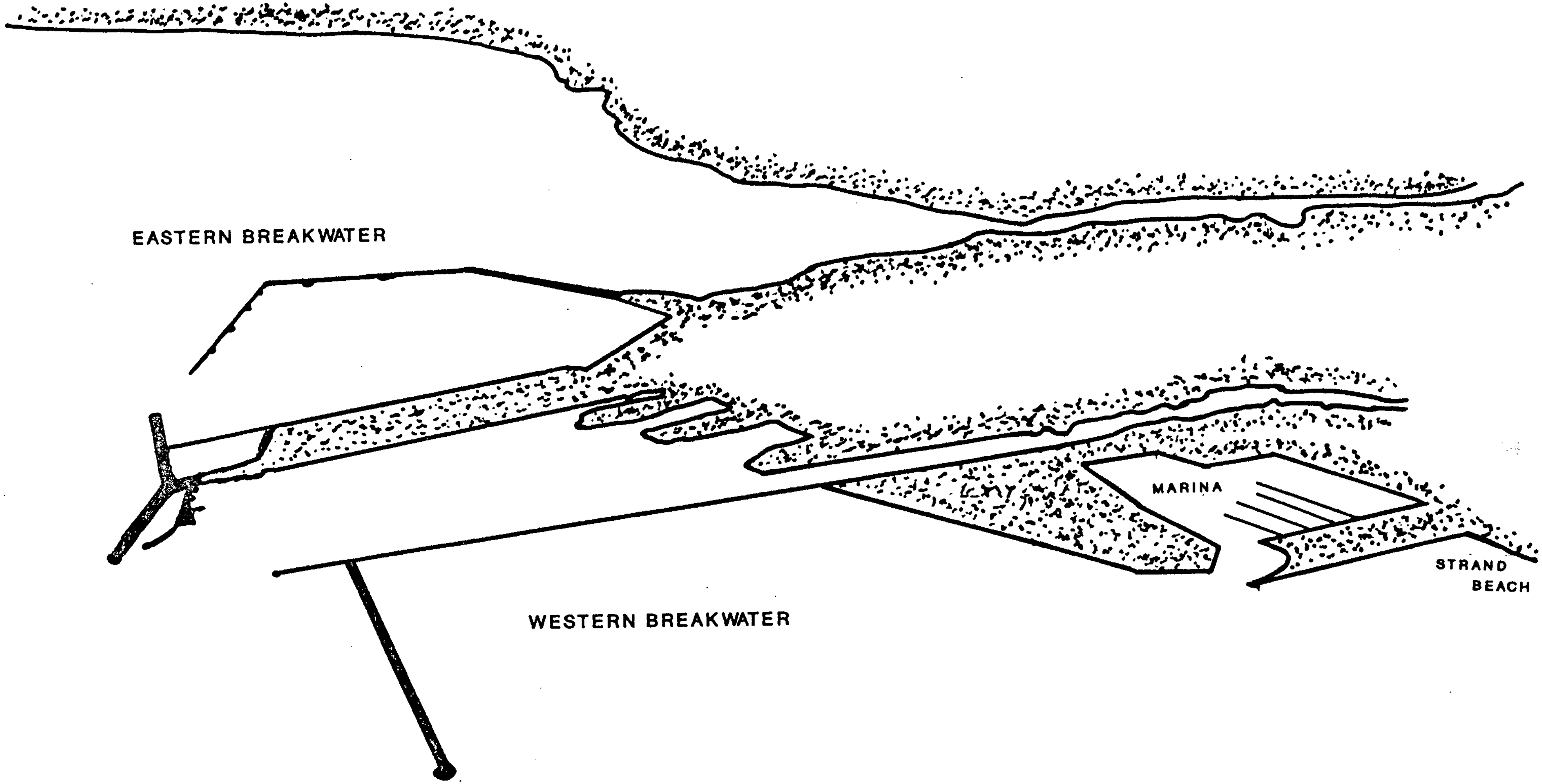
AUSTRALIA
20°
147°
FLORENCE BAY
MAGNETIC ISLAND
A
CAPE CLEVELAND
LONG BEACH
CLEVELAND BAY
19° 15'S
A
C
B
TOWNSVILLE
3 KM
147° E

Figure 3.2

Photograph of the Townsville Harbour and surrounding region, facing southeast. The western breakwater/marina complex is in the foreground adjacent to the Strand Beach (bottom right of photograph). The eastern breakwater complex is situated further back in the photograph. Cleveland Bay and Cape Cleveland are shown in the background (see Fig. 3.3 for further details). (Photograph courtesy of the Townsville Port Authority).

Figure 3.3

Important collecting localities in the Townsville Harbour region.


EASTERN BREAKWATER
MARINA
STRAND BEACH
WESTERN BREAKWATER

4.0 STATOLITH ANALYSIS AND GROWTH RING VALIDATION

4.1 INTRODUCTION

The techniques employed in this chapter to determine age and growth parameters in tropical squids parallel work being undertaken on growth ring analysis of fish otoliths. Fish have three pairs of calcareous otoliths which all appear to grow concentrically. Pannella (1971) first discovered the daily periodicity of these microstructural growth rings. Since then a plethora of information has been published on otolith microstructural research (see Campana & Neilson, 1985; and Jones, 1986 for reviews of the relevant literature). Growth ring analysis has provided a means to evaluate age structures and growth rates in young fishes, and constitutes a powerful tool for population analysis. However, while otolith growth ring analysis has focused primarily on larval and juvenile fishes, it has been of little use in ageing older fish or adults (Beamish & McFarlane, 1987), due predominantly to compression of the rings in older fish.

Cephalopods also possess internal calcareous balance organs (statoliths), which appear to be physiologically analogous to fish otoliths (Radtke, 1983). While fish possess three pairs of otoliths, cephalopods possess only one pair of statoliths. Similar 'otolith-like' growth increments have also been observed within the statolith microstructure of many squid species.

The majority of cephalopod species appear to be short-lived and exhibit rapid growth rates (Packard, 1972; Saville, 1987). The ability to age cephalopods is critical to understanding life history phenomena and population dynamics. Despite the presence of statolith microstructures, there have been few attempts to use this information to develop a better comprehension of demographic events in the Cephalopoda.

Statolith growth rings have been observed in a number of oegopsid squids; *Illex illecebrosus* (Hurley & Beck, 1979; Lipinski, 1978,1981; Radtke, 1983; Balch *et al.*, 1988, see also Dawe, 1981b); *Illex argentinus* (Lipinski, 1981; Rodhouse & Hatfield, 1990a, 1990b); *Illex oxygonius, Illex coindetii, Martialia hyadesi, Histioteuthis macrohista, Moroteuthis ingens, Todarodes angolensis,* (Lipinski, 1981); *Todarodes sagittatus* (Rosenberg *et al.*, 1981); *Todarodes pacificus* (Murata, 1990; Nakamura & Sakurai, 1990) and *Gonatus fabricii* (Kristensen, 1980); in the myopsid squids *Alloteuthis subulata* (Lipinski, 1986); *Photololigo edulis* (Natsukari *et al.*, 1988); *Heterololigo bleekeri* (Kinoshita, 1989); *Sepioteuthis lessoniana* (this study; Jackson, 1990a); *Loliolus noctiluca* (this study; Jackson, 1990b); *Loligo pealei* (Lipinski, 1981); *Loligo forbesi* (Martins, 1982); *Loligo opalescens* (Spratt, 1979); *Loligo gahi* (Rodhouse & Hatfield, 1990a) and *Loligo chinensis* (this study; Jackson, 1990b). Growth rings have also been shown to exist in the sepioids *Rossia glaucopis* (Kristensen, 1980) and *Idiosepius pygmaeus* (this study; Jackson, 1989). Concentric rings do not appear to be laid down in octopus statoliths (Boyle, 1983b). Moreover, using current preparation techniques, growth rings have also not been detected in cuttlefish statoliths (Natsukari, personal communication; personal observation). Squid statolith growth rings can be observed and counted in the largest individuals (this is a considerable advantage as opposed to the situation with adult fish otolith growth rings discussed previously). It is thus possible to obtain precise size-at-age data from the full size range of an individual species.

A considerable amount of work has been focused on validating the periodicity of teleost otolith growth increments. The majority of studies have concluded that the rings in fish otoliths are laid down on a daily basis (see Campana & Nielson, 1985; Jones, 1986). However, despite the fact that squids possess very similar laminae within the statolith microstructure, there have only been several studies which have attempted to validate the periodicity of these rings. Previous to this study, daily periodicity in statolith growth ring formation has been demonstrated in three northern hemisphere squid species based on chemical staining of the statoliths, *I. illecebrosus* (Hurley *et al.*, 1985; Dawe *et al.*, 1985) *A. subulata* (Lipinski, 1986) and *Todarodes pacificus* (Nakamura & Sakurai, 1990). Yang *et al.* (1986) demonstrated daily periodicity in juvenile individuals of *L. opalescens* of known age, which were raised in captivity. In a recent review of statolith growth ring analysis, Rodhouse & Hatfield (1990a) support the view that statolith rings are formed daily. These conclusions were based on validated species maintained in captivity, along with circumstantial evidence from non-validated species.

Based on the evidence suggesting daily periodicity in statolith ring formation, there was a compelling research background to further explore statolith ring periodicity, especially in tropical cephalopods. The five cephalopod species in the Townsville region, which were the focus of this study, had distinct bipartite growth rings within the statolith microstructure. These rings closely resembled the rings reported in the other cephalopods, and the periodic laminae present in fish otoliths. There was thus the potential for statolith growth rings in these tropical species to be used as chronological time marks.

The aims of this chapter were threefold:

1) to develop techniques necessary to visualise and enumerate growth rings in each of the study species,

2) to determine statolith ring periodicity by *in situ* chemical staining of the statoliths of squids and sepioids maintained in captivity, and

3) to gain an understanding of growth and lifespan parameters by obtaining length-at-age data for field captured individuals.

4.2 MATERIALS AND METHODS

The two primary components of this study involved firstly, the collection of individuals which were maintained alive and stained with tetracycline or calcein to determine statolith ring periodicity, and secondly, the collection of field specimens which were fixed shortly after capture and subsequently used for age analysis.

Regular sampling was undertaken for *I. pygmaeus* from March 1988 to November 1989 (see chapter 5). Aged individuals used for this chapter were captured in March 1988 and 1989. Regular trawl sampling was also carried out for *L. chinensis* and *Loligo* sp. 1 from February 1988 to November 1989. However, specimens of *L. chinensis* and *Loligo* sp. 1 used in age analysis were taken from trawls on January 1 and November 9, 1989 respectively. Similarly, aged specimens of *L. noctiluca* were collected in shallow water near Cape Cleveland in the vicinity of 19°13'S, 146°59'E from May to July 1989 with juveniles collected from the

Strand Beach, Townsville in June 1989. The growth curve for *S. lessoniana* was constructed from aged juveniles captured off the eastern and western breakwaters in May and September 1989, while adults were jigged at night off the Picnic Bay jetty (19°11'S, 146°50'E) on Magnetic Island and at the Australian Institute of Marine Science jetty, south of Townsville (19°17'S, 147°03'E). Night jigging took place between November 1988 and February 1989. All lengths refer to dorsal mantle length (DML).

4.2.1 Statolith analysis

Statoliths are paired calcareous structures, with each statolith situated within a statocyst cavity at the posterior base of the cephalic cartilage. Statoliths of *I. pygmaeus* were removed by severing the head at the head mantle margin and carefully teasing the statocysts from the cephalic cartilage, which usually resulted in the statoliths falling free from the statocysts. To remove the statoliths from the other larger species, the cephalic cartilage containing the statocysts was dissected out and teased apart under a dissecting microscope to reveal the statoliths. With increasing experience, statoliths could be removed by making an incision through the cephalic cartilage behind the eye, and removing the exposed statoliths with forceps.

Whole, unground statoliths of *I. pygmaeus* were originally mounted in the synthetic mountant dibutyl-phthalate-polystyrene-xylene (D.P.X.) (Jackson, 1989). The statolith was placed on a microscope slide, rinsed with ethanol and allowed to dry. The statolith was then irrigated with xylene and then immediately covered with D.P.X. The xylene assisted in decreasing the viscosity of the D.P.X. and facilitating the penetration of the mountant into the statolith. A coverslip was then gently lowered on to the D.P.X. to spread the mountant out evenly. This mounting technique produced a high degree of increment resolution within the statolith microstructure of *I. pygmaeus*. This technique had the disadvantage, in that statoliths could not be manipulated or repositioned after mounting. Furthermore, some statoliths were broken as the D.P.X. dried.

Subsequently, use of the thermoplastic cement, Crystal Bond, was also found to have excellent optical qualities as a mounting medium. After drying the statoliths on a microscope slide, a small piece of Crystal Bond was then placed over the statolith and the slide was placed on a hot plate. The cement rapidly melted and coated the statolith. Moreover, while the cement was still warm and pliable, the statolith could be manipulated before the glue hardened. The cement could also be remelted for further manipulation of the statolith. This technique had the advantage, in that the angle of the statolith could be changed within the cement. For example, the discernment of growth rings in statoliths of *I. pygmaeus* were often greatly enhanced by turning over the statolith. Statoliths were also protected within the hardened Crystal Bond. This prevented the breakage problems encountered with mounting statoliths under coverslips in D.P.X.

Crystal Bond was subsequently used as a mountant for statoliths of the larger squid species. Due to its convenience of being able to be remelted, the larger statoliths which required grinding and polishing could be easily manipulated. Furthermore, hardened Crystal Bond was relatively soft which facilitated grinding of larger statoliths.

The statoliths of individuals of *I. pygmaeus, L. noctiluca* and some juvenile *S. lessoniana* were small and translucent enough that ring definition could be discerned on whole-mounted specimens. However, the statoliths of *L. chinensis*, *Loligo* sp.1 and adult *S. lessoniana* generally required grinding and polishing to reveal the microstructure. Grinding was carried out by hand with wet 200 grade carborundum paper. Scratches from the grinding were removed by hand polishing on wet suede with 0.05 μm alumina powder or by using a modified gem polishing machine equipped with a 16 cm rotating disc to which was attached a wet felt (Leco Lecloth) impregnated with alumina powder. Ground statoliths were either held by hand against the rotating polishing disc or lowered onto the disc with a specially made microscope slide holding arm.

The statoliths of *L. chinensis* and *Loligo* sp. 1 were considerably opaque and required grinding and polishing on both the anterior and posterior surfaces to produce a thin section with visible growth rings. Growth rings within the statoliths of *S. lessoniana* were generally best visualised by grinding and polishing on the anterior (concave) surface only, however, some particularly opaque or thick statoliths required grinding on both surfaces to increase the transparency and enhance the growth increments. The statolith size and shape of a newly hatched *S. lessoniana* was determined from a specimen which was hatched from an egg trawled up in Cleveland Bay. This specimen further provided information on DML at hatching (5.3 mm).

Growth rings were observed with an Olympus BH compound microscope equipped with polarising filters. Counts were predominantly carried out by enumerating rings with a hand counter while following the ring sequence with a pencil via a camera lucida. Specimen age in days (ie. statolith ring number) was established by taking the mean of at least three counts that deviated less than 10% from the mean. The age determined from the replicate counts was rounded to the nearest whole number. For a small number of the statoliths of *I. pygmaeus*, age was determined when there were two identical replicate ring counts rather than taking a mean of three counts.

4.2.2 Maintenance and tetracycline staining

4.2.2.1 Collection of live individuals

Collection techniques and maintenance of the sepioids and squids varied according to the habitat of each species. Live individuals of *I.pygmaeus* and juvenile *S. lessoniana* were dip-netted off mangrove mud banks and breakwaters. Live individuals of *L. noctiluca* were captured using 1 mm and 8 mm mesh beach seine nets off the Strand Beach, Townsville. These species were easily transported back to the laboratory in 20 l plastic buckets. The majority of *L. chinensis* were killed during trawling, however, any squids that were in good condition when trawled were placed immediately in a 98 l tub with flow-through seawater. Although mortality was high, some individuals usually survived during the course of the day. No specimens of *Loligo* sp. 1 were able to be maintained alive.

4.2.2.2 Chemical staining of statoliths

The chemicals tetracycline or calcein were used to place chemical marks on the statoliths, to thereby produce a time mark to calibrate statolith ring periodicity. Statoliths of *I.pygmaeus*, *L. noctiluca* and *S. lessoniana* were stained by exposing the squid to an ambient solution of tetracycline-seawater (250 mg per litre for 2 h). A minimum of 2 h was found to be needed for the tetracycline to be incorporated into the statolith. In one experimental treatment, five individuals of *S. lessoniana* were exposed to 100 mg of calcein per litre of seawater for 1.5 h. A number of individuals of *S. lessoniana* and several individuals of *I. pygmaeus* were also stained a second time with an interval of between 9 and 20 days between stainings. Individuals of *L. chinensis* were more sensitive to handling and exposing them to an ambient solution of tetracycline-seawater was not suitable, therefore an injection method was used. Squids brought back from trawling were injected with a tetracycline-seawater solution (6 mg per ml) at the base of arm I. Previous trials with injections indicated that tetracycline was incorporated into the statolith within at least 15 hours of injection, (eg. an individual which was injected in the evening of the day of trawling and found dead the following morning had already taken up the tetracycline into its statolith).

4.2.2.3 Maintenance

Individuals of *I.pygmaeus* were small enough to be maintained in small aquaria, although 33 l white plastic trays proved most satisfactory as the opaque walls prevented the sepioids from damaging themselves. The walls of the tray were lined with a black 1 cm plastic mesh. This served to increase the visibility of the walls and allowed a structure for individuals to attach to with their adhesive organ. Captive individuals of *I. pygmaeus* were fed *ad libitum* with the sergestid shrimp *Acetes sibogae australis.*

The larger squid species (*L. noctiluca, S. lessoniana* and *L. chinensis*) were maintained in either a 308 l, 1500 l or 2500 l circular, fiberglas tank. Food organisms consisted of juvenile penaeids and *Acetes*, and fishes of the families Ambassidae, Mugilidae and Sillaginidae. Feeding also was *ad libitum*, although in several instances captive *S. lessoniana* were induced to eat previously frozen prawns.

All the aquaria and tanks were located outside to provide natural diel periodicity and were connected to a closed circulating seawater system. To reduce stress in the captive squids, no detailed records of growth were maintained for captive individuals.

4.3 RESULTS

4.3.1 *Idiosepius pygmaeus*

Idiosepius pygmaeus appears to be predominantly a shallow water estuarine species. No specimens have been captured from benthic sampling on the nearby continental shelf (P. Arnold, personal communication). Three-hundred and twenty-two one-half hour oblique Tucker Trawl plankton samples taken at a number of stations across the Central Great Barrier Reef lagoon (Jackson and Hartwick, unpublished data) yielded only 19 specimens of *I. pygmaeus,* even though numerous other cephalopod paralarvae were captured. In comparison, up to 171 individuals were dip-netted during one two hour collecting trip along 1.72 km of breakwater in the vicinity of Townsville Harbour.

4.3.1.1 Statolith structure and microanatomy

The statoliths of *I. pygmaeus* are complex three-dimensional structures. Because of the limited depth of field for one plane of focus, some rings near the nucleus were not discernible when photographed (Fig. 4.1A; classification is after Clarke, 1978). Growth rings were most clearly seen in the lateral region near the rostrum. A specimen stained with tetracycline twice, 17 days and 8 days before death, showed considerable statolith growth over a relatively short period of time

(Fig. 4.1B). In many instances a prominent discontinuity (check) within the statolith corresponded to the time of staining. This feature was particularly useful for subsequent ring counts under the light microscope. A tetracycline stained statolith was selected and photographed under both white light, to identify the daily ring structure (Fig. 4.1C) and UV light to identify the point of staining (Fig. 4.1D) from which the subsequent daily rings were laid down. Daily ring periodicity was documented in a total of six individuals of *I. pygmaeus* which were exposed to tetracycline and successfully maintained (Table 4.1).

Experimental treatments were carried out in June 1986 (data from Jackson, 1986) and between March and May 1988. Daily rings were validated in the lateral statolith region of four individuals, and on the dorsal dome and rostrum of the other two individuals (Table 4.1).

Statolith checks were also observed within the ring sequence of some field captured specimens of *I. pygmaeus* (Fig.4.1A, arrow). Statolith checks were considerably more prominent than the other rings due to a greater degree of transparency in the check region producing enhanced visibility under the light microscope. The degree of enhanced visibility often varied between checks.

Tetracycline produced a distinct fluorescent band on the statoliths of each species studied including *I. pygmaeus*, when viewed under ultraviolet irradiation (Leitz Dialux UV compound microscope with kp500 filter and ultra high pressure mercury lamp). Tetracycline produced a yellow fluorescence while calcein (used with *S. lessoniana*) produced a green fluorescence. The inner edge of the band corresponded to the growth increment deposited during the time of staining.

4.3.1.2 Sexual dimorphism and growth

Idiosepius pygmaeus showed considerable sexual dimorphism, with females achieving a much greater length and weight than males. During the entire study period, the largest male captured was 159 mg in weight and 10.3 mm in length, while the largest female weighed 655 mg and was 17.6 mm in length. The majority of females captured were less than 15 mm in length while the majority of males captured were greater than 10mm in length (see chapter 5).

The reasons for the considerable size related sexual dimorphism in *I.pygmaeus* could be ascertained by ageing individual males and females. A total of 19 males and 25 females were aged to explore the relationship between length and age (Fig. 4.2). Females achieved a greater length predominantly by growing at a much greater rate than males and to a lesser extent by growing older than males. Moreover, length was an unreliable index of age, particularly in females, as individuals of similar ages varied considerably in length (Fig. 4.2)

Jackson (1989) reported that maturity in males took place as young as 42 and 60 days in males and females respectively. However, more detailed analysis of age at maturity over the course of several years found that maturity parameters varied considerably throughout the year (see chapter 6).

Based on statolith age analysis determined from the present sampling regime, the lifespan of *I. pygmaeus* was found to be quite short, with the life cycle for both males and females being completed in less than three months. Furthermore, individuals reached adult sizes in less than two months.

4.3.2 Sepioteuthis lessoniana

The notable characteristics in the statoliths of *S. lessoniana* were a rounded dorsal dome and a relatively long thin rostrum (Fig.4.3B). Unlike *I. pygmaeus* in which rings were most obvious in the lateral region of the statolith (Jackson, 1989) or *Photololigo edulis* (Natsukari *et al.*, 1988) which have a clearly countable ring sequence in the rostrum, statolith rings of *S. lessoniana* rings were most discernible in the dorsal dome. Rings were very difficult to discern in the rostrum.

The ring sequence within the statolith commenced from a prominent check although some faint ring structure was visible inside this check. The position of this check corresponded closely to the outer margin of the statolith of the newly hatched *S. lessoniana* (which also had some ring structure at hatching), indicating that this ring represented a hatching check. This feature has also been documented in the loliginid squids *Alloteuthis subulata* (Lipinski, 1986) and *Photololigo edulis* (Natsukari *et al.*, 1988).

4.3.2.1 Tetracycline staining

The growth ring sequence could be clearly visualised and counted in seven of the tetracycline or calcein stained specimens maintained in captivity. The counts of these specimens corresponded to a daily periodicity in ring formation (Table 4.2). Rings were generally more easily counted between two stain marks (Fig. 4.3C) than from the stain mark to the edge, as the edge rings were the most difficult to discern. As was reported with *I. pygmaeus,* a statolith check was often induced within the statolith which corresponded to the date of capture and staining (Figs. 4.3A, 4.3C). Multiple staining of the statolith of *S. lessoniana* provided a clear record of statolith growth. Likewise, the tetracycline band induced at the time of staining provided a clear outline of the statolith size and shape. Considerable statolith growth took place over the 38 d of maintenance (Fig. 4.3A), with major changes in shape attributed to the elongation of the rostrum and the expansion of the dorsal dome region.

When using high magnification or very sharp focus, numerous less prominent sub-daily rings could be discerned which often made counting of daily rings difficult. This was especially true in areas of the statolith where rings were quite thick (wide). Using a lower magnification or changing the plane of focus, helped to delineate the true daily rings which were superimposed over the numerous sub-daily rings. A similar phenomena has been shown to exist in the otoliths of the freshwater fish *Coregonus* spp. (Eckmann & Rey, 1987).

4.3.2.2 Age and growth

A total of eleven males, six females and nine paralarvae/juveniles were aged to produce a growth curve (Fig. 4.4) and to ascertain maturity. Growth in *S. lessoniana* was rapid with a large size reached in less than six months. Maturity was found to take place as young as 67 d and 69 d in males and females respectively.

4.3.3 *Loliolus noctiluca*

Loliolus noctiluca is a small loliginid which tends to be littoral and estuarine in habitat (Lu *et al.*, 1985). The small size and robustness (due to its tolerance of inshore waters) facilitated the maintenance of this species. The statoliths of this species were exceptionally translucent with very clear ring definition (Fig 4.5A). The microstructural growth rings were the clearest yet observed and the easiest to enumerate. Processing of statoliths was enhanced by the fact that no grinding or polishing techniques were necessary. Individuals maintained for statolith ring periodicity experiments, adjusted well to confinement and grew considerably during the course of maintenance.

4.3.3.1 Tetracycline staining

Squids captured and stained with tetracycline in June and July 1989 were maintained alive until sacrificed after 30 and 31 d, respectively, although there was some mortality during the course of the maintenance. Three squids captured in June were transferred to the 308 l round tank and allowed to grow until one died after 77 d and the other two died after 83 d. These experimental treatments resulted in the validation of daily statolith growth rings in nine individuals (Table 4.3). Due to the generally greater number of rings involved in the experimental treatments of *L. noctiluca*, ring number was established by three consecutive ring counts. The mean value obtained from replicate growth ring counts from the tetracycline mark to the statolith edge corresponded to, or was very close to, the number of days the squids were maintained. The experiment with the three specimens which were maintained alive for up to 83 days provided evidence that daily growth rings occur regularly over an extended period of growth and not just in juveniles. The degree of correspondence between days maintained and ring number decreased, and the among-count variance increased, with the length of time maintained. This reflects the problems associated with counting large numbers of relatively narrow rings.

4.3.3.2 Age and growth

A total of eight males, six females and eight juveniles were aged to explore the relationship between length and age, and to determine the form of growth and probable lifespan of this species (Fig. 4.6). *Loliolus noctiluca* also exhibited rapid growth and a short lifespan. Of all the specemens aged, only the very large female (90mm DML, 112 d) which is the largest specimen yet recorded (see Lu et. al., 1985) was mature. Males matured as young as 59 d and 59 mm in length. This species thus grew extremely fast and reached maturity and adult sizes in the same time span as was seen in *I. pygmaeus*.

4.3.4 *Loligo chinensis*

4.3.4.1 Tetracycline staining

Due to the greater degree of difficulty in collecting and maintaining live individuals of *L. chinensis*, only two male specimens were maintained for any length of time after collecting and injecting with tetracycline. However, these two individuals provided useful information for ring periodicity in this species. These squids survived for 21 and 25 days respectively. The outermost rings could be visualised on the dorsal dome of the statolith of the larger *L. chinensis* without any polishing or grinding. However, the second squid's statolith required grinding and polishing on both sides to enhance the visibility of the growth rings. This resulted in the growth rings being most easily delineated on the rostrum. The mean value obtained from six replicate growth ring counts for each squid, from the tetracycline mark to the statolith edge corresponded to the number of days the squids were maintained (Table 4.4). The validation of these larger individuals was relevant since they demonstrated that daily statolith rings were laid down in adult squids. Both individuals were sexually mature and reproductively active, with numerous spermatophores at the end of the penis.

4.3.4.2 Age and growth

After grinding and polishing of the statoliths on both sides, the complete ring structure could be clearly seen in the statoliths of field captured individuals of *L. chinensis* (Fig. 4.5B). A total of 19 males, 19 females and 4 juveniles were aged to consider the relationship between length and age. Based on age information, *L. chinensis* also exhibited very rapid growth. The length-at-age data also suggested that the lifespan was short, with the life cycle being completed in less than four months (Fig 4.7). Males matured as young as 76 d and 102 mm while females matured as young as 83 d and 110 mm. Males were somewhat larger (longer) than females. Their greater length was obtained by growing at a greater rate (rather than living longer) than their female counterparts.

4.3.5 *Loligo* sp. 1

As this species was only captured intermittently, there were few opportunities to collect individuals for tetracycline age analysis. Hence it was not possible to validate the periodicity of statolith ring formation. However, after grinding and polishing, this species had a very clear and regular ring structure within the statolith microstructure, which was very similar in appearance to *L. chinensis*. For the purposes of this study, it was assumed that these rings were laid down daily as has been demonstrated for the other four species.

4.3.5.1 Age and growth

A total of ten males and nine females were aged to explore the age-length relationship in this species (Fig. 4.8). *Loligo* sp. 1 showed a considerable degree of sexual dimorphism with males growing longer than females. As in the other sexually dimorphic species such as *I. pygmaeus* and *L. chinensis*, this length difference was primarily due to a greater growth rate of the larger sex. Males matured as young as 112 d and 134 mm, while females matured as young as 123 d and 126 mm. It is interesting to note that the sexual dimorphism was reversed between *I. pygmaeus* (in which the females were larger) and the two *Loligo* species in which the males were the larger sex. Aged individuals of *Loligo* sp. 1 were older than aged individuals of *L. chinensis*, with males and females reaching 160 d. However, the sample of *Loligo* sp. 1 was captured at a different time of the year (November) than was the *L. chinensis* sample (January). This could have influenced the growth rates (see chapter 5). More comprehensive sampling would be needed to confirm whether the different ages were species characteristic or a result of time differences between samples.

4.3.6 Form of growth and comparison with other species

Adult sizes of the five species spanned an order of magnitude (Table 4.5). *Idiosepius pygmaeus* was much smaller than the other species with females attaining mantle lengths of up to 17.6 mm. The largest individual of *L. noctiluca* was the exceptionally large 90 mm female. *Sepioteuthis lessoniana, L. chinensis* and *Loligo* sp. 1 all had mantle lengths greater than 100 mm. The greatest lengths recorded in the present study were 213, 180 and 230 mm respectively. These larger individuals were males.

The length-at-age data used in this study covered the full length range that the sampling regime could detect. While it is possible that larger individuals of *L. chinensis*, *S. lessoniana* and *Loligo* sp. 1 were present in the study area, it is unlikely in the case of *I. pygmaeus* and *L. noctiluca*, as it was possible to comprehensively sample the habitat of these small near-shore cephalopods. In the case of *L. noctiluca* the 90 mm specimen was the largest record for this species. The maximum recorded sizes of *S. lessoniana* and *L. chinensis* were considerably larger than those taken in the present study (Table 4.6).

For the length range sampled in this study, the relationship between age in days and mantle length was linear for each species. For *L. noctiluca* (Fig. 4.6) and *S. lessoniana* (Fig 4.4) there was evidence of a curvilinear (asymptotic) relationship but this was due to a single large individual in each case. It is possible that if age data from large individuals of *L. chinensis*, *S. lessoniana* or *Loligo* sp. 1 were included in the age-length graphs, an asymptotic plot could have resulted. However, none were located and the upper length range for these species were similar to those obtained from other regular sampling programs (Rao, 1954; Chan & Karim, 1986) in which very large individuals were rare.

Although daily statolith ring periodicity could be demonstrated to exist in four of the five study species, being able to compare length-at-age data from aged specimens to length-at-age data from cultured specimens would further strengthen the validity of using statolith rings to age field captured individuals. This information was available for *S. lessoniana* which has been cultured both in Japan and in the Philippines (Segawa, 1987) and in the U.S.A. (Hanlon, 1990). Length-at-age data for a female *S. lessoniana* grown in tropical waters in the Philippines (113 d, 143 mm) and for two specimens which were raised under artificial conditions at Galveston, Texas (125 d, 164 mm and 200 d, 217 mm) (R. Hanlon, personal communication) fitted well within the length-age correlation for aged specimens from Australia (Fig. 4.9). This suggests that the results obtained from statolith ageing of field captured individuals were providing accurate estimates of individual age.

4.4 DISCUSSION

4.4.1 The statolith as a tool for squid growth studies

Evidence for daily periodicity in statolith growth rings, derived from chemical marking experiments, now exists from 51 individuals out of six species of squids (two oegopsids and four myopsids) and one sepioid (Table 4.5). The tropical species in this study provides further evidence for the one-ring : one-day hypothesis and suggests that daily statolith growth rings are a widespread phenomenon among cephalopod species.

Statolith growth ring analysis promises to be the most useful method for establishing squid age. However, the technique is only of value when it can be assured that there is a high level of accuracy in the ring counts. Statolith ring counting requires a certain level of experience and familiarity with the ring structure of the species studied. For example, validation of daily rings in *S. lessoniana* also highlighted the presence of sub-daily rings, which if counted would lead to an overestimation of squid age. Because of the specificity involved in ring counting, it is often difficult to obtain independent counts from multiple observers. It is therefore important to avoid observer bias. This is most easily achieved by using a hand counter during counts so the observer is not biased by previous trials. In addition, replicate counts should be made of each statolith to provide estimates of variance in ring numbers. It would be of use in future work to establish a core of cephalopod workers with expertise and experience in statolith ring counting. In this way both the intra- and inter-observer counting biases could be systematically addressed. The author intends to address this in future collaborative work.

The growth rings observed in the statoliths of the study species are very similar to those found in fish otoliths. Having the ability to obtain accurate age estimates from fishes has proven to be valuable to the understanding of their biology (eg. Campana & Neilson, 1985). It is becoming increasingly apparent that squid statoliths can also be used in a similar way to ascertain important biological and demographic parameters that would be difficult to obtain by other means (eg. Natsukari *et al.*, 1988). However, the results obtained are only tentative until the periodicity of statolith growth rings can be validated. Validation of statolith ring periodicity in the four cephalopod species in this study has emphasised the usefulness of using statoliths for tropical squid demography.

Techniques used in the analysis of fish otolith growth ring data can be applied to the study of statolith microanatomy. These include delineation of growth rings using scanning electron microscopy on polished and etched statolith surfaces (Radtke, 1983; Hurley & Beck, 1985; Lipinski, 1986). Alternatively, light microscopy has been used to observe growth rings in whole untreated statoliths (Balch *et al.*, 1988; this study) or in statoliths that have been ground and polished using various techniques (Kristensen, 1980; Rosenberg *et al.*, 1981; Natsukari *et al.*, 1988; this study).

Artificially inducing a chemical time mark on the statolith is perhaps one of the most convenient methods for the validation of daily statolith ring periodicity. Culturing squids from hatching (thereby obtaining individuals of known age) is the only other means to calibrate ring periodicity (eg. Yang *et al.*, 1986). This method however, is often quite difficult and time consuming. Exposing squids to an ambient solution of tetracycline or calcein-seawater is most easily used with small individuals which can easily be maintained in relatively small confines during the staining process, with minimal damage (eg. *I. pygmaeus*, *L. noctiluca* and juvenile *S. lessoniana*). However, larger more active species such as the larger loliginids are too sensitive for this type of method, thus other techniques such as injection (Lipinski, 1986) or inclusion of the statolith staining drug in the food (Dawe *et al.*, 1985; Hurley *et al.*, 1985) have been used.

There is considerable scope to extend ring validation work to other cephalopod species, as the species which have been worked on represent only a small portion of the total cephalopod fauna. It is of interest to note that microstructural growth rings are not a widespread phenomenon across all groups of the Cephalopoda. The Nautiloids do not have a pair of single statoliths, but rather a cluster of ovate-shaped calcium carbonate crystals loose within each statocyst cavity (Morris, 1989). Of the other groups which possess paired statoliths (octopus, squids and sepioids), only the squids appear to consistently lay down growth rings. Concentric rings have not yet been observed in octopus statoliths (Boyle, 1983b; Tait, 1980). Furthermore, the presence of periodic growth laminae does not appear to be consistently laid down in the statoliths of all sepioids. Although rings have been observed in the idiosepioid (*I. pygmaeus*) and in the sepiolid (*Rossia glaucopis*) (Kristensen, 1980) they have not been detected in the sepiids (ie. cuttlefish) (personal observation; Natsukari, personal communication). Currently it is not known how widespread microstructural statolith growth rings are within the Sepioidea. The mechanism of ring formation along with the factors influencing statolith growth is an area which deserves further research.

It may be possible that periodic laminae in other structures such as the eye lens (Boyle, 1983b) may be a means to age specimens in which growth rings cannot be detected within the statoliths. Furthermore, recent developments in the detection of possible daily rings within the squid gladius by Russian scientists (R. Hanlon, personal communication) suggests that these structures may also prove to be useful for obtaining age data. Collaborative research is commencing on the comparison of the number of daily statolith growth rings to the number of rings in the gladius of *S. lessoniana.*

Ring validation work on the larger oceanic squids is particularly needed as statolith growth ring analysis promises to be one technique which can be used to establish important biological parameters for many of these species. Furthermore, it is necessary to increase the sample size of validated individuals to extend age validation work beyond the scope of preliminary findings.

It can only be assumed that the statolith ring deposition process which has been observed in the species observed in this study, under these artificial conditions is similar to what occurs in the natural environment. The maintenance conditions were especially suitable for providing a good environment for statolith incremental growth, since the tanks were located outside and thereby exposed to the natural diel light regime.

4.4.2 Growth in tropical Australian near-shore squids

The growth curves resulting from the age analysis provided an insight into the life history strategies of these tropical squids and sepioids. This research suggests that tropical cephalopods probably have considerably greater growth rates and shorter lifespans than what previous population analysis has suggested. Despite the order of magnitude difference in the size range of the five species studied, the shape of the growth curves developed from age analysis were very similar in appearance.

The determination of the form of growth in squids has in the past been plagued with uncertainties, with every major type of growth curve being fitted to available data (Hixon, 1980). This has also been reiterated by Mangold (1983) and Pauly (1985). A major reason for the existence of this situation has been that data has been assembled from indirect methods such as length frequency analysis (often incorporating fish growth models). The length-at-age data for the five study species appear to be linear or curvilinear in form, with extremely fast growth and short lifespans. Extrapolation of the data to the smaller size range (paralarval/juvenile phases) appears to suggest initial slow growth, followed by a more rapid growth phase coincident with sexual maturity. More detailed analysis of growth modelling for several of the study species is considered later (chapter 7).

There is little comparative ecological data for *I. pygmaeus*, *L. noctiluca* or *Loligo* sp. 1. However, *L. chinensis* and *S. lessoniana* are widespread in the Indo-Pacific and some demographic and ecological research has been carried out with these species.

The comparative culture data for *S. lessoniana* from the Philippines and in the U.S.A. suggests that statolith ageing techniques are providing an accurate description of squid growth for the Australian population. However, growth data for this species based on length frequency analysis from other localities suggests a very different pattern of growth. Earlier work in India (Rao, 1954) has estimated that this species grows beyond three years. This has also been further supported by ELEFAN 1 computer program analysis of Rao's (1954) length frequency data (Longhurst & Pauly, 1987).

Length frequency analysis of *L. chinensis* in the Gulf of Thailand using ELEFAN 1 computer analysis has resulted in similar findings to that of *S. lessoniana* (Rao, 1954; Longhurst and Pauly, 1987), namely a perennial lifespan of more than three years with relatively slow asymptotic growth.

The results obtained from *S. lessoniana* and *L. chinensis* age analysis in Australia contradict the previous results from the more classical length frequency analysis. The question is raised as to whether this is a locality difference or a reflection of different methodologies in analysis of growth rates. Does statolith age analysis compound the problem of the confusing background of the contradictory growth models previously described for squid (Hixon, 1980)? Longhurst and Pauly (1987) have suggested that these seemingly contradictory statements about cephalopod growth can be resolved by analysing length frequency data with a growth model incorporating an asymptotic growth pattern with superimposed seasonal growth oscillations. However, length frequency analysis produces results very different from validated statolith growth ring analysis. The sampling region off Townsville provided an opportunity to test length frequency analysis with populations of the same species in which growth had already been determined from ageing research. Sequential sampling of *I. pygmaeus* and *L. chinensis* was undertaken to obtain length frequency data and to consider this data in the light of the age and growth parameters already determined for these species (see chapter 5).

Table 4.1: Age validation information for *Idiosepius pygmaeus*. (L: lateral region; R: rostrum; DD: dorsal dome).

Mantle length (mm)	Sex	Date stained	Date experiment terminated	Number of days	Number of increments	Area of statolith where rings viewed
7.6	M	28 May 86	3 June 86	6	6	L
11.1	F	14 March 88	26 March 88	12	12	R
7.3	F	22 April 88	28 April 88	6	6	L
8.1	M	10 June 88	13 June 88	3	3	L
12.9	F	14 March 88 25 March 88	31 March 88	10 (between staining)	9	DD
14.4	F	22 April 88 1 May 88	9 May 88	9 (betweeen staining)	9	L

Table 4.2: **Age validation information for *Sepioteuthis lessoniana*.**

Mantle length (mm)	Stain 1	Stain 2	Date experiment terminated	Number of days	Number of increments	Comments
53	11 May 88	-	20 May 88	9	9	
50*	18 May 88	-	27 May 88	9	9	
Not taken	14 October 88 (calcein)	25 October 88	8 November 88	11	11	between stains
52	14 October 88 (calcein)	25 October 88	9 November 88	11	11	between stains
73	14 October 88 (calcein)	25 October 88	20 November 88	26	26	from 2nd stain to edge
67	20 October 88	9 November 88	28 November 88	20	20	
50	8 February 89	20 February 89	20 February 89	12	11	died during 2nd staining

* development of hectocotylus observed on this specimen

Table 4.3: **Tetracycline staining and statolith ring counts for *Loliolus noctiluca* (SD = standard deviation).**

Sex	Mantle Length (mm)	Date Stained	Date Experiment Terminated	Number Of Days	Replicate Statolith Ring Counts	Mean	SD
F	35.0	6 June 1989	20 June 1989	14	14, 14, 13	14	0.58
J	19.8	6 June 1989	21 June 1989	15	15, 14, 16	15	1.00
F	38.0	7 June 1989	7 July 1989	30	30, 29, 28	29	1.00
M	32.0	7 June 1989	7 July 1989	30	28, 27, 28	28	0.58
F	34.0	7 June 1989	7 July 1989	30	29, 29, 31	30	1.15
M	30.0	7 July 1989	7 August 1989	31	30, 30, 31	30	0.58
M	38.0	7 June 1989	23 August 1989	77	79, 75, 75	76	2.31
F	45.0	7 June 1989	30 August 1989	83	86, 82, 89	86	3.51
F	54.0	7 June 1989	30 August 1989	83	77, 82, 84	81	3.61

Table 4.4: **Tetracycline injection and statolith ring counts for *Loligo chinensis*. (SD = Standard Deviation).**

Sex	Mantle Length (mm)	Date Injected	Date Experiment Terminated	Number Of Days	Replicate Statolith Ring Counts	Mean	SD	Area Of Statolith Observed
M	81	13 July 1989	7 August 1989	25	24, 26, 26 26, 23, 24	25	1.33	Rostrum
M	111	13 July 1989	3 August 1989	21	20, 20, 20 22, 21, 22	21	0.98	Dorsal Dome

Table 4.5: **Summary of species and number of individuals used in chemical staining of statoliths for the determination of ring periodicity.**

Species	Number of individuals	Chemical used	Technique employed	Reference
Illex illecebrosus	4	strontium	given with food	Hurley *et al.*, 1985
Illex illecebrosus	8	strontium-tetracycline	with food, force feeding	Dawe *et al.*, 1985
Alloteuthis subulata	11	tetracycline	injection	Lipinski, 1986
Idiosepius pygmaeus	6	tetracycline	ambient exposure	Jackson, this study
Sepioteuthis lessoniana	7	tetracycline-calcein	ambient exposure	Jackson, this study
Loliolus noctiluca	9	tetracycline	ambient exposure	Jackson, this study
Loligo chinensis	2	tetracycline	injection	Jackson, this study

Table 4.6: **Maximum sizes attained for the study species from this sampling program, and as recorded from the literature.**

Species	Maximum Size this Study (mm)	Maximum Size Recorded	Author
Idiosepius pygmaeus	Male 10.3 Female 17.6	Male 12.0 Female 14.0	Voss, 1963
Loliolus noctiluca	Male 58.0 Female 90.0	Male 62.0 Female 83.0	Lu *et al.*, 1985
Loligo chinensis	Male 180.0 Female 120.0	380*	Winstanley *et al.*, 1983
Sepioteuthis lessoniana	Male 213.0 Female 184.0	360*	Roper *et al.*, 1984

* Sex not determined

Figure 4.1

Light micrographs of *Idiospeius pygmaeus* statoliths. (A) Whole statoliths from male age 36 days, mantle length 7.2mm, showing complex shape and ring structure, DD = dorsal dome, LD = lateral dome, R = rostrum, arrow indicates prominent check. Scale bar = 24 μm. (B) Whole statolith stained with tetracycline twice 17 days and eight days before death (viewed under incident UV light). Scale bar = 25 μm. (C) Ring sequence from specimens maintained in aquarium for six days poststaining. Scale bar = 5 μm. (D) Same specimen as 1C under UV light to highlight fluorescent band.

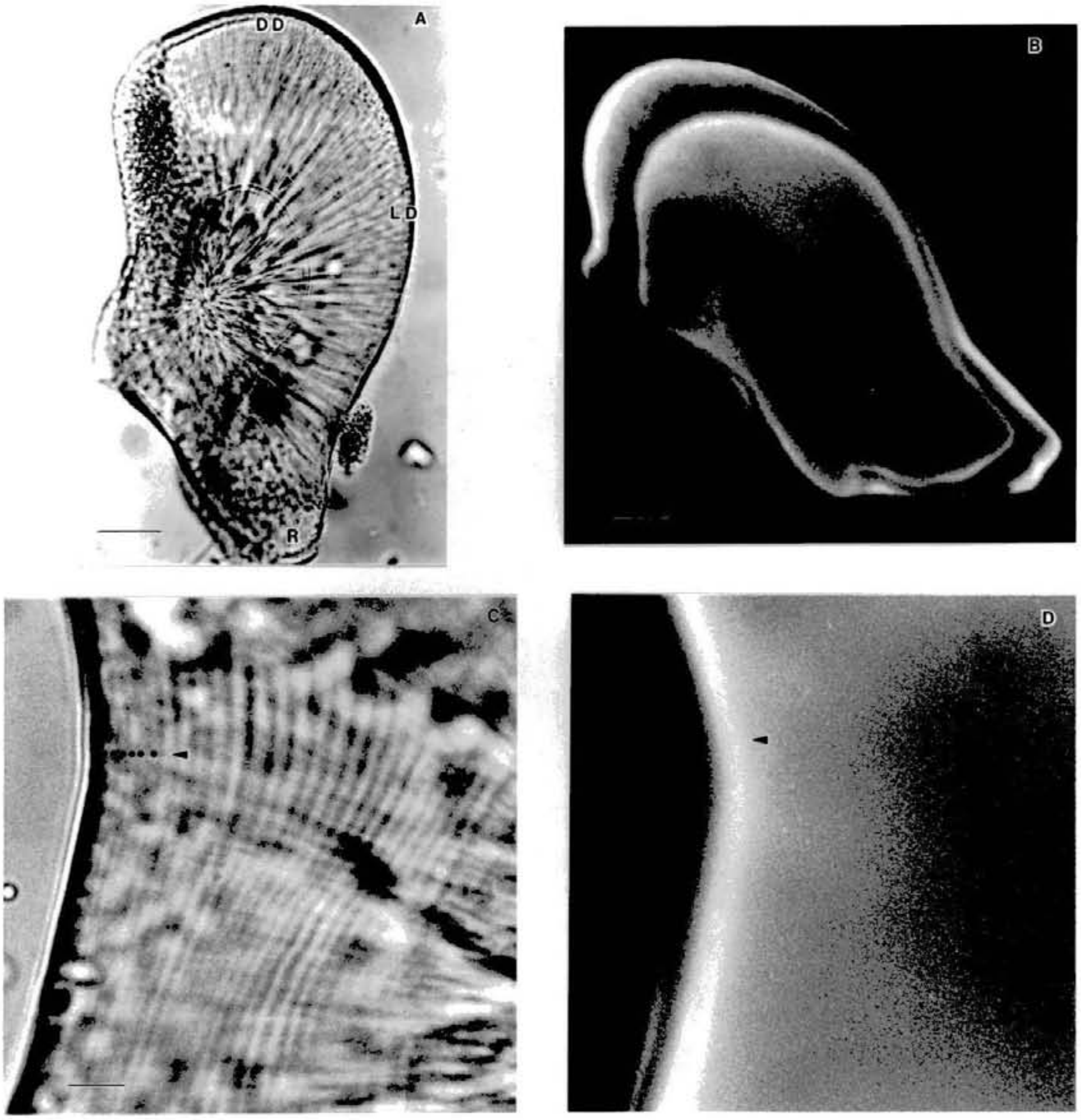

D D
A
L D
R
B
C
D

Figure 4.2

Relationship between age (statolith ring number) and mantle length for field captured male and female *Idiosepius pygmaeus*.

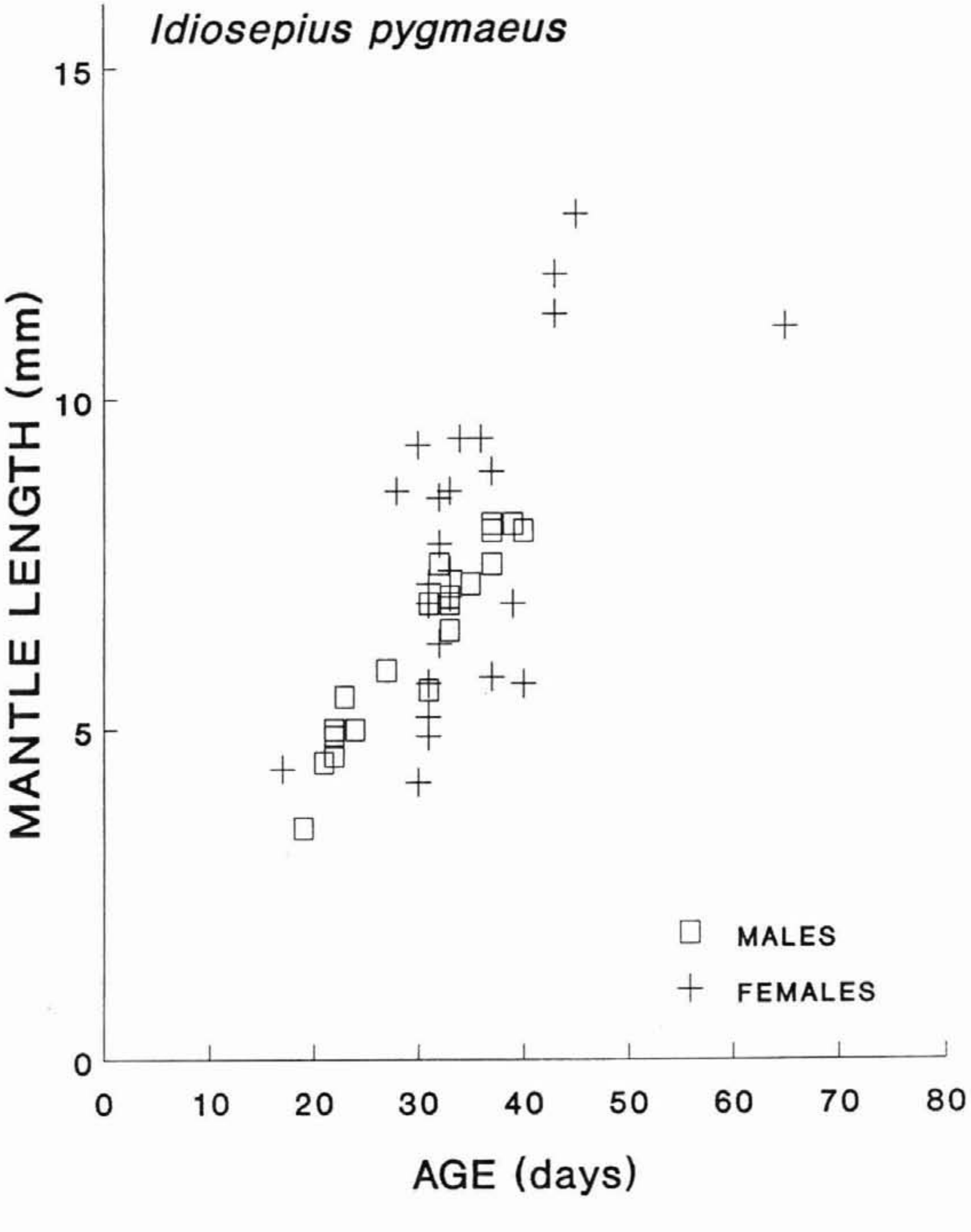

Idiosepius pygmaeus
MANTLE LENGTH (mm)
0
5
10
15
AGE (days)
0
10
20
30
40
50
60
70
80
☐ MALES
+ FEMALES

Figure 4.3

Light micrographs of *Sepioteuthis lessoniana* statoliths. (A) UV micrograph of statolith from specimen (age 68 days, mantle length 67 mm) stained with tetracycline twice at a 20-day interval and then allowed to grow for a further 18 days. Scale bar = 100 μm. (B) Light micrograph of same statolith in 4.3 A, ground and polished on both anterior and posterior surfaces. Scale bar = 100 μm. (C) Light micrograph of lateral region of dorsal dome (indicated by arrow in Fig. 4.3 B). Arrows indicate checks that correspond to tetracycline stainings. Scale bar = 50 μm.

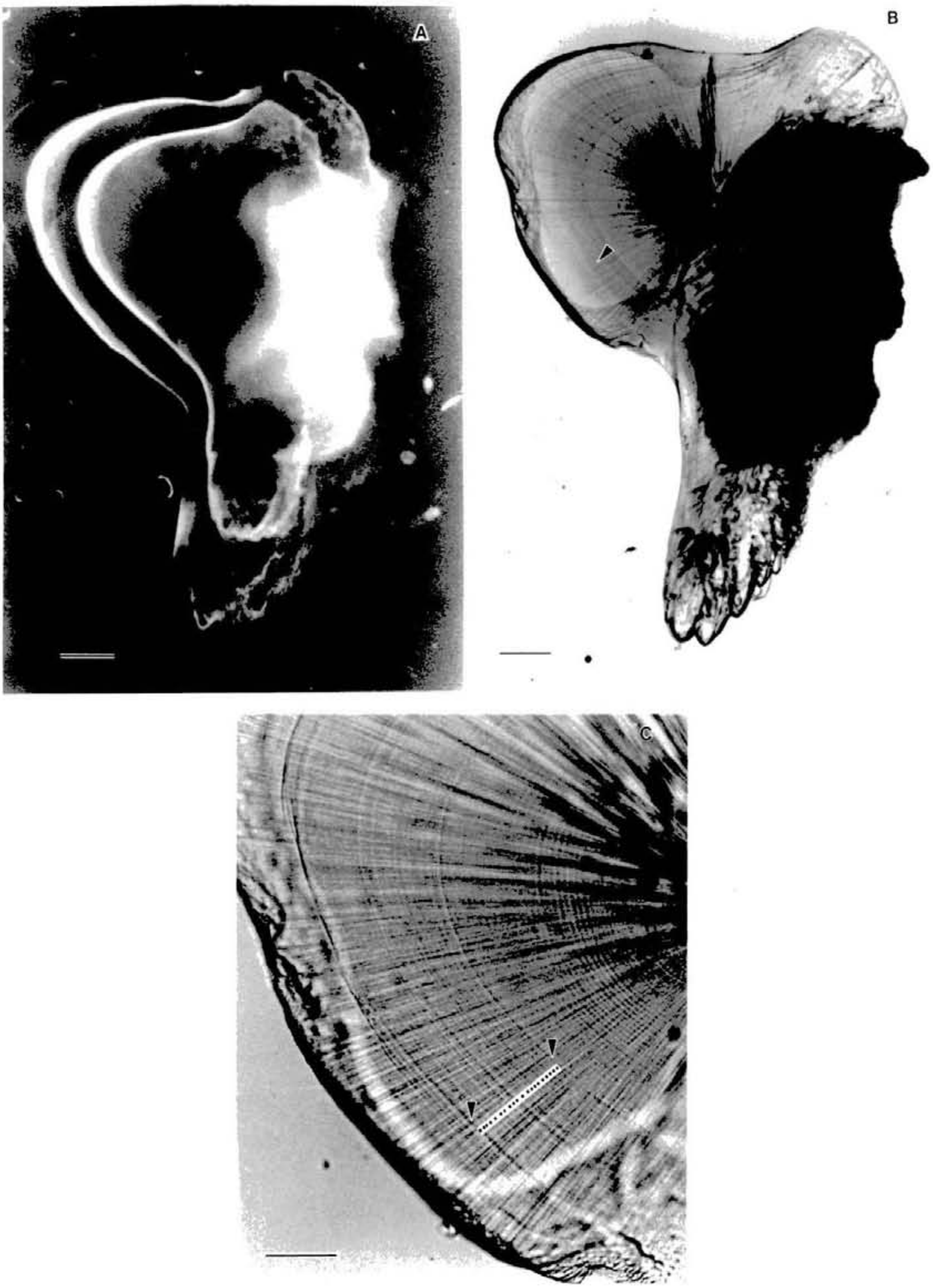

A
B
C

Figure 4.4

Relationship between age (statolith ring number) and mantle length for field captured male, female and juvenile *Sepioteuthis lessoniana* specimens.

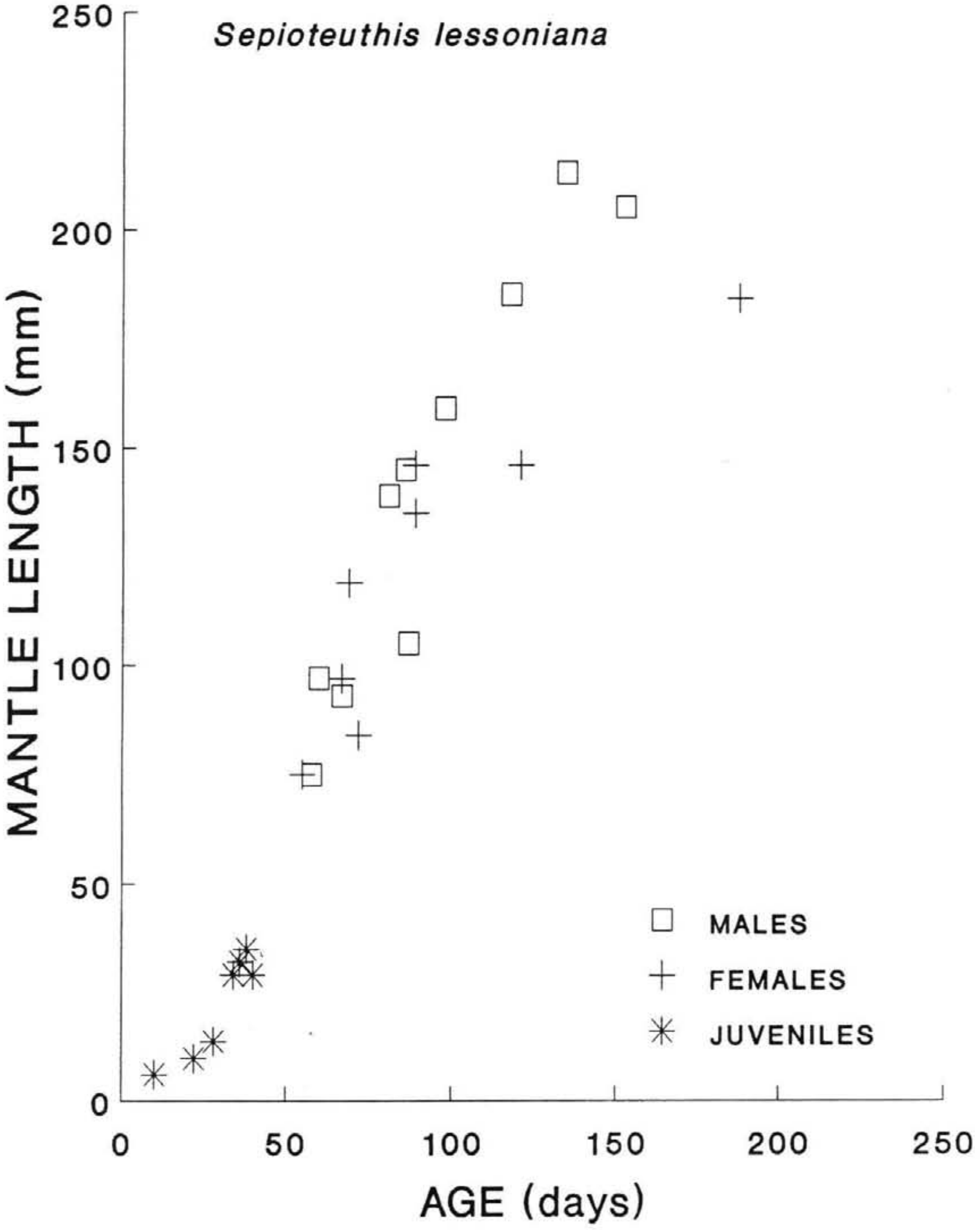
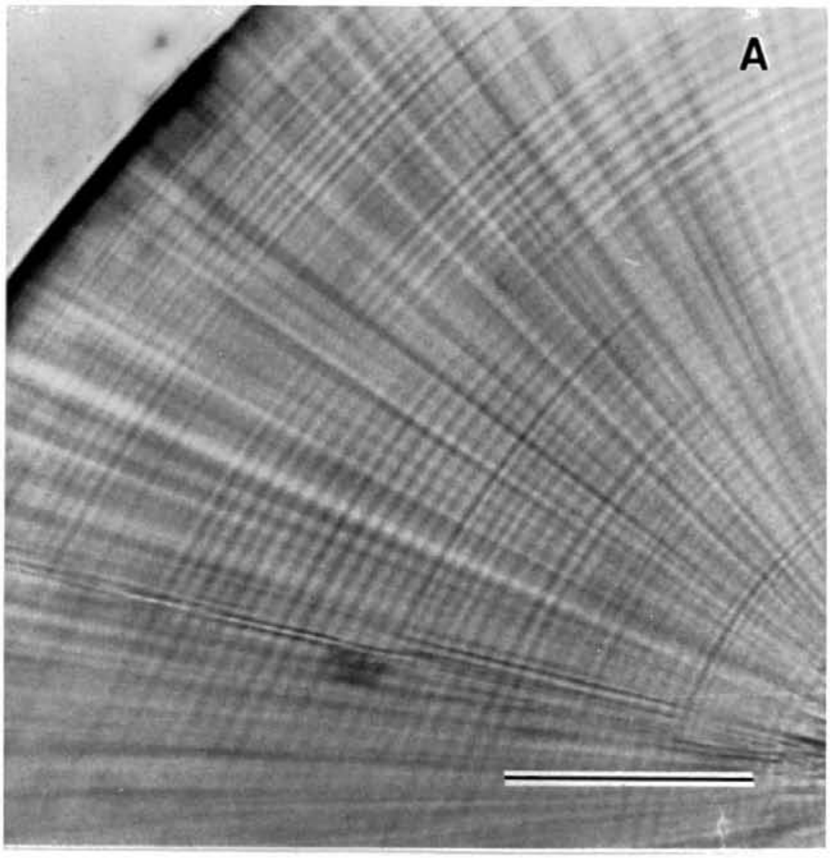

Sepioteuthis lessoniana
MANTLE LENGTH (mm)
250
200
150
100
50
0
0
50
100
150
200
250
AGE (days)
MALES
FEMALES
JUVENILES

Figure 4.5

(A) Daily growth rings in a whole statolith of a field captured *Loliolus noctiluca* female (age 59 days, mantle length 52mm) mounted in thermoplastic cement. Scale bar = 50 μm. (B) Daily growth rings in a statolith of a field captured *Loligo chinensis* male (age 78 days, mantle length 110mm) which has been ground and polished on both sides to produce a thin section. Scale bar = 100 μm.


A

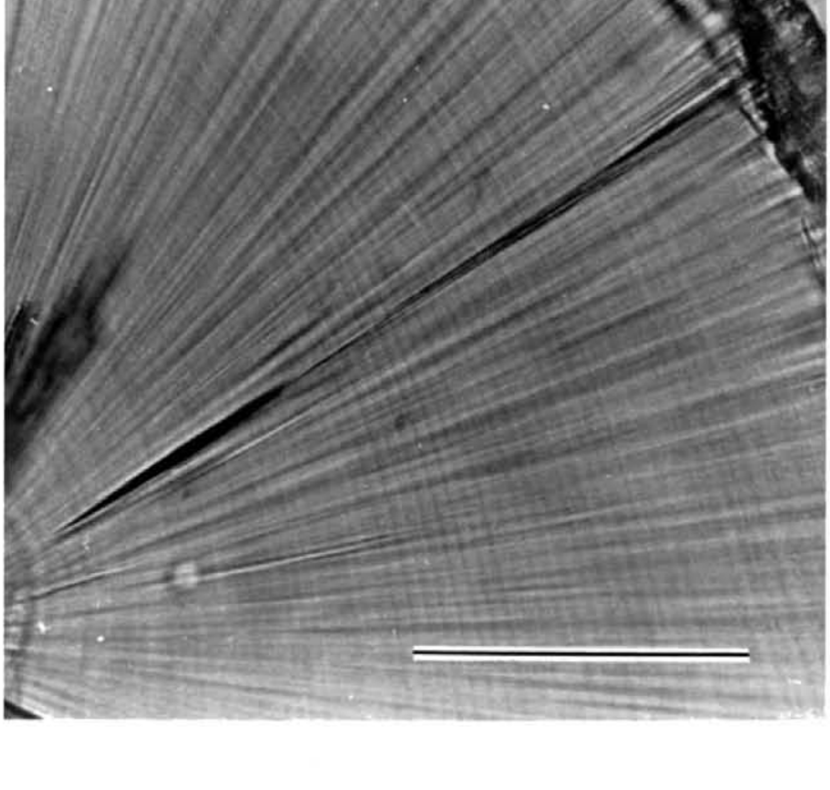

B

Figure 4.6

Relationship between age (statolith ring number) and mantle length for field captured male, female and juvenile *Loliolus noctiluca* specimens.

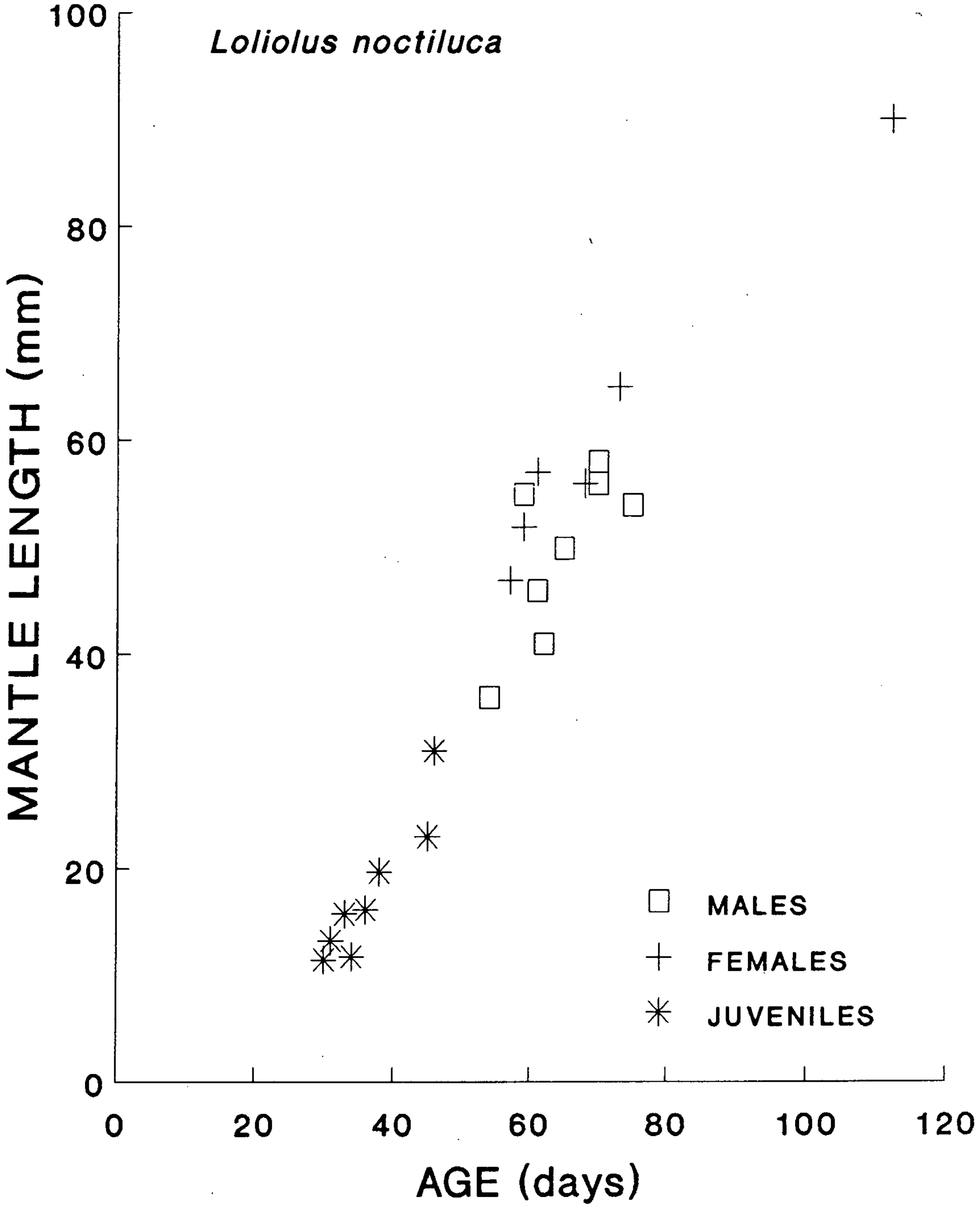

Loliolus noctiluca
MANTLE LENGTH (mm)
100
80
60
40
20
0
0
20
40
60
80
100
120
AGE (days)
MALES
FEMALES
JUVENILES

Figure 4.7

Relationship between age (statolith ring number) and mantle length for male, female and juvenile field captured *Loligo chinensis* specimens.

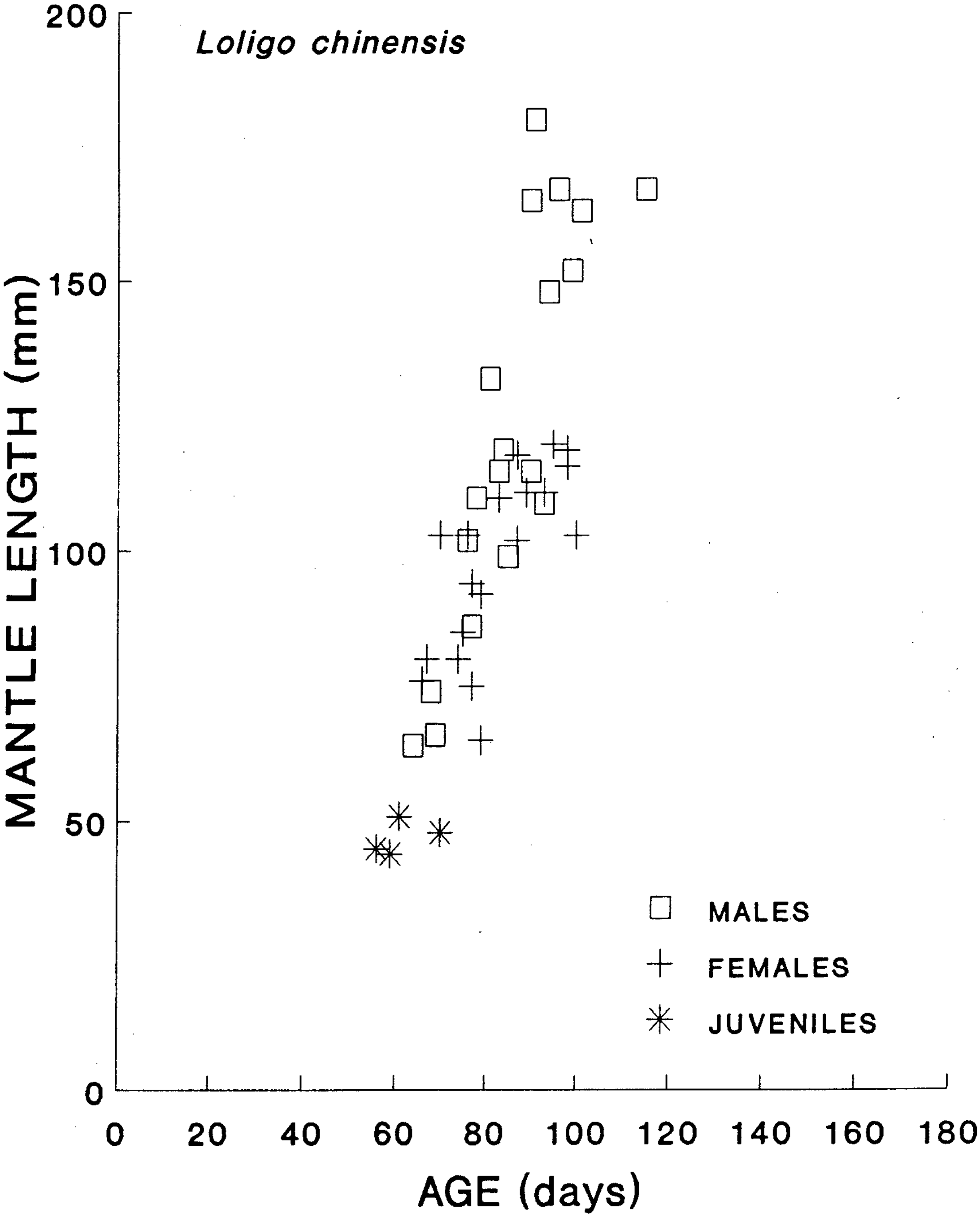

Loligo chinensis
MANTLE LENGTH (mm)
200
150
100
50
0
0
20
40
60
80
100
120
140
160
180
AGE (days)
MALES
FEMALES
JUVENILES

Figure 4.8

Relationship between age (statolith ring number) and mantle length for field captured male and female *Loligo* sp. 1.

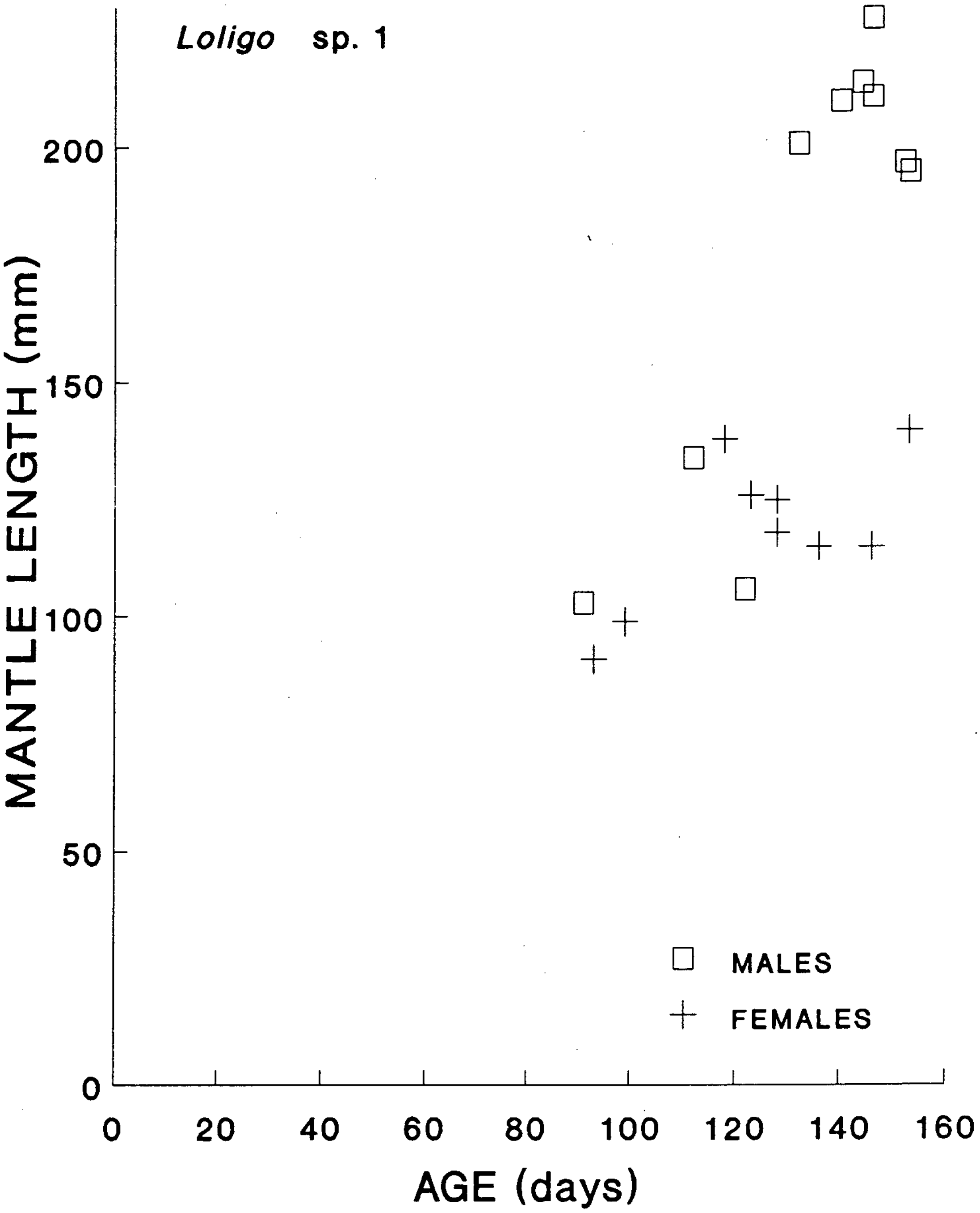
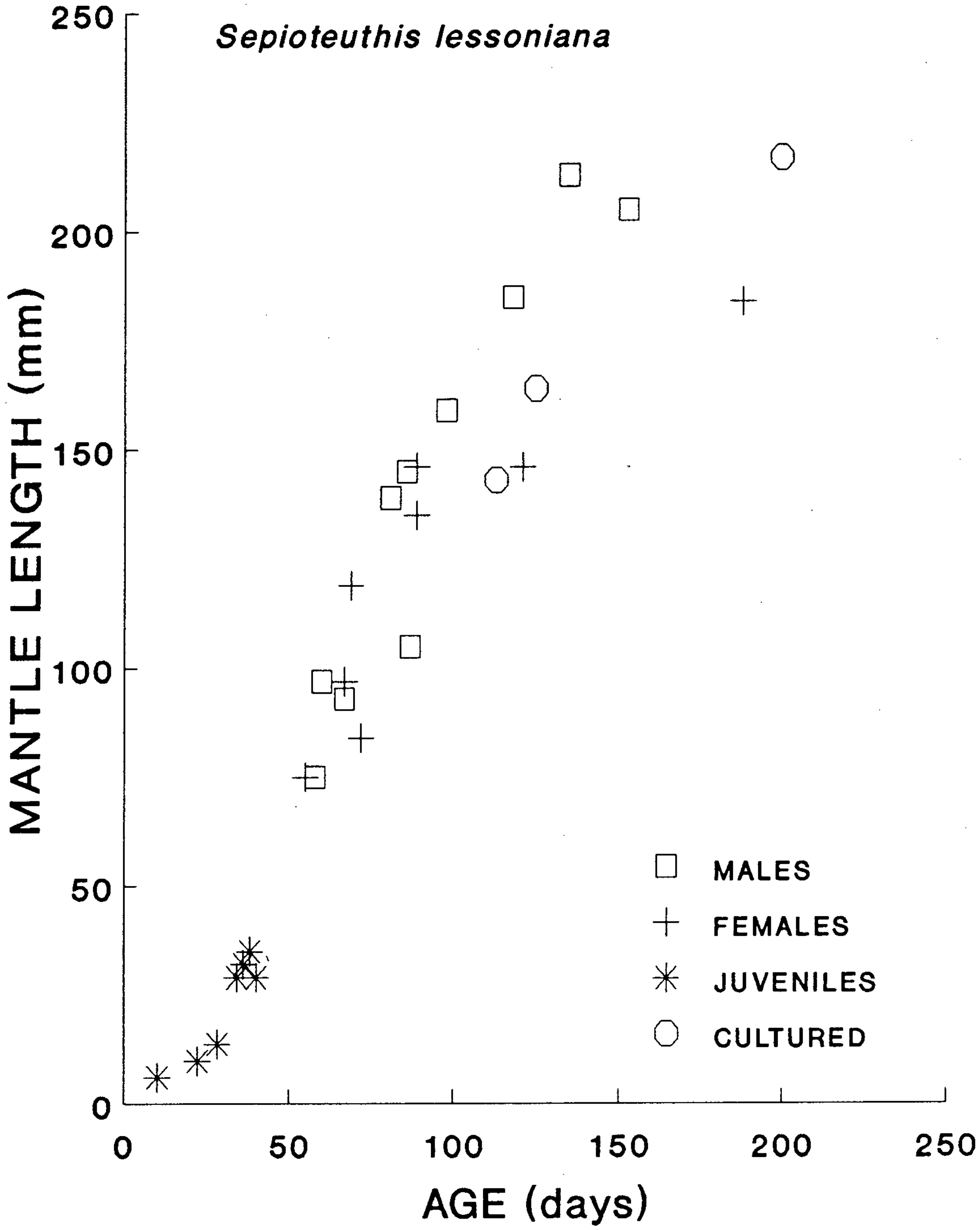

Loligo sp. 1
MANTLE LENGTH (mm)
0
50
100
150
200
0
20
40
60
80
100
120
140
160
AGE (days)
MALES
FEMALES

Figure 4.9

Length-at-age data for male, female and juvenile *Sepioteuthis lessoniana* from this study shown with length-at-age data for cultured *S. lessoniana* specimens of known age from Segawa (1987) and R. Hanlon (personal communication). See text for details.



Sepioteuthis lessoniana
MANTLE LENGTH (mm)
250
200
150
100
50
0
AGE (days)
0
50
100
150
200
250
□ MALES
+ FEMALES
✳ JUVENILES
○ CULTURED

5.0 THE PATTERN OF AGE AND SEASONAL SPECIFIC EVENTS

5.1 INTRODUCTION

With a background now developed for obtaining accurate size-at-age data for tropical squids, field research was expanded to look at population variability over time. This was related to the dynamics in the size structure of populations and trends in seasonal abundance. The aims of this chapter were to use more traditional approaches to infer growth based on cohort analysis, and to test the robustness of these methods with validated length-at-age data.

Pelagic cephalopods show many similarities to fishes (Packard, 1972; Calow, 1987). However, recent studies on the physiology and activity patterns of cephalopods have suggested that such comparisons should be made with caution given the fundamentally different biochemical and metabolic organisation of cephalopods and teleosts (O'Dor & Webber, 1986).

Trends in population abundance and growth in pelagic cephalopods is of topical interest especially in relation to growth dynamics of fishes. At this point in time growth modelling in squids is still in its infancy, and most previous work in growth dynamics has utilised existing finfish models. However, there are differing opinions with regards to the application of fish growth models to cephalopods. Pauly (1985) and Longhurst & Pauly (1987) have argued that existing finfish models are appropriate for accommodating and describing cephalopod growth. Nevertheless, information is increasing that cephalopods have distinctive demographic patterns (Forsythe & Hanlon, 1988, 1989), more complex reproductive strategies (Saville, 1987) and more ephemeral life histories (Amaratunga, 1987) than do most finfish.

A number of estimates of growth rate and lifespan are now available for pelagic cephalopods from a range of environments. Two general trends have been observed. For a number of species there is strong evidence for rapid growth and short lifespan with the form of the growth curve being linear or exponential (see review, Rodhouse & Hatfield, 1990a; also Jackson, 1989). Other studies suggest relatively slower growth rates, a more extended lifespan and an asymptotic growth curve (see review, Mangold, 1983; also Rao, 1954; Nesis, 1983; Worms, 1983a; Pauly, 1985; Longhurst & Pauly, 1987; Supongpan, 1988). Most of the material to date is derived from cold water and subtropical species although information on tropical squids is now becoming available.

Three main methods have been used to obtain estimates of squid growth rates and to infer longevities. These are analyses of length frequency data (eg. Hixon, 1980; Pauly, 1985; Longhurst & Pauly, 1987; Supongpan, 1988; Ueta & Jo, 1989), maintenance experiments in which growth is monitored in cultured individuals (LaRoe, 1971; Yang *et al.*, 1983; Yang *et al.*, 1986; Segawa, 1987; Forsythe & Hanlon, 1989; Hanlon *et al.*, 1989) and size-at-age estimates derived from the analysis of statolith microstructures (see Rodhouse & Hatfield, 1990a). At present, results from the different methods are confounded because analysis has been carried out with species inhabiting different environments. Most estimates of growth in tropical squids are derived from length frequency analysis (which may be biased, due to size or age-specific migrations out of the sampled population) while maintenance experiments have focused on temperate and subtropical species.

The interpretation of the growth phenomena in pelagic cephalopods is complicated by a high degree of temperature dependency in growth (O'Dor & Wells, 1987; Forsythe & Hanlon, 1989). Moreover, growth is highly variable even within particular temperature regimes (Lipinski, 1986; Natsukari *et al.*, 1988). The use of validated daily rings in statoliths is a convincing tool for determining both the rate of growth and its variance. As mentioned previously (chapter 4) a particular advantage of cephalopod statolith research is that daily increments may be reliably observed in the largest individuals sampled, a strikingly different situation from that seen in teleosts, in which rings are difficult or impossible to see in large fish (Beamish & McFarlane, 1987). However, this aspect of cephalopod biology is at an early stage of development and more validations of daily increments from different species, from different habitats and at different times are required.

The questions addressed in this chapter include; 1) What is the seasonal abundance pattern of *I. pygmaeus* and *L. chinensis* and how can this be understood in light of age information? 2) What growth estimates can be derived from length frequency data of tropical squids? 3) How does length frequency analysis based on fin fish models compare to ageing techniques? 4) What can statolith ageing information tell us about sex and season-specific growth performance in tropical squids? 5) What useful ecological data can be derived from statolith-soma relationships?

5.2 MATERIALS AND METHODS

The aims of this component of the research were to obtain regular population samples through time, firstly to determine trends in abundance throughout the year and secondly, to use information collected during regular sampling episodes to discern any pattern of growth from cohort analysis. Two environments were monitored, the offshore region of Cleveland Bay, to obtain collections of loliginid squids, and the inshore breakwater areas of the near-shore Townsville region to obtain regular samples of *Idiosepius pygmaeus.*

5.2.1 Offshore sampling for loliginid squids

Offshore trawling was carried out with the university vessel R.V. James Kirby. Sampling was taken using paired otter trawls (40 mm mesh) between February 1988 and November 1989. Only six samples, spread throughout the year, were obtained during 1988, whereas monthly samples were obtained from December 1988 to November 1989. All samples were taken for approximately 20 minutes, towed between 2 and 2.5 knots except for the last three dates in which trawls were taken at about three knots after an engine refit. Because of time and financial constraints, trawling was targeted at areas where the most reliable squid catches could be obtained. Since the distribution of squids was patchy and since squids often constituted only a small proportion of the catch, the trawling program each month could be considered more exploratory rather than regimented. However, despite this, the trawling program did provide an estimate of seasonal squid abundance within Cleveland Bay, as a considerable number of areas were targeted within the bay during any one sampling period.

Squids were also obtained from other university research and teaching trips during May and July 1988 and May and August 1989. This data was included for length frequency analysis and catch composition data, however, it was not used in subsequent calculations of average number of squid caught per trawl. Squids for each trawl were preserved separately and later enumerated. However, during October 1988, two exceptionally large catches of squids from subsequent trawls were pooled together due to space constraints. The total number of squids of each species were later counted and divided equally between both trawls when calculating the mean number of squid per trawl. The standard error of the mean of these two samples was thus somewhat reduced.

As mentioned previously (chapter 4) all squid were preserved in 10% buffered seawater formalin and subsequently transferred to 70% ethanol. All weight and length measurements were taken using preserved specimens. Individual length was taken as dorsal mantle length (DML) using a graduated ruler to the nearest millimetre. For length frequency analysis, data was grouped into 10 mm length classes.

5.2.2 Statolith growth analysis of *Loligo chinensis*

Squids used to analyse variability in growth based on length-at-age data were obtained from samples taken in two seasonal periods, summer (November and January) and winter (July and August). Length-at-age data covering the full length range of squids sampled were derived from samples obtained on 12 January 1989 (summer) and 13 July 1989 (winter). Length-at-age data from individuals of a selected length range were obtained from 9 November 1989 and 16,17 August 1989.

Parameters measured for each squid used in these analysis include DML, preserved wet weight (blotted with paper towelling prior to weighing) and individual age (ie. number of statolith growth rings). Statolith length (the distance from the top of the dorsal dome to the end of the rostrum (after Clarke, 1978) was taken from statoliths of aged specimens of the January and July samples. This measurement was taken using an eyepiece micrometer.

A methodological problem was encountered with the statolith analysis of preserved specimens. Under normal conditions of preservation for periods greater than two months, the extreme margins of the statolith tended to become opaque, probably as a result of adhering tissue which could not be removed from the edge of the statolith. For samples of *L. chinensis* used in seasonal growth analysis 61% of the statoliths could be read in their entirety. For the remainder, growth rings in marginal areas had to be extrapolated. In order to validate this procedure, rings were counted in the marginal areas of clear statoliths and this count was compared with the count of rings in equivalent widths adjacent to marginal areas. The number of rings were similar in each of the areas counted. There was no evidence that the pattern of spacing in marginal areas differed from that in submarginal areas (Table 5.1). This was done for both summer and winter sampled squids. For the 39% of statoliths with opaque margins, ring number was extrapolated from equivalent widths of submarginal areas. For all the statoliths treated this way (ie. those in table 5.1, plus all othersquids which were aged using the extrapolation technique) the actual area of marginal extrapolation was small, consisting of approximately 8-16% of the total statolith radius from the nuclear area to the edge.

5.2.3 Inshore population analysis of *Idiosepius pygmaeus*

As individuals of *I. pygmaeus* normally resided at the surface and were often dark with expanded chromatophores, they were easy to count and collect along the usually calm protected waters within the breakwaters.

Both visual and dip-net census methods were incorporated to monitor changes in the populations of *I. pygmaeus* through time. The western breakwater/marina complex (see Figs. 3.2, 3.3) was used for visual monitoring. This consisted of walking along 3.2 km of breakwater and noting the presence of any individuals of *I.*

pygmaeus. The population of sepioids was left undisturbed except for a few individuals collected on several occasions for experimental maintenance purposes. Counts were taken approximately monthly from July 1987 and generally fortnightly throughout 1988 and 1989.

The eastern breakwater complex (see Figs. 3.2, 3.3) was used to obtain regular population samples of *I. pygmaeus* for length frequency, age and reproductive analysis. Because the collecting technique involved dip-netting of individual sepioids, only a limited number of sepioids could be captured during any one collecting period along the breakwater. To increase the sample size, a method was employed which incorporated systematic collection of individuals along approximately 1.72 km of breakwater. The resulting sample was obtained by pooling all individuals captured from two collecting trips, predominantly from an afternoon sampling followed by one the next morning. The numbers of sepioids collected on the eastern breakwater during the paired sampling trips were roughly equivalent to the numbers counted during one visual census taken on the western breakwater, as the distance covered on the eastern breakwater (1.72 km x 2 trips) was roughly equivalent to the distance covered on the western breakwater (3.21 km).

5.2.4 *Acetes* analysis

The abundance of *Acetes sibogae australis* was observed to fluctuate considerably during the course of the year. Therefore, the abundance of *Acetes* was monitored during 1989 to see if there was any relationship between fluctuations of *Acetes* and *I. pygmaeus*. Since *Acetes* often schooled in very close proximity to the breakwater, a monthly dip-net estimation technique was developed to determine the relative abundance of *Acetes* throughout the year. A total of 25 stations were selected spread regularly along the eastern breakwater where *I. pygmaeus* was sampled. At each station three replicate dip-nettings were taken at close proximity giving a total of 75 samples. The total number of *Acetes* in each dip was assigned a rank number (Table 5.2). To obtain a relative *Acetes* abundance index (RAAI), the rank numbers of all dip-nettings were summed for each monthly sampling.

5.2.5 Statolith age analysis of *I. pygmaeus*

Specimens of *I. pygmaeus* collected throughout the year along the eastern breakwater were also selected for age analysis. Individuals which were aged and used for growth analysis were collected in March 1988, 1989; May 1988; June 1988, 1989 and August/September 1988. The individuals which were aged included specimens from the entire size range for each sampling date.

5.2.6 Length frequency analysis

The length frequencies obtained from the population sampling of *I. pygmaeus* and *L. chinensis* were analysed to see if growth curves could be derived from this data. The data was analysed with the ELEFAN (version 1.02) software package. The best growth curve was determined by manipulating parameters such as $L\infty$ and K within the automatic search routine to obtain a growth curve which best fitted the data (ie. the curve with the highest Rn value).

For *L. chinensis*, males, females and juveniles were pooled for the ELEFAN analysis, and the length class interval was set at 10 mm. Although females of this species do not grow as large as the males, very few large males were captured.

Because sexual dimorphism was not pronounced over the size range sampled, and there were only small catches for many of the months, this did not warrant constructing separate length frequencies based on sex. The data for the length frequency analysis was taken from trawling trips between February 1988 and February 1989 (9 samples, 2090 individuals). Alternatively, male and female *I. pygmaeus* were analysed separately due to the greater degree of sexual dimorphism exhibited by this species. Length class interval was set at 1 mm. The data used for the analysis was from ten fortnightly collections taken on the eastern breakwater consisting of a total of 847 individuals. As no regular population samples could be obtained for *S. lessoniana,* data from the Indian beach seine fishery (Rao, 1954 p. 47) was used. *Sepioteuthis lessoniana* in Indian waters was previously referred to as *S. arctipinnis* (see Lu & Tait, 1983). All specimen lengths given refer to DML.

5.3 RESULTS

5.3.1 Loliginid abundance

The majority of the cephalopod catch in Cleveland Bay consisted of *Loligo chinensis* predominantly and *Loligo* sp. 1. Although cuttlefish, some sepiolids and some individuals of *Loliolus noctiluca* were captured, numbers were usually low for these species. *Loligo chinensis* was captured consistently throughout the year with catches of *Loligo* sp. 1 occurring somewhat more sporadically and in lesser numbers (Fig. 5.1).

The average number of squid obtained for each trawling episode provided a means to discern the relative abundance of squid throughout the year (Fig. 5.2). The patchiness in the squid distribution can be appreciated by the large standard errors, especially for months when considerable numbers were captured. For both *L. chinensis* and *Loligo* sp. 1 the greatest catches were obtained during the summer periods (October to February for *L. chinensis* and October to December for *Loligo* sp. 1). Very few individuals of either species were captured over the winter periods, from April to September.

The distribution of *Loligo* sp. 1 was very patchy both spatially and temporally. For example, the largest catch of this species was in October 1988, while no individuals were captured in October 1989. Furthermore, the majority of individuals captured in October 1988 were obtained from only two trawls out of eleven, as indicated by the large standard error for this month (Fig. 5.2).

5.3.2 Length frequency of *Loligo chinensis*

The percentage length frequency data for *L. chinensis* was difficult to interpret. There was no clear pattern of modal progression over time, which made it difficult to follow any cohort through time (Fig. 5.3). The low numbers captured between March and June 1989 also made the detecting of patterns difficult. The general pattern was very little change in the modes with often consistent patterns shown from month to month. For example, throughout most of the percentage frequencies between August 1988 and March 1989 the largest peaks in abundance were in the 60 to 70 mm length class with no major shift in the modal size. Some months did show a bimodal pattern in squid abundance, eg. February, April, May 1988 and April, September, October 1989. Only a very small percentage of the catch was greater than 150mm DML.

The largest influx of juveniles captured was during May 1988, in which the 40 to 50 mm length class accounted for a large percentage of the catch. Other significant catches of juveniles also occurred during October and November 1989.

5.3.3 *I. pygmaeus* abundance

The abundance of *I. pygmaeus* based on visual counts on the western breakwater showed considerable variability in abundance, interspersed between periods of absence or very low abundance (Fig. 5.4). This was especially noteworthy during the summer period between December and February. Generally no individuals, or very few, were observed over the summer months during all three years. This pattern is even more accentuated if the total abundance from collected specimens on the eastern breakwater is superimposed over the western breakwater visual census (Fig. 5.5). During 1988 and 1989 the same pattern of abundance was noted along the eastern breakwater, with very few individuals captured during the summer months and large catches of individuals during the winter periods.

It was difficult to directly compare trends in abundance for specific months between both breakwaters because sampling was generally taken on alternate weeks for each site. However, it was interesting to note that during the same periods, the pattern in abundance was very similar on both breakwaters. For example, the drop in *I. pygmaeus* abundance during October to November 1988 followed by a very low abundance and subsequent rise in abundance during February 1989 was very similar for both sites. Furthermore, there was also a very similar pattern in abundance for both breakwaters during the period between July and November 1989, where a slight increase in sepioid abundance during the months of September and October 1989 was parallelled at both sites. This suggests that the populations of *I. pygmaeus* on both breakwaters were responding in a similar way to some external environmental factor influencing abundance.

As *I. pygmaeus* has been shown to feed readily on *Acetes* (Jackson, 1986; Jackson, 1989) and *Acetes* abundance was observed to fluctuate over time during 1987 and 1988, the *Acetes* sampling regime was designed to see if the pattern shown by the fluctuations in the *Acetes* abundance had any relationship to the abundance pattern of *I. pygmaeus*. However, when the relative *Acetes* abundance index (RAAI) was compared to the pattern of *I. pygmaeus* abundance on the eastern breakwater (Fig. 5.6), no parallel in the abundance pattern was noted. The *Acetes* population showed a more regular fluctuation in abundance which was superimposed over a more erratic abundance of *I. pygmaeus*. This suggests that other factors were responsible for governing the trends in sepioid abundance observed at both breakwaters.

5.3.4 Length frequency of *I. pygmaeus*

Since individuals of *I. pygmaeus* were captured over the same distance of breakwater each fortnight, it was possible to visualise both abundance and length frequency concurrently (Fig. 5.7). Males covered a much narrower range in length than females, with all individuals captured less than 10 mm DML and the majority ranging in length between 5 and 8 mm DML. In contrast, females grew much larger (greater than 17 mm DML) and consequently showed a greater spread in length frequencies.

As with *L. chinensis,* it was difficult to interpret any pattern of cohort growth from the length frequencies, even though samples were taken on a fortnightly periodicity. Males generally showed the greatest peak in abundance in the 6 mm length class (eg. in most of the samples taken between March and June 1988).

Standard

24 × 13 312

[illegible]

14.5 667

However, on certain dates, peaks occurred in the 7 to 8 mm length classes (eg. May, June, August 1988 and February, March 1989). Only a few individuals were captured which were greater than 8 mm DML.

In contrast, the abundance of females cover a much greater spread of length classes, with large catches in the 5 to 10 mm length classes, and in several instances prominent peaks of abundance in the larger length classes (eg. June 1988, May 1989). Based on visual analysis of the modes, there was some suggestion of cohort growth detected from several of the sequential length frequencies, although the peaks were often based on few individuals. These sequential modes could be used to estimate average growth rates over these periods. In 1988, the observed modal progressions occurred in late March to early April, 8 → 13 mm (growth rate=0.36 mm d^{-1}) and mid to late June, 5 → 10 mm and 10 → 13 mm (growth rate=0.36 and 0.21 mm d^{-1}). In 1989, observed modal progressions occurred from early to late May, 6 → 11 → 14 mm (growth rate=0.29 mm d^{-1}).

For both males and females, the length frequency analysis appeared to suggest a regular influx of individuals into the sampling area during periods when these sepioids were abundant. Low numbers of individuals captured throughout many months of the year (especially much of 1989) also made interpretation of length frequencies difficult.

5.3.5 Use of ELEFAN for length frequency analysis

Statolith ageing techniques have shown that all squid and sepioid species in this study exhibited rapid growth and short lifespans (see chapter 4) with sexual maturity reached in a matter of months and with no individual found older than 200 d. Furthermore, small species such as *I. pygmaeus* appeared to be able to complete their life cycle in less than three months.

The collection of length frequency data for *L. chinensis* and *I. pygmaeus* provided a unique opportunity to compare growth and lifespan estimates from cohort analysis with statolith ageing techniques. The growth curves established with ELEFAN for the three species were very different to growth curves resulting from age analysis (Fig. 5.8). The goodness of fit (Rn values) from the ELEFAN analysis, ranged from relatively low values of 0.227 for *I. pygmaeus* females (age estimate of up to ten years) to the highly significant value of 0.605 for *I. pygmaeus* males.

The growth curve for *I. pygmaeus* males suggested a lifespan of 2½ to 3 years. A similar growth curve was indicated for *L. chinensis* with a lifespan of three to four years for the largest individuals in the study area. Analysis of Rao's (1954) *S. lessoniana* data also produced an asymptotic curve with an estimated four to five years to reach 200 mm DML (Fig. 5.8).

The best growth estimates from the ELEFAN length frequency analysis indicated slow asymptotic growth and a perennial life history. This is in contrast to the length-at-age data which indicated rapid linear or exponential growth over a very short period of days or months. Statolith ageing techniques appear to be suggesting a very different pattern of growth than more traditional length frequency analysis. This may reflect a sampling bias in that larger and older members were not collected by the sampling methods. This is especially true for *L. chinensis* and *S. lessoniana* which are reported to reach larger sizes (Roper *et al.*, 1984). However, an examination of the recent literature (Table 5.3) suggests that lifespans of between 12 and 24 months are the norm for pelagic cephalopods. Furthermore, a review of the literature of growth rate estimates was also undertaken for comparative purposes[1]. Although a number of growth estimates were available, comparisons were difficult due to different protocols and periods over which measurements were taken. Estimates of growth were available from length frequency analysis, maintenance experiments and counts of statolith growth rings. There was also a single estimate based on mark-recapture (Araya, 1983). Growth is expressed as rate per day (mm d^{-1})

The greatest growth rates recorded were from statolith ageing, *Todarodes sagittatus* 2.5 mm d^{-1} (Rosenberg *et al.*, 1981), *Photoligo edulis* 5.0 mm d^{-1} (Natsukari *et al.*, 1988) maintenance, *Loligo plei* 1.97 mm d^{-1} (Hixon, 1980) and mark recapture, *Ommastrephes bartrami* 2.7 mm d^{-1} (Araya, 1983). Most of the recorded growth estimates were obtained from length frequency analysis. While these included a number of relatively high growth rates ranging from 1.33 mm d^{-1} *Ommastrephes bartrami*, *Nototodarus sloani* (Araya, 1983; Hatanaka *et al.*, 1985), 1.50 mm d^{-1} *Nototodarus* sp. (Mattlin *et al.*, 1985) and 1.67 mm d^{-1} *Todarodes eblanae* (Dunning & Brandt, 1985), the majority of estimates were below 1.0 mm d^{-1}. These included *Loligo pealei* (Hixon, 1980), *Loligo forbesi* (Holme, 1974) and *Sthenoteuthis oualaiensis* (Suzuki *et al.*, 1986). Ageing research is thus suggesting that growth rates of squids are probably faster than what has been determined in the past by other means.

5.3.6 Incorporation of ageing methods to determine seasonal influences on growth

As squids and sepioids were accessible throughout many months of the year,

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[1] Growth rates were either given in the paper or calculated from information given in the paper. Growth rates from ageing and maintenance were calculated as average daily growth rate, based on known age-at-length. Growth rate from length frequency data was calculated from modal progression over time.

it was of interest to be able to utilise ageing methods to determine if any differences in age or size-specific growth patterns could be detected. Since the greatest number of specimens were collected for *I. pygmaeus* and *L. chinensis* throughout the year, these species were used in the assessment of any seasonal influences of growth.

5.3.6.1 *Loligo chinensis*

The initial length-at-age plot developed for *L. chinensis* was based on a summer population (chapter 4) which suggested that growth was very rapid (oldest individual 115 d), therefore growth of a winter population was also analysed to determine if there were any seasonal differences in growth.

A total of 42 (summer) and 33 (winter) squids were aged and scatter plots were constructed for both length-at-age and weight-at-age (Fig. 5.9). Length-at-age regression equations were determined for males and females for both seasons. Four juveniles were not included in the summer regression and six juveniles were not included in the winter regressions. Linear regressions were highly significant (Table 5.4). Furthermore, an estimate of growth rate (mm d^{-1}) over the length range sampled could then be estimated from the slope of the regression of mantle length (mm) on age in days (Table 5.5). There was evidence of both season and sex-specific growth patterns. The greatest growth rate occurred in the summer sample of males, while the lowest growth rate was displayed by the winter females. The growth rate of males was considerably greater than the females during summer. However, during winter growth rates of males and females were similar.

Growth regressions for *L. chinensis* for each season were considerably different with no overlap between winter and summer growth curves (Fig 5.10). There was evidence of both season and sex-specific growth patterns with males showing evidence of rapid growth in summer. Covariance analysis confirmed that the slope of the regression line for summer samples of males was significantly greater than that of females and males from winter samples (Table 5.6). The regression slopes (ie. an expression of the growth rate in mm per day) of females from summer samples was not significantly different from that of either males or females in winter. However, for a given age the mean length of females in summer was significantly greater than that of either males or females in winter (significant differences in elevation). For winter samples no significant differences could be detected in the rate or pattern of growth between males and females. Males from the winter sample attained a greater age but it is possible that this might have been a sampling artefact.

Data for the regression analyses were obtained from single periods during the summer and winter. In order to examine the generality of these results an analysis was carried out involving squid which were aged from each seasonal period. This provided an opportunity to compare the magnitude of within and among season variation in length-at-age. As the number and length range of *L. chinensis* from each sampling period was not sufficient to allow for a balanced analysis of covariance, an analysis of variance on the age estimates of squids from a length range between 90 and 110 mm was used (Table 5.7).

The data were analysed by a two factor analysis of variance with sampling times nested within seasons. The only significant term in the model was 'season', which reflected the higher growth rates observed from samples collected during the summer period. The term 'times' (within seasons) was non-significant (Table 5.7). Future research involving further subsampling within each season would undoubtedly reveal greater within-season variability in length-at-age. Furthermore, the November subsample was very early within the summer period. Future subsampling over the warmer summer period (January to February) would most likely reveal a more marked seasonal difference in growth rates.

Although a linear model adequately described growth, extrapolation to the smaller sizes suggests a curvilinear model of growth may be more appropriate. This is explored in more detail elsewhere (see chapter 7). The weight-at-age data for *L. chinensis* suggested an extended period of juvenile growth with little change in weight. The growth pattern could be divided into two equal parts; an extended paralarval/juvenile phase in which very little increase took place and an adult period characterised by an abrupt increase in weight.

5.3.6.2 *Idiosepius pygmaeus*

For *I. pygmaeus* the relationship between length and age was described by a linear function (Fig. 5.11). Fitting exponential equations to the female length-at-age data did not improve the fit. Data were available for four time periods, March, May, June, and August/September. The scatter plots of the length-at-age relationship suggested; a) for older sepioids, females at a given age were larger than males and attained greater lengths, and b) seasonal differences in growth, with the slowest growth rates occurring in the August/September period (Fig. 5.11). Regression analyses confirmed the considerable variation in the length-at-age relationship for both sexes. Despite the variation, all regressions were significant with the exception of male *I. pygmaeus* in May (Table 5.8).

Covariance analysis was not attempted on this data set because of the substantial variation (ie. poor fits) in several of the regression relationships. However, the mean growth rate data indicated that except for March, females had a higher growth rate than males (Fig. 5.11). For females the greatest growth was recorded during the periods of May and June. For males the rate of growth was greatest in March and lowest in August/September. Future research incorporating more intensive sampling during these periods and greater sample sizes would be needed to confirm these trends.

5.3.7 Statolith length analysis

Despite the noted usefulness and potential of statolith growth ring analyses in tropical squids, the measurement of statolith length was taken to discern if any biological information could be obtained from growth of the statolith. Data was obtained from both the summer and winter populations of *L. chinensis* (Fig. 5.12). For both the relationship of statolith length versus mantle length and statolith length versus age, the relationship for the summer population was curvilinear whereas it was linear for the winter population (Fig 5.13). The plotted curves (Fig. 5.13) were constructed as a means to visualise the different relationships between the two seasonal periods. However, due to the complex differences between the two data sets (curvilinear versus linear) an analysis of covariance could not be applied to these data sets. However, a paired t-test was carried out to test the significance of seasonal variation in statolith length. Statolith length values were selected from individual males and females of similar lengths (mantle lengths between 90 and 110 mm, n=24) from both January (summer) and July (winter). Squid statoliths from the July sample were significantly longer than statoliths from the January sample ($p<0.05$).

5.3.7.1 Statolith length versus mantle length

The limited scatter in the correlation (Fig. 5.12) suggested that statolith growth was a function of somatic growth, however, the fact that the summer curve was curvilinear suggests that somatic growth eventually outstrips statolith growth (ie. in larger squids the mantle was increasing in length faster than the statolith was). There was also a consistent relationship in that for any given length, a winter squid had larger statoliths than its summer counterpart.

5.3.7.2 Statolith length versus age

There were also considerable seasonal differences in the growth of the statolith with age. There was a rapid curvilinear increase in statolith length in summer over a relatively short period from 60-100 d. In contrast, statolith growth was much slower in the winter, with the statolith gradually increasing in length from 80-170 d. However, statoliths eventually reached a greater length in the older, winter population squids. This feature was only a factor of age as winter squids were no longer than summer squids. In comparing similar aged squids between seasons, for any given age, a summer squid had a larger statolith than its winter counterpart.

5.4 DISCUSSION

5.4.1 Population variability over time

5.4.1.1 Offshore

Currently little is known of the possible movement or migrations of loliginid squids in Barrier Reef waters or in near-shore localities such as Cleveland Bay. Roper *et al.* (1984) has noted that *L. chinensis* forms large aggregations at certain periods of the year, especially in spring and autumn in the northern hemisphere. The best comparative work on loliginid abundance has been carried out with semitropical and temperate species in the Gulf of Mexico. Hixon (1980) has recorded seasonal abundance for the three loliginid species in the Gulf, *Loligo pealei* (predominantly temperate), *Loligo plei* (predominantly tropical) and *Loliguncula brevis* (predominantly semitropical). The highest abundance for all three species was in the spring/summer period, whereas the periods of lowest abundance were in autumn for *Loliguncula brevis* and in winter for the other two species. Voss & Brakoniecki (1985) have also reviewed catch data for squid species in the Gulf of Mexico and have noted that during winter, *L. plei* tended to be restricted to warmer southwestern Florida waters and virtually absent elsewhere in the northern Gulf. However, with seasonal warming of the water, this species migrated westward into the northern Gulf.

This migration pattern suggests that warm water loliginid squids do move according to environmental influences, most probably temperature. It is possible that the low abundance of *L. chinensis* during winter may have been due to squids migrating offshore to avoid the cooler, more shallow inshore water. Furthermore, food abundance may have also had some influence on seasonal abundance or movements of tropical loliginids. The patchy distribution of *Loligo* sp. 1 and the fact that the majority of individuals were adults, suggests that this squid was only a transitory species in Cleveland Bay, possibly moving inshore to spawn.

On only one occasion was there any suggestion of a large aggregation of squid in Cleveland Bay. In October 1988 some very large catches of both loliginid species were trawled in the vicinity of Florence Bay, Magnetic Is. In this area the trawls were predominantly squid, which is unusual as squid usually only accounted for a small proportion of the catch. It could have been an aggregation associated with spawning, especially for *Loligo* sp. 1, as most individuals captured were adults. However, there were also a considerable number of smaller *L. chinensis* captured. The waters in this area of Cleveland Bay are often clearer than other regions of the Bay (J. Collins, personal communication). Clearer water might have allowed for greater recognition of conspecifics and promoted schooling. On other dates when large numbers of squid were trawled (eg. December and January 1989, Fig. 5.2), individuals were more widespread across the bay (eg. good catches were taken at a number of trawling locations).

5.4.1.2 Inshore

Very little ecological data existed for *I. pygmaeus* before the discovery of large populations of this species in the Townsville region (Jackson, 1986; Jackson, 1989). The seasonal fluctuations observed in the population is of interest, especially considering that the trend is opposite to that observed for the offshore loliginids (ie. a greater abundance in winter and a virtual absence during the summer periods).

The inshore habitat of this sepioid and its abundance in estuarine/mangrove tributaries suggests that it is tolerant of a wide range in salinity and water quality. Low salinity tolerance is unusual for cephalopods although *Lolliguncula brevis* (Hendrix *et al.*, 1981) and *Loliolus noctiluca* (Lu *et al.*, 1985) have been found to be more euryhaline than other species. Further research into the salinity tolerance of *I. pygmaeus* would prove useful as the environment inhabited by this species suggests that cephalopods may be more plastic in their environmental tolerance than was previously thought.

The regular absence of *I. pygmaeus* in inshore waters during warm periods is not yet fully understood. *Idiosepius pygmaeus* has been caught in considerable numbers around light traps in the vicinity of Lizard Is. (northern Barrier Reef waters) in early January (Jackson & McCormick, unpublished data) while they were virtually absent from the breakwater habitat at Townsville.

Very little field data is published on the ecology of very small tropical cephalopods, although some data is available for the related sepiolids. Singley (1983) has reported that *Euprymna scolopes,* a tropical reef sepiolid lives for a matter of months and that various sized individuals are captured throughout the year. Furthermore, Boletzky (1983a) found no evidence of seasonality in populations of *Sepiola robusta* in the Mediterranean. However, these species are more closely associated with the substrate and apparently inhabit a greater depth range than *I. pygmaeus*.

The close association of *I. pygmaeus* with surface waters may have been one factor which placed constraints on its observed temporal distribution along the breakwaters. The absence of *I. pygmaeus* during summer periods may have been due to inshore surface temperatures rising above its physiological tolerance. It is possible that high surface water temperatures forced *I. pygmaeus* to migrate to deeper waters. Since this sampling program consisted of a visual sampling regime, there would be no way to detect movement into deeper water. Furthermore, juvenile fish and baitfish (especially herrings) [illegible] were especially abundant along the breakwaters during the summer periods, as many new recruits enter inshore waters in summer. Kubota & Uyeno (1970) did observe that Lancetfish *Alepisaurus* *ferox* fish preyed on *I. paradoxa* in Suruga Bay, Japan. As the fluctuations of *I. pygmaeus* did not appear to be related to *Acetes* abundance, physical factors such as temperature, or biological factors such as increased predation, may have accounted for the apparent low numbers during summer.

5.4.2 Length frequency analysis

In determining growth rates and lifespans of squids, biologists have mainly used either length frequency analysis, maintenance of individuals under artificial conditions or ageing techniques. Due to difficulties in maintaining individuals alive and the fact that the use of validated statolith growth rings is a relatively recent technique, the majority of growth estimates have come from length frequency analysis.

The considerable differences found between the two methods of estimating squid growth (ie. length frequency analysis versus direct ageing) raises questions as to the accuracy of previous analysis techniques used in describing squid growth, especially tropical squid growth. Fishery biologists have extensively used the asymptotic von Bertalanffy growth equation (which is incorporated into the ELEFAN package) to model fish growth. Although there are a number of different growth equations describing fish growth, the von Bertalanffy model has proven most successful in modelling fish growth in that it satisfies the two most important criteria - "it fits most of the observed data on fish growth, and can be incorporated readily into stock assessment models" (Gulland, 1985).

There has been some discussion as to whether traditional fish growth models can be applied to squids as well. Pauly (1985) and Longhurst & Pauly (1987) have promoted the apparent usefulness of ELEFAN modelling of squid length frequency data. They have suggested that the application of this type of length frequency analysis would help alleviate the present dearth of knowledge on squid growth and "help researchers obtain a 'feel' for the growth performance of squids similar to that which biologists now have for fishes" (Pauly, 1985). Boyle (1990) has recently supported this line of thought, suggesting that inaccuracies developed from length frequency analysis were due to the modes being detected by eye rather than the result of statistically objective procedures. However, based on the analysis of the growth of *L. chinensis* and *I. pygmaeus*, inaccuracies persist in growth curve analysis despite using stringent statistical procedures. The problem appears to be in the nature of the statistical package (incorporating an asymptote into the model) and the nature of tropical squid length frequency data (ie. rapid growth and turnover of generations thus creating confusion in modal frequencies). In contrast to Boyle's (1990) statement, establishing growth rates by detecting modes by eye for *I. pygmaeus* females were actually quite close to values obtained from statolith ageing (0.21 to 0.36 mm d^{-1} length frequency versus 0.174 to 0.294 mm d^{-1} age analysis).

The appropriateness of applying asymptotic growth models to squids has been questioned (Saville, 1987; Forsythe & Van Heukelem, 1987; Forsythe & Hanlon, 1989). The comparisons between the length-at-age plots and the length frequency analyses in this study appear to confirm the above raised arguments that asymptotic growth models are inappropriate for describing squid growth.

The possibility exists that if large individuals of *L. chinensis* and *S. lessoniana* were included in the plots an asymptotic plot could have resulted. However, none were located by the sampling regime and the upper size range for these species were similar to those obtained from other regular sampling programs (Chan & Karim, 1986;

Rao, 1954) in which very large individuals were rare. For example, although *S. lessoniana* is reported to reach 350 mm in length, out of 2105 individuals measured by Rao (1954) over a two year study period, only 12 specimens were larger than 210 mm, and the largest specimen recorded was less than 260 mm.

There is a further problem associated with length frequency analysis, regardless of what model is applied to the data. Sampling of cephalopod populations may be biased, in that larger animals may migrate out of the population (eg. Dawe, 1981a; Dawe *et al*, 1981; Rowell *et al*, 1985, Patterson, 1988). If there is a size or age-dependent migration, sequential sampling, and analysis of length frequency data may suggest that growth is asymptotic, when in reality, the larger individuals are leaving the sampling region. Furthermore, length frequency analysis may also imply asymptotic growth if some individuals in the population are very fast growing. Very fast growing squids have been shown to grow, mature, spawn and die in advance of other members within the same cohort (Rodhouse *et al*, 1988; Rodhouse & Hatfield, 1990b). This phenomena would also incorrectly imply that there is a slowing in growth rate at the larger sizes. Assigning asymptotic growth to squids may not only be due to the application of inappropriate growth models, but also due to a more fundamental problem of length frequency analysis not being an appropriate technique with which to analyse squid population data.

5.4.3 Using ageing research to interpret seasonal changes in growth

5.4.3.1 Seasonal growth patterns of *L. chinensis*

The rapid growth and short lifespan of *L. chinensis* suggests that this species would be vulnerable to environmental changes. The two most probable factors influencing growth rate would be food supply and temperature, with temperature being the main factor influencing growth when food is abundant (Forsythe & Van Heukelem, 1987). At this stage the fluctuations in the abundance of possible prey items of *L. chinensis* is not known, however, temperature in Cleveland Bay and across the continental shelf does fluctuate and has been monitored (Kenny, 1974; Walker, 1981, Milward & Hartwick, 1986). Surface water temperatures in the middle of Cleveland Bay have been shown to seasonally fluctuate between 19.3°C and 30.9°C (Walker, 1981) with summer maxima occurring in January and winter minima occurring in July. Temperature profiles taken across the shelf have shown little temperature stratification (Milward & Hartwick, 1986). The summer and winter collections of *L. chinensis* analysed for length-at-age and weight-at-age correspond to these peaks in temperature extremes.

The different growth patterns of *L. chinensis* in the summer and winter is of interest. These field results agree with recent experimental growth trials for captive cephalopods. Forsythe & Hanlon (1988) have found similar differences in growth curves for the Californian littoral octopus *Octopus bimaculoides* by culturing this species throughout its life cycle at two different temperatures, 18°C and 23°C. They found that individuals reared at the higher temperature had an increased growth rate (up to three times greater), earlier maturity, and a shorter lifespan than did individuals reared at the lower temperature. The age-weight curve showed no overlap between the two experimental treatments. More significant to this study are the results of growth trials at different temperatures with the loliginid squid *Loligo forbesi* (Forsythe & Hanlon, 1989). This species was found to be extremely sensitive to temperature fluctuations and is capable of sustained exponential growth for at least three to four months, with a 1 °C temperature difference capable of changing the growth rate by 2% BW d^{-1}, while a 3.5 °C rise in temperature increased the growth rate by approximately 4% BW d^{-1}.

Extrapolating these studies to the field situation suggests that the seasonal temperature difference in Cleveland Bay (greater than 10 °C) could account for the different growth rates of the summer and winter populations of *L. chinensis.*

Cephalopod growth appears to be more temperature dependent than does growth of teleost fishes. For example, the tropical anchovy *Stolephorus purpureus,* which has a lifespan of less than one year and appears to have linear growth shows only slight seasonal differences in its growth rate (Struhsaker & Uchiyama, 1976). This might be a reflection of the biochemical and metabolic differences between squid and fish, as squids have a minimal ability to store energy reserves as compared to fish and therefore less ability to regulate their growth (O'dor & Webber, 1986).

5.4.3.2 Seasonal growth patterns of *I. pygmaeus*

Growth of *I. pygmaeus* was characterised by a large amount of variability for both sexes throughout the year. This study has shown that individual variability in growth rates appear to be the norm for tropical squids and sepioids, and agrees with previous ageing work which has documented the same phenomena (Lipinski, 1986; Natsukari *et al.*, 1988). This feature would be expected to be even more accentuated in *I. pygmaeus* due to the greater degree of environmental heterogeneity in the near-shore littoral areas this species inhabits. The inshore sampling areas were shallow (many individuals were captured in a water depth of less than 1 m), this would subject the sepioids to significant changes in temperature, salinity and water quality.

Furthermore, considerable changes in the environment would be encountered over the period of the tidal cycle each day. For example, the water often became substantially more turbid during periods of low tide.

Previous work on the influence of temperature on cephalopod growth rate (Forsythe & Hanlon, 1988,1989), suggests that temperature fluctuation would no doubt be the prominent influence on the observed differences in growth rates encountered for *I. pygmaeus* between March (early autumn) and August/September (early spring). However, the situation is possibly even more complex than for *L. chinensis* due to greater short-term fluctuations in environment and food supply in the near-shore habitat.

5.4.4 Somatic versus statolith growth

Squid statolith microstructural research is continuing along very similar lines to that of fish otolith research. The applications of microstructural research for both squids and fish is perhaps one of the most profound examples of anatomical similarities between two very different organisms living under similar environmental constraints. Current research into fish somatic-otolith growth comparisons provides possible explanations for mechanisms underlying somatic-statolith growth relationships for *L. chinensis*. The relationship between statolith length and mantle length, and statolith length and age shows striking similarities to otolith length versus fish length and age studies. Secor & Dean (1989), Reznick *et al.* (1989) and Wright et al.(1990) have all shown that slower growing fish have larger otoliths than similar sized faster growing fish. Furthermore, Secor & Dean (1989) and Wright *et al.* (1990) have also shown with Striped Bass (*Morone saxatilis)* and salmon parr (*Salmo salar)* respectively, that generally, although slower growing fish have larger otoliths at any given size, faster growing fish have larger otoliths at any given age. This relationship holds true for the statolith-mantle relationship for *L. chinensis.*

These teleost studies help to explain the growth of the squid statolith. The slower growing individuals of *L. chinensis* (winter population) had larger statoliths because they were in reality much older than similar sized faster growing squids (summer population). Alternatively, when statolith length and individual age was compared, faster growing squids had larger statoliths than slower growing squids for a given age because the individual itself was considerably larger (eg. in the summer, squids were reaching adult sizes at around 80 d whereas in the winter 80 d squid were still juveniles).

Morris & Aldrich (1985) have suggested that statolith length may be a better descriptor of squid age than ring number since they observed less variation in the mantle length : statolith length correlation than in the mantle length : age correlation in *I. illecebrosus.* However, the seasonal difference in the relationship between the statolith and the soma of *L. chinensis* due to ambient temperature variation, suggests that this technique should be used cautiously until further research into temperature influences is carried out. The uncoupling of statolith growth and somatic growth in squid is certainly one area that deserves more research.

Statoliths are one structure which has accentuated both the differences and similarities between cephalopods and fish. The ring structure and the growth of the statolith in relation to the squid soma has shown remarkable similarities between the ring structure in fish otoliths and in otolith-soma growth relationships (indeed the photograph of the salmon parr otolith in Wright *et al.* (1990) would be indistinguishable from a photograph of the ring structure in the rostrum of a loliginid squid). In contrast, the enumeration of growth rings in both otoliths and statoliths have accentuated very different growth strategies and life histories of two organisms which are biologically very different, but nevertheless show many similarities.

Table 5.1: *Loligo chinensis*. **Comparison of the number of rings extrapolated from submarginal areas of the statolith compared with the actual rings counted at the marginal areas of equivalent width in clear statoliths. (S) Summer specimens (W) Winter specimens**

Sex	Season	Mantle Length (mm)	Age (d)	Extrapolated Area of Statolith Radius (%)	Number of Rings Extrapolated from Sub-marginal Areas	Mean	Actual Ring Number in Marginal Areas	Mean
M	S	109	93	11.4	13,14,13	13	13,14,12	13
M	S	110	78	9.1	10,10,10	10	10,11,11	11
M	S	102	76	7.9	9,10,10	10	9,9,9	9
F	S	94	77	7.5	12,11,11	11	13,13,13	13
F	S	102	87	7.7	9,9,10	9	10,10,11	10
F	S	110	83	10.4	12,11,11	11	11,12,13	12
M	W	104*	119	7.0	11,11,11	11	12,11,13	12
M	W	109*	132	7.3	12,11,11	11	10,10,11	10
F	W	106*	119	6.1	11,10,10	10	10,10,10	10
F	W	108*	107	6.25	11,11,11	11	11,11,12	11

* mantle length of fresh specimens

Table 5.2: ***Acetes*** **abundance estimates applied to each dip-net sample used in obtaining a Relative *Acetes* Abundance Index.**

Number of *Acetes* Dip-netted	0	1	2-10	11-25	25-50	50-100	>100
Ranking	0	1	2	3	4	5	6

Table 5.3: Recent published information on squid lifespans.

Species	Lifespan	Method	Author
Alloteuthis subulata	~1yr	Length frequency	Rodhouse et al., 1988
Loligo gahi	1yr	Length frequency	Patterson, 1988
Photololigo edulis	1yr	Statoliths	Natsukari et al., 1988
Loligo pealei	1-1½yr	Length frequency + culture	Hixon, 1980
Loligo plei	1-1½yr	Length frequency + culture	Hixon, 1980
Lolliguncula brevis	1-1½yr	Length frequency + culture	Hixon, 1980
Loligo forbesi	1-2yr	Culture	Hanlon et al., 1989
Loligo opalescens	<1yr	Culture	Yang et al., 1986
Sepioteuthis lessoniana	~1yr	Culture	Segawa, 1987
Illex illecebrosus	1yr	Statoliths	Radtke, 1983
Gonatus fabricii	1½-2yr	Statoliths	Kristensen, 1980
Todarodes sagittatus	1½yr	Statoliths	Rosenberg et al., 1980
Todarodes pacificus	~1yr	Mark recapture + fishery statistics	Okutani, 1983

Table 5.4: **Regression equations for the length-at-age data for specimens of *Loligo chinensis* sampled during January (Summer) and June (Winter), by sex.**

Period	Sex	n	Equation	r^2	Anova F
Summer	Male Female	25 19	$y = -107.32 + 2.65x$ $y = -0.22 + 1.19x$	0.81 0.52	p<.001 p<.001
Winter	Male Female	18 16	$y = -40.97 + 1.10x$ $y = -32.29 + 1.04x$	0.80 0.67	p<.001 p<.001

Table 5.5: ***Loligo chinensis*. Mean growth rates expressed as mm day^{-1} for summer and winter samples of male and female *L. chinensis*.**

Season	Sex	Mean Growth mm day^{-1}	±95%C.I.	No. Days	Length Range (mm)
Summer	Male	2.65	0.54	59	74-180
	Female	1.19	0.59	35	69-118
Winter	Male	1.10	0.30	103	51-167
	Female	1.04	0.43	57	52-109

Table 5.6: ***Loligo chinensis*. Summary of the comparisons of regression equations based on analysis of covariance**

Summer	Male vs Female	Slope F = 10.3	Pr>F 0.0027	Significant
Winter	Male vs Female	Slope F = 0.07 Elevation F = 0.08	Pr>F 0.795 Pr>F 0.777	ns ns
Female	Winter vs Summer	Slope F = 0.209 Elevation F = 62.72	Pr>F 0.650 Pr>F 0.0001	ns Significant
Male	Winter vs Summer	Slope F = 26.43	Pr>F 0.0001	Significant

Table 5.7: ***Loligo chinensis*. Mean length and age ± standard error of squids between 90 and 110 mm mantle length for males and females collected at two times within each season. The age data were subjected to analysis of variance. Sex and season are fixed factors, times within season a random factor. Variable is age within the specified length class. n = 44.**

	Summer T_1	**Summer T_2**	**Winter T_1**	**Winter T_2**
Females				
Mean Length	102.2 ± 3.3	95.2 ± 1.5	100.5 ± 2.5	100.0 ± 2.1
Mean Age	80.7 ± 2.6	98.7 ± 3.5	120.5 ± 6.1	123.2 ± 2.6
Males				
Mean Length	105.0 ± 2.7	100.5 ± 1.3	98.2 ± 2.5	100.2 ± 1.8
Mean Age	83.0 ± 3.8	93.7 ± 1.5	132.2 ± 9.5	126.0 ± 2.3

Analysis of variance table for age data.

Source	**df**	**ms**	**F**	**Pr>1**
Sex	1	108.8	1.11	0.403
Season	1	11063.3	25.47	0.037
Times (Season)	2	434.4	4.42	0.184
Sex x Season	1	205.0	2.09	0.285
Sex x Times (Season)	2	98.3	1.12	0.342
Residual	24	87.86		

Table 5.8: ***Idiosepius pygmaeus*. Regression equations for the length-at-age data sampled from different seasonal periods, by sex.**

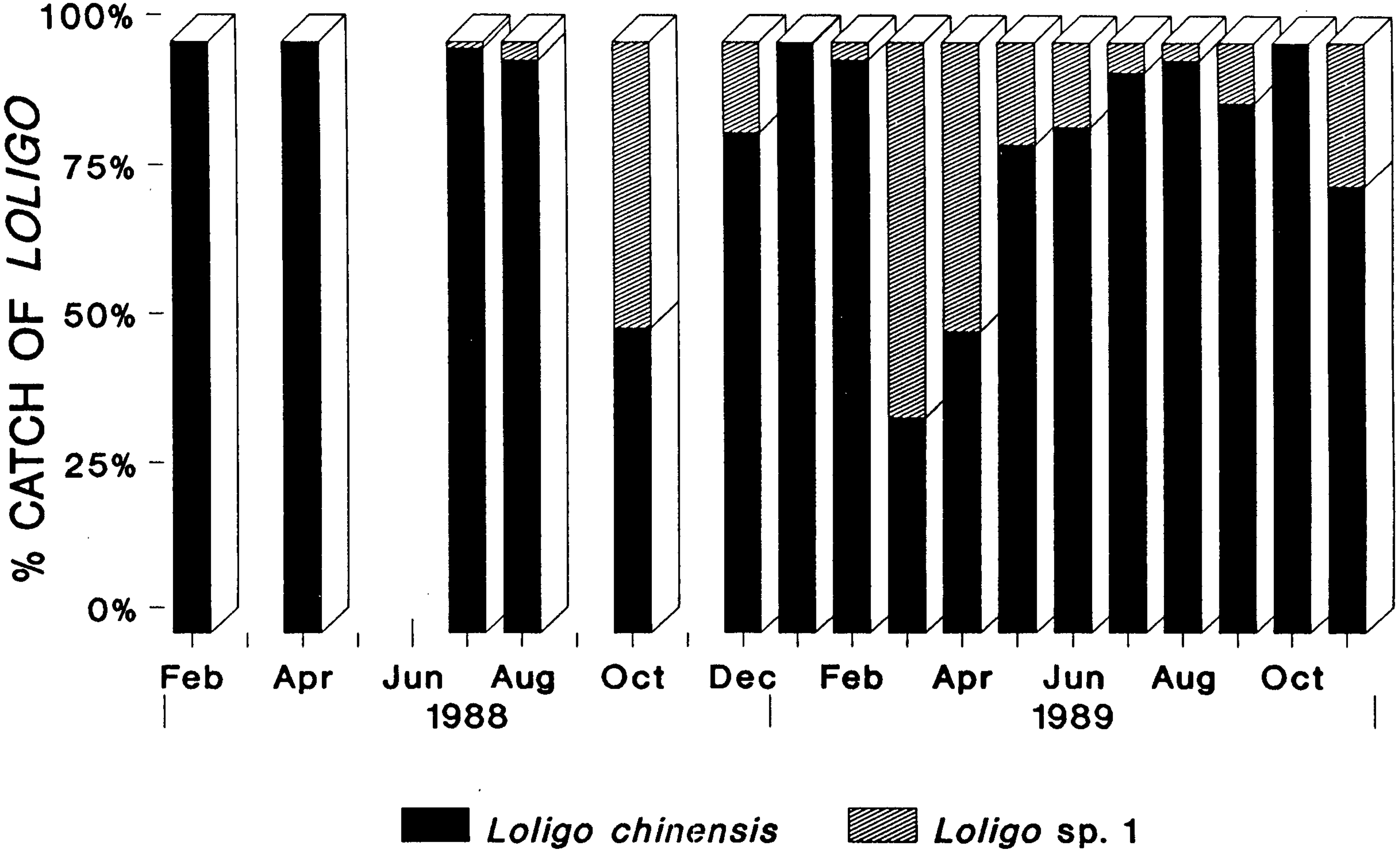
Period	Sex	n	Equation	r^2	Anova F
March	Male	22	$y = 0.41 + 0.20x$	0.93	$p<.001$
	Female	25	$y = 1.72 + 0.17x$	0.39	$p<.001$
May	Male	18	$y = 2.96 + 0.12x$	0.22	ns
	Female	17	$y = -1.27 + 0.29x$	0.56	$p<.001$
June	Male	20	$y = 2.35 + 0.12x$	0.47	$p<.01$
	Female	22	$y = -1.09 + 0.23x$	0.41	$p<.01$
Aug/Sept	Male	23	$y = 3.02 + 0.08x$	0.39	$p<.01$
	Female	17	$y = 0.74 + 0.17x$	0.51	$p<.001$

Table 5.9: ***Idiosepius pygmaeus*. Mean growth rates expressed as mm day $^{-1}$ for each combination of sex and sampling period.**

Period	Sex	Mean growth mm d^{-1}	± 95% C.I.
March	Male	0.195	.037
	Female	0.174	.093
May	Male	0.121	.119
	Female	0.294	.141
June	Male	0.122	.064
	Female	0.230	.128
Aug/Sept	Male	0.083	.047
	Female	0.167	.086

Figure 5.1

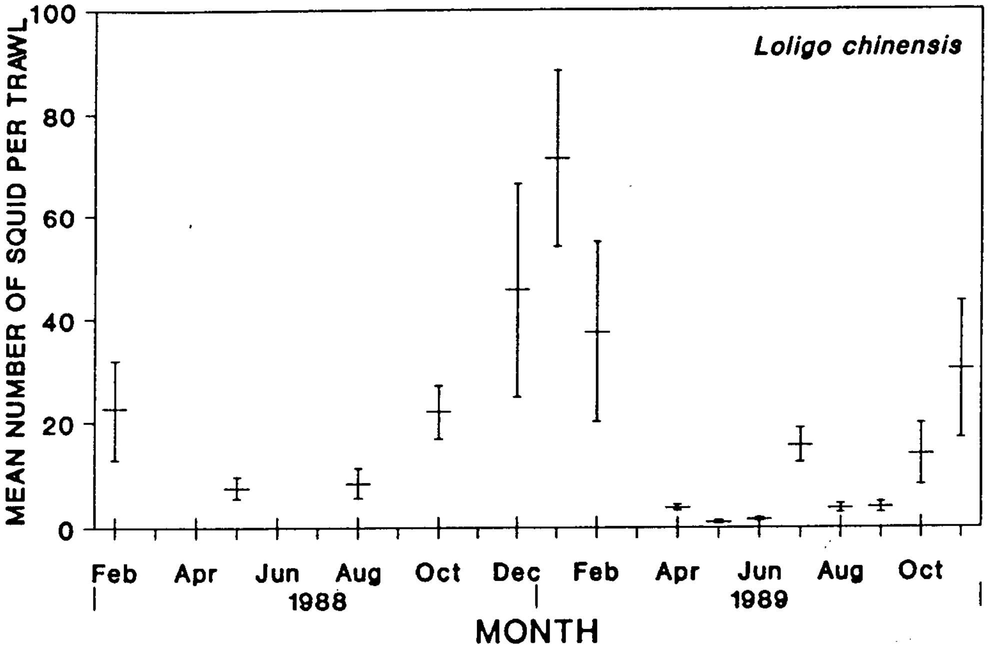
Total percentage catch of loliginid squids (*Loligo chinensis* and *Loligo* sp. 1) captured in trawl samples during each sampling trip.



% CATCH OF *LOLIGO*
100%
75%
50%
25%
0%
Feb
Apr
Jun
Aug
Oct
Dec
Feb
Apr
Jun
Aug
Oct
1988
1989
Loligo chinensis
Loligo sp. 1

Figure 5.2

Mean number of individuals of *Loligo chinensis* and *Loligo* sp. 1 captured per trawl for each sampling trip. Bars represent standard errors. Note, no individuals of *Loligo* sp. 1 captured during February 1988 and October 1989.


Loligo chinensis
MEAN NUMBER OF SQUID PER TRAWL
0
20
40
60
80
100
Feb
Apr
Jun
Aug
Oct
Dec
Feb
Apr
Jun
Aug
Oct
1988
1989
MONTH

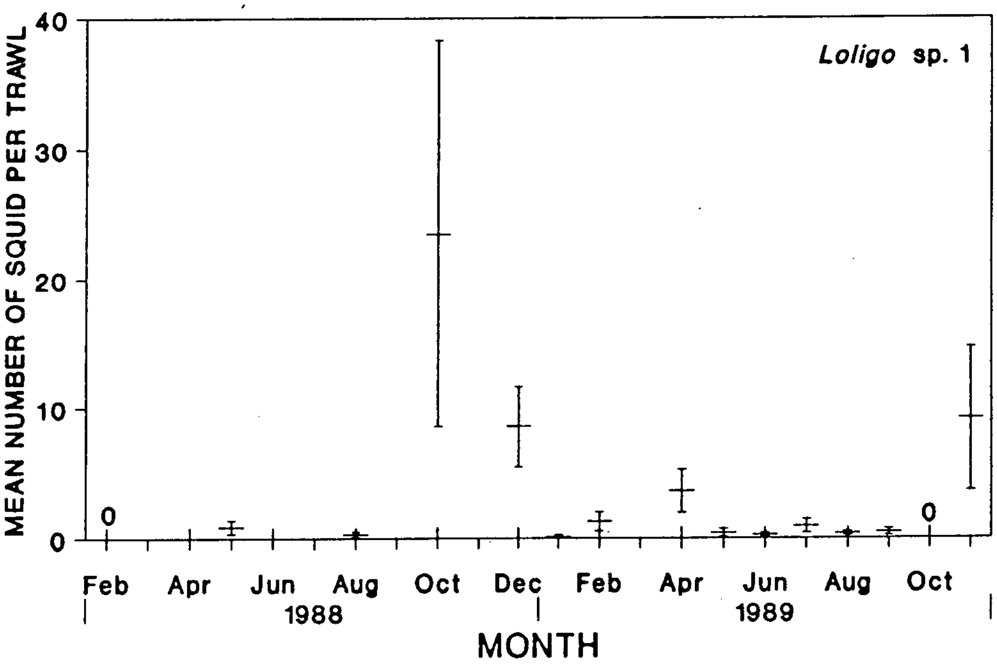
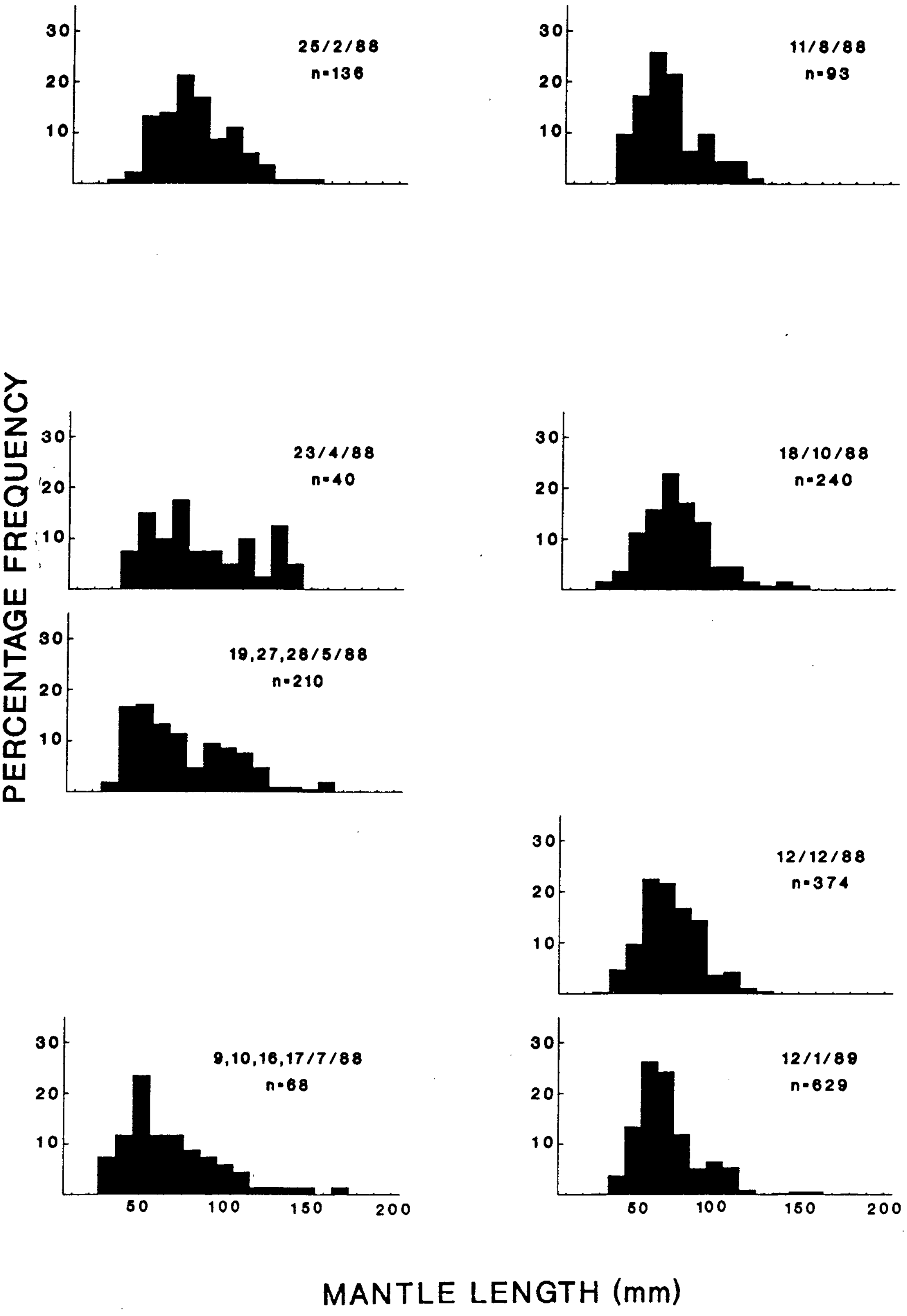

Loligo sp. 1
MEAN NUMBER OF SQUID PER TRAWL
0
10
20
30
40
0
0
Feb
Apr
Jun
Aug
Oct
Dec
Feb
Apr
Jun
Aug
Oct
1988
1989
MONTH

Figure 5.3

Percentage length frequency distributions for all individuals of *Loligo chinensis* captured during each sampling trip.


25/2/88
n=136
11/8/88
n=93
23/4/88
n=40
18/10/88
n=240
19,27,28/5/88
n=210
12/12/88
n=374
9,10,16,17/7/88
n=68
12/1/89
n=629
PERCENTAGE FREQUENCY
10
20
30
50
100
150
200
MANTLE LENGTH (mm)

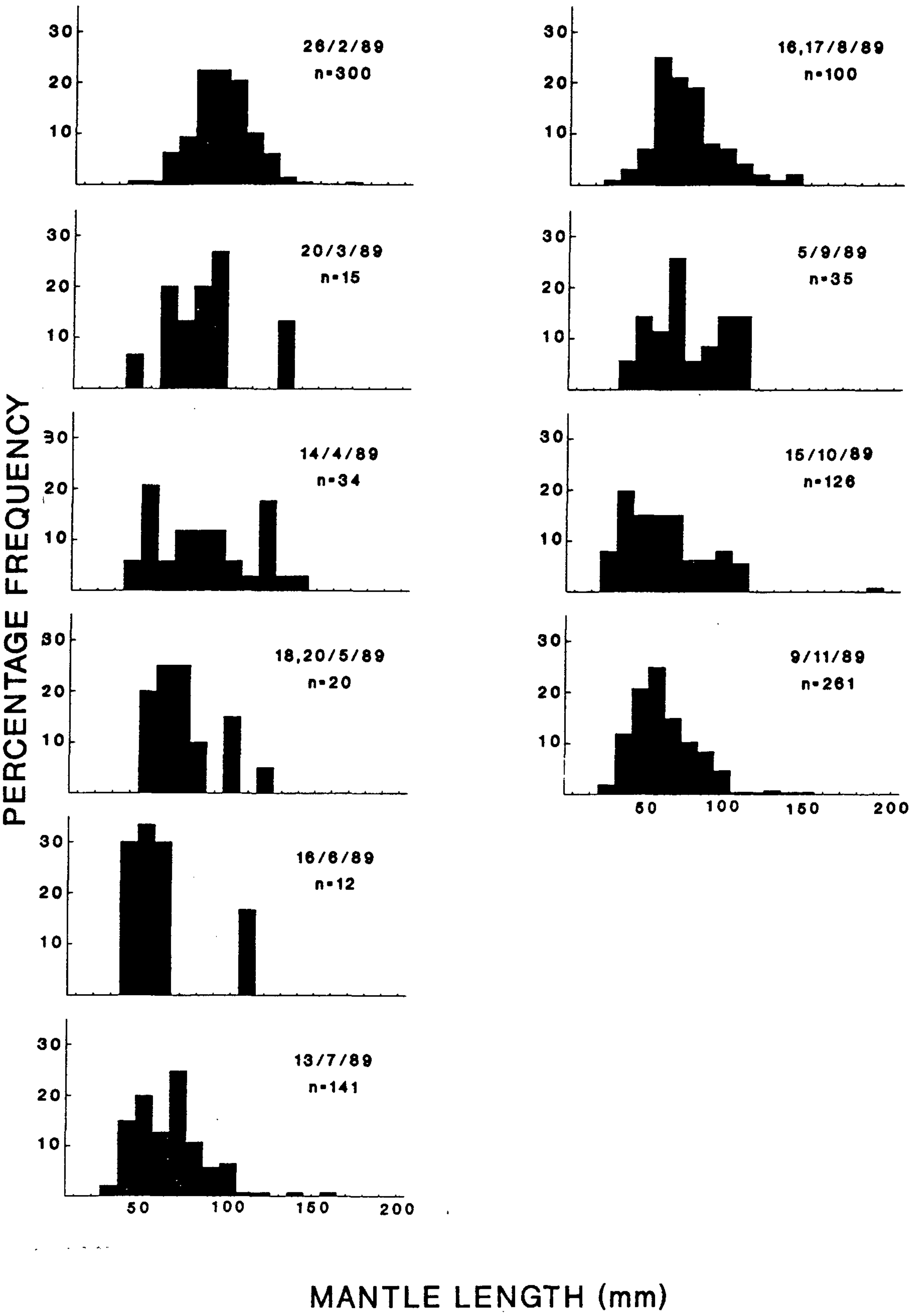

26/2/89
n=300
20/3/89
n=15
14/4/89
n=34
18,20/5/89
n=20
16/6/89
n=12
13/7/89
n=141
16,17/8/89
n=100
5/9/89
n=35
15/10/89
n=126
9/11/89
n=261
30
20
10
50
100
150
200
PERCENTAGE FREQUENCY
MANTLE LENGTH (mm)

Figure 5.4

Numbers of individuals of *Idiosepius pygmaeus* counted along the western breakwater.

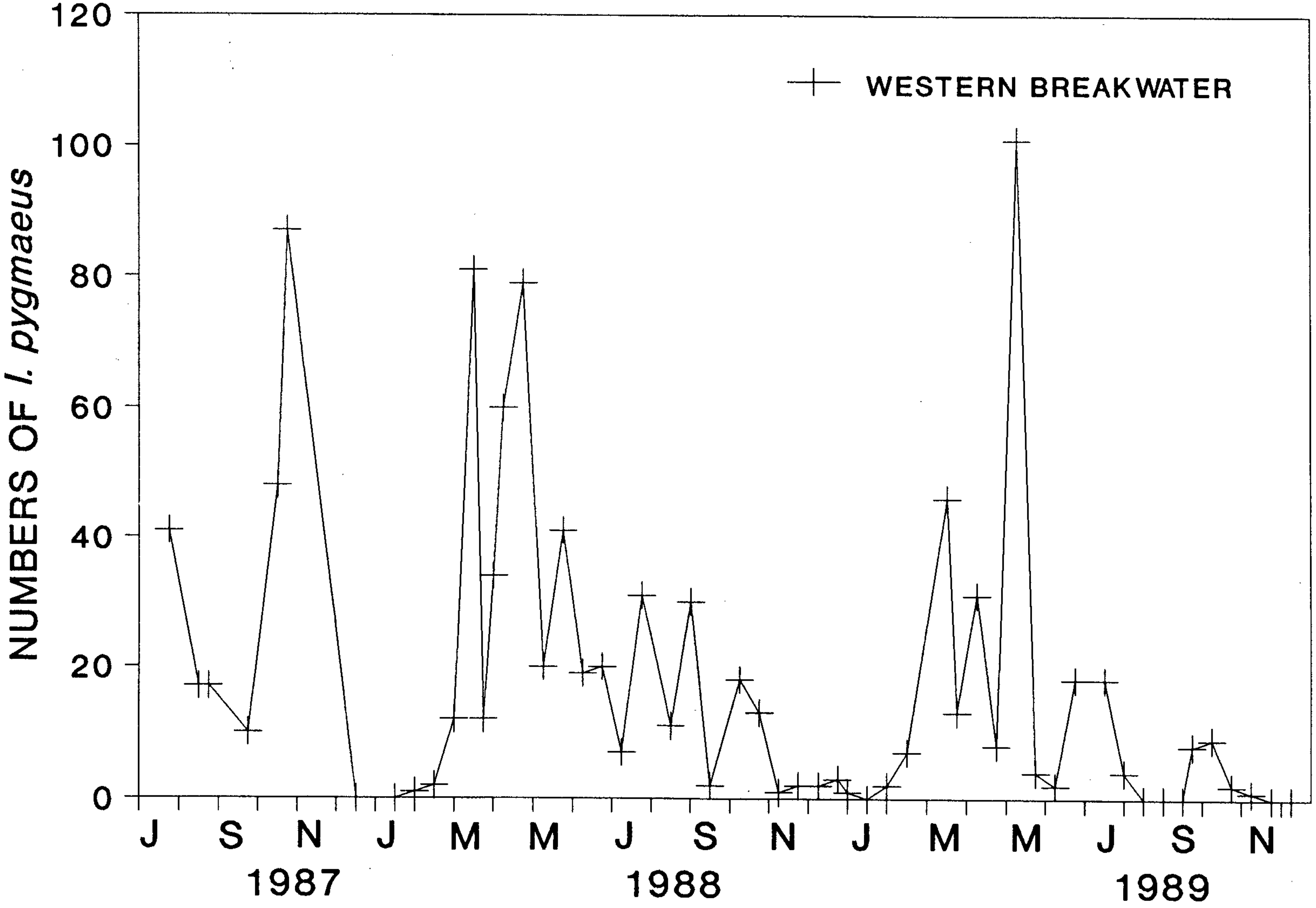

WESTERN BREAKWATER
NUMBERS OF *I. pygmaeus*
120
100
80
60
40
20
0
J
S
N
J
M
M
J
S
N
J
M
M
J
S
N
1987
1988
1989

Figure 5.5

Numbers of individuals of *Idiosepius pygmaeus* collected along the eastern breakwater superimposed over the numbers of *I. pygmaeus* counted along the western breakwater.

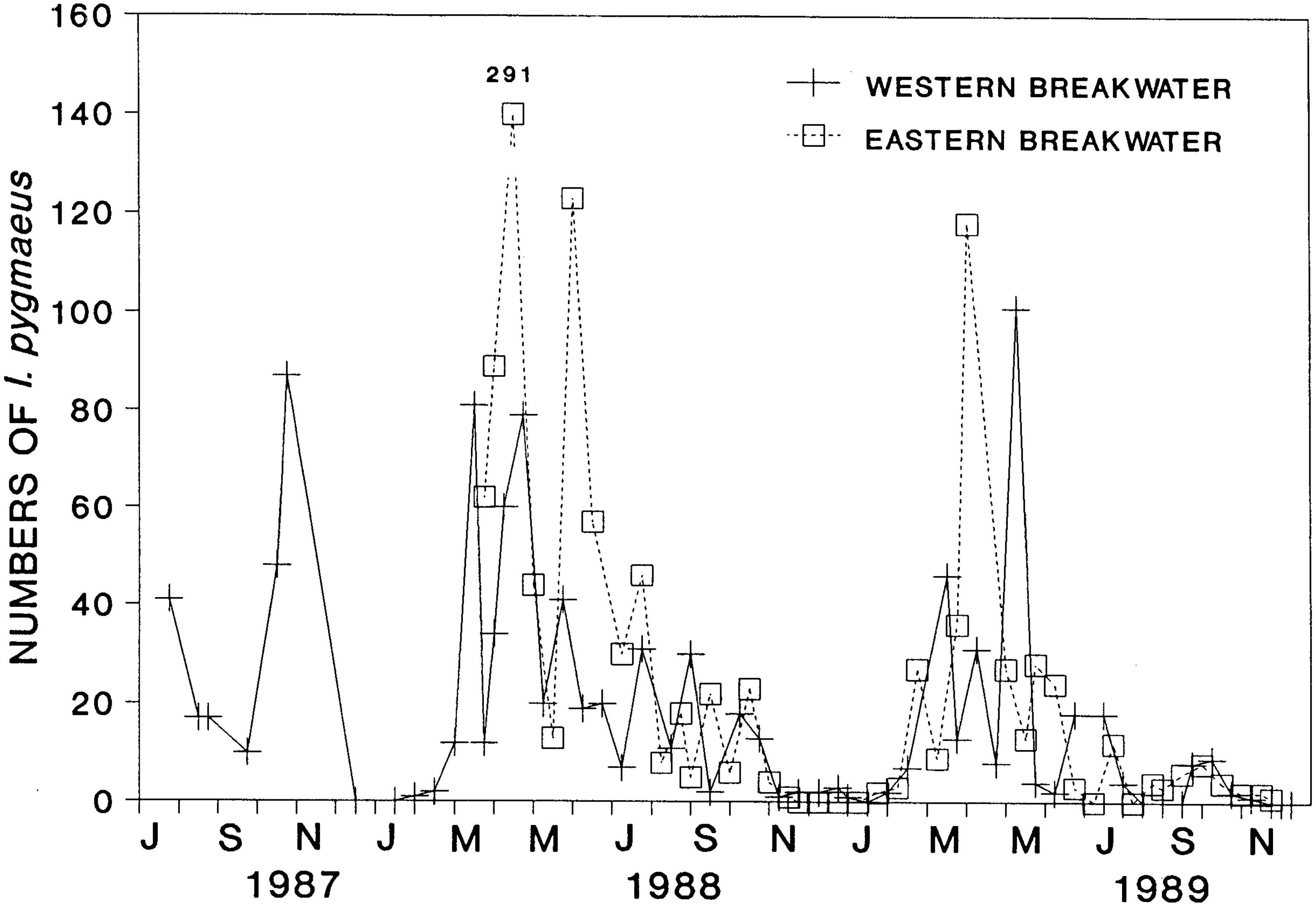

NUMBERS OF *I. pygmaeus*
160
140
120
100
80
60
40
20
0
291
WESTERN BREAKWATER
EASTERN BREAKWATER
J
S
N
J
M
M
J
S
N
J
M
M
J
S
N
1987
1988
1989

Figure 5.6

The Relative *Acetes* Abundance Index calculated for one sampling trip each month, superimposed over the numbers of individuals of *Idiosepius pygmaeus* collected along the eastern breakwater.

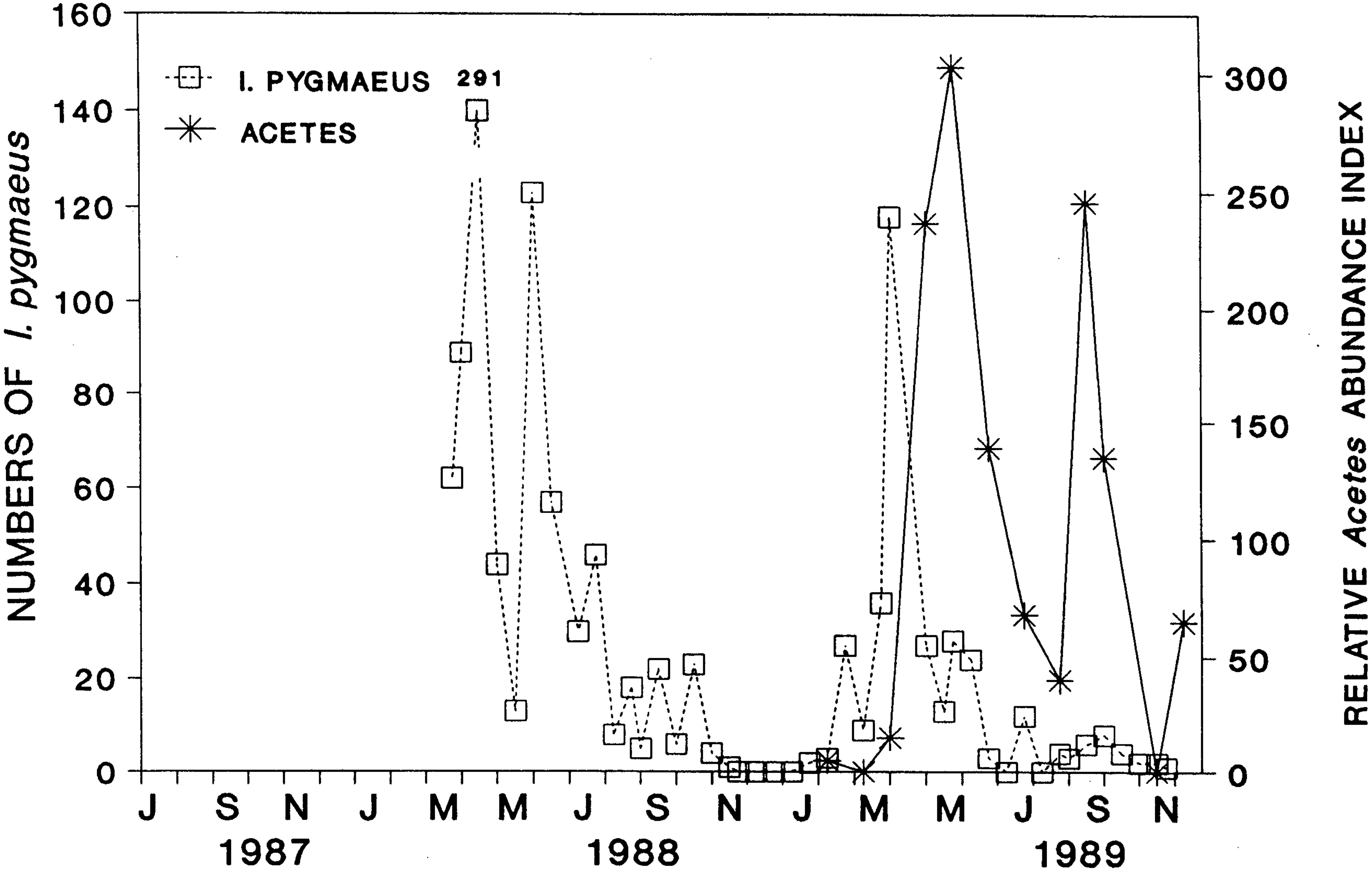
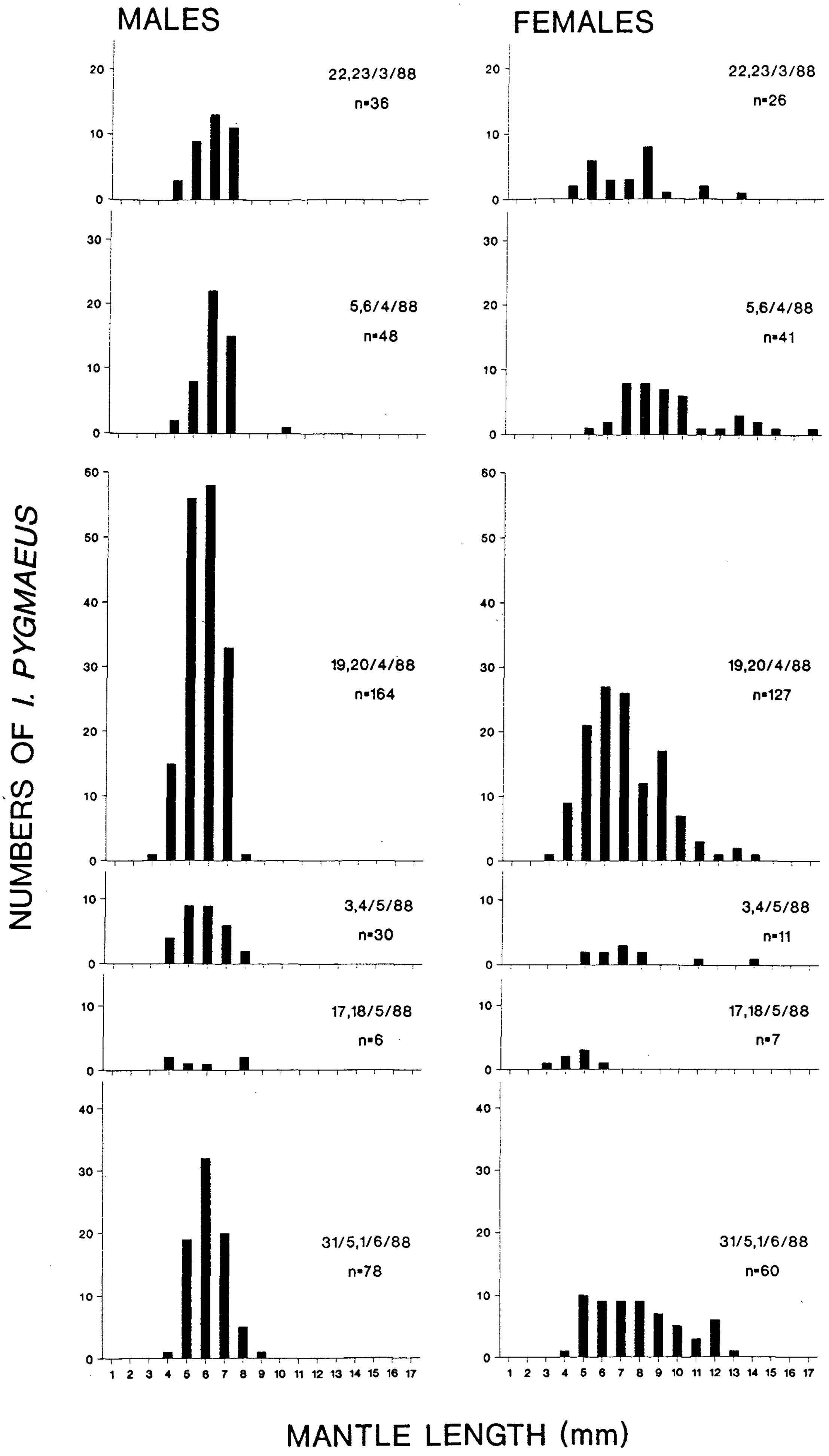
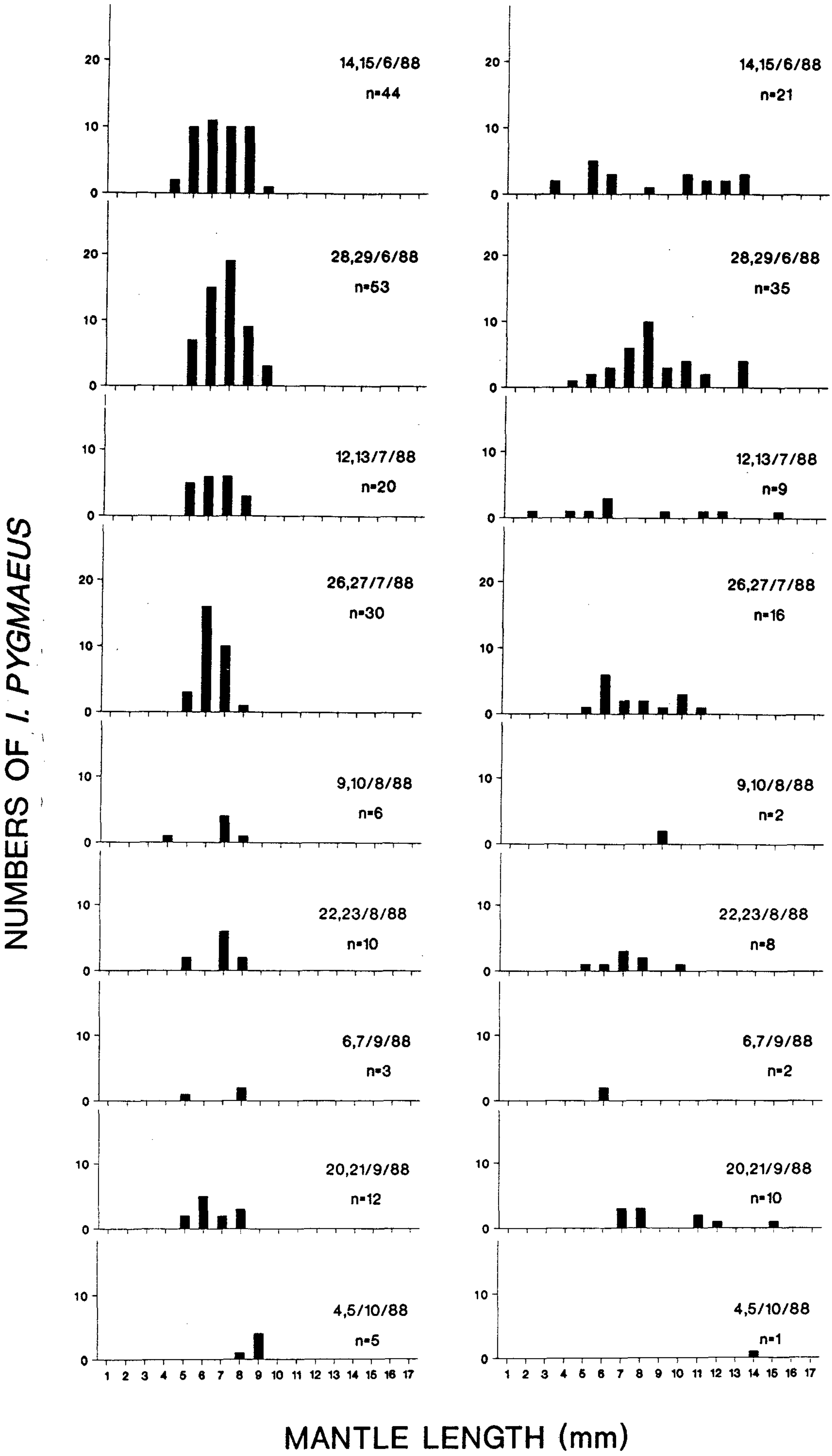
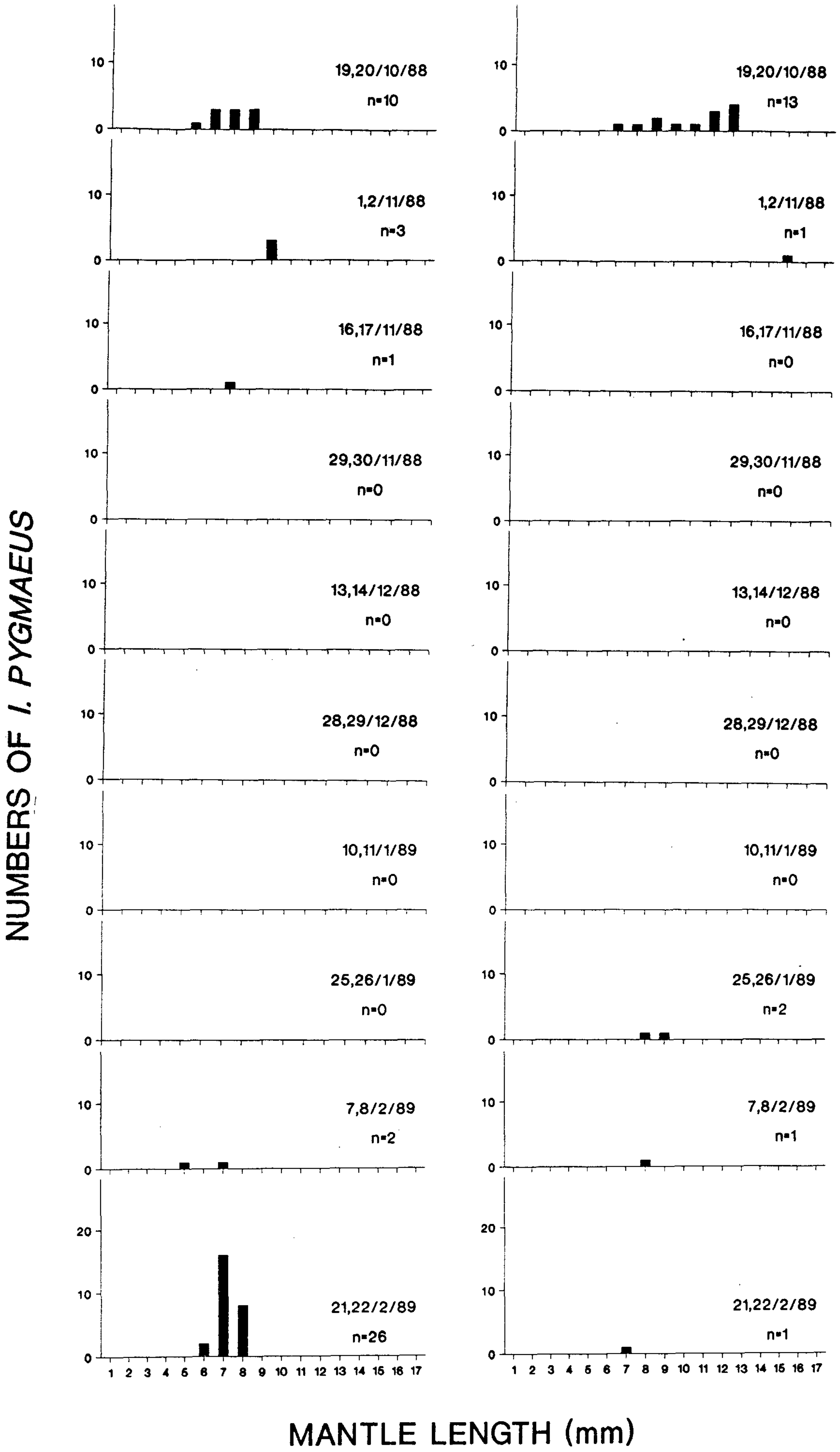

I. PYGMAEUS
ACETES
291
NUMBERS OF *I. pygmaeus*
RELATIVE *Acetes* ABUNDANCE INDEX
0
20
40
60
80
100
120
140
160
0
50
100
150
200
250
300
J
S
N
J
M
M
J
S
N
J
M
M
J
S
N
1987
1988
1989

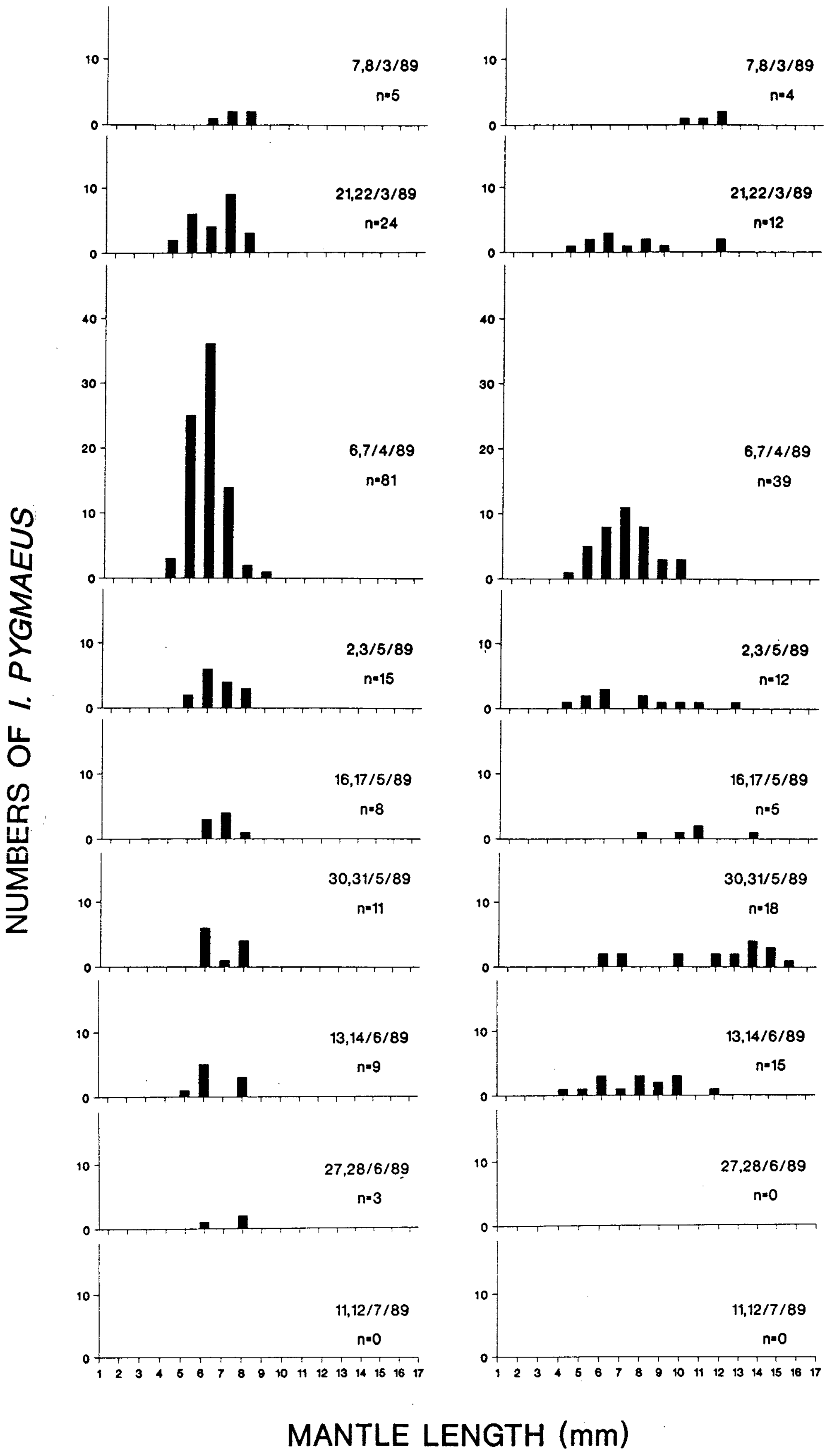
Figure 5.7

Length frequency distribution for male and female individuals of *Idiosepius pygmaeus* captured along the eastern breakwater. Asterisk (October 1989 sample) indicates data from only one sampling trip.


MALES
FEMALES
22,23/3/88
n=36
22,23/3/88
n=26
5,6/4/88
n=48
5,6/4/88
n=41
19,20/4/88
n=164
19,20/4/88
n=127
3,4/5/88
n=30
3,4/5/88
n=11
17,18/5/88
n=6
17,18/5/88
n=7
31/5,1/6/88
n=78
31/5,1/6/88
n=60
0
10
20
30
40
50
60
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
NUMBERS OF I. PYGMAEUS
MANTLE LENGTH (mm)


14,15/6/88
n=44
14,15/6/88
n=21
28,29/6/88
n=53
28,29/6/88
n=35
12,13/7/88
n=20
12,13/7/88
n=9
26,27/7/88
n=30
26,27/7/88
n=16
9,10/8/88
n=6
9,10/8/88
n=2
22,23/8/88
n=10
22,23/8/88
n=8
6,7/9/88
n=3
6,7/9/88
n=2
20,21/9/88
n=12
20,21/9/88
n=10
4,5/10/88
n=5
4,5/10/88
n=1
0
10
20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
NUMBERS OF I. PYGMAEUS
MANTLE LENGTH (mm)


NUMBERS OF I. PYGMAEUS
MANTLE LENGTH (mm)
19,20/10/88
n=10
19,20/10/88
n=13
1,2/11/88
n=3
1,2/11/88
n=1
16,17/11/88
n=1
16,17/11/88
n=0
29,30/11/88
n=0
29,30/11/88
n=0
13,14/12/88
n=0
13,14/12/88
n=0
28,29/12/88
n=0
28,29/12/88
n=0
10,11/1/89
n=0
10,11/1/89
n=0
25,26/1/89
n=0
25,26/1/89
n=2
7,8/2/89
n=2
7,8/2/89
n=1
21,22/2/89
n=26
21,22/2/89
n=1
0
10
20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17


NUMBERS OF I. PYGMAEUS
7,8/3/89
n=5
7,8/3/89
n=4
21,22/3/89
n=24
21,22/3/89
n=12
6,7/4/89
n=81
6,7/4/89
n=39
2,3/5/89
n=15
2,3/5/89
n=12
16,17/5/89
n=8
16,17/5/89
n=5
30,31/5/89
n=11
30,31/5/89
n=18
13,14/6/89
n=9
13,14/6/89
n=15
27,28/6/89
n=3
27,28/6/89
n=0
11,12/7/89
n=0
11,12/7/89
n=0
0
10
20
30
40
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
MANTLE LENGTH (mm)

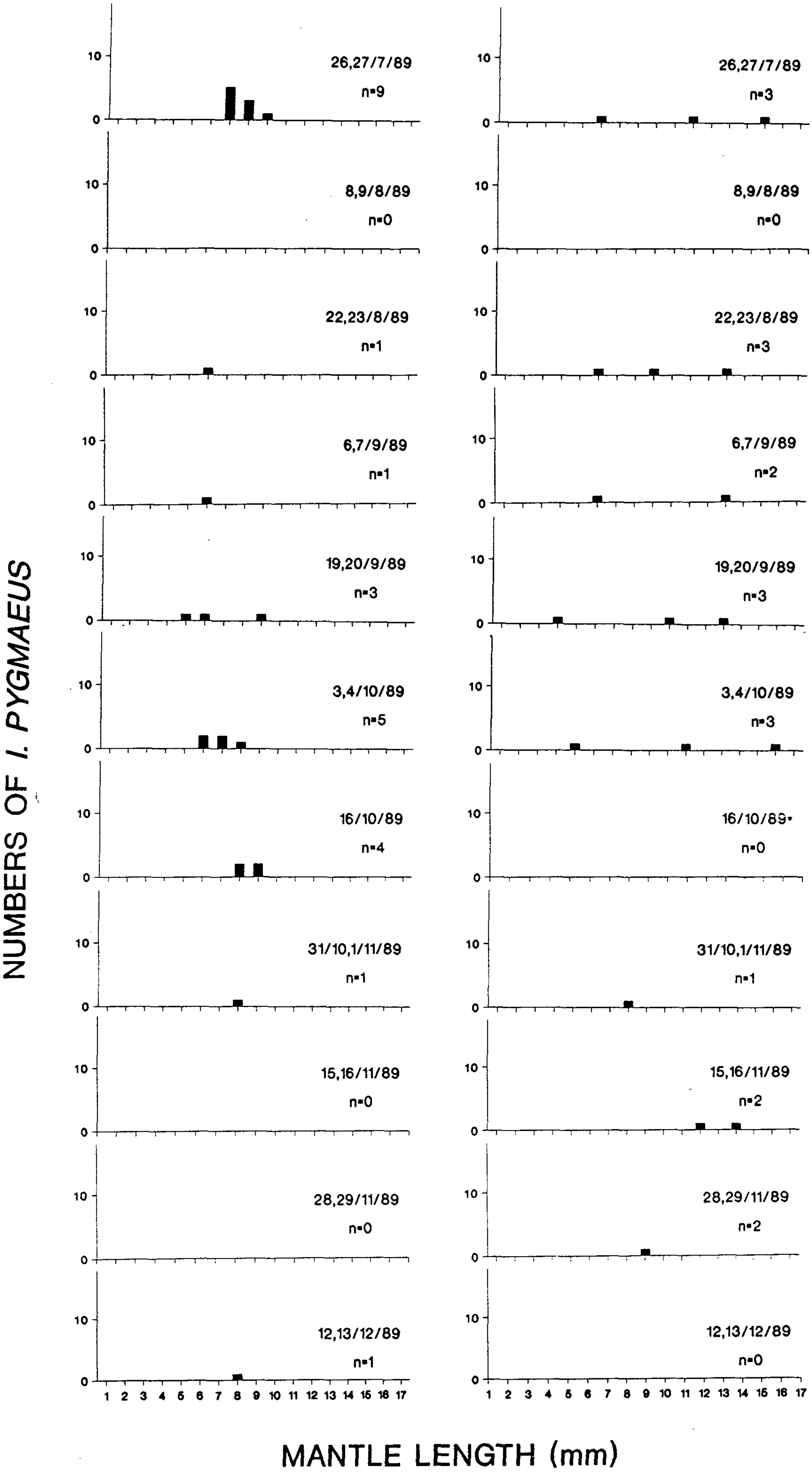

NUMBERS OF *I. PYGMAEUS*
MANTLE LENGTH (mm)
26,27/7/89
n=9
26,27/7/89
n=3
8,9/8/89
n=0
8,9/8/89
n=0
22,23/8/89
n=1
22,23/8/89
n=3
6,7/9/89
n=1
6,7/9/89
n=2
19,20/9/89
n=3
19,20/9/89
n=3
3,4/10/89
n=5
3,4/10/89
n=3
16/10/89
n=4
16/10/89
n=0
31/10,1/11/89
n=1
31/10,1/11/89
n=1
15,16/11/89
n=0
15,16/11/89
n=2
28,29/11/89
n=0
28,29/11/89
n=2
12,13/12/89
n=1
12,13/12/89
n=0
0
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

Figure 5.8

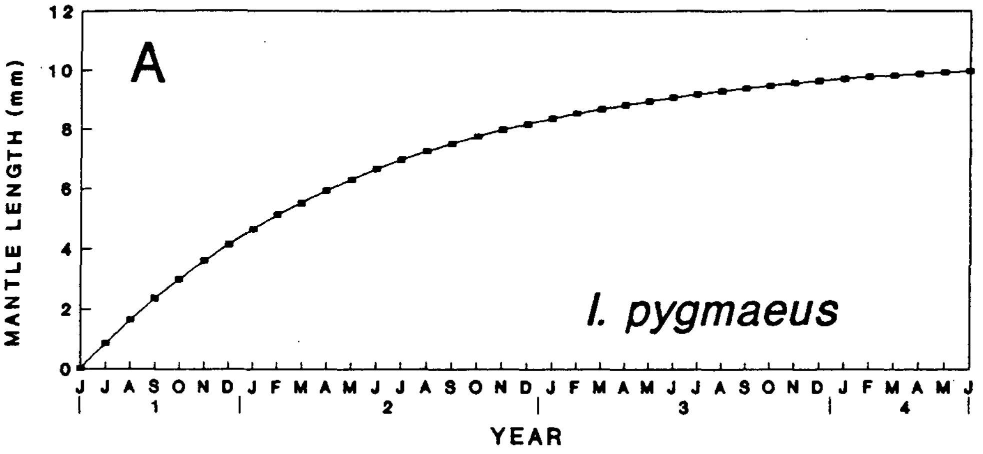
Growth curves constructed for ELEFAN analysis of squid length frequency.

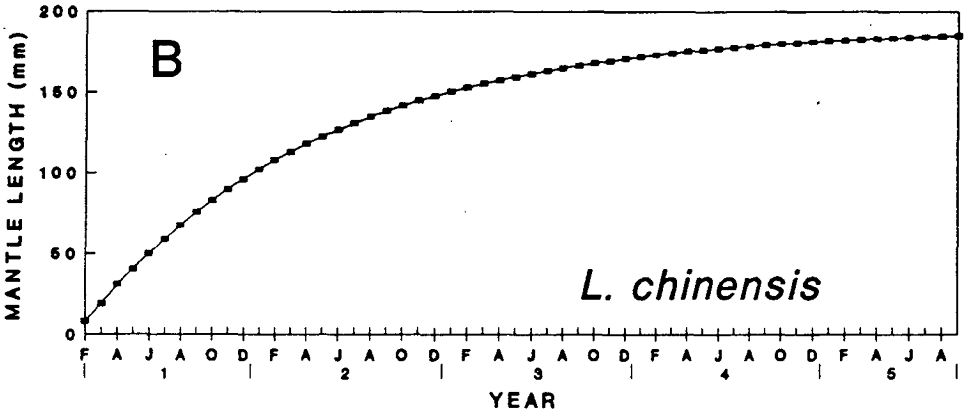
(A) Male *Idiosepius pygmaeus;* K 1.0; L∞ 10.5 mm; Rn 0.605.

(B) *Loligo chinensis;* K 0.8; L∞ 10.5 mm; Rn 0.308.

(C) *Sepioteuthis lessoniana* (data from Rao, 1954); K 0.4; L∞ 268 mm; Rn 0.333.

Note that the curve has a slight seasonal oscillation with a winter point of 0.9 and an amplitude set at 0.15.


A
I. pygmaeus
MANTLE LENGTH (mm)
0
2
4
6
8
10
12
J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J
1
2
3
4
YEAR


B
L. chinensis
MANTLE LENGTH (mm)
0
50
100
150
200
F A J A O D F A J A O D F A J A O D F A J A O D F A J A
1
2
3
4
5
YEAR

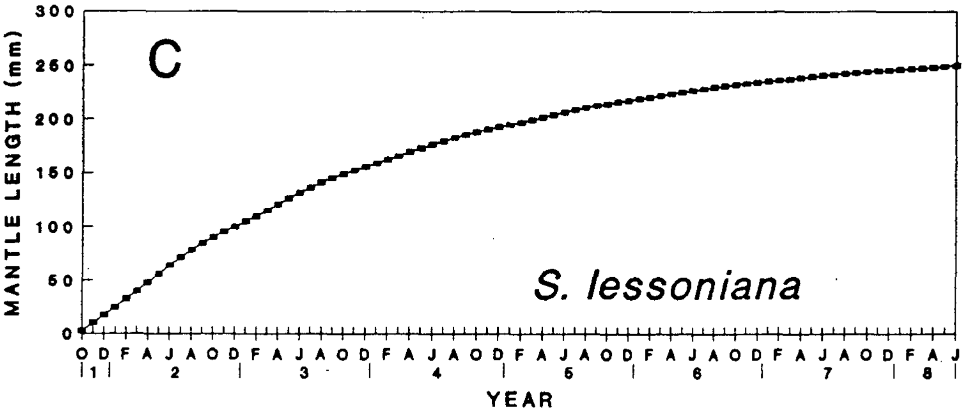

C
S. lessoniana
MANTLE LENGTH (mm)
0
50
100
150
200
250
300
O D F A J A O D F A J A O D F A J A O D F A J A O D F A J A O D F A J A O D F A J
1
2
3
4
5
6
7
8
YEAR

Figure 5.9

(A) Relationships between mantle length and age for two samples of individuals of *Loligo chinensis* collected during summer (January) and winter (July). Lines are least squares regressions calculated for each sex. Solid lines represent female regressions, dashed lines represent male regressions. (B) Relationships between weight and age for the same samples.

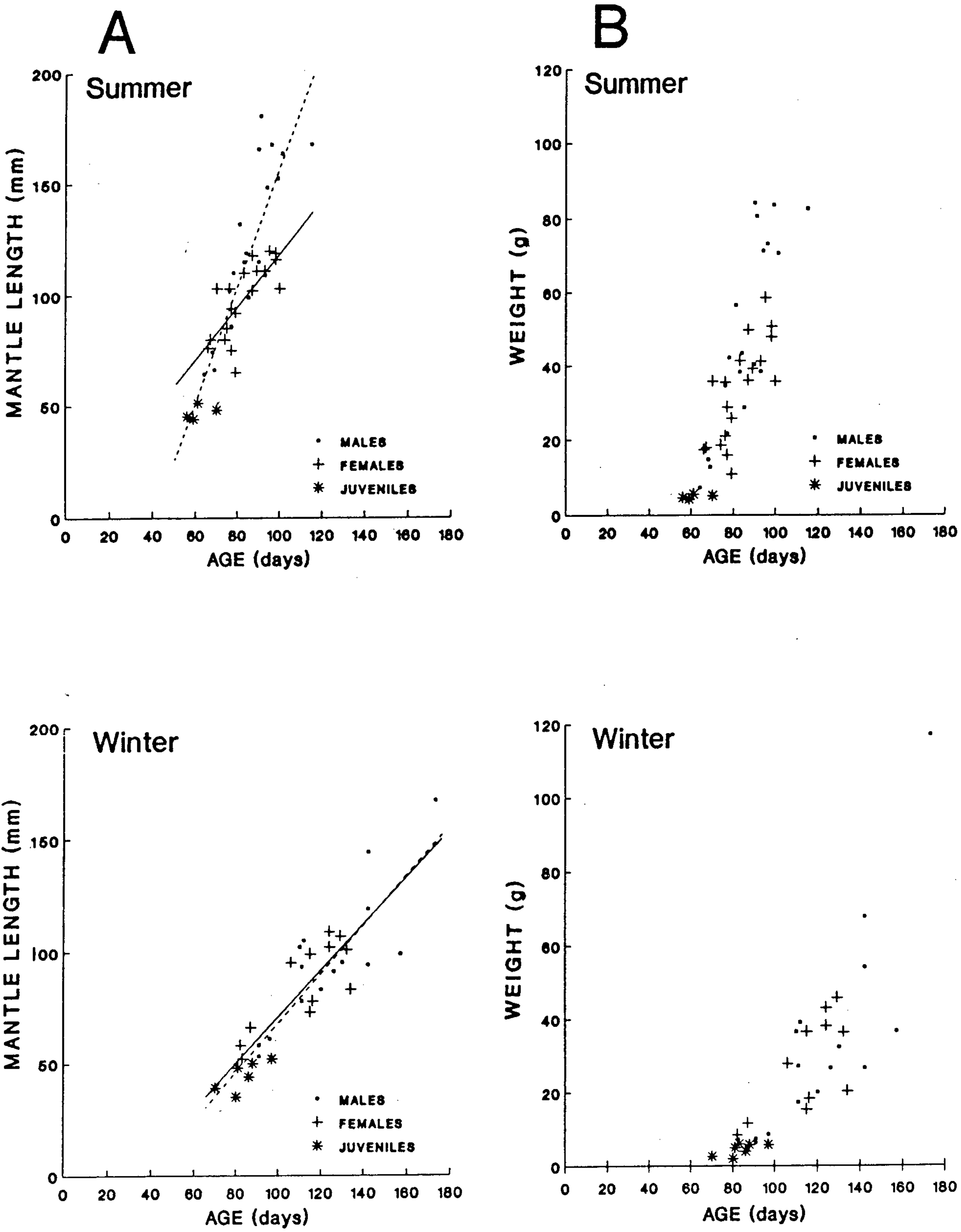

A
B
Summer
Summer
Winter
Winter
MANTLE LENGTH (mm)
WEIGHT (g)
AGE (days)
0
20
40
60
80
100
120
140
160
180
50
150
200
MALES
FEMALES
JUVENILES

Figure 5.10

Least squares regression lines for the relationship between mantle length and age for both the summer (January) and winter (July) samples of *Loligo chinensis*.

Loligo chinensis

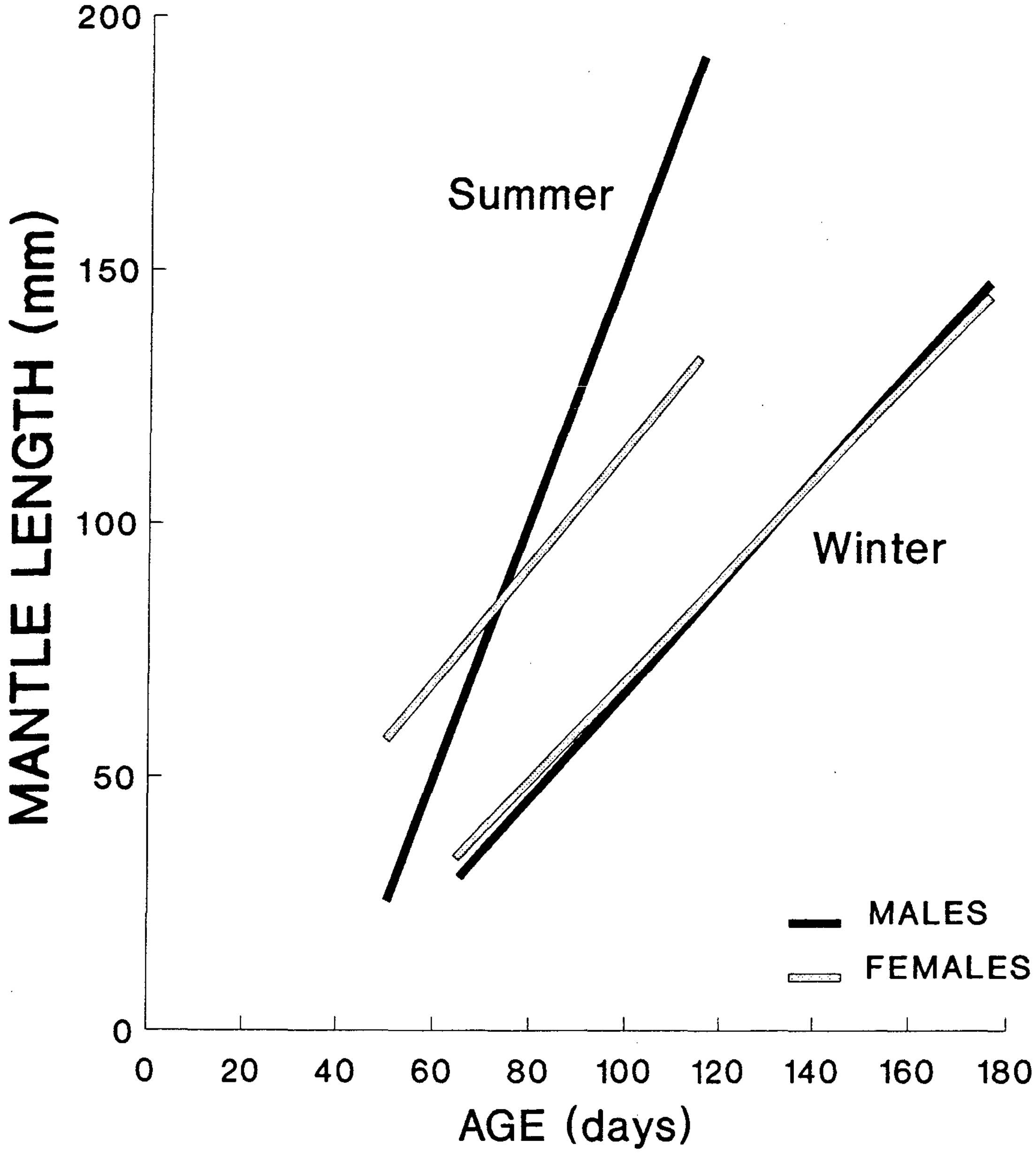
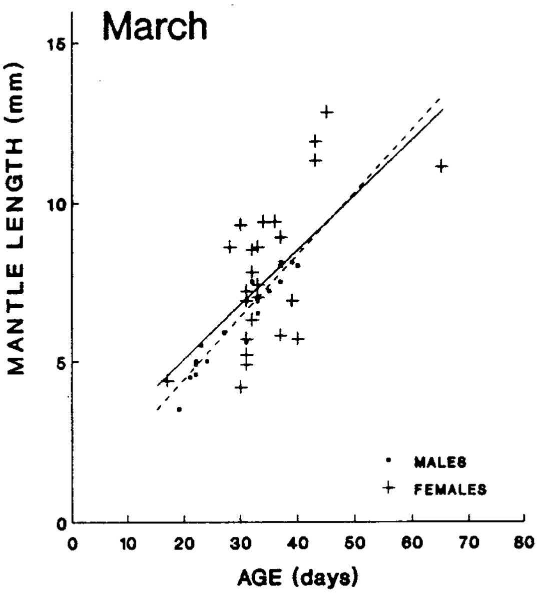
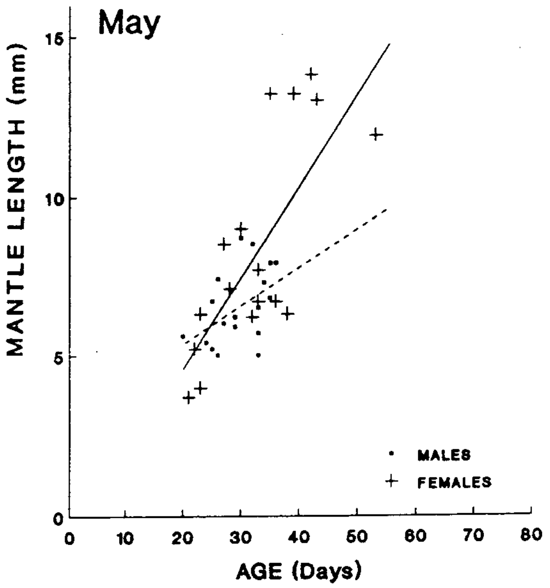
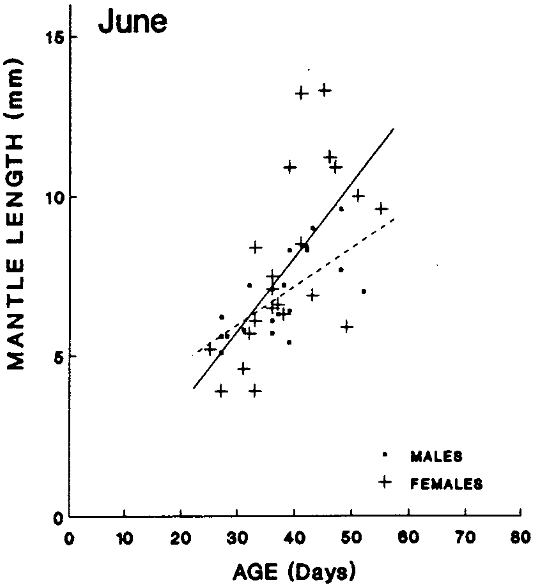


Figure 5.11

The relationship between mantle length and age for individuals of *Idiosepius pygmaeus* collected from four time periods. Lines are least squares regressions for each sex. Solid lines represent females, dashed lines represent males.


March
MANTLE LENGTH (mm)
0
5
10
15
0
10
20
30
40
50
60
70
80
AGE (days)
• MALES
+ FEMALES


May
MANTLE LENGTH (mm)
0
5
10
15
0
10
20
30
40
50
60
70
80
AGE (Days)
• MALES
+ FEMALES


June
MANTLE LENGTH (mm)
0
5
10
15
0
10
20
30
40
50
60
70
80
AGE (Days)
• MALES
+ FEMALES

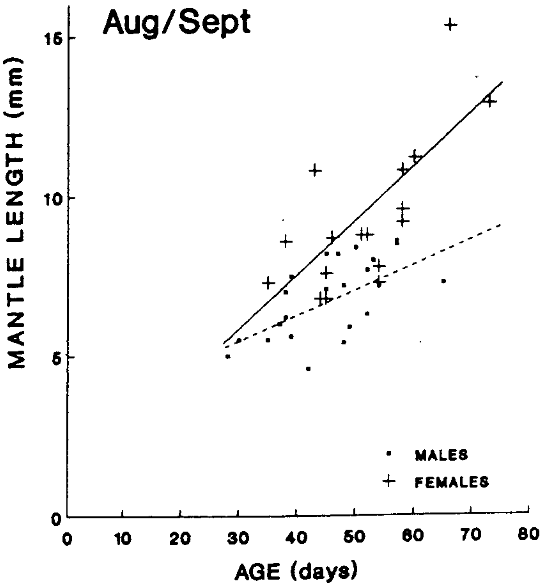
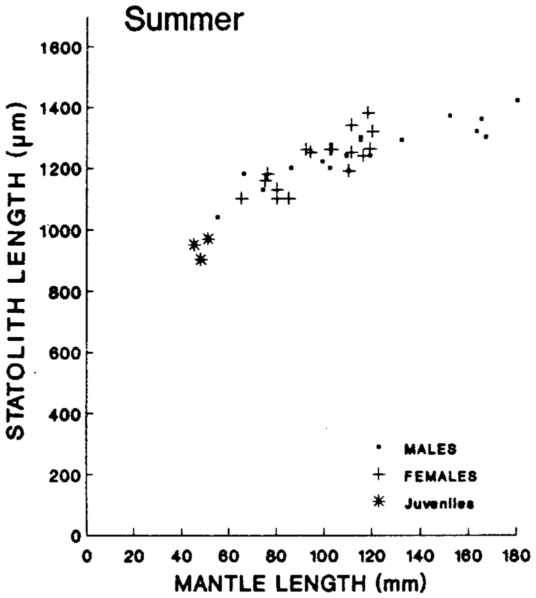
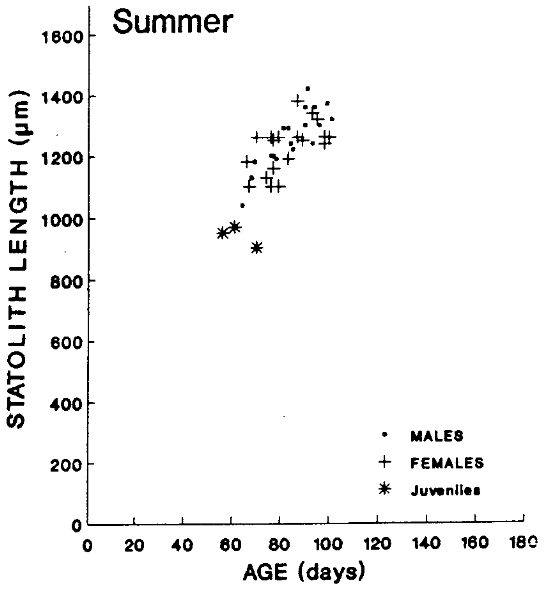
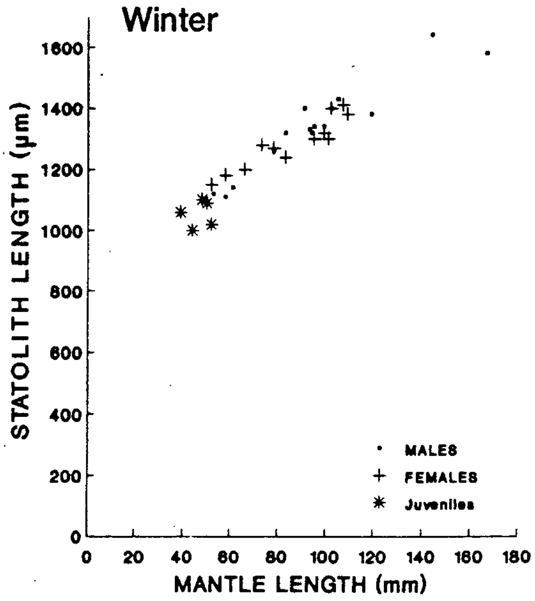

Aug/Sept
MANTLE LENGTH (mm)
0
5
10
15
0
10
20
30
40
50
60
70
80
AGE (days)
• MALES
+ FEMALES

Figure 5.12

The relationship between statolith length and mantle length, and statolith length and age for summer (January) and winter (July) samples of *Loligo chinensis*.


Summer
STATOLITH LENGTH (µm)
1600
1400
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
140
160
180
MANTLE LENGTH (mm)
• MALES
+ FEMALES
✱ Juveniles


Summer
STATOLITH LENGTH (µm)
1600
1400
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
140
160
180
AGE (days)
• MALES
+ FEMALES
✱ Juveniles


Winter
STATOLITH LENGTH (µm)
1600
1400
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
140
160
180
MANTLE LENGTH (mm)
• MALES
+ FEMALES
✱ Juveniles

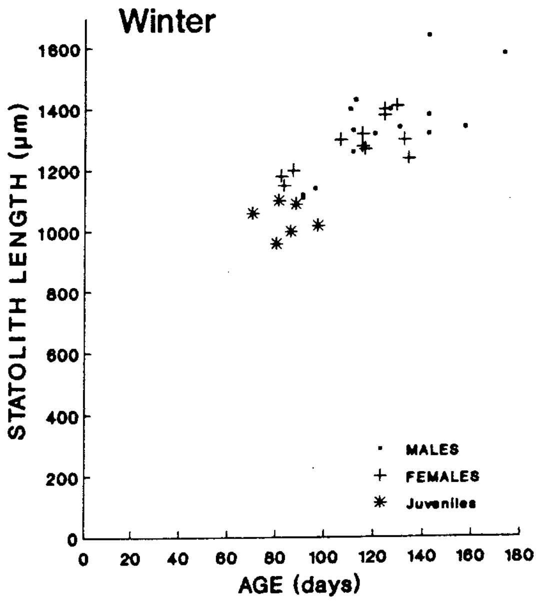
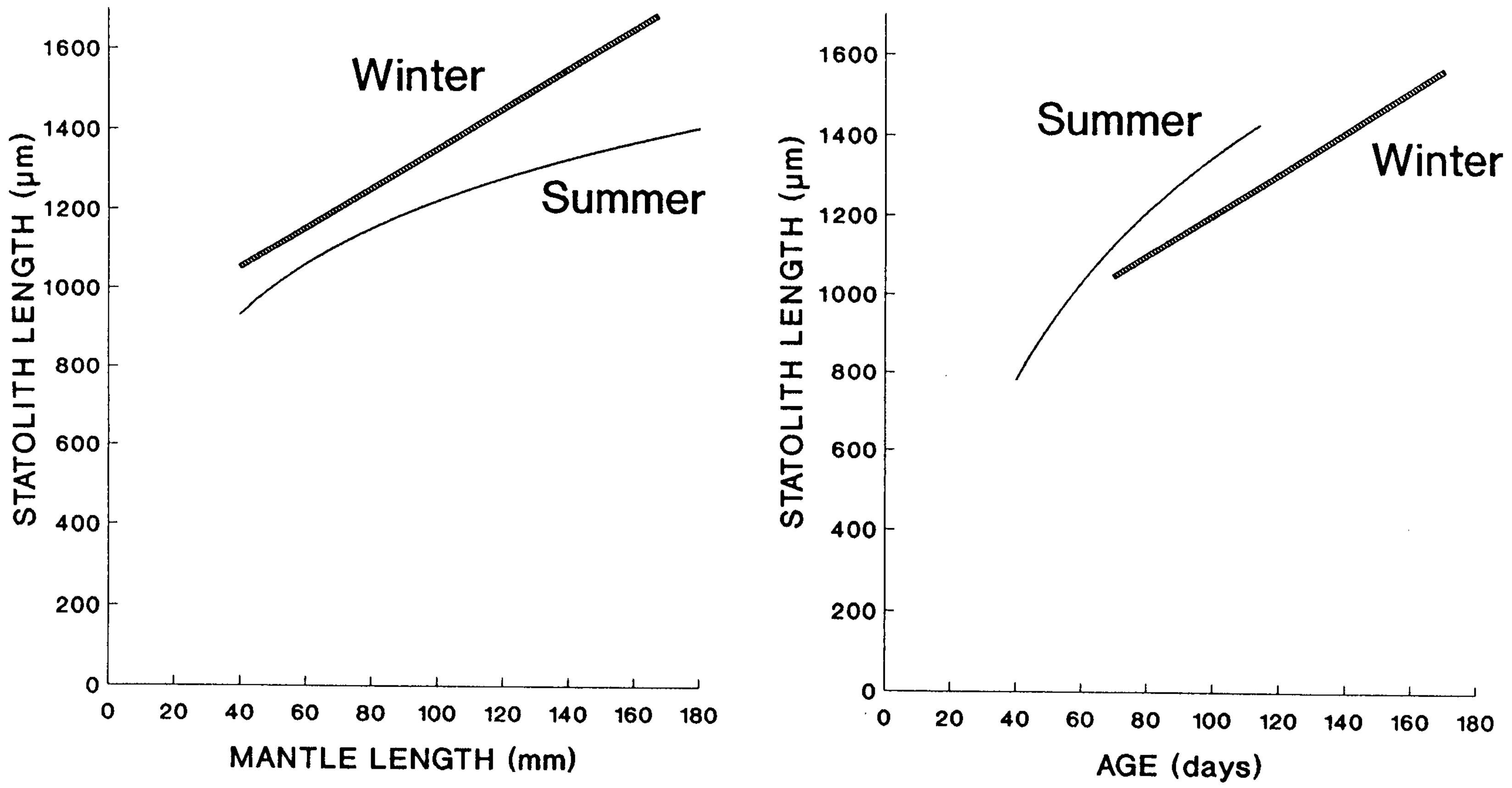

Winter
STATOLITH LENGTH (µm)
1600
1400
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
140
160
180
AGE (days)
• MALES
+ FEMALES
✱ Juveniles

Figure 5.13

Fitted curves and regressions for the relationship between statolith length and mantle length, and statolith length and age for *Loligo chinensis*


STATOLITH LENGTH (µm)
1600
1400
1200
1000
800
600
400
200
0
Winter
Summer
0
20
40
60
80
100
120
140
160
180
MANTLE LENGTH (mm)
STATOLITH LENGTH (µm)
1600
1400
1200
1000
800
600
400
200
0
Summer
Winter
0
20
40
60
80
100
120
140
160
180
AGE (days)

6.0 REPRODUCTIVE BIOLOGY

6.1 INTRODUCTION

Cephalopod reproductive strategies are complex. In reviewing reproductive strategies of cephalopods, Mangold (1987) has emphasised that there are at least as many open questions as there are established facts, and that there are thus many gaps in our knowledge as well as contradictory statements. As research is continuing, we are finding that lifespans are considerably shorter than many estimates made over the last several decades. It appears that this past confusion has led to a poor understanding of the reproductive tactics of cephalopods. Clearly any ideas regarding the lifespan of an organism will influence ideas regarding reproductive events in the individual.

While terminal spawning has often been suggested as a typical model for cephalopods (Arnold & Williams-Arnold, 1977; Arnold, 1990; Boyle, 1990) evidence is increasing that many species are in fact able to reproduce more than once. This evidence has come from culture experiments (Hixon, 1980; Rodaniche, 1984; Boletzky, 1987) as well as size analysis of ovarian oocytes (Boucher, 1983; Harmon *et al.*, 1989). However, other evidence does suggest that for some species, spawning is terminal with death following shortly after spawning. For example, Knipe & Beeman (1978) have shown through histological examination of *Loligo opalescens* that in spawning animals the gonad appears to break down. They found that spawning females had no oogonia in the ovaries and that spawning males also showed considerable breakdown of the testicular tissue. Death in other species after spawning has also been observed in *Todarodes pacificus* from field sampling (Juanico, 1983) and *Illex illecebrosus* in captivity (O'Dor, 1983). However, Boyle (1987) has emphasised that while semelparity may not be universal, there is still confusion over the duration of breeding activity, and that the large majority of all cephalopods die shortly after their 'first breeding episode'.

Statolith ageing techniques have the potential for disentangling some of the discrepancies with regards to the reproductive tactics of tropical near-shore squids and sepioids. By analysing individual age and maturity status, it is possible to construct time specific schedules of gonad growth. It is thus possible to estimate directly, age at maturity and the percentage of the adult lifespan that an individual spends as reproductively active.

The fact that *I. pygmaeus* has a short lifespan, lays benthic eggs and is easy to maintain in captivity, suggests that this species could also be useful for measurement of female reproductive output by direct counting of eggs produced.

The questions addressed in this chapter include: A) What type of information can determining individual age provide us with in regards to growth of the gonad and maturity processes in tropical squids and sepioids? B) How does age-gonad relationships compare to individual size-gonad relationships? C) Can analysis of growth of secondary sexual characteristics tell us anything about maturation processes in tropical squids? D) What type of reproductive strategy does *I. pygmaeus* possess given the fact that it has such a short lifespan? E) What further information can be gleaned on reproductive strategies of tropical cephalopods based on histological examination of gonads?

6.2 MATERIALS AND METHODS

The reproductive aspect of this research focused on populations of *I. pygmaeus* and *L. chinensis*. Because populations of these species could be obtained consistently throughout many months of the year, it was possible to analyse seasonal differences in gonad growth.

Analysis of reproductive structures was carried out with aged individuals of *L. chinensis* captured on 12 January 1989 (summer) and 13 July 1989 (winter). Although aged individuals of *I. pygmaeus* were available from at least four different periods of the year, gonad analysis was carried out with aged individuals captured during March 1988, 1989 (autumn) and August/September 1988 (spring), as the greatest difference in growth rates and population age structure was observed between these two seasons (see chapter 5).

6.2.1 Analysis of reproductive structures

Ovary and testis weights were taken for each aged squid and sepioid. As mentioned previously, specimens of *L. chinensis* were fixed in buffered 10% seawater-formalin and later transferred to 70% alcohol, while specimens of *I. pygmaeus* were preserved immediately in 70% alcohol. Gonads were dissected out, blotted with paper towelling (*L. chinensis*) or filter paper (*I. pygmaeus*) and immediately weighed. Other parameters taken included dorsal mantle length, as well as hectocotylus and nidamental gland length for male and female specimens of *L. chinensis* respectively. Measurements were taken with an eyepiece micrometer (*I. pygmaeus*) or with either callipers or a graduated ruler (*L. chinensis*). Maturity was determined by the presence of mature oocytes in the ovary along with large nidamental glands in females, and the presence of spermatophores in males.

Due to the large amount of scatter in many of the correlations (especially with *I. pygmaeus*) and the complex curvilinear relationship between many of the relationships, regression analyses were not carried out. Furthermore, this prevented the carrying out of any detailed analysis of covariance.

6.2.2 Gonadosomatic and nidamental gland length / mantle length indices

The seasonal trends in growth of the gonad was also considered for *L. chinensis*. The status of maturity was determined for individuals taken from trawl samples between February 1988 and November 1989. To determine the trend in the maturity process, individuals were selected from each sample within the size range in which maturity was reached. Only males and females which were greater than 100 mm in mantle length were selected for gonad analysis. For most samples, all the individuals within the adult size range were used in the gonad analysis, except for several summer samples in which a very large number of individuals were captured.

Parameters measured for each squid were, mantle length, body weight, gonad weight and nidamental gland length for females. The gonadosomatic index (GSI) was calculated for each specimen as:

$$\frac{gonad\ weight\ (g)}{total\ body\ weight\ (g)}\ x\ 100$$

for females, the nidamental gland length / mantle length (NGL/ML) index was also calculated as:

$$\frac{\text{nidamental gland length (mm)}}{\text{mantle length (mm)}} \times 100$$

6.2.3 Ovary analysis

Oocyte diameter (ie. the longest axis) was measured for all oocytes in the ovary of two female individuals of *I. pygmaeus,* to determine their length frequency distribution. This was carried out by completely teasing apart the ovaries to separate the oocytes and spreading them out in a Bogorov tray. Oocyte diameters were taken with a binocular microscope using an eyepiece micrometer.

6.2.4 Egg production

Idiosepius pygmaeus females were maintained outside in separate aquaria covered on three sides with black plastic or in 33 l plastic trays lined with 1 cm plastic mesh (see chapter 4) to monitor egg output. Females were fed *ad libitum* with *Acetes sibogae australis.* Unglazed clay tiles (approximately 11 cm square) were placed in the aquaria for an egg attachment medium. Aquaria were checked regularly and all eggs were removed and counted. Eggs were frequently laid on the tiles or sometimes on the aquarium wall or on the seawater intake hose.

6.2.5 Histological examination of ovaries

Histological examination was carried out on ovary samples from all the study species, *I. pygmaeus, L. noctiluca, Loligo* sp. 1, *L. chinensis* and *S. lessoniana* to determine the oocyte size structure. Whole specimens of female *I. pygmaeus* were embedded in paraffin wax, while smaller blocks were taken from the middle region of the ovaries of the larger study species and embedded. Histological sections were cut at 6 μm and stained with Mayer's Haematoxylin followed by a counterstaining with Young's Eosin-Erythrosin.

6.3 RESULTS

6.3.1 Growth of the gonad of *L. chinensis* with age

There were considerable differences in the growth of the gonad with age for the two samples of *L. chinensis* taken within different seasonal periods (Fig. 6.1). Although the analysis of maturity was carried out on a number of individuals for each sampling date, the data reflects trends based on only one sample within each seasonal period. The fact that age analysis has highlighted rapid growth and a short lifespan for this species, suggests that changes in gonad growth and maturation rates can be rapid. Due to these facts, seasonal differences in age-maturity relationships can only be considered preliminary in scope. Future work would need to focus on more intensive sampling within each seasonal period to be able to understand the general trend in the age-maturity process. Such intensive sampling, along with establishing age-specific maturity from a larger sample pool was beyond the scope and resources of this study. Notwithstanding this, the detailed age-specific maturity analysis obtained from the summer and winter samples, which were based on a number of specimens within each period, did suggest that very different processes were governing growth of the gonad in winter and in summer.

6.3.1.1 Males

The form of the relationship between testis weight and age had similarities between both seasons, with the main difference being the shift in the July curve to the right along the age axis. Because of seasonally induced differences in somatic growth, males matured later (older) in July (winter). The relationship between age and testis weight had a greater variability in July, which suggested that age at maturity was less well defined in the winter with a slower rate of maturity in some individuals.

In both July and January (summer), males had spermatophores present just above a testis weight of 0.100 g. However, there was variability in age at maturity in both seasons with apparent immature males with a testis weight of between 0.307 and 0.335 g. The youngest mature male for summer and winter was 83 d and 115 mm in length and 111 d and 78 mm in length respectively. The oldest immature males for summer and winter were 90 d and 115 mm in length and 130 d and 95 mm in length respectively.

6.3.1.2 Females

There were considerable seasonal differences between the maturity patterns of the females based on ovary weight. In January, maturity was rapid with the ovary reaching a large size from 65 to 85 d. However, there were several anomalies. Two of the older specimens (including the oldest female aged in the summer) were immature. This was especially apparent in the oldest individual (100 d) which also had very undeveloped nidamental glands.

The winter trend in growth of the ovary was somewhat unexpected as all squids had very small gonads and associated nidamental glands. No mature females or females approaching maturity were found in the July sample. This was very different to the pattern observed in the males, which reached maturity in July. The fact that no mature females were found in July suggests that female maturity was out of phase with the males during this period of the year.

All females aged in January with an ovary weight greater than 1.161 g were mature and had ovaries packed with oocytes, which filled much of the mantle cavity. In January, the youngest mature female was 83 d and 110 mm in length, while the oldest immature female was the oldest female aged (100 d, 103 mm in length).

6.3.2 Gonad soma relationships of *L. chinensis*

Comparing gonad weight to individual size (mantle length) (Fig. 6.2) resulted in a very different pattern than in the age-gonad weight analysis. Without having an understanding of individual age it would be very difficult to determine the seasonal dynamics of gonad growth. Despite the fact that there was a noticeable difference in the slopes of testis weight with age, due to the greater age of the winter population, this was completely masked with the gonad weight : mantle length relationship, with both seasons' data points falling within the same correlation. Thus gonad increase was proportional to the squid size rather than its age. Based on squid size alone, it would not be possible to distinguish these seasonal differences in maturity.

A very different pattern (based on squid size) also emerged when comparing gonad weight : mantle length correlations for female *L. chinensis*. Although the lack of maturity was still obvious in winter females, the gonad weight : mantle length relationship for both seasons resulted in a single curvilinear relationship, suggesting that maturity took place rapidly between 100 and 120 mm DML regardless of age. However, larger winter squids would need to be collected to clarify this.

Furthermore, although this ageing study indicated that a large proportion of the winter females were older than their summer counterparts, many of these immature females were actually smaller than the squids captured in the summer. As with the males, this suggests that squid size rather than age may have the greatest influence on maturity.

6.3.3 Reproductive indices of *L. chinensis*

Although it was possible to determine the ages of only a small proportion of individuals over two time periods of the year, it was of interest to consider changes in the gonad weight : soma weight relationship throughout the year. For both males and females, a seasonal trend could be detected for the GSI values (Fig. 6.3). Values were relatively small for males, with less than 1.2% of the total weight consisting of gonad. In contrast, the values for females had considerably more variation and were generally much higher, with gonad accounting for as much as 8% of the total body weight.

Mature males were found throughout all months sampled. However, a regular seasonal ocellation in the relative gonad size was apparent (Fig. 6.3A). There was a general increase in the relative gonad weight from February to October in both years. Over the two years the testis accounted for the greatest percentage of body weight in October, while the lowest values were recorded in July.

A similar pattern of fluctuation also existed with the female GSI values (Fig. 6.3B). Females consistently had GSI values in October which were considerably higher than the other months. The trends during other periods of the year were less clear, although, as recorded for the males, the lowest values obtained were in the winter period, especially during July. Mature females were present in all samples except July 1989. As discussed previously, females sampled during January 1989 showed a considerable range in gonad size and level of maturity, despite similarities in both size and age. This feature was also reflected in the female GSI values, in that the mean values had large standard errors (Fig. 6.3). This was due to the fact that for many months, a proportion of the individuals were immature.

The seasonal trend in the NGL/ML index was very similar to the female trend in the GSI (Fig 6.4). This index also indicated a greater investment in reproduction during October with lowest values in July. Furthermore, standard errors were less for this index than for the GSI.

The possibility existed that the higher values in October along with the low values in July may have been influenced by the body size of individuals captured during these times of the year (eg. larger individuals captured in October), rather than actual trends in relative gonad sizes. To explore this possibility the monthly mean mantle length for the males and females were plotted for the two year period (Fig. 6.5). Although there was some variation in the mantle lengths for the different samples, these could not be related to the seasonal peaks or troughs in the GSI values. For example, the largest females were captured from February to July 1988 (Fig. 6.5B). However, the GSI values dropped considerably over this period. Furthermore, mean mantle length was not highest in October for each year, and although 1989 July values were slightly lower than the other months, the July 1988 values were not. The observed changes in relative gonad weight can thus be considered to not be biased by individual size but rather a response to changes in the ambient environment.

Since *L. chinensis* grows and matures very rapidly, obtaining more samples (with a more intensive sampling program) would provide a better picture of the fluctuation in relative gonad growth, especially within monthly periods.

6.3.4 Growth of the gonad of *I. pygmaeus* with age

In contrast to *L. chinensis,* age-specific trends and seasonal differences in maturity patterns for *I. pygmaeus* could be determined with a greater degree of accuracy and a greater understanding of individual variability. This was possible because of the greater number of replicate sub-samples taken during each seasonal period. The autumn sample consisted of individuals captured during four sampling periods over two years; 22, 23 March 1988 and 21, 22 March 1989. The Spring sample was derived from sepioids captured during six sampling trips over 2 months; 10, 23, 24 August and 7, 20, 21 September 1988.

The seasonal pattern of gonad growth over time was very different for *I. pygmaeus* than for *L. chinensis.* The seasonal influence on gonad growth and maturation may have been somewhat less for *I. pygmaeus* compared to *L. chinensis,* as individuals of *I. pygmaeus* were virtually absent from the collecting sites during summer. However, individuals captured in autumn (March) would have grown over the warmer period at the end of summer, while the spring sample (August/September) would have grown and matured through the colder period, at the end of the winter period.

As would be expected, based on the size-age relationships for this species (Chapter 5) there was also considerable scatter in the gonad weight-age relationship (Fig. 6.6). While gonad size-age relationships for *L. chinensis* resulted in very different plots separated on the age continuum (x axis), the pattern was modified differently for *I. pygmaeus* for the two seasons (ie. both seasons data points produced one curvilinear relationship). While individuals of *I. pygmaeus* did generally reach a greater age in the spring sample, their gonads reached a proportionally greater weight than did the warmer autumn individuals.

6.3.4.1 Males

The relationship for testis weight versus age was the same for both seasons, that is, roughly a single linear relationship for both sets of data, with the exception of one 41 d individual which fell considerably outside the cluster of data points. The major difference in the seasonal component of the data was a clustering of data points for each season at opposite ends of the testis weight : age continuum, with cooler season (spring) individuals reaching a greater age and possessing proportionally heavier testes than their warmer season (autumn) counterparts.

The youngest mature males in spring and autumn were 22 d and 37 d respectively, while the oldest immature males for both seasons were 32 d and 38 d respectively.

6.3.4.2 Females

A similar relationship to the males also existed in the ovary weight : age relationship, in that, both seasons data points tended to cluster along one ovary weight : age continuum. However, the scatter was considerably greater due to the fact that in both seasons there were individuals with very small ovaries. For example, in both seasons, individuals between 40 to 60 d showed a considerable range in ovary weight. However, as with the males, the cooler season (spring) females also had the heaviest ovaries.

Although a number of females had considerably developed ovaries, none of the specimens examined had any mature ova present. It is possible that due to this species short lifespan and rapid growth, that ovum maturation could take place very rapidly just before egg deposition. Therefore, it could be difficult to detect mature oocytes unless it was just prior to egg deposition.

6.3.5 Gonad soma relationships of *I. pygmaeus*

One possibility for the much greater weight of the gonad in the spring versus autumn individuals of *I. pygmaeus* could have been due to the possibility of larger individuals being captured in the autumn. However, plotting gonad weight against individual size (mantle length) for both sexes (Fig. 6.7) revealed that there was no difference in size of males between seasons and only one spring female was larger than the other specimens. Although there is some overlap in the data for the smaller individuals for both seasons, in the larger sizes, slower growing spring individuals did in fact eventually produce larger gonads than their autumn counterparts. This relationship does bear some resemblance to the statolith length : mantle length relationship for summer and winter populations of *L. chinensis* (see chapter 5, Figs. 5.12, 5.13).

6.3.6 Secondary sexual characteristics of *L. chinensis*

Nidamental gland length was perhaps the most useful organ to measure in female squids. The relationship of nidamental gland and mantle length and age of female specimens of *L. chinensis* (Fig 6.8) closely resembled the gonad weight : mantle length and age relationships for this species. For example, two separate relationships were apparent when nidamental gland length was plotted against age, whereas both seasons data points produced one curvilinear relationship for nidamental gland length versus mantle length. As with the ovary data, this data suggests that nidamental gland length was more closely related to size than to individual age.

Similarly for males, hectocotylus length versus mantle length and age had roughly the same pattern that was observed in the testis weight : length and age relationships (Fig 6.9). For example, in winter individuals there was a shift in the data points along the age axis producing a separate correlation to the summer hectocotylus length : age data points. The pattern shown in the hectocotylus length : mantle length data was also similar to testis weight : mantle length. However, there was some indication that at large sizes, faster growing (summer) squids have a shorter hectocotylus than slower growing (winter) squids. This relationship bears resemblance to seasonal differences in statolith length : mantle length relationships in this species (chapter 5) and gonad weight : mantle length relationships for *I. pygmaeus* mentioned above.

6.3.7 Ovary analysis/egg production

Ovaries of the study species were examined to determine the oocyte size distribution. Earlier examination of *I. pygmaeus* female ovaries revealed that they contained a number of different sized oocytes. To quantify this, the oocyte diameters of all oocytes within the ovaries of two females were measured (Fig 6.10). In the female specimen which possessed mature eggs (Fig 6.10A) only very few oocytes were actually mature. The greater diameters noted in some of the immature oocytes as compared to the mature ones in this specimen was due to considerable distortion of some of the immature oocytes which were packed tightly within the ovarian structure.

The oocyte size distributions suggested that *I. pygmaeus* was a serial spawner rather thansemelparous. Therefore a number of females were captured in the field and maintained in separate aquaria to monitor reproductive output over time until each individual died. These experiments indicated that *I. pygmaeus* does in fact produce mature eggs over a period of time in repeated batches (Fig. 6.11). Although repeated spawnings took place over a relatively short time period (less than 15 d), egg output was measured in the hundreds, with the most fecund female producing a cumulative egg output of 922 eggs (Fig. 6.11, experiment 2). Although this egg production is considerable, it does not take into account the possibility of eggs being laid before capture, suggesting that egg output could even be greater than observed.

Oocyte size distribution was examined for all the study species using histological techniques to determine possible reproductive strategies of the other loliginid squids. This also revealed variable size distribution in oocyte diameter with all ovaries possessing oocytes at various stages of development (Fig. 6.12). This suggests that a serial spawning strategy is possessed by all these near-shore squid and sepioid species

6.4 DISCUSSION

Having the ability to age squids is an important step in gaining a greater understanding of the maturity processes in cephalopods. It is especially useful in the light of the fact that size may not be closely related to age. Furthermore, in the past there has been considerable confusion over maturity in many species of squids. For example, in some oceanic species very large individuals can be immature while much smaller ones can be mature (Dunning, personal communication) Although this study was only a preliminary consideration of the maturation processes of tropical squids in the light of age information, these techniques should prove useful in providing a time scale on squid maturation processes and disentangling the problems previously encountered with the wide variation in size at maturity.

Macy (1982) found very large individuals of *Loligo pealei* off Rhode Island, U.S.A., that had gonads which appeared to be regressing (an apparently rare phenomenon in cephalopods). Although the squids were large and had gonads similar in size and coloration to smaller mature individuals, they were thin and had no obvious eggs or spermatophores. Because these specimens could not be aged, it was not possible to substantiate that the larger immature or 'regressed' squids were actually older than their smaller counterparts. Applying statolith ageing techniques to this species would help to clarify the apparent maturity paradox in this species. The application of ageing techniques to *L. pealei* to understand the maturity cycle in this species is to be carried out in a future study (W. Macy, personal communication).

The biotic and abiotic influences on maturity are complex. Factors such as light (day length), temperature and food availability can all affect age at, and rate of maturity. Gonad development is directly under hormonal control which appears to be influenced by the optic gland (Mangold, 1987; Boyle, 1990). However, the process of maturity is not completely understood and may also be controlled to a certain degree by individual genetic factors, apart from outside influences. For example, under culture conditions, individuals of the octopus *Eledone moschata* which hatched within three days of each other from an egg batch from a single female, were shown to become mature at different sizes and ages, although kept under strictly controlled identical conditions (Mangold, 1983). Currently though, it is not known if culture under artificial conditions may disrupt maturity processes. Mangold's (1983) observation does however, agree with observed variation in maturity in *L. chinensis* and *I. pygmaeus.*

6.4.1 *Loligo chinensis*

Age data for *L. chinensis* in Cleveland Bay revealed that there was a strong seasonal influence on both age at maturity and rate of gonad development with winter slowing both growth and maturity processes.

The fact that some of the older summer female squids were very immature despite their relatively advanced aged is of interest in the light of previous observations. Hixon (1980) found that some immature *Lolliguncula brevis* females were as large as other fully mature individuals. Considerable variation has also been shown to exist in *L. opalescens* with females maturing by 81 mm DML while other females remain immature until 140 mm DML (Hixon, 1983). Similar discrepancies in size at maturity have also been documented for *Sepia officinalis* (Boletzky, 1983b) and *Dosidicus gigas* (Nesis, 1983). As mentioned previously, Mangold (1983) has also demonstrated that cultured octopuses reared from the same egg mass reached maturity independent of sibling size or age. Variability in size at maturity appears to be a common phenomenon with cephalopods. The age data for the summer *L.*

chinensis females showed that some of the immature females were actually older and not simply larger than other females of the population.

Although male and female *L. chinensis* matured in the same age range in January, a very different situation existed in July (winter population), suggesting that maturity in the female population in July was out of phase with the males. This situation has been noted with *Todarodes pacificus* in which males reach maturity three to six months earlier than females (Okutani, 1983). This may not be an ecological constraint considering the reproductive strategies of cephalopods. In many species of cephalopods, males mate with immature females (Mangold, 1987). Furthermore, females do have the ability to retain spermatophores until they spawn, as shown with the *I. pygmaeus* fecundity experiments, in which individuals produced repeated batches of fertilised eggs over several weeks in the absence of males. Furthermore, an extended interval between mating and spawning has also been noted in octopods, with up to a 114 d interval between spermatophore transfer and spawning (Boyle, 1990).

Alternatively, the apparent absence of mature females in the winter may have been due to inadequate sampling , since the oldest aged female captured in winter was 134 d whereas males as old as 173 d were captured. Given the fact that maturation was rapid (eg. females matured in less than 80 d in summer) it is possible that older mature females existed in winter but that none were captured. Notwithstanding this, the data did suggest that maturity in at least a considerable proportion of the female population in July, was out of phase with the males.

The fact that gonad maturation closely corresponded to squid size rather than to squid age, suggests that there might be some physical or physiological mechanisms controlling maturation apart from age. Some female cephalopods have been shown to not develop eggs unless a minimum threshold in body size is achieved (Mangold, 1987).

Based on previous work on the effect of temperature on growth rate (eg. Forsythe & Hanlon, 1988, 1989 see chapter 5) it is reasonable to assume that seasonal differences in water temperature would also account for the lack of gonad development in *L. chinensis* during winter. As age of field specimens in relation to maturity processes has not previously been considered, only aquarium culture experiments are available for comparison. Richard (1966, cited in Mangold, 1987) has shown that males and females of the cuttlefish *Sepia officinalis* from the English Channel which were raised at 20°C attained sexual maturity at seven months and 140 mm DML while conspecifics raised at 10°C were totally immature at the same age and were only 50 mm in length. Moreover, Richard (1970, cited in Mangold, 1987) also found that female *S. officinalis* raised at 18°C had a gonad index of 8% at 270 d, while it took 480 d for females raised at 13°C to reach the same value.

Temperature not only had a profound influence on growth rate and therefore the size-at-age relationship in *L. chinensis* (see chapter 5) but was probably the primary influence on the rate of gonad development and the onset of sexual maturity (especially in females) as well. It appears that it simply took longer for a squid to reach sexual maturity during the cooler winter months.

6.4.1.1 Seasonality of reproductive investment

The fluctuations of the GSI and NGL/ML index indicated that ambient environmental conditions were influencing both the maturity process and the energy

L. chinensis partitioned into reproduction. Although individuals were often mature (eg. the majority of males analysed) there were time periods when the ovary and testes accounted for a greater percentage of total body weight. This was consistently recorded for both sexes in the month of October (spring). This would coincide with the spring warming of water in Cleveland Bay after low winter temperatures which begin to rise in August/September (Kenny, 1974; Walker,1981). Highest GSI values were also reported for male and female *L. vulgaris* during the spring period in the Mediterranean (Worms, 1983a).

The interpretation of the fluctuations in the GSI and NGL/ML index is complex due to a variety of factors: i) *Loligo chinensis* is very fast growing and has a short lifespan, therefore annual data sets reflect a number of different generations of squids. ii) Because of rapid growth and the observed variability in gonad growth (especially in females), greater sample numbers would be needed to adequately describe gonad growth fluctuations on a smaller scale (eg. intra-monthly variability). iii) Due to the tropical nature of the environment, squids (especially males) are mature throughout most months of the year and therefore gonad indices are generally reflecting periods of greater investment in reproductive structures rather than periods of immaturity versus maturity.

The data peaks in October were unexpected, especially since water temperatures were considerably higher later in the summer period. This phenomenon may have been due to the increasing day length in spring stimulating the optic gland to produce increased hormonal levels which accelerates gonad growth (see Mangold, 1987). Since day length would be shortened over the winter period (June-August) the increasing day length during the spring period along with increasing water temperatures may produce physiological responses leading to maximal gonad growth. More intensive sampling (eg. fortnightly) over the late spring and early summer period would no doubt provide a clearer picture of relative gonad growth over this period.

In relation to indices involving nidamental gland length, Worms (1983a) suggested that the relationship between mantle length and nidamental gland length could constitute a good maturity index for females, since the relationship between NGL and ML was closer than the relationship between ML and gonad weight. Data of *L. chinensis* females from this study suggests that the NGL/ML index provides very similar results to values of the GSI. Maturity parameters based on the NG length could constitute a rapid and more convenient means to consider maturity for tropical squids.

6.4.2 *Idiosepius pygmaeus*

The pattern of gonad maturation in tropical squids andsepioids becomes much more complex when the pattern of seasonal maturation of *I. pygmaeus* is also considered. While gonad maturation patterns could be reasonably explained in terms of temperature for *L. chinensis* (eg. lower winter temperature → slower growth rate → decreased rate of gonad maturation with a longer time needed for gonads to reach comparable sizes), the pattern was considerably modified with *I. pygmaeus*. As with *L. chinensis, I. pygmaeus* reached a greater age in the cooler (spring) season. However, gonad maturation was modified in a very different way. For example, in both seasons, males (including mature individuals) had similar sized testes at an age of 30 to 40 d suggesting that no seasonal effects were evident up to 40 days. Nevertheless, as the cooler season males continued to grow, the gonad continued to reach a relatively greater size compared to warm season males. Thus environmental constraints (such as temperature as well as possibly food availability or light levels) produced a different allometric gonad-soma relationship in this species which was not apparent in *L. chinensis.*

The physiological reasons responsible for these different allometric relationships are not understood although noticeable differences in relative gonad weights for similar sized octopus species have been observed. Individuals of *Octopus vulgaris* and *Eledone cirrhosa* captured from both the North Sea and the Mediterranean, and held in captivity over a period of time have been shown to have larger gonads than similar sized octopuses captured at sea (Mangold, 1987).

Idiosepius pygmaeus thus appears to be employing a 'trade-off' in its seasonal reproductive strategy, that is, benefiting from one process bought at the expense of another (Begon *et al.*, 1986). During the cooler period of the year, growth is slowed (presumably as a result of metabolic responses to temperature), therefore lifespan is increased as a necessity, since individuals take longer to reach adult size. As a result, *I. pygmaeus* appears to change its strategy by partitioning a greater amount of energy into gonads over the longer lifespan. Although a longer time period is taken to reach maturity, there would be a reproductive advantage, in that possessing larger gonads would increase reproductive output. A similar situation has been shown to exist with teleost fishes. Stearns (1976) has provided evidence from several species of teleosts, showing that the ratio of ovary weight to body weight (as a measure of reproductive effort) increased with age.

The fact that this phenomenon occurs in *I. pygmaeus* and not the larger *L.*

chinensis may be due to the need for *I. pygmaeus* to maximise its reproductive chances because of the greater near-shore habitat variability, or because of the greater constraints this species faces due to its small body size.

6.4.3 Secondary sexual characteristics of *L. chinensis*

6.4.3.1 Nidamental gland length

The fact that the length of the nidamental gland (NG) of *L. chinensis* bears a very close resemblance to the growth of the gonad, highlights the close association this organ has with maturation and egg development. The nidamental gland serves the function of producing a gelatinous matrix which encases the cephalopod egg (Roper *et al.*, 1984).

Previous studies have used the NGL/ML ratio as a convenient means to assess maturity. Temperate loliginids have been shown to possess mature oocytes when this ratio is greater than 0.2 (Yang *et al.*, 1986; Hanlon *et al.*, 1989). This relationship generally holds well for *L. chinensis* females. Out of 112 females (captured throughout the two year sampling period) analysed for this ratio, all the mature females had a NG/ML ratio greater than 0.2. The majority of immature females were below this value. However, six individuals (which were immature) out of the 112 analysed, did have a ratio greater than 0.2. However, the values were only slightly higher than 0.2, with the highest value of only 0.26. This parameter therefore appears to be useful for tropical loliginids as well. The 0.2 NG/ML ratio does appear to be the minimum parameter for mature females.

Nidamental gland measurements are thus useful for providing a rapid and convenient means of assessing the level of maturity in tropical loliginids. The nidamental gland can also provide useful information about the past history of a female squid. The fact that some of the larger, older, immature females captured during January had small underdeveloped nidamental glands, was one means to ascertain that these females had actually not yet matured and had not regressed from a previously mature condition.

6.4.3.2 Hectocotylus length

The male hectocotylus is an important reproductive structure employed by the male to pass spermatophores to the female during copulation. It is also extremely useful for quick sexual identification of preserved specimens. Coelho *et al.* (1985) carried out a detailed study of the growth of the hectocotylus of *Illex illecebrosus* to determine if the degree of hectocotylisation of the fourth arm could be related to maturity. However, no close relationship was found between maturity and the degree of hectocotylisation. A partial explanation for this lack of relationship between these parameters was attributed to the fact that the squids examined might have included individuals which hatched at different localities and which had developed under different temperature regimes (eg. some squids could have been immigrants from a more southerly population exposed to warmer temperatures). This could well account for considerable confusion in the *Illex* data, as individuals of *L. chinensis* from different seasons showed a very different relationship between hectocotylus length and mantle length, and hectocotylus length and age. Seasonal somatic growth patterns which were temperature related, did have an influence on the rate of development of the hectocotylus, with slower growing individuals eventually possessing a larger hectocotylus than their faster growing counterparts. Hectocotylus length thus provided similar ecological information to analysis of seasonal statolith growth patterns (see chapter 5). For example, different sized hectocotyli on similar sized squids could indicate that the squids had developed under different growth rates. This also suggests that the size ratio of many other structures to body size may also be influenced by the ambient temperature in which the squid develops. Furthermore, parameter ratios and indices used for taxonomic purposes should be cautiously employed, at least for near-shore squids, and that whenever possible parameters should be measured in individuals captured under similar environmental conditions.

6.4.4 Tropical squid and sepioid reproductive patterns

The variable oocyte size frequencies within the ovaries of the study species, along with the fecundity experiments with *I. pygmaeus* all indicate that these tropical species produce repeated batches of eggs rather than a single terminal spawning. Repeated spawning has been reported for cuttlefish (Boletzky, 1987) and sepiolids (Gabel-Dieckert, 1990) and several tropical Hawaiian oceanic squids (Boucher, 1983; Harmon *et al.*, 1989).

The reproductive strategy displayed by *I. pygmaeus* agrees with previous work with *I. paradoxus* in Japan. Natsukari (1970) noted that after capture, female *I. paradoxus* laid eggs in several batches with the number of eggs in each clutch ranging from 25 to 64, and with death following the final egg laying. More recently, Yamamoto (1988) also reported that *I. paradoxus* was observed to produce 30 to 80 eggs every 2 to 7 d for more than a month. Although total egg output was not reported, egg production was reported as almost equal between six females maintained, with a mean daily egg production rate of 13.7 ± 0.4.

Repeated spawning appears to be a common phenomena with sepiolids as well. The Mediterranean species *Sepiola affinis, S. rondeleti* and *Sepietta owieiana* have been shown to produce a total of 349, 302 and 428 eggs over a two month period. The fastest egg production was noted for *S. owineiana,* which produced the 428 eggs in 18 d (Gabel-Dieckert, 1990).

Idiosepius pygmaeus is clearly capable of a considerable egg output over a very short period of time (922 eggs over 15 d). This is the greatest egg output yet reported for any sepioid. This high egg output is somewhat surprising considering the small size of *I. pygmaeus* (Boletzky, personal communication). This strategy is probably related to its small size, short lifespan, tropical environment and variable near-shore locale which it inhabits. This study suggests that tropical species are not only fast growing with extremely short lifespans but also probably have a much greater reproductive output than previously thought. Furthermore, fecundity estimates based on ovary oocyte counts (eg. Voss, 1983; Boyle, 1990) are probably incorrect.

The histological evidence for the study species also suggests that they spawn repeatedly rather than releasing all their eggs at once. This is in agreement with other data on oocyte size distributions within the ovary of other tropical oceanic species in Hawaiian waters. The oocyte size distribution of *Pterygioteuthis microlampas, Pyroteuthis addolux, Abraliopsis* sp. (Boucher, 1983) and *Stenoteuthis oualaniensis* (Harmon *et al.*, 1989) have all suggested that these species spawn repeatedly. Data on *S. oualaniensis* has shown that it would be impossible for all oocytes within the ovary to mature at once based on size constraints of the squid. Furthermore, this species matures over a relatively narrow size range, with all specimens mature after 200 mm. As mature specimens as large as 318 mm were captured, this indicates that a considerable amount of growth takes place during the mature reproductive phase. This agrees with the age data of this study (see chapter 5) which suggested that squid growth continues or even accelerates with the onset of sexual maturity, rather than slowing or stopping. Furthermore, the findings of this study and those of Harmon et. al. (1989) contradict statements by Boyle (1990) that different sizes in mature cephalopods are achieved by different individuals reaching maturity at different sizes, and that asymptotic growth is coincident with maturity.

Harmon *et al.* (1989) suggested that multiple spawning may prove to be a common strategy among tropical squids. Research on the tropical near-shore Australian species is supporting their hypothesis. However, as the growth dynamics of tropical squids is only just beginning to be understood, much more research is also needed into the reproductive strategies and the possible fecundities of tropical as well as temperate species.

Based on the discussions currently in the literature, it is difficult to adequately give a description to the reproductive events in cephalopods. Descriptions such as iteroparity (repeated spawnings), semelparity (spawning once), chronic, intermittent and multiple spawning have all been used to describe reproductive events. However, there are ecological problems encountered if descriptors are not used precisely or if terms used are not adequately explained, especially when dealing with short lived species such as cephalopods.

6.4.5 Semelparity or iteroparity?

Fritz *et al.* (1982) discussed problems associated with the terms iteroparous and semelparous in relation to insect strategies. They suggested that these terms should be defined by the temporal pattern of egg maturation and deposition based on the generation time of the organism, rather than an arbitrary period such as an annual cycle. They have pointed out that the distribution of clutches of single eggs over a relatively short lifespan was analogous to annual reproduction in longer lived vertebrates. On the other hand, Kirkendall & Stenseth (1985) have pointed out that according to the rationale of Fritz *et al.* (1982), most annual plants could be considered iteroparous since they also have a short lifespan and produce multiple 'clutches' over a period of several weeks to several months. This raised several important questions that is, "should annual plants be considered iteroparous, or should insects be considered semelparous, or are such questions meaningless to ask?" Kirkendall & Stenseth (1985) offered a solution to this problem by proposing a more precise terminology, incorporating both short lived and longer lived organisms.

In many ways, the situation encountered with the Cephalopoda (excluding Nautilus) is very similar to terrestrial insects which have a short lifespan with a variable period of time devoted to reproduction. Recent descriptions of cephalopod reproductive strategies have been confusing in the light of the current terminology problems. Boyle (1983a) in the summary chapter of a major volume of comparative life histories of cephalopods, commented that "most authors have presumed that a single period of breeding activity, semelparity, is the norm". However, two sentences later he highlighted the fact that there is some variation in the duration of egg laying, with the entire spawning output being deposited in hours, days or up to a month.

The argument has been somewhat further complicated by Calow (1987) who, in commenting on the same volume of work as Boyle (1983a), suggested that apart from Nautilus, iteroparity is rare in cephalopods, and that with one exception (an octopus that was reported to spawn twice six months apart), semelparity is ubiquitous, based on the evidence on all other species of cephalopods reviewed. However, in the previous paragraph, Calow (1987) referred to semelparous as "breed once and die" and iteroparous as "repeated breeding". Both authors have referred to cephalopod species as semelparous despite evidence which suggests that they might be repeated spawners.

Rodaniche (1984) suggested that *Octopus chierchiae* was iteroparous, based on aquarium observations of several females which produced repeated batches of eggs, with two females producing three batches of eggs each in less than 200 d. Rodhouse *et al.* (1988) referred to the data of Rodaniche (1984) as being an exception to semelparity in extant coeloid cephalopods.

It can be appreciated that the term semelparity has been used quite loosely in the cephalopod literature and apparently includes the reproductive strategy of a female producing repeated batches of eggs over an extended period of time. The term iteroparity used by Rodaniche (1984) could quite justifiably be also used to describe many annual plants according to Kirkendall & Stenseth (1985). Boletzky (1987) has somewhat cleared the argument by referring to repeated batches of eggs laid by a captive cuttlefish over almost four months as "intermittent" or "chronic spawning", and suggested that whether this was referred to as iteroparity or intermittent spawning is a matter of taste, unless iteroparity was reserved to refer to seasonal spawning.

Harmon *et al.* (1989) have further suggested that the distinction is unclear between extended semelparous spawning and multiple spawning in cephalopods and reserved the term "multiple spawning" for all types of non-semelparous reproduction.

Based on the previous discussion, it appears that the terms iteroparity and semelparity have caused confusion in the past and these are best not used in referring to cephalopods or other organisms which may live less than 1 year. For clarification it would be more accurate and less confusing to refer to species which appear to spawn only once and die, for example *Loligo opalescens* (Knipe & Beeman, 1978) (a strategy probably uncommon in cephalopods) as "terminal spawners" or "uniseasonal-uniparous" (Kirkendall & Stenseth, 1985), and species which produce multiple batches of eggs as "multiple spawners" as proposed by Harmon *et al.* (1989) or "uniseasonal-iteroparous" (Kirkendall & Stenseth, 1985).

Figure 6.1

The relationship between gonad weight and age for male and female individuals of *Loligo chinensis* collected in summer (January) and winter (July).

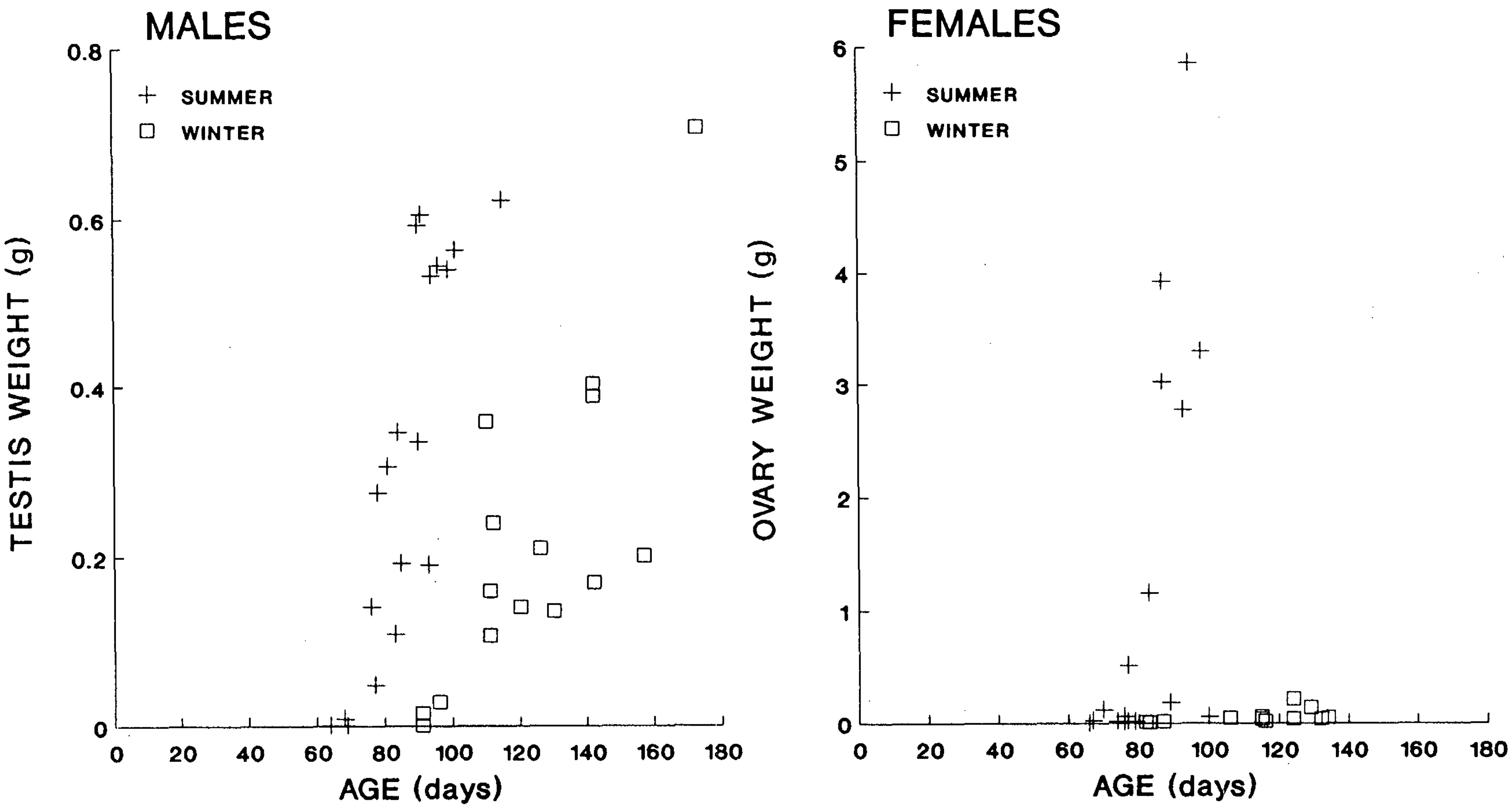

MALES
TESTIS WEIGHT (g)
0.8
0.6
0.4
0.2
0
+ SUMMER
□ WINTER
0
20
40
60
80
100
120
140
160
180
AGE (days)
FEMALES
OVARY WEIGHT (g)
6
5
4
3
2
1
0
+ SUMMER
□ WINTER
0
20
40
60
80
100
120
140
160
180
AGE (days)

Figure 6.2

The relationship between gonad weight and mantle length for individuals of male and female *Loligo chinensis* collected in summer (January) and winter (July).

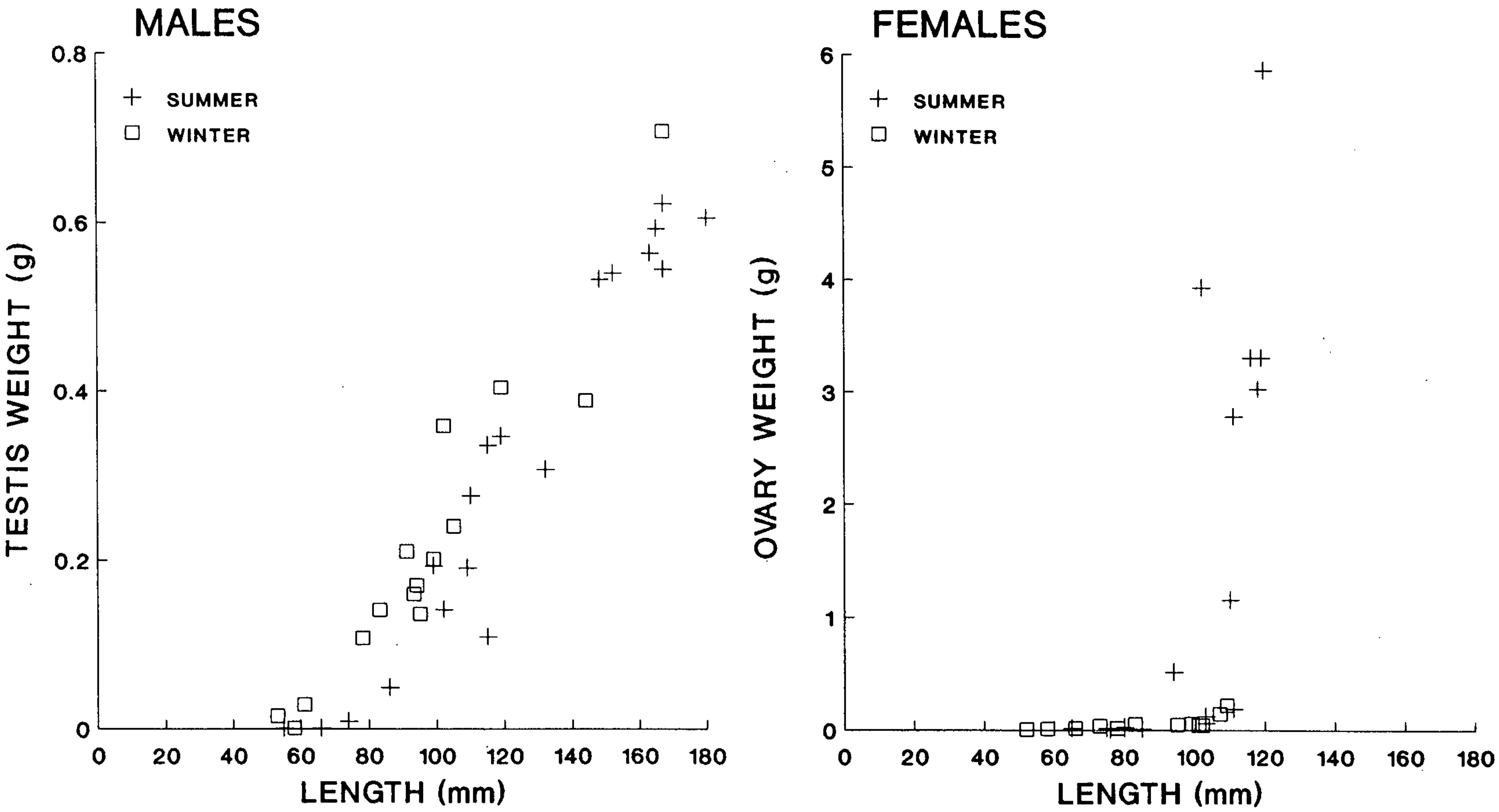
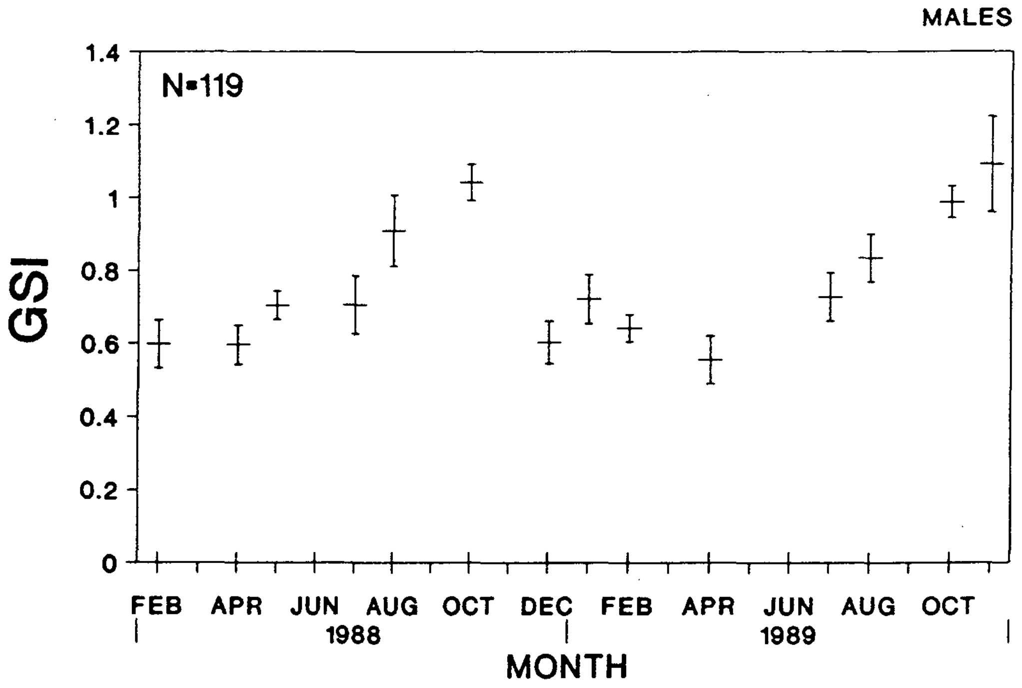

MALES
FEMALES
+ SUMMER
□ WINTER
TESTIS WEIGHT (g)
OVARY WEIGHT (g)
LENGTH (mm)
0
0.2
0.4
0.6
0.8
1
2
3
4
5
6
20
40
60
80
100
120
140
160
180

Figure 6.3

Mean monthly gonadosomatic index for *Loligo chinensis* over the study period for males and females. Bars represent standard errors.


MALES
N=119
GSI
1.4
1.2
1
0.8
0.6
0.4
0.2
0
FEB
APR
JUN
AUG
OCT
DEC
FEB
APR
JUN
AUG
OCT
1988
1989
MONTH

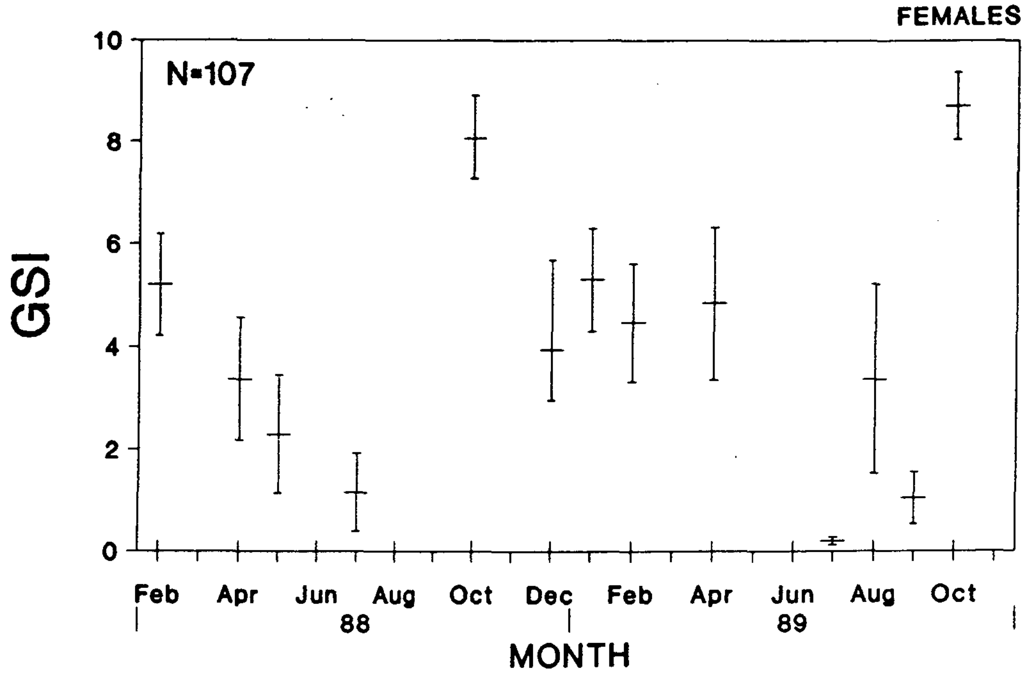
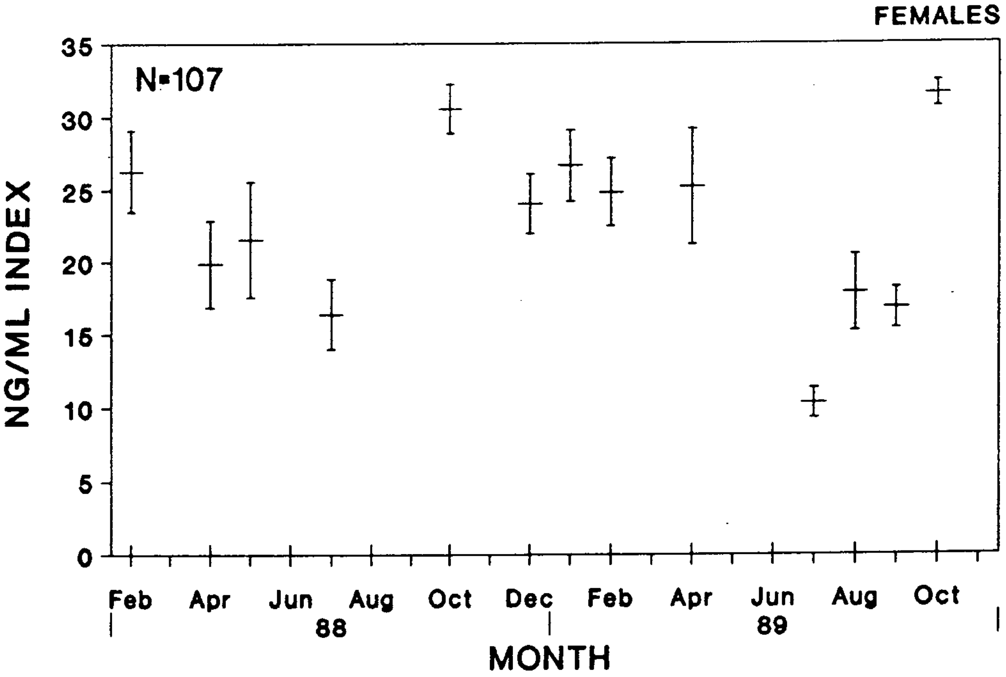

FEMALES
N=107
GSI
10
8
6
4
2
0
Feb
Apr
Jun
Aug
Oct
Dec
Feb
Apr
Jun
Aug
Oct
88
89
MONTH

Figure 6.4

Comparison of the nidamental gland/mantle length index with the gonadosomatic index for female individuals of *Loligo chinensis* collected over the study period. Bars represent standard errors.


FEMALES
N=107
NG/ML INDEX
35
30
25
20
15
10
5
0
Feb
Apr
Jun
Aug
Oct
Dec
Feb
Apr
Jun
Aug
Oct
88
89
MONTH

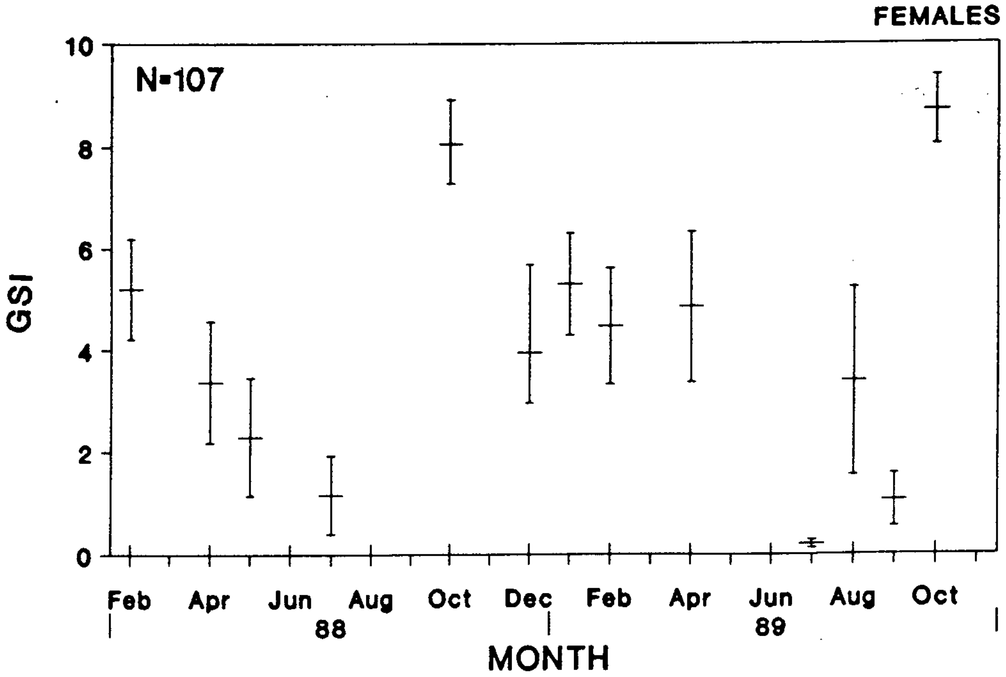
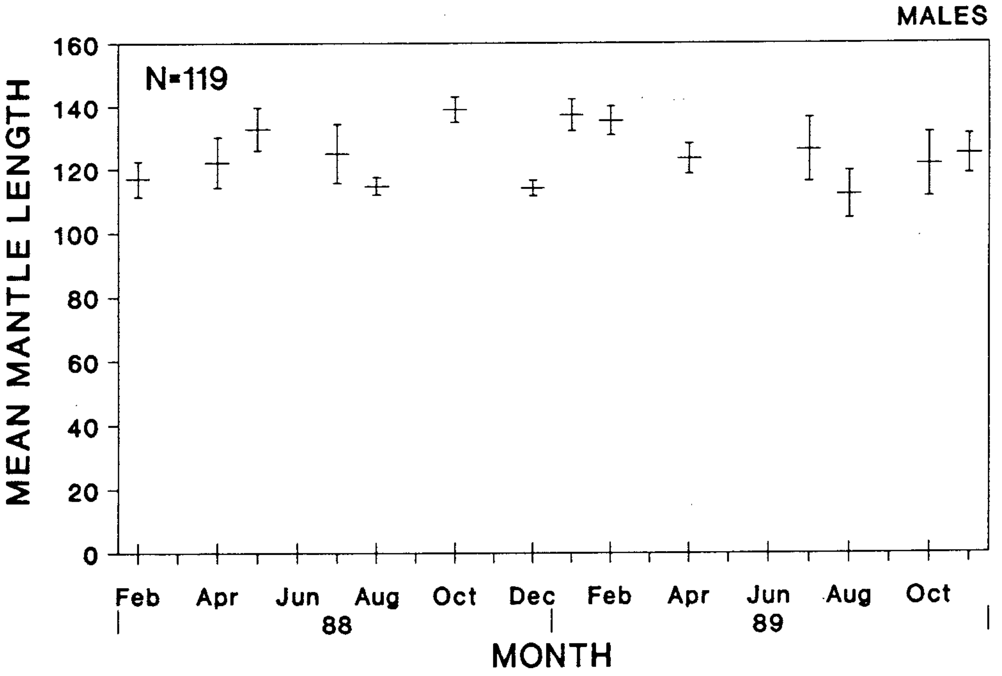

FEMALES
N=107
GSI
10
8
6
4
2
0
Feb
Apr
Jun
Aug
Oct
Dec
Feb
Apr
Jun
Aug
Oct
88
89
MONTH

Figure 6.5

Mean mantle length for males and females of *Loligo chinensis* used in gonad weight analysis (Figs. 6.3, 6.4). Bars represent standard errors.


MALES
N=119
MEAN MANTLE LENGTH
0
20
40
60
80
100
120
140
160
Feb
Apr
Jun
Aug
Oct
Dec
Feb
Apr
Jun
Aug
Oct
88
89
MONTH

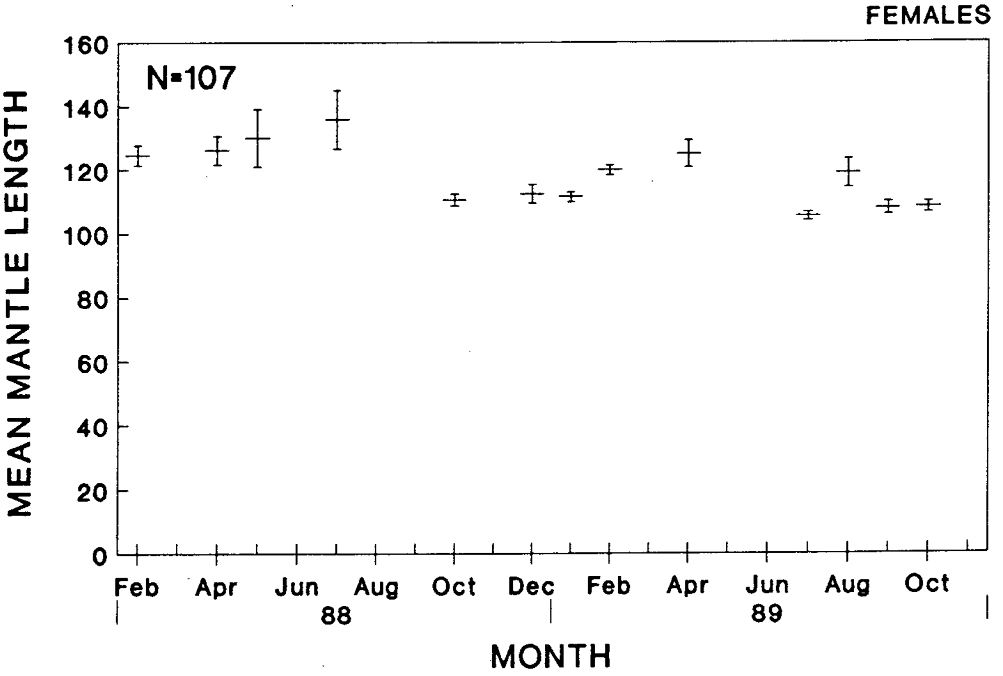
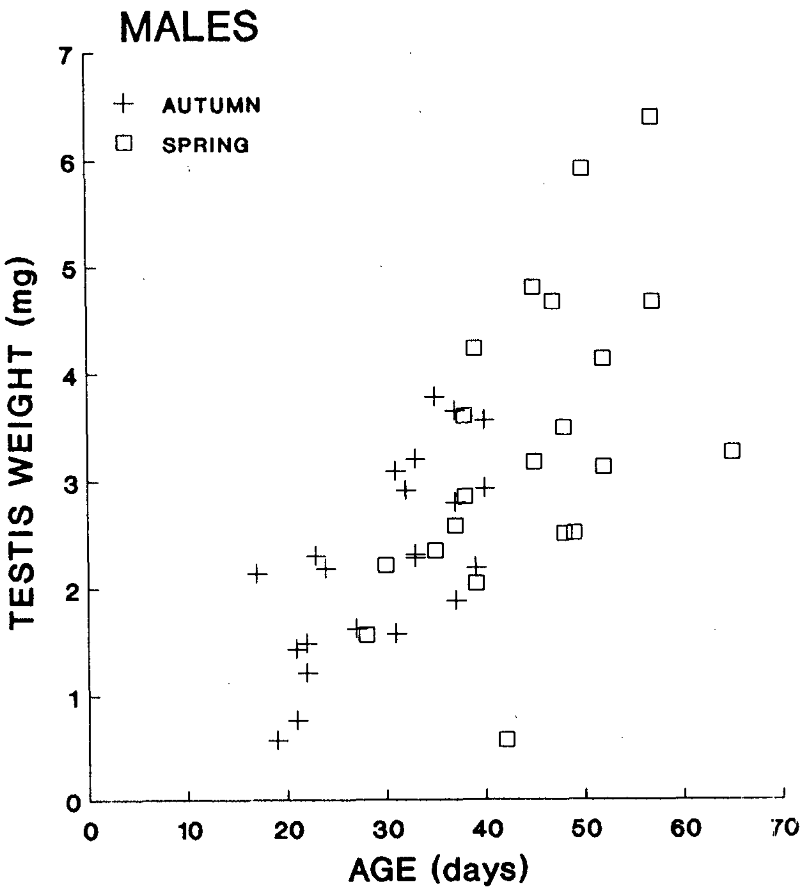

FEMALES
N=107
MEAN MANTLE LENGTH
0
20
40
60
80
100
120
140
160
Feb
Apr
Jun
Aug
Oct
Dec
Feb
Apr
Jun
Aug
Oct
88
89
MONTH

Figure 6.6

The relationship between gonad weight and age for male and female individuals of *Idiosepius pygmaeus* collected in autumn and spring.


MALES
+ AUTUMN
□ SPRING
TESTIS WEIGHT (mg)
0
1
2
3
4
5
6
7
AGE (days)
0
10
20
30
40
50
60
70

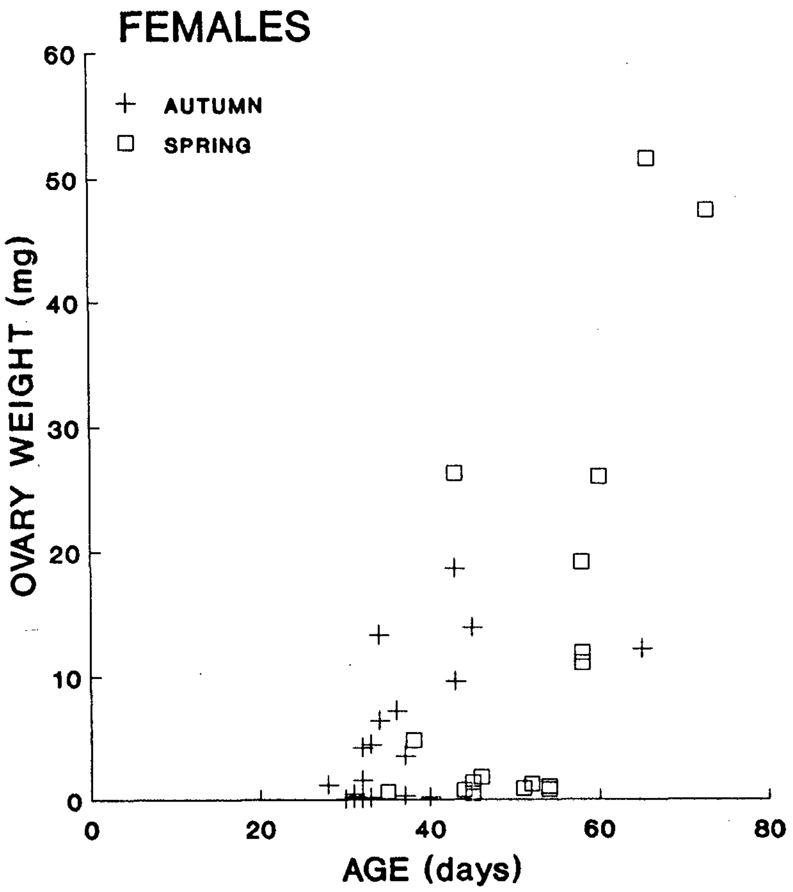

FEMALES
+ AUTUMN
□ SPRING
OVARY WEIGHT (mg)
0
10
20
30
40
50
60
AGE (days)
0
20
40
60
80

Figure 6.7

The relationship between gonad weight and mantle length for male and female individuals of *Idiosepius pygmaeus* collected in autumn and spring.

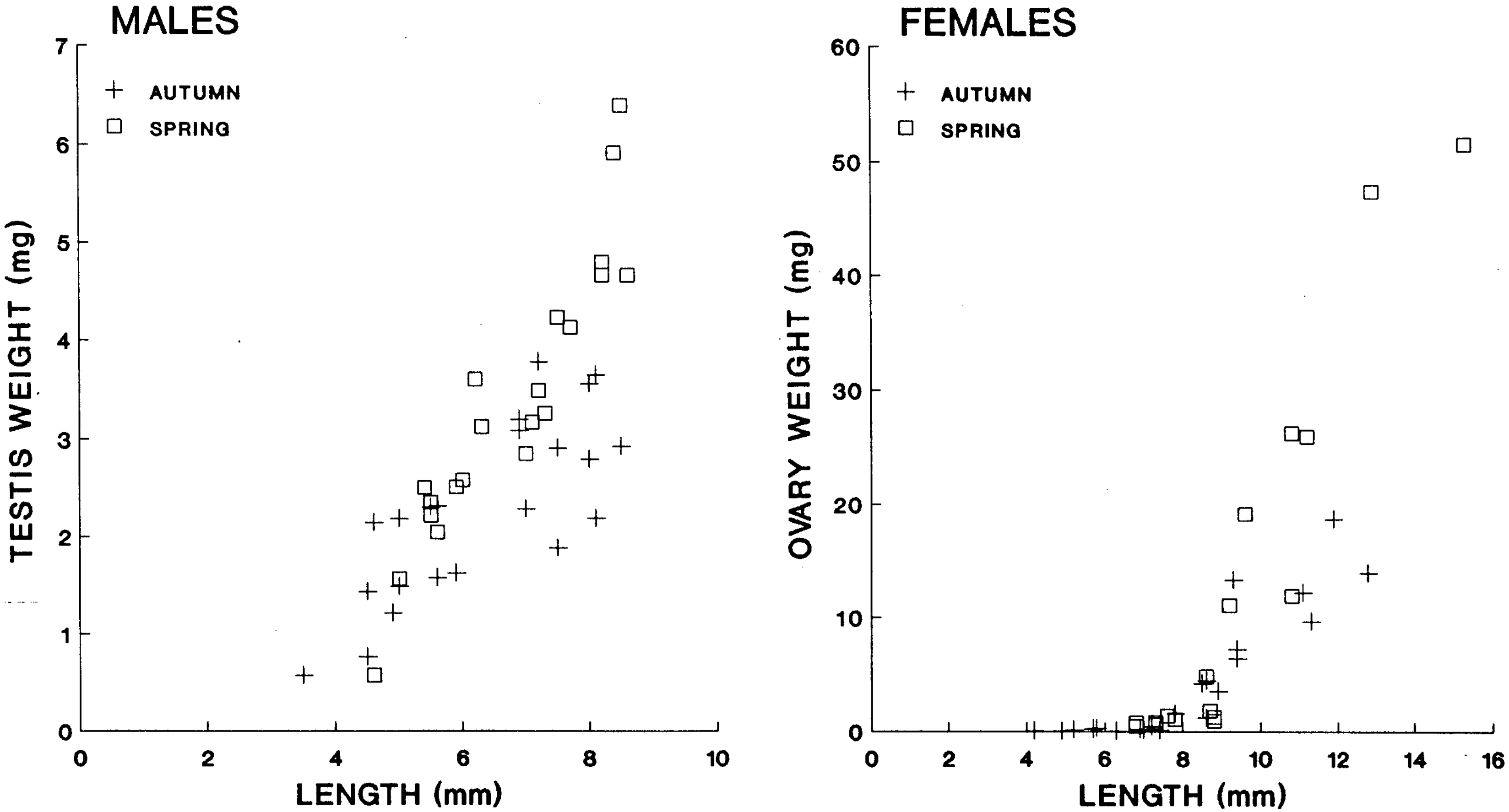
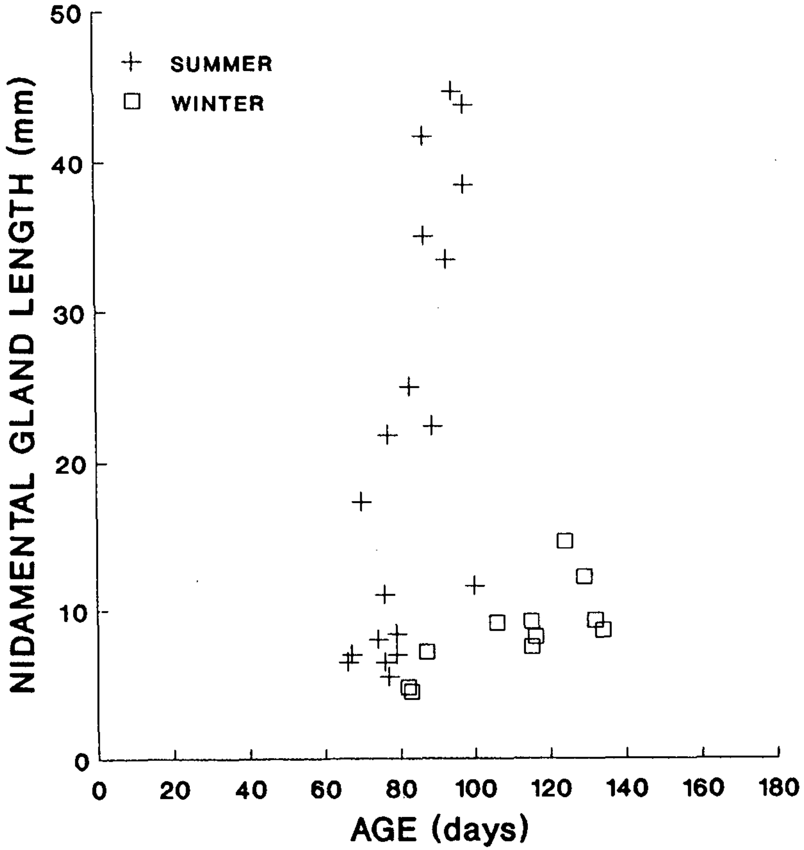

MALES
TESTIS WEIGHT (mg)
LENGTH (mm)
AUTUMN
SPRING
0
1
2
3
4
5
6
7
0
2
4
6
8
10
FEMALES
OVARY WEIGHT (mg)
LENGTH (mm)
AUTUMN
SPRING
0
10
20
30
40
50
60
0
2
4
6
8
10
12
14
16

Figure 6.8

The relationship between nidamental gland length and age; and nidamental gland length and mantle length for female individuals of *Lologo chinensis* collected during summer (January) and winter (July).


SUMMER
WINTER
NIDAMENTAL GLAND LENGTH (mm)
0
10
20
30
40
50
0
20
40
60
80
100
120
140
160
180
AGE (days)

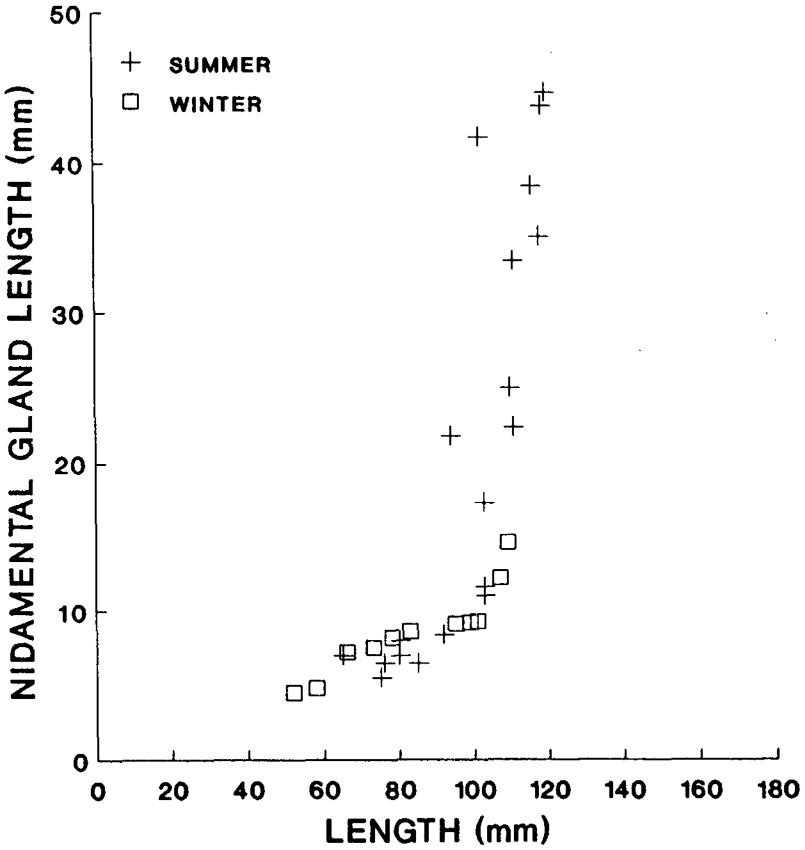
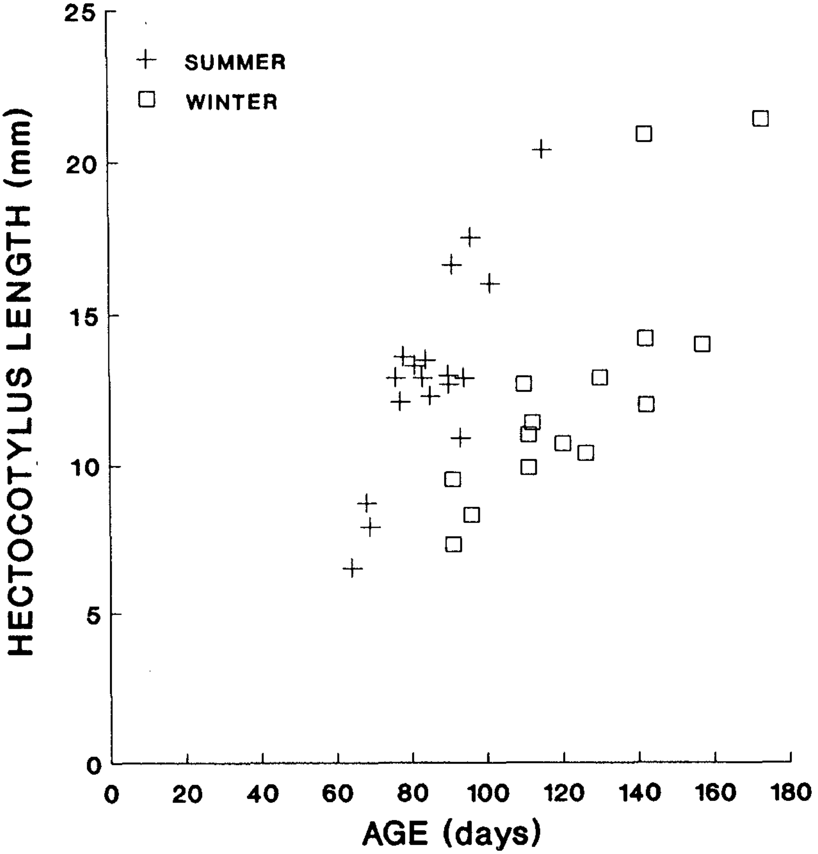

SUMMER
WINTER
NIDAMENTAL GLAND LENGTH (mm)
0
10
20
30
40
50
0
20
40
60
80
100
120
140
160
180
LENGTH (mm)

Figure 6.9

The relationship between hectocotylus length and age; and hectocotylus length and mantle length for male individuals of *Loligo chinensis* collected during summer (January) and winter (July).


HECTOCOTYLUS LENGTH (mm)
25
20
15
10
5
0
+ SUMMER
□ WINTER
0
20
40
60
80
100
120
140
160
180
AGE (days)

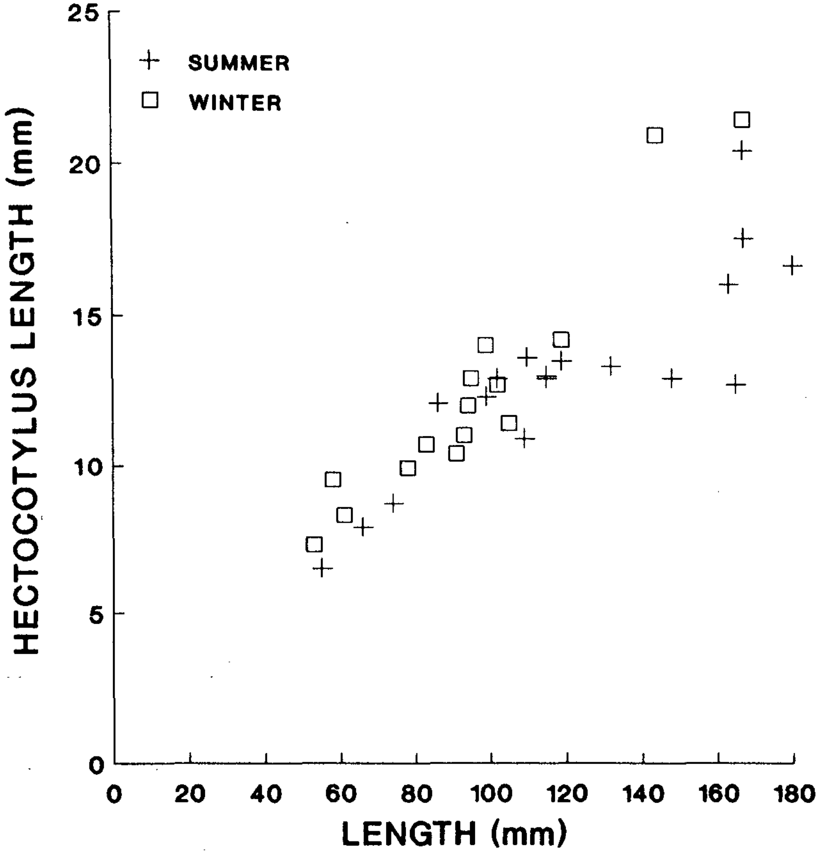
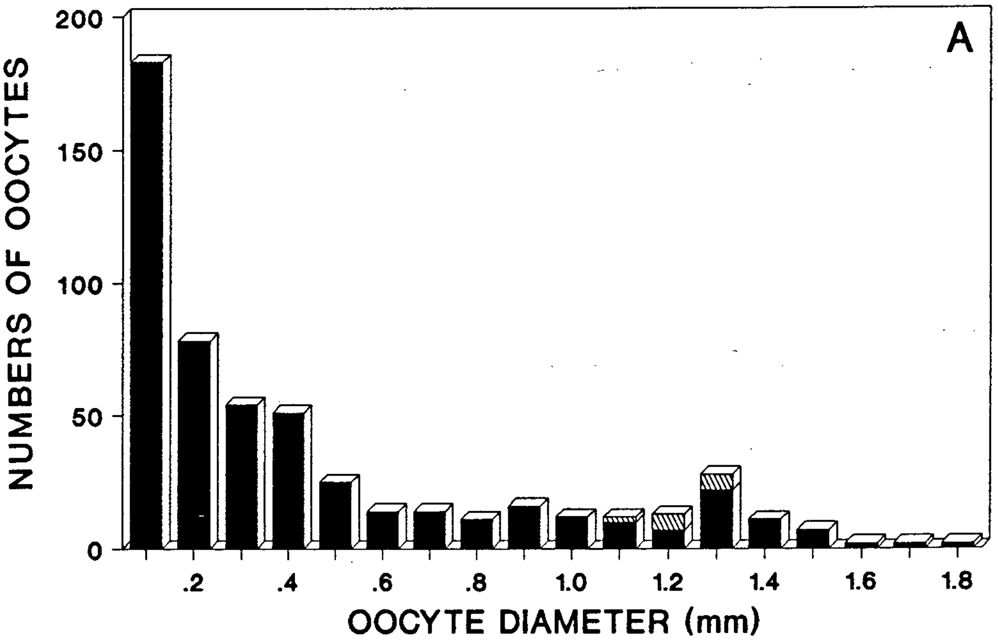

HECTOCOTYLUS LENGTH (mm)
25
20
15
10
5
0
+ SUMMER
□ WINTER
0
20
40
60
80
100
120
140
160
180
LENGTH (mm)

Figure 6.10

Oocyte diameter within the ovary of two female individuals of *Idiosepius pygmaeus*. Solid bars represent immature oocytes, lined bars represent mature oocytes. (A) Female captured 10 July 1986, age 60 days, total number of oocytes 376. (B) Female captured 20 May 1987, age 59 days, total number of oocytes 282.


A
NUMBERS OF OOCYTES
200
150
100
50
0
.2
.4
.6
.8
1.0
1.2
1.4
1.6
1.8
OOCYTE DIAMETER (mm)

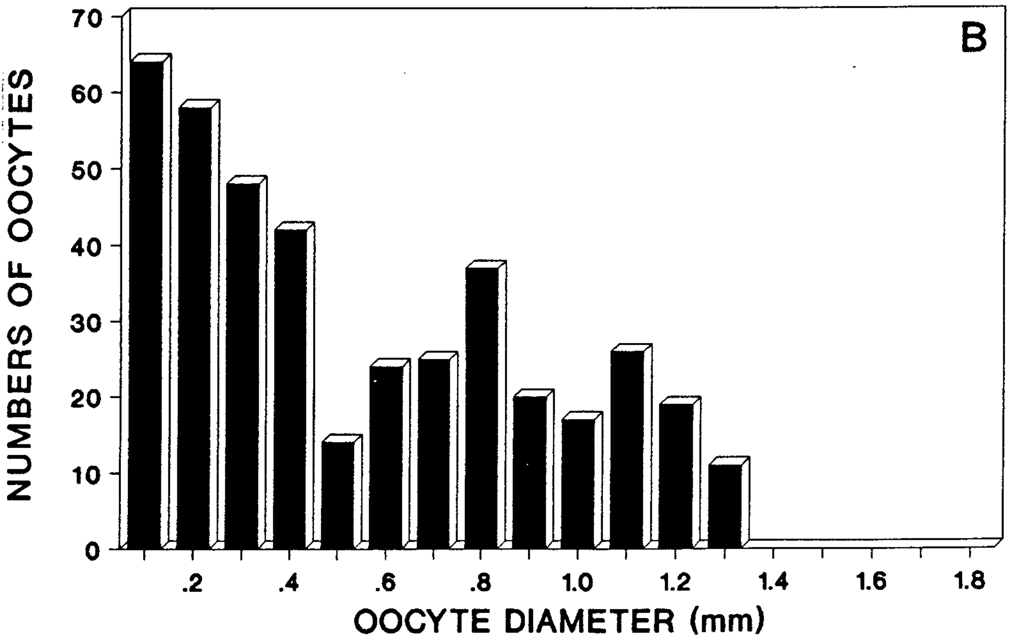

B
NUMBERS OF OOCYTES
70
60
50
40
30
20
10
0
.2
.4
.6
.8
1.0
1.2
1.4
1.6
1.8
OOCYTE DIAMETER (mm)

Figure 6.11

Cumulative egg output for female individuals of *Idiosepius pygmaeus* maintained in captivity. Each experiment represents the egg output of one female.

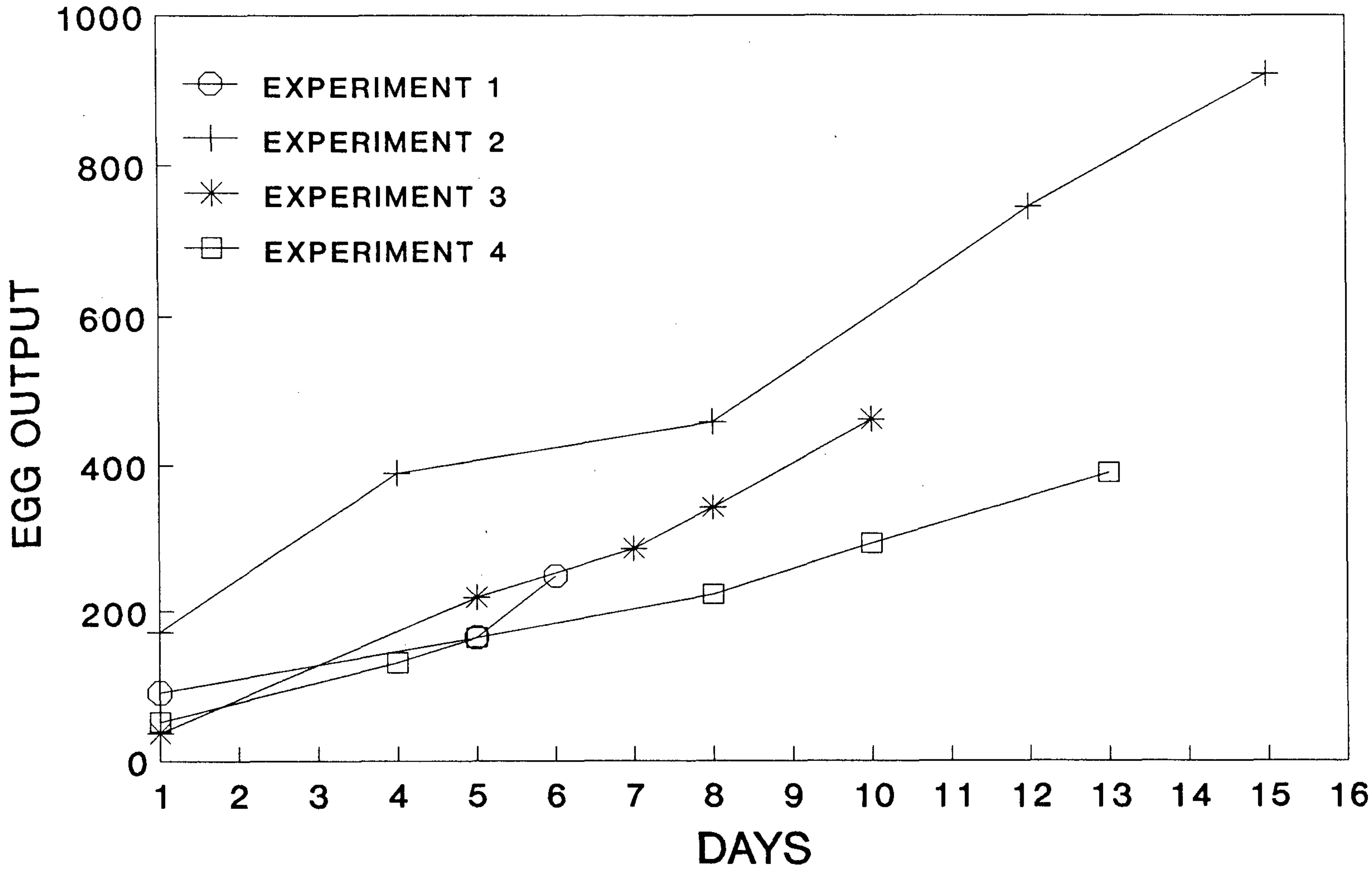
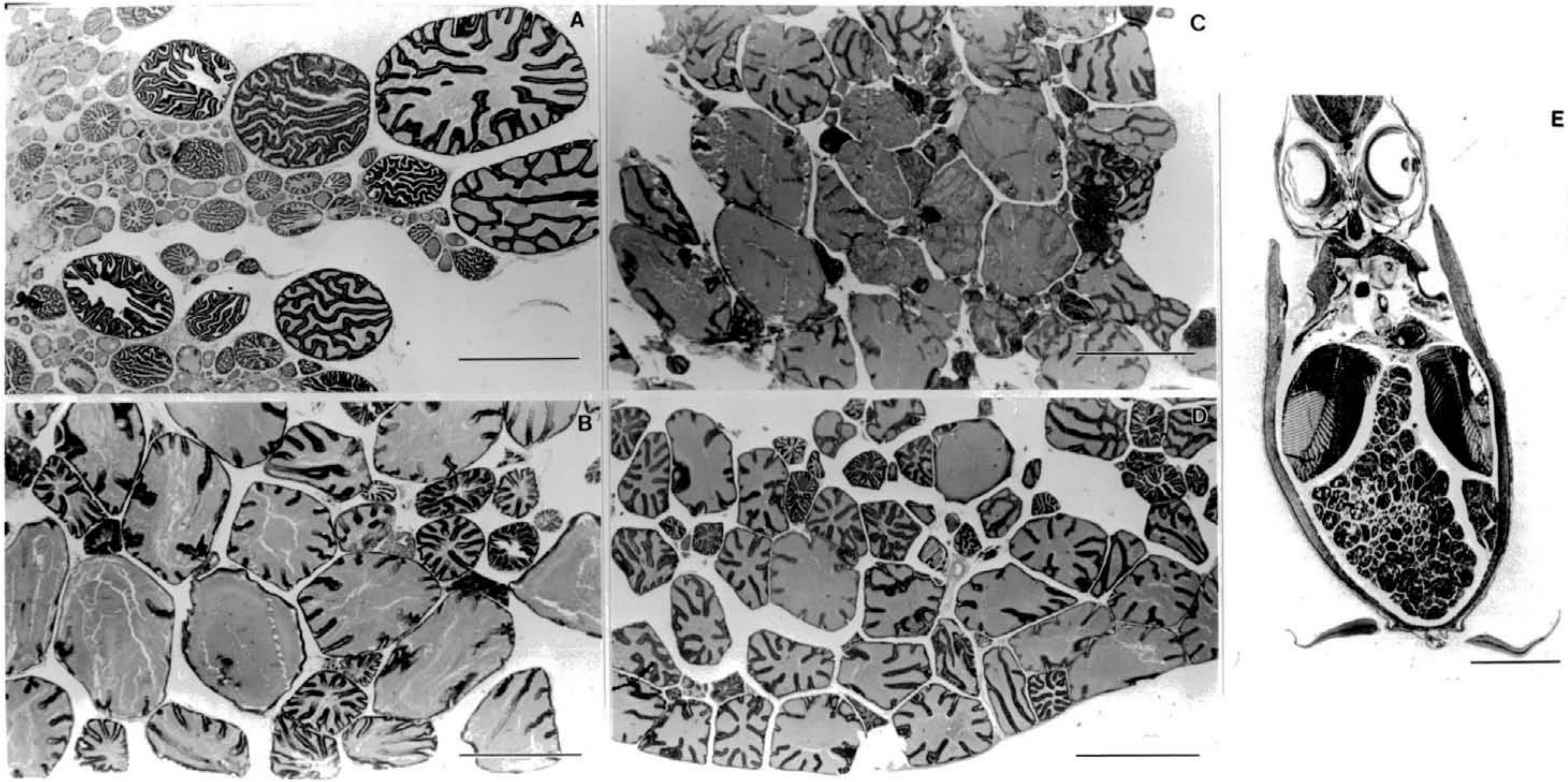

1000
800
600
400
200
0
EGG OUTPUT
EXPERIMENT 1
EXPERIMENT 2
EXPERIMENT 3
EXPERIMENT 4
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
DAYS

Figure 6.12

Histological sections of ovaries of the five study species, showing a range in oocyte size. (A) *Sepioteuthis lessoniana.* Scale bar = 500 μm. (B) *Loliolus noctiluca.* Scale bar = 500 μm. (C) *Loligo chinensis.* Scale bar = 500 μm. (D) *Loligo* sp. 1. Scale bar = 500 μm. (E) *Idiosepius pygmaeus,* section of whole specimen showing ovary and large nidamental glands. Scale bar = 3.5 mm.


A
C
E
B
D

7.0 ALLOMETRY AND GROWTH MODELLING

7.1 INTRODUCTION

Research on cephalopod growth and lifespans has greatly increased over the last decade. In the past most work on squid growth was restricted to length frequency analysis of regular samples from field populations (eg. Squires, 1967; Summers, 1971). However, though these analyses are still widely used, they can often result in misleading conclusions (see chapter 5). Furthermore, attempting to develop growth models from such techniques lacks precision. At best, only vague estimates of growth can be established, and the accuracy of such results remains in question.

Recent advances in both culturing and ageing research have opened up new avenues for obtaining accurate descriptions of cephalopod growth. Culturing individuals throughout their life cycle has enabled direct observation of growth at all levels of development (see reviews, Boletzky & Hanlon, 1983; Hanlon & Hixon, 1983; Hanlon, 1987). Furthermore, continuing work with validation of statolith growth rings (see chapter 4) and obtaining age information from field captured specimens has provided a means to obtain size-at-age information and to subsequently use this information to construct growth curves (see review, Rodhouse & Hatfield, 1990a).

Until recently cephalopod biology has been plagued with uncertainties with regards to the form of growth of cephalopods. For example, all major types of growth models have been applied to one genus, *Loligo* (see Hixon, 1980; Mangold, 1983). Continuing research with culture experiments and statolith ageing should help to more accurately determine the appropriate form of growth for cephalopods. This work will allow the distinguishing of whether cephalopods exhibit a variety of very different growth curves or whether growth can be encompassed within one equation with a variety of parameters. Detailed background research into the biology and physiology of fishes has enabled the development of such a generalised model for these organisms (eg. the von Bertalanffy growth equation). However, there is still considerable ground to cover before enough data is available to develop such a generalised growth function for cephalopods. Detailed work on the form of growth, and spatial and temporal variation in the growth of specific species will help to alleviate the current lack of knowledge in this area.

The aims of this chapter were:

1) To consider the change in the length-weight relationship (allometry) of the five study species and to compare the length-weight relationships of these tropical species to published information on other species.

2) To develop mathematical growth models for two of the study species (*L. chinensis* and *S. lessoniana)* in which adequate size-at-age data were available over the entire size range and to compare culture data with statolith age data for the Californian squid *Loligo opalescens*.

3) To review the recent literature dealing with the development of growth models for squids based on culture experiments and statolith ageing, to consider the potential usefulness of ageing research for other squid species (including the giant squid *Architeuthis*) and to discuss current ecological theory in the light

of what new information squid growth modelling is giving us in regards to life cycles and reproductive tactics in these organisms.

7.2 MATERIALS AND METHODS

7.2.1 Allometry

The relationship between length and weight was determined for each of the five study species. The relationship between length (L) and weight (W) is best expressed by the power equation $W=aL^b$ where a is the y intercept and b is the slope or power component (Forsythe, 1984).

Least squares regression analyses were carried out on the log-transformed length and weight data for each sex of each species. The male and female regressions for *S. lessoniana* and *L. noctiluca* also included data for unsexed juveniles. Analysis of covariance was used to determine if there were any differences in the slopes or elevations of the log-transformed length-weight (hereafter referred to as L-W) relationships between the sexes of each species. Length-weight data for each of the study species except *S. lessoniana* were taken over similar seasonal periods, March 1988, 1989 and April to May 1988 for *I. pygmaeus;* May to July for *L. noctiluca;* February 1988 and January 1989 for *L. chinensis* and November 1989 for *Loligo* sp. 1. *Sepioteuthis lessoniana* juveniles were captured during May and September 1989, while adults were captured between November 1988 and February 1989.

7.2.2 Growth modelling

Analyses were carried out to fit growth equations to size-at-age data determined from statolith ageing studies. For all the study species except *S.*

lessoniana there were very few aged individuals from the paralarval/juvenile size range. To obtain a better description of growth for *L. chinensis,* paralarval squids were obtained from night light trapping (carried out by S. Thorrold, AIMS) during December 1989, from Bowling Green Bay, east of Cape Cleveland. Although it is currently not possible in North Queensland waters to identify paralarval squids to species, they could be ascertained to be of the genus *Loligo.* Due to the fact that *L. chinensis* is the most abundant species in near-shore waters, they were assumed to be paralarvae of this species for the purpose of growth modelling. Since paralarvae were obtained during the summer period, they were incorporated into the summer (January) age data already obtained for *L. chinensis.*

An estimate of length and weight for hatchlings of *L. chinensis* were determined from a hatchling obtained from an egg mass which was laid by a female *L. chinensis* jigged off Horseshoe Bay, Magnetic Island, and maintained alive overnight. Unfortunately, immediately after the hatching of this individual, the other developing squids in the egg mass died. This one individual was immediately preserved in 70% ethanol and was later weighed and measured. Similarly, hatchling length and weight for *S. lessoniana* were obtained from a paralarval *S.. lessoniana* which was hatched out of an egg which was trawled in Cleveland Bay. Paralarval *S. lessoniana* are easy to distinguish from other local loliginid paralarvae due to their large body size and heavy pigmentation.

For *S. lessoniana*, only data for juveniles and males were used in the growth curve analysis, as there were too few aged females, especially in the upper size range which was represented by only one individual.

Age-weight and age-length data for juveniles, males and females; and juveniles and males were analysed for *L. chinensis* and *S. lessoniana* respectively.

Both weight and length data were logged to improve the age-size relationships.

7.2.3 *Loligo opalescens* age analysis

Length-at-age data were also obtained from specimens of the temperate Californian squid *Loligo opalescens*. Fresh specimens were purchased from a fish market at Moss Landing, California on the 20th June, 1990. Consultation with the fisherman indicated that they were captured the previous night between Monterey Point and Fort Bragg.

Statoliths were prepared as described for *L. chinensis* (see chapter 4). Individual age was taken as the mean of 2 counts that varied less than 10%.

7.2.4 *Architeuthis* age analysis

A statolith was obtained (from C.C. Lu, Museum of Victoria) from a juvenile of the giant squid *Architeuthis* sp. to see if periodic growth rings were present within the microstructure of this species. The *Architeuthis* specimen (female, 422 mm DML) was captured on 30 of January 1982 on a cruise of the CSIRO FRV 'Soela' using an IYGPT midwater trawl. The trawl was taken between 1845hr-1950hr off New South Wales, Australia, 33°44'S 153°00'E, from the surface to 600 m. Total water depth of the locality was approximately 2000 m. Important parameters were measured immediately on the freshly captured squid and the statoliths were removed before preservation of the specimen. The statoliths were immediately placed in 70% ethanol and examined in January 1990. Preparation of the statolith for growth ring analysis was as for *L. chinensis* (see chapter 4). The years of preservation in ethanol did not appear to adversely damage the statoliths.

7.2.5 Digitising of published growth data

Size-at-age data for other species from the literature were obtained by the use of the digitising program Sigma Plot, in conjunction with a mouse and digitising board. Statolith derived length-at-age data were taken from scatter plots of male and female *Photololigo edulis* (warm-season brood) (Natsukari *et al.*, 1988, p.183). Mean length-at-age and the length range was also digitised from a growth plot for cultured individuals of *L. opalescens* (Yang *et al.*, 1986) (L.O. 1981 experiment p. 783).

7.3 RESULTS

7.3.1 Allometry

The length-weight relationship provides a good description of the relative growth of an organism. The slope (*b*) or power exponent gives an indication of whether growth is isometric or allometric. When $b=3$ weight increases as the cube of length (isometric growth) (Forsythe, 1984). When the slope is not equal to three growth is allometric, that is, the body proportions or overall body shape does not remain constant (Forsythe & Van Heukelem, 1987).

Taking log-transformations of the L-W data provides a convenient means to compare the slopes of this relationship between species and between sexes of the same species (Figs. 7.1-7.5). Males and females of *I. pygmaeus, L. noctiluca* and *S. lessoniana* had similar L-W relationships (ANCOVA $p>0.05$). Males and females of *L. chinensis* and *Loligo* sp. 1 had significantly different slopes ($p<0.05$).

Moreover, of the other three species only *I. pygmaeus* showed any significant difference in elevation ($p<0.05$) (Table 7.1).

All five species had allometric growth with slopes less than three, although *L. noctiluca* was close to isometric growth. The lower value of *b* indicated that length was increasing at greater rate relative to weight. This is especially apparent in *Loligo* sp. 1 males. While the female slope value lies within a range similar to the other species ($b = 2.274$), the male slope value was exceptionally low ($b = 1.420$) (Table 7.2). This reflected the very different body proportions of the males as compared to the females. The males of this species grew considerably longer than their female counterparts. Moreover, they grew primarily in length while maintaining a very thin mantle. Some of the males of *Loligo* sp. 1 were exceptionally thin as displayed in the non-logged scatter plot (Fig. 7.4A) with considerable scatter along the length axis. Despite the very different body proportions of these males they have been identified as the same species (C.C. Lu, personal communication).

Data available from other localities provided some indication on geographic variability in the L-W differences displayed by *S. lessoniana.* The slopes obtained for *S. lessoniana* in Australia were considerably less than those recorded elsewhere. Values for *S. lessoniana* in Australia were near two ($b=2.075$ males, 2.08 females). Values of *b* for both wild caught and cultured individuals of *S. lessoniana* ranged between 2.553 and 2.675 (Segawa, 1987), while in Indian waters these values were 2.45 and 2.66 for males and females respectively (Rao, 1954). Slopes of the males for Australia and India and for both sexes of cultured and wild caught specimens in Japan, indicate that the Australian population has a very different length-weight relationship than this species in other parts of the world (Fig. 7.6).

7.3.2 Growth modelling

7.3.2.1 *Loligo chinensis*

Obtaining smaller paralarvae from the light traps provided a more comprehensive description of growth in both length and weight for *L. chinensis* (Fig. 7.7). Obtaining a hatchling from an egg mass laid by an identified *L. chinensis* female further aided the growth modelling by providing precise information on the length and weight at hatching (although the range in hatchling size is currently not known).

Both the length and weight data appeared to be exponential in form with no suggestion of an asymptote being reached (Fig. 7.7). Plotting the data on a semi-log graph revealed that the curve could be adequately described by an exponential function (ie, a straight line was produced). Subsequent regression analyses revealed high r^2 values for both males and females for both age-length and age-weight curves. Equations for male and female growth in weight were:

males $y=0.00392\text{x}10^{(0.04608x)}$ $r^2=0.9262$

females $y=0.00346\text{x}10^{(0.04679x)}$ $r^2=0.9206$

while equations for male and female growth in length were:

males $y=3.085\text{x}10^{(0.01863x)}$ $r^2=0.9486$

females $y=2.910\text{x}10^{(0.01820x)}$ $r^2=0.9125$

where y is weight in grams or mantle length in millimetres and x is age in days. Analysis of covariance revealed that there was no significant difference in the slopes or elevations between the growth curves of males and females for both length and weight (Table 7.3).

The plotting of the growth curves enabled a better visualisation of the form of growth during the summer period for *L. chinensis*. Both growth in length and weight showed an initial extended paralarval/juvenile growth phase, followed by achievement of a large body size over a relatively short period of time, coincident with the adult maturing phase. This was especially accentuated with the form of growth in weight (Fig. 7.8B) where there was negligible weight gain for over half of the estimated lifespan, with the majority of the weight increase taking place in the last 20% of the estimated lifespan.

7.3.2.2 *Sepioteuthis lessoniana*

Sepioteuthis lessoniana males (captured during the summer period) and paralarvae/juveniles was the other data set in which size and age data was available for a full size range from hatchling to adult (Fig. 7.9). The data set for adult females was too limited to attempt curve fitting with any degree of accuracy. Plotting the data for both length and weight-at-age on semi-log and log-log graphs did not result in a linear relationship. However, the semi-log plot revealed an initial exponential phase to approximately 70 d (appearing curvilinear on an arithmetic plot and linear on a semi-log plot) and an adult linear phase thereafter to 153 d (appearing linear on an arithmetic plot and curvilinear on a semi-log plot) (Figs. 7.9, 7.10). Therefore, these two phases of male growth were described by separate equations. The equation for growth in length during the initial exponential phase (data points up to day 67) was:

$$y=4.726\times10^{(0.02082x)} \qquad r^2=0.9512$$

while growth in length for the linear phase (data points between 81 to 153 d) was:

$$y=1.25x + 29.075 \qquad r^2=0.7986$$

Growth in weight was described for the initial exponential phase by the equation:

$$y=.1459\times10^{(0.0378x)} \qquad r^2=0.9486$$

while growth in weight over the subsequent linear phase was described by the equation:

$$y=3.85x - 200.55 \qquad r^2=0.8441$$

where y is body weight in grams, or mantle length in millimetres and x is age in days.

Plotting these growth equations revealed a more disjunct form of growth in *S. lessoniana* (Figs. 7.11A+B) compared to the more regular exponential growth of *L. chinensis* (Figs. 7.8A+B). Growth in weight was more regular in *S. lessoniana* with less of a demarcation between the exponential and the linear phase (Fig. 7.11B) than was growth in length (Fig. 7.11A).

The growth model developed for male *S. lessoniana* bears some resemblance to the growth model of *L. chinensis* in that there is an initial extended paralarval/juvenile phase, followed by achievement of adult size over a relatively short time period. Furthermore, there was no obvious asymptote or levelling off of growth for both species. However, adult *L. chinensis* individuals appeared to grow more rapidly than adult *S. lessoniana* individuals (exponential versus linear adult growth) and the juvenile phase of *S. lessoniana* was less extended than that of the summer population of *L. chinensis*.

7.3.3 Comparisons of growth models with other research

7.3.3.1 *Loligo chinensis, Loligo forbesi and Loligo opalescens*

Growth modelling with other loliginids has been carried out with culture experiments. Forsythe & Van Heukelem (1987) provided data for average daily growth in weight for *L. opalescens* and *L. forbesi* based on culture data. Comparing the growth curves of these temperate species with that of *L. chinensis* allowed a comparison between them (Fig. 7.12). All three species show a similar growth form with an initial slow juvenile phase followed by a rapidly increasing adult phase concurrent with maturity. However, the juvenile phase of the summer population of *L. chinensis* is considerably shortened compared to the other species with a greater weight being achieved in 30 to 50% of the time taken for the other species to reach equivalent weights.

The rapid growth of *L. chinensis* in comparison to the other loliginids was of interest in the light of comparative squid sizes. *L. opalescens* is relatively small, less than 200 mm in mantle length whereas *L. forbesi* is very large, reaching 900 mm and 410 mm in length for males and females respectively (Roper *et al.*, 1984). The size of the near-shore *L. chinensis* population is similar to the size range of *L. opalescens*.

These growth models are derived from different species which come from different environments. Furthermore, since the models are derived using two different techniques, (size-at-age of field captured individuals versus culture experiments), it would be a decided advantage to be able to compare both techniques on the same species faced with similar environmental constraints. This was possible with *L. opalescens* as data was available from both aged field-captured specimens and culture experiments. Growth data for *L. opalescens* from culture experiments (Yang *et al.*, 1986) were superimposed on length-at-age data from aged individuals captured in the vicinity of Monterey Bay, California (Fig. 7.13). Unfortunately only relatively large adult individuals were available for age analysis. Statolith aged wild squids appeared to be somewhat faster growing then the cultured squids, although some of the wild squids fell within the length range of the cultured squids.

7.3.3.2 *Sepioteuthis lessoniana and Sepioteuthis sepioidea*

Growth data was also available for cultured individuals of the Caribbean reef squid *Sepioteuthis sepioidea* (Forsythe & Van Heukelem, 1987). Although this species is smaller (maturity can be reached at 90 mm) (Roper *et al.*, 1984) it is of interest to compare the form of growth in this species with *S. lessoniana* as they are in the same genus and both inhabit tropical waters. When the growth models of these two species were superimposed and scaled for weight, they showed a close similarity in the form of growth based on age (Fig. 7.14). Both growth models indicated a very similar pattern of growth with approximately equal proportions of time spent in the paralarval/juvenile phase (with only negligible growth taking place) and the rapidly increasing adult phase. Both species showed little increase in weight up to 50 to 60 d, followed by a rapid period of growth into the adult phase up to 140 to 150 d.

7.3.3 Cuttlefish

Growth models derived from culture experiments (Forsythe & Van Heukelem, 1987) for other semi-pelagic cephalopods, the cuttlefish, show a form of growth similar to squids (Fig. 7.15). All three species of *Sepia* also have a prolonged paralarval/juvenile phase in which little increase in weight takes place, followed by a rapid growth phase coincident with the adult phase and reproductive maturity. The culture experiments with these cuttlefish also indicate that after the initial extended juvenile phase, growth is extremely rapid with adult size reached in less than a year. This gives further evidence that life cycles of cephalopods (excluding *Nautilus*) in general are probably much shorter than previously estimated.

7.3.4 Growth of the giant squid *Architeuthis*

Although considerable knowledge has accumulated on near-shore cephalopods which are easily obtained and cultured, many species which inhabit open oceanic waters, are rarely ever cultured or observed alive. Growth modelling for these species should be possible if accurate size-at-age estimates can be obtained from statoliths. Cephalopods are unusual in that they show a huge range in size from the tiny *Idiosepius* to the huge *Architeuthis* the largest of all invertebrates. The possibility of obtaining age information from the giant squid at the far end of the cephalopod size continuum would greatly increase knowledge of the growth dynamics of the group as a whole.

The preparation of the statolith from the juvenile *Architeuthis* revealed that this species has a very clear periodic ring sequence within the statolith microstructure (Fig. 7.16). Subsequent ring counts resulted in little variation between counts: 154, 154, 150, mean = 153, standard deviation = 2.31. These rings were similar in structure to rings observed in other species which have been validated as occurring daily. If these rings are daily in occurrence then it is possible that *Architeuthis* may have a very short life cycle as well. More data on size-at-age of other specimens covering the size range of this species would give some indication of the growth dynamics of this huge species.

7.4 DISCUSSION

7.4.1 Allometry

Allometry can be considered the study of size and its consequences, or the way in which shape changes as the magnitude of the organism changes (Gould, 1966). Constancy in shape with change in size is termed isometric growth while departure from the geometric similarity is termed allometry (LaBarbera, 1989).

The logged length-weight relationships of the five study species provided a useful means to compare differences in their relative body proportions, covering an order of magnitude of difference in body size. Although this technique has been criticised, it is considered appropriate in the majority of cases, and its simplicity and convenience has made it the method of choice for most workers (LaBarbera, 1989).

While many octopods exhibit isometric growth ($b=3$), loliginid squids generally do not. Loliginid squids generally have slopes less than 2.7, while the larger more robust ommastrephids have slopes more similar to octopods (2.7 or higher). This has been interpreted in terms of the general morphological differences of the two groups. Ommastrephids have very thick muscular mantle walls, while loliginids are smaller and lighter, with much thinner mantle walls. The resulting differences in L-W slopes arises from ommastrephids increasing in weight more rapidly per unit increase in mantle length than do the loliginids (Forsythe & Van Heukelem, 1987).

The values obtained from the four loliginids and *Idiosepius* agree with the previous suggestions, as all species have slopes very near or less than 2.7. Except for *Loligo* sp. 1 and *L. chinensis* ,the L-W relationships are similar for both sexes of the other three species (slopes ranging between 2.08-2.68) despite very different body shapes and sizes.

The biogeographic differences in the L-W relationship shown in the different populations of *S. lessoniana* revealed that different populations of squids may show different allometric relationships. The evidence suggested that northern hemisphere populations of *S. lessoniana* in Japan and India were heavier per unit length than the population in Australia. Statistically significant morphometric differences have also been noted for other parameters (mantle width, fin length, fin width, length of arms I-IV, and tentacle club length) for Australian, Indonesian and Philippine populations of *S. lessoniana* (Lu & Tait, 1983). Lu & Tait (1983) attributed these morphological differences to not only distance factors but also to areas of deep water separating populations of this species. It is therefore not surprising that the populations of *S. lessoniana* from Australia, Japan and India show different length-weight relationships. This situation may be attributable to the great degree of geographic separation between these three populations.

In contrast to the biogeographic L-W data for *S. lessoniana*, *L. chinensis* appears to show less plasticity in this relationship. Length-weight values for *L. chinensis* in the Gulf of Thailand were closer to values obtained for this species in Australia. Supongpan (1988) reported that values of *b* for *L. chinensis* of Thailand were close to 2.0 with a range of mean monthly values of 1.4 to 2.09, while Ratana-anan (cited in Supongpan, 1988) reported values of 1.87 and 2.09 for males and females respectively. Thus the values for *L. chinensis* are more similar over these two geographic localities. However, the situation for this species is opposite to that obtained for *S. lessoniana,* as the Australian population of *L. chinensis* was slightly heavier per unit length than the Gulf of Thailand population. Since the length-weight relationship can change over time for one species (eg. Dawe, 1988) found both intra- and inter-yearly variation in length-weight values for *I. illecebrosus*), more detailed analysis from different seasonal periods would aid in the understanding of the change in the relationship between length and weight over time for these tropical species. .

Although two of the study species (*I. pygmaeus* and *Loligo* sp. 1) showed considerable sexual dimorphism, they both had very different L-W relationships between the sexes. *Idiosepius pygmaeus* females reached a considerably greater size than the males and showed a significant difference in the elevations of the slopes. However, the slopes were very similar (2.3, 2.5 for males and females respectively) indicating that the extreme sexual dimorphism does not effect the relative body proportions of each sex. In contrast, males and females of *Loligo* sp. 1 had very different L-W relationships with significantly different slopes. The thinness of the males can be appreciated by the very low value of b (1.42). Thus males were growing considerably in length but proportionally less in weight than were the females. To a certain extent this was probably a reflection of a greater proportion of growth being allotted to gonads in females than in males.

It is unclear what advantages there might be in the males of *Loligo* sp. 1 having a more pronounced body elongation than females. This feature is also shown in other loliginids, especially of the genus *Alloteuthis*. For example, *A. subulata* males have an extended posterior mantle with males reaching 200 mm and females reaching only 120 mm. Similarly *A. africana*, displays very extreme body elongation with males having a long thin 'tail' region of equal length to the mantle. Males of this species reach 190 mm in length while females reach only 90 mm (Roper *et al.*, 1984). Allometric studies would prove useful in these very thin species to compare the extent of differences between the sexes, as well as variation in form within the loliginids generally.

7.4.2 Modelling of squid growth

There is currently no reliable basis in physiology or ontogeny for constructing a general growth model for squids. The describing of squid growth in the past has been plagued with uncertainties and a variety of growth equations have been used to describe growth. These have included linear, asymptotic, cyclic, exponential and sigmoidal equations (see Hixon, 1980; Mangold, 1983). The majority of these squid growth curves have resulted from studies of regular sampling of field populations, such as length frequency analysis, often employing the von Bertalanffy equation (eg. Worms, 1983; Pauly, 1985; Supongpan, 1988; Ueta & Jo, 1989). However, the application of length frequency analysis to cephalopod data must be used cautiously to avoid reaching wrong conclusions (see chapter 5).

The fact that cephalopods grow faster than previously thought has probably been a major factor leading to erroneous conclusions based on length frequency analysis. Furthermore, because length frequency analysis lacks precision, it is advantageous to be able to develop squid growth models using more direct means. These includes culture experiments and statolith age analysis.

7.4.3 Growth models derived from culture experiments

There have been recent advances in information on growth of cephalopods in captivity since the development of advanced squid culturing techniques, particularly at the Marine Biomedical Institute, Galveston, Texas (see Hanlon & Hixon, 1983; Hanlon *et al.*, 1983; Yang et al., 1986). This institute has been first to culture many cephalopods through their life cycle, including several of the loliginid squid species. Many of these culturing experiments have focused on the derivation of growth models.

Squid culture work has shown in a direct way that cephalopods are capable of rapid growth, early reproduction and short lifespans. Growth over the paralarval/juvenile and sub-adult phase of the life cycle can be adequately described by exponential equations; *L. vulgaris* to 140 d (Turk *et al.*, 1986), *L. pealei* to 6 months (Hanlon *et al.*, 1987), and *L. opalescens* to 8 months (Yang *et al.*, 1983).

However, with further success in culturing a number of individuals throughout the life cycle, it was found that growth was often best described in two phases, an initial exponential phase (in which a plot of weight or length verses age produces a straight line on semi-log paper) and a second logarithmic phase (producing a straight line on log-log paper) described by the power equation (Forsythe & Van Heukelem, 1987).

More detailed analysis of growth of *L. opalescens* revealed that growth in length and weight could accurately be described by an initial exponential phase up to day 60, followed by a logarithmic phase from 60 to 243 and 248 days based on two different experiments (Yang *et al.*, 1986).

Growth for *L. forbesi* was found to be somewhat different. While growth in length was described in two phases (exponential for the first 19 to 100 d and logarithmic to 323 and 400 d in two separate experiments), growth in weight could be described as exponential throughout its entire lifespan.

More recently *S. lessoniana* has also been raised throughout its lifespan and has been shown to grow phenomenally fast, with a maximum size of 360 mm ML and 2200 g reached on only 184 d at 25°C (Hanlon, 1990). Description of growth of this species under various temperatures is currently in preparation (R. Hanlon, personal communication).

Similar growth models of an exponential phase followed by a logarithmic phase have also been described for the benthic octopods *Octopus joubini* (Forsythe, 1984), *O. digueti* (DeRusha *et al.*, 1987) and *O. bimaculoides* (Forsythe & Hanlon, 1988).

Although culture conditions may or may not reflect growth in the field, it does give insights into the growth capabilities of these organisms. These recent culture experiments all suggest that cephalopod growth is rapid and continuous with termination of the lifespan before any obvious asymptote is reached.

7.4.4 Growth models derived from statolith ageing

Growth modelling based on statolith age estimates provides a good basis for comparison with growth parameters determined from culture experiments, since size-at-age can be determined from field captured individuals. However, there is still some uncertainty in the general application of statolith growth rings to ageing studies until further validation work can be carried out with more species.

Growth was found to be linear for the boreal squid *Gonatus fabricii*, with a lifespan of up to 1½ years (Kristensen, 1980). Based on Kristensen's data, growth appears to be very regular (linear) over the duration of the lifespan, although these conclusions are based on only ten data points. However, the scatter of points cover a wide length range from very small individuals (8 mm) to adults up to 150 mm in length.

Analysis of age data for *I. illecebrosus* in two separate analyses resulted in a curvilinear relationship between age and length (Hurley & Beck, 1979; Lipinski, 1981), best described by a power function. These showed that growth was rapid in this species and non-asymptotic.

More recently detailed growth modelling has been carried out with two Japanese loliginids *Photololigo edulis* (Natsukari *et al.*, 1988) and *Heterololigo bleekeri* (Kinoshita, 1989). Both of these studies were based on statolith ring counts of a large number of individuals, although as of yet no ring validation experiments have been carried out with these species. Although the lifespans of these species were estimated to be short (< 1 year) these researchers fitted a logistic curve to the data. The data for the females of both species suggest that a logistic curve might be appropriate, however, the data for the males do not appear to be logistic in form. Digitised data from Natsukari *et al.* (1988) was therefore analysed to determine the adequacy of fitting a logistic curve to the data.

7.4.5 Analysis of Natsukari's method

The data given for *P. edulis* by Natsukari and co-workers is very similar in form to that of *L. chinensis* in this study, that is, an extended period of juvenile growth followed by a more rapid increase in size coincidental with the adult phase and maturity. Furthermore, there was considerable scatter in the data indicating a wide variation in individual growth rate. Natsukari *et al.* (1988) stated that growth curves could not be derived directly from the data. They therefore divided the data in to 22, ten increment classes and calculated the mean mantle length for each increment class. The data was further smoothed by taking moving averages of five classes. These moving averages were then used for fitting to a logistic equation.

The applicability of this technique is questionable with organisms which have a very large scatter in age for any given length. Data points representing x and y values for older individuals can be truncated off from the data set and used to create an 'artificial' data point on the graph, which is subsequently used to fit a curve. More correctly, fitting a curve through any set of data points (at a given mantle length) should ideally take into account the entire range in length-at-age.

It is unclear why Natsukari and co-workers stated that growth curves could not be derived directly from the data. On the contrary, plotting the digitized data of the male (warm-season brood) on a semi-log plot resulted in a straight line, indicating that the data was exponential in form. An exponential curve could thus be fitted to the data (Fig. 7.17) with the resulting equation being:

$$y=10.71\mathrm{x}10^{(0.005x)} \qquad r^2=0.793$$

where y is mantle length in millimetres and x is age in days.

Plotting of female digitised data of the warm-season brood on a semi-log plot did not result in a linear relationship over the whole data set. However, the initial phase of growth (up to 180 d) resulted in a straight line on the semi-log plot while the remaining data points (180 to 360 d) resulted in a straight line on a log-log plot. This indicated that growth was in two phases, an initial exponential phase followed by an adult phase which is logarithmic. Thus growth in *P. edulis* can be described based on techniques used for other cultured species (eg. Yang *et al.*, 1986; Forsythe & Van Heukelem, 1987). Growth models which incorporate all the data are preferable to other techniques such as taking moving averages. This is especially relevant to organisms such as squids which display a wide variation in size-at-age.

7.4.6 Growth of *L. chinensis* and *S. lessoniana*

The resulting growth models for both *L. chinensis* and *S. lessoniana* demonstrated both the similarities and differences in models of cephalopod growth. The data for both species agreed with the bulk of the literature in displaying an initial extended phase of paralarval/juvenile growth followed by subsequent rapid development into mature adults. However, there were differences in the growth models developed. Although the summer population of *L. chinensis* conformed to an exponential model throughout its lifespan, growth of *S. lessoniana* could be compartmentalised into two different phases similar to cultured species previously discussed. The growth model for *S. lessoniana* bears similarity to earlier models described by Forsythe (1984) and Yang *et al.* (1986) with the major difference being that the adult phase was best described by a linear function rather than a power function.

There is currently no simple model to describe cephalopod growth. The overall trend appears to be curvilinear growth with either an exponential model, or a two-phase model consisting of an exponential plus a subsequent non-asymptotic phase. Alternatively, linear models may be appropriate in some instances. More research is needed to unequivocally show if logistic growth is an appropriate model for the growth of some species.

7.4.7 Future considerations

Further comparative work using both cultured and aged field specimens of the same species will prove useful in gaining an understanding of squid growth. Growth rates in the field may be different than those obtained from culture experiments as suggested by Yang *et al.* (1983, 1986). Culture environments may also extend the life of cephalopods (Hanlon *et al.*, 1989). *Loligo opalescens* has been shown to mature precociously in captivity (Yang *et al.*, 1986). This feature would account for the observed difference in the length-at-age data from the cultured versus field aged specimens of this species (Fig. 7.13). Alternatively, length-at-age data for two cultured specimens of *S. lessoniana* have shown that growth of the Australian population conforms closely to results obtained from cultured Japanese individuals (see chapter 4, Fig 4.9). It would be of interest to age field specimens of *L. forbesi* to compare the resulting growth curve with the extensive culture data base of this species (Forsythe & Hanlon, 1989; Hanlon *et al.*, 1989).

An intriguing aspect of squid demography is the period of extended juvenile growth evident in age-weight relationships (see Fig. 7.8B). Data for other species also suggests an extended juvenile period, *Todarodes sagittatus* (Rosenberg *et al.*, 1981), *L. opalescens* (Yang *et al*, 1986), *P. edulis* (Natsukari *et al.*, 1988), *L. forbesi* (Forsythe & Hanlon, 1989) and *H. bleekeri* (Kinoshita, 1989). Despite rapid achievement of adult body size during the maturing,adult phase, squids appear to spend a large proportion of their lives as small individuals. This contrasts with the general pattern for teleost fishes, in which there is initial rapid juvenile growth followed by an asymptotic adult phase. The observed time lag in the growth of juvenile cephalopods contradicts current ecological theory of cephalopod growth and reproduction. O'Dor & Wells (1987) suggested that small squid should be able to feed and grow more rapidly to reach a size in which they are accessible to fewer predators. Furthermore, Calow (1987) and Hiller (1990) have supported the theory that high juvenile growth rates would reduce juvenile mortality by "speeding individuals through a vulnerable phase". This in turn is proposed to counteract early mortality in the adults, since survival of the offspring would be high. However, further developments and refinements of squid growth models in this study and in the literature have accentuated the fact that in comparison to the adult phase, the juvenile phase is an extended component of the life cycle. Growth is in fact diminutive during the paralarval/juvenile phase in comparison to the adult phase of growth in squids.

As more precise and accurate data are becoming available from statolith ageing and culture studies, it should be possible to further refine growth models for a variety of temperate and tropical squid species. This will in turn provide the needed information to better apply ecological theories to interpret squid growth.

Table 7.1: **Summary of the comparisons of logged length-weight regression equations for each of the study species based on analysis of covariance.**

Idiosepius pygmaeus	Male vs Female	Slope F=2.21 Elevation F=5.14	Pr>F 0.1396 Pr>F 0.0251	ns Significant
Loliolus noctiluca	Male vs Female	Slope F=0.31 Elevation F=0.27	Pr>F 0.5823 Pr>F 0.6074	ns ns
Loligo chinensis	Male vs Female	Slope F=14.4	Pr>F 0.0002	Significant
Loligo sp. 1	Male vs Female	Slope F=7.96	Pr>F 0.0075	Significant
Sepioteuthis lessoniana	Male vs Female	Slope F=0.22 Elevation F=0.04	Pr>F 0.6420 Pr>F 0.8426	ns ns

Table 7.2: Values of the constants *a* and *b* from the log-transformed length-weight regression analysis.

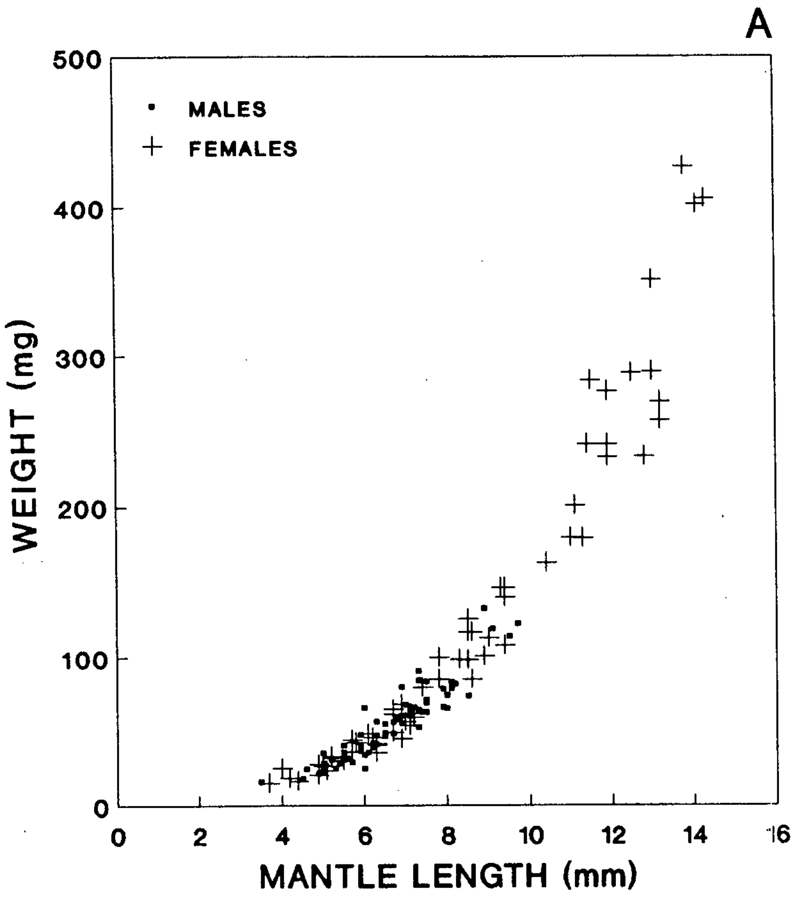
Species	*a*	*b*	r^2
Idiosepius pygmaeus			
males	-0.206	2.336	0.8865
females	-0.323	2.512	0.9721
Loliolus noctiluca			
males	-3.861	2.707	0.9943
females	-3.827	2.680	0.9989
Loligo chinensis			
males	-2.823	2.167	0.9710
females	-3.249	2.399	0.9790
Loligo sp. 1			
males	-1.482	1.420	0.8622
females	-3.213	2.274	0.8826
Sepioteuthis lessoniana			
males	-2.409	2.075	0.9461
females	2.411	2.080	0.9328

Table 7.3: Summary of the comparisons of regression equations for male and female *Loligo chinensis* for age versus log-weight and age versus log-length based on analysis of covariance.

Age versus log weight	Male versus Female	Slope F=0.06 Elevation F=0.02	Pr>F 0.8071 Pr>F 0.8879	ns ns
Age versus log length	Male versus Female	Slope F=0.16 Elevation F=0.18	Pr>F 0.6901 Pr>F 0.6724	ns ns

Figure 7.1

Length-weight relationship for *Idiosepius pygmaeus*. (A) Arithmetic plot. (B) Logarithmic plot.


A
MALES
FEMALES
WEIGHT (mg)
500
400
300
200
100
0
0
2
4
6
8
10
12
14
16
MANTLE LENGTH (mm)

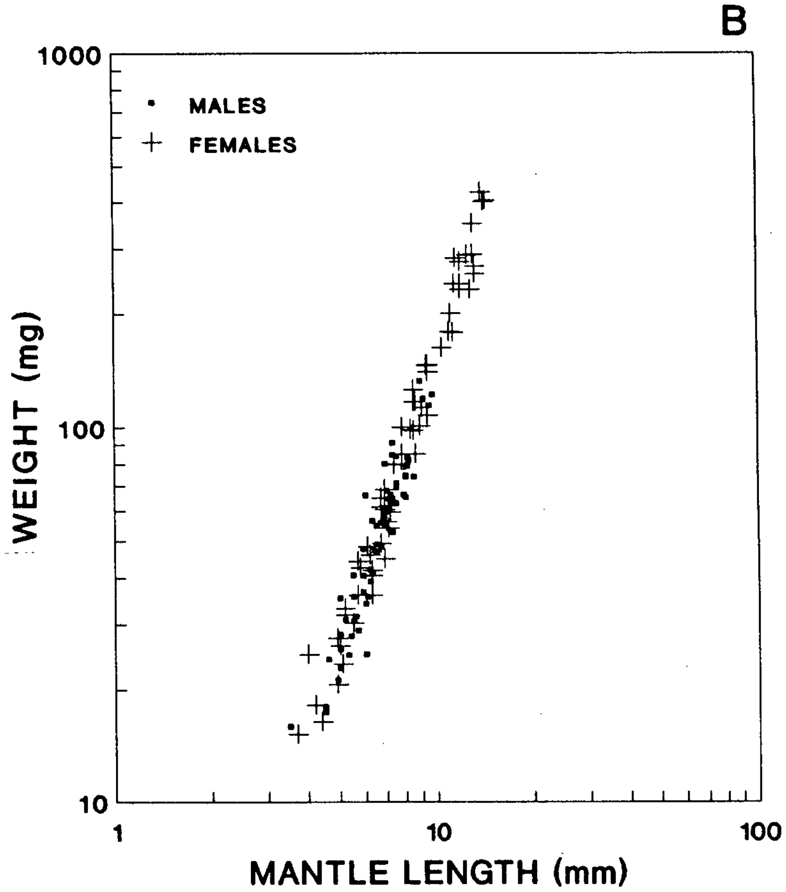

B
MALES
FEMALES
WEIGHT (mg)
1000
100
10
1
10
100
MANTLE LENGTH (mm)

Figure 7.2

Length-weight relationship for *Loliolus noctiluca.* (A) Arithmetic plot. (B) Logarithmic plot.

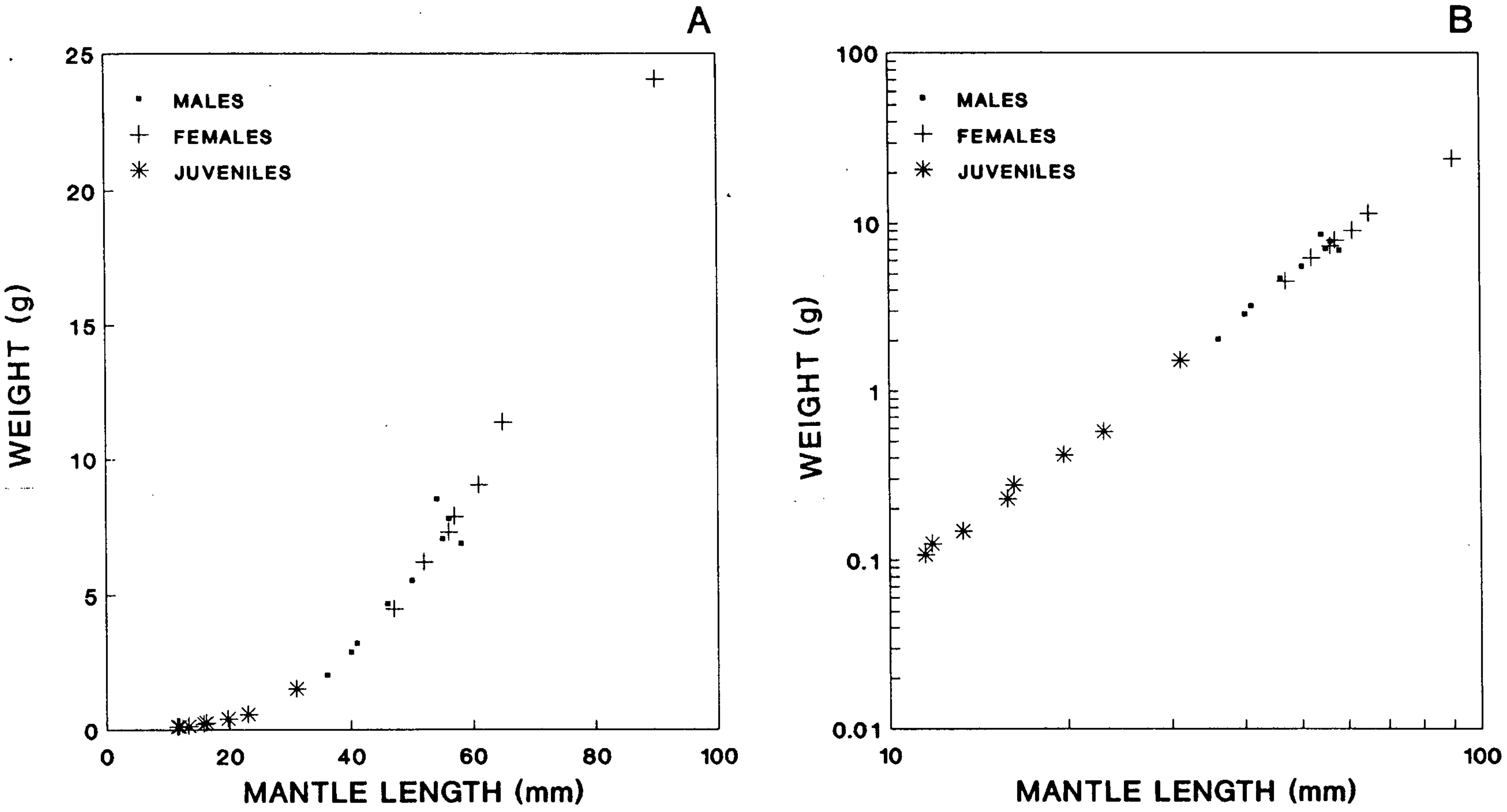

A
B
MALES
FEMALES
JUVENILES
WEIGHT (g)
MANTLE LENGTH (mm)
0
5
10
15
20
25
0
20
40
60
80
100
0.01
0.1
1
10
100
10
100

Figure 7.3

Length-weight relationship for *Loligo chinensis*. (A) Arithmetic plot. (B) Logarithmic plot.

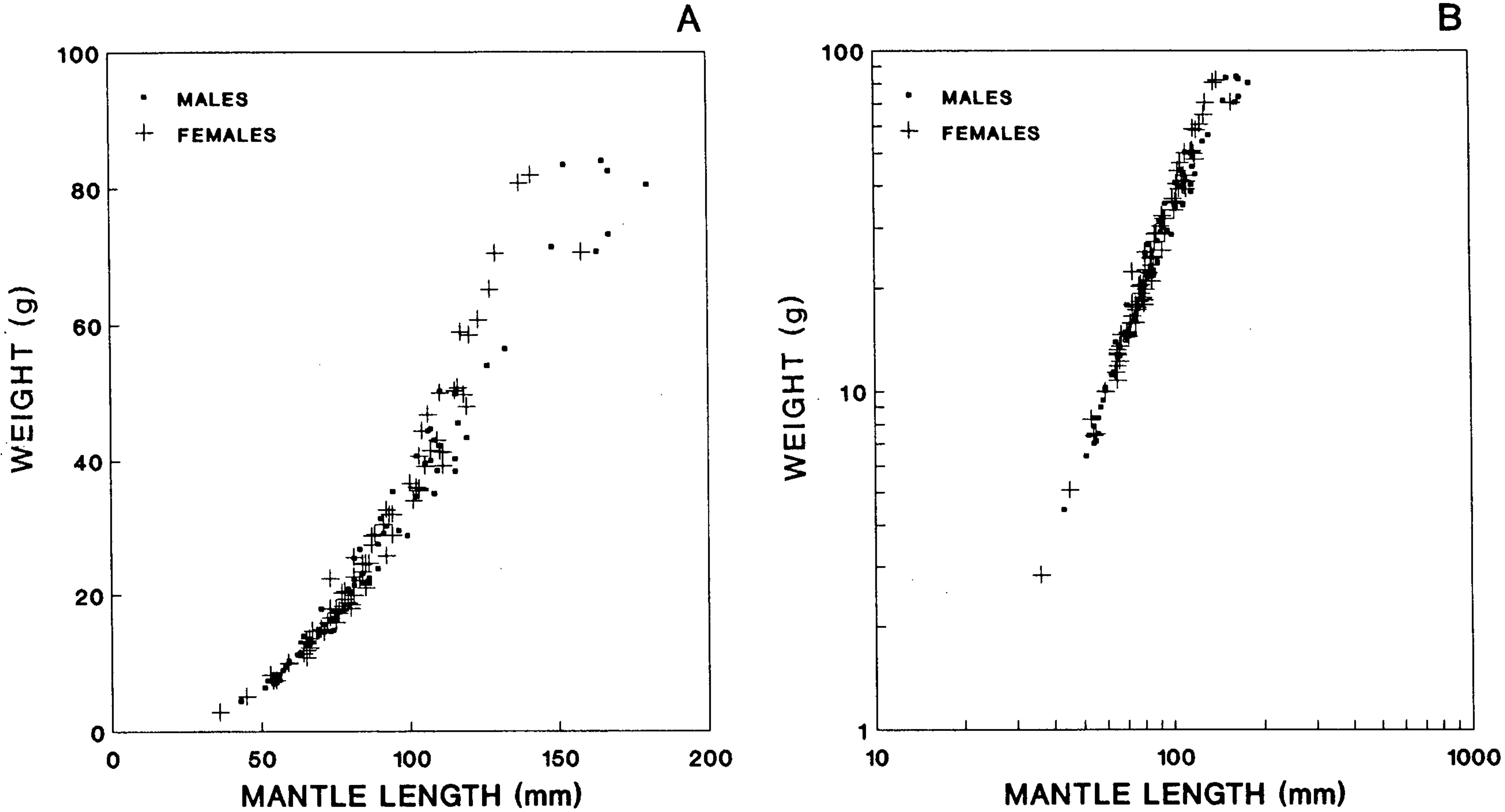

A
MALES
FEMALES
WEIGHT (g)
100
80
60
40
20
0
0
50
100
150
200
MANTLE LENGTH (mm)
B
MALES
FEMALES
WEIGHT (g)
100
10
1
10
100
1000
MANTLE LENGTH (mm)

Figure 7.4

Length-weight relationship for *Loligo* sp. 1. (A) Arithmetic plot. (B) Logarithmic plot.

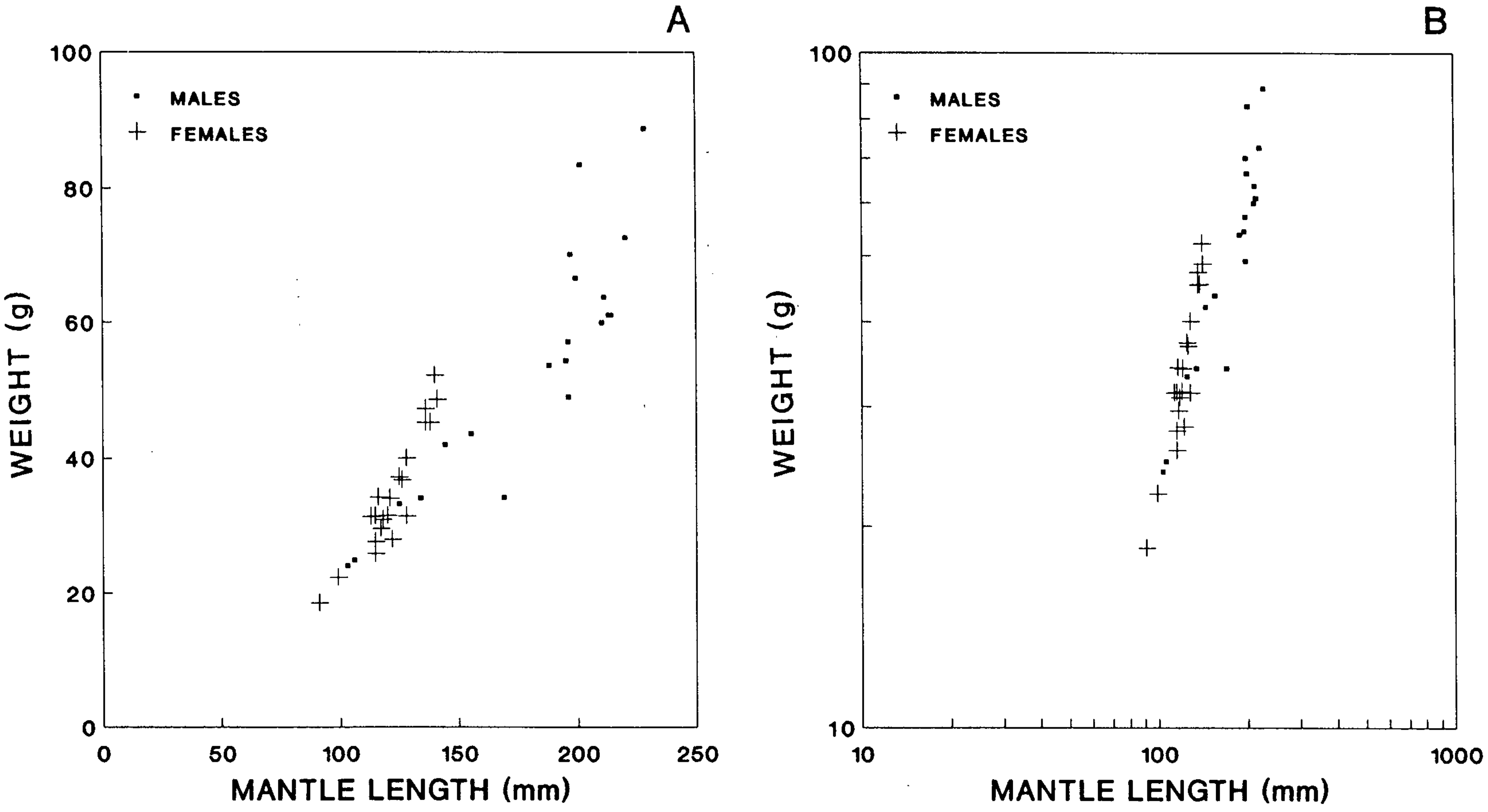
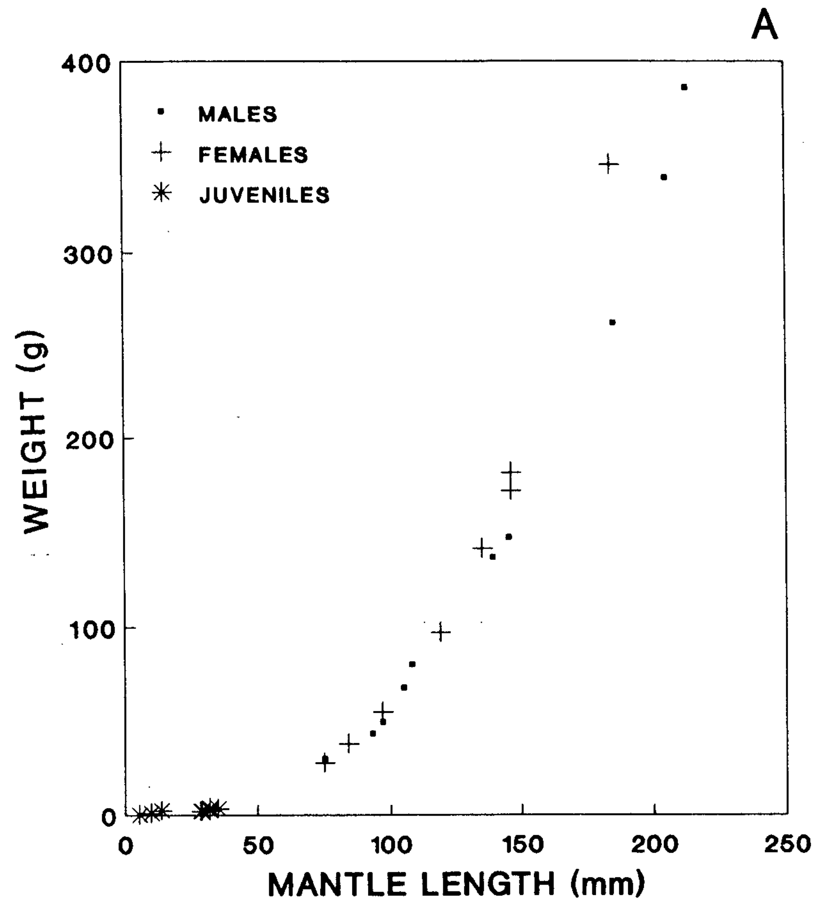

A
MALES
FEMALES
WEIGHT (g)
0
20
40
60
80
100
MANTLE LENGTH (mm)
0
50
100
150
200
250
B
MALES
FEMALES
WEIGHT (g)
10
100
MANTLE LENGTH (mm)
10
100
1000

Figure 7.5

Length-weight relationship for *Sepioteuthis lessoniana.* (A) Arithmetic plot. (B) Logarithmic plot.


A
WEIGHT (g)
400
300
200
100
0
MALES
FEMALES
JUVENILES
0
50
100
150
200
250
MANTLE LENGTH (mm)

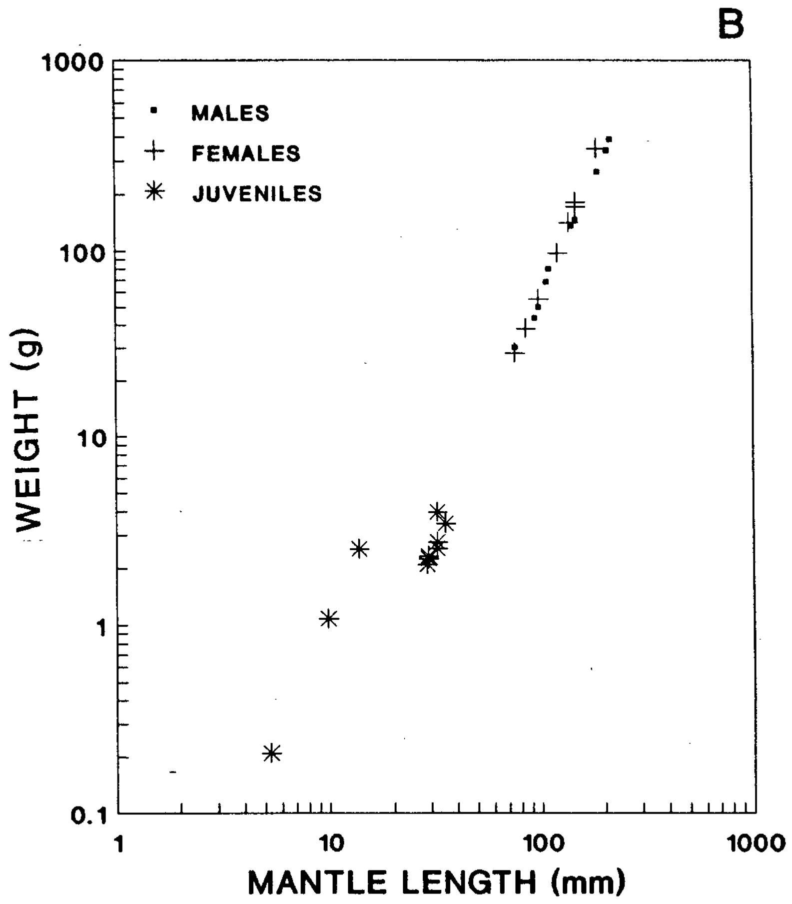

B
WEIGHT (g)
1000
100
10
1
0.1
MALES
FEMALES
JUVENILES
1
10
100
1000
MANTLE LENGTH (mm)

Figure 7.6

Length-weight relationship for *Sepioteuthis lessoniana* from Australian, Indian, Japanese field and Japanese cultured populations (see text for details).

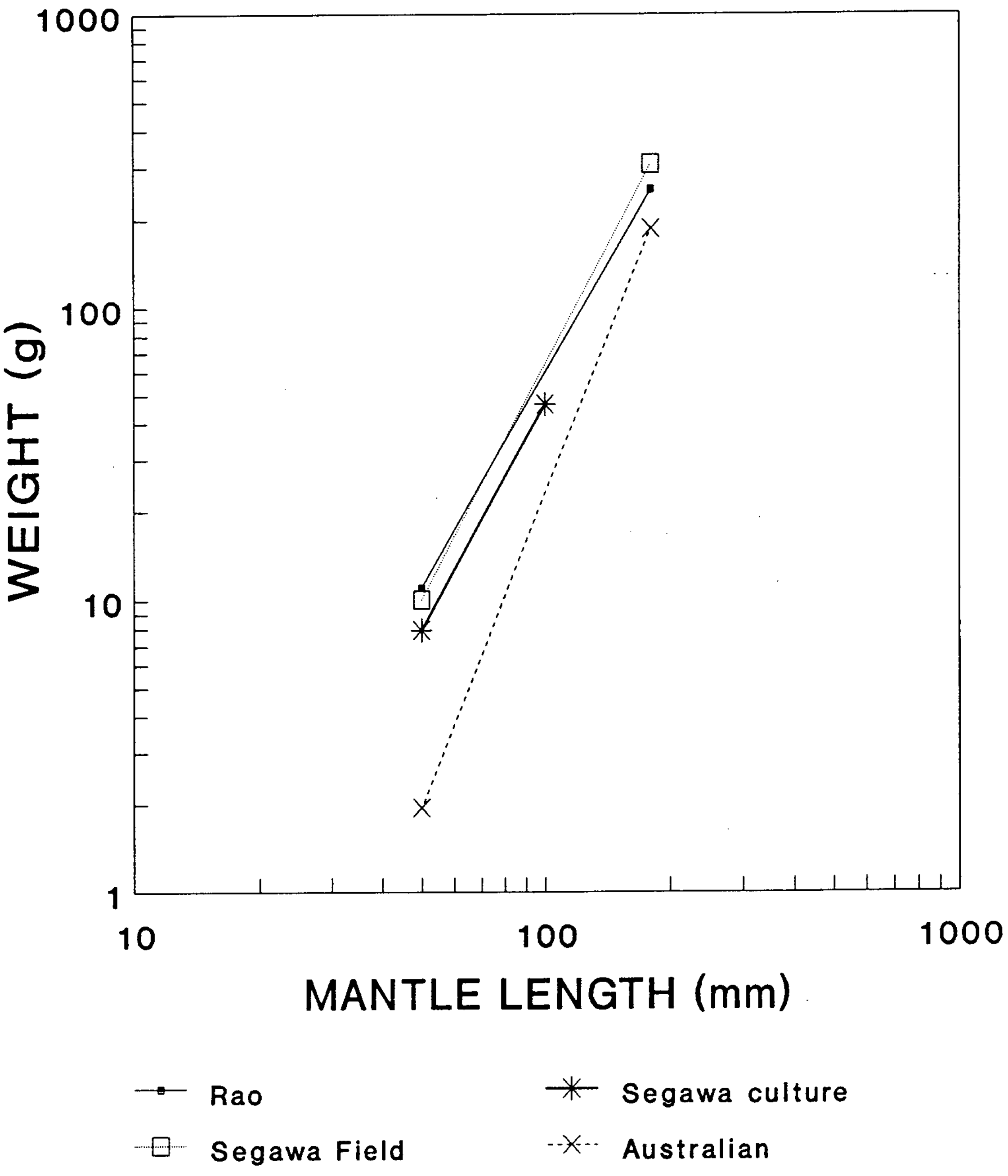

1000
100
10
1
WEIGHT (g)
10
100
1000
MANTLE LENGTH (mm)
Rao
Segawa Field
Segawa culture
Australian

Figure 7.7

Relationship between (A) mantle length and age, and (B) weight and age, for individuals of *Loligo chinensis* (captured in January 1989), paralarval *Loligo* (captured in light traps during December 1989) and a newly hatched *Loligo chinensis*.

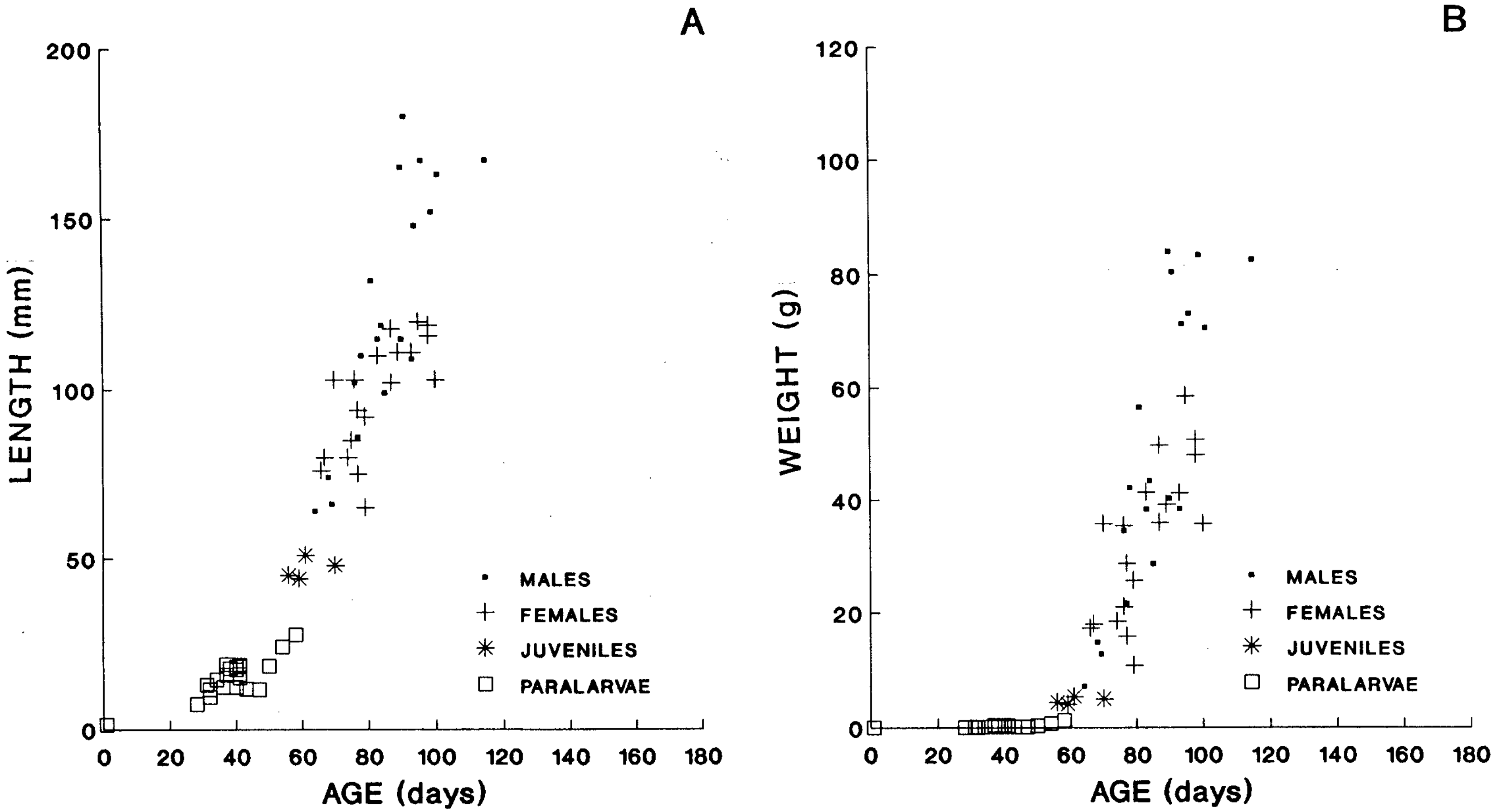

A
200
150
100
50
0
LENGTH (mm)
0
20
40
60
80
100
120
140
160
180
AGE (days)
MALES
FEMALES
JUVENILES
PARALARVAE
B
120
100
80
60
40
20
0
WEIGHT (g)
0
20
40
60
80
100
120
140
160
180
AGE (days)
MALES
FEMALES
JUVENILES
PARALARVAE

Figure 7.8

Exponential growth curves determined for *Loligo chinensis,* for both growth in (A) mantle length, and (B) weight.

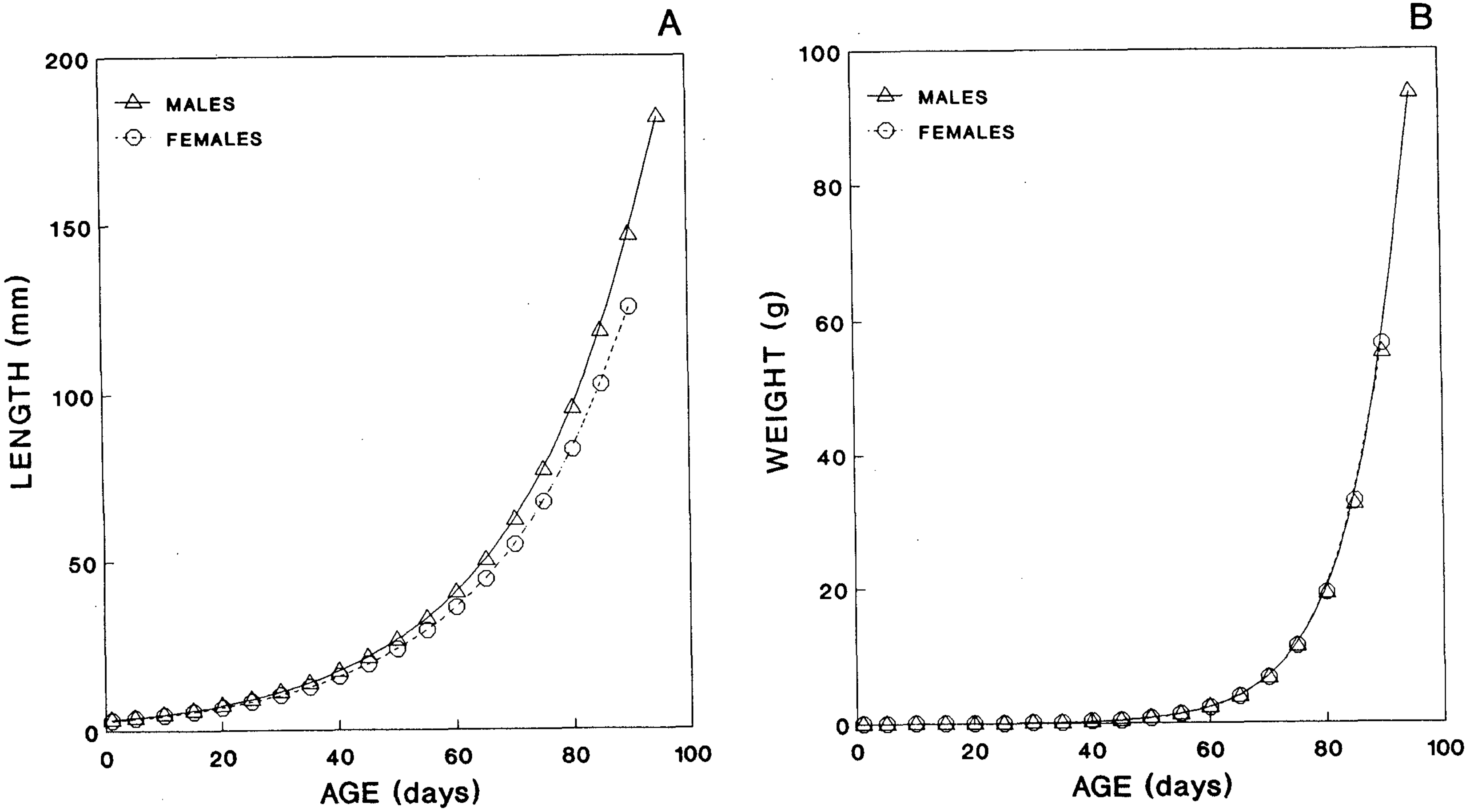
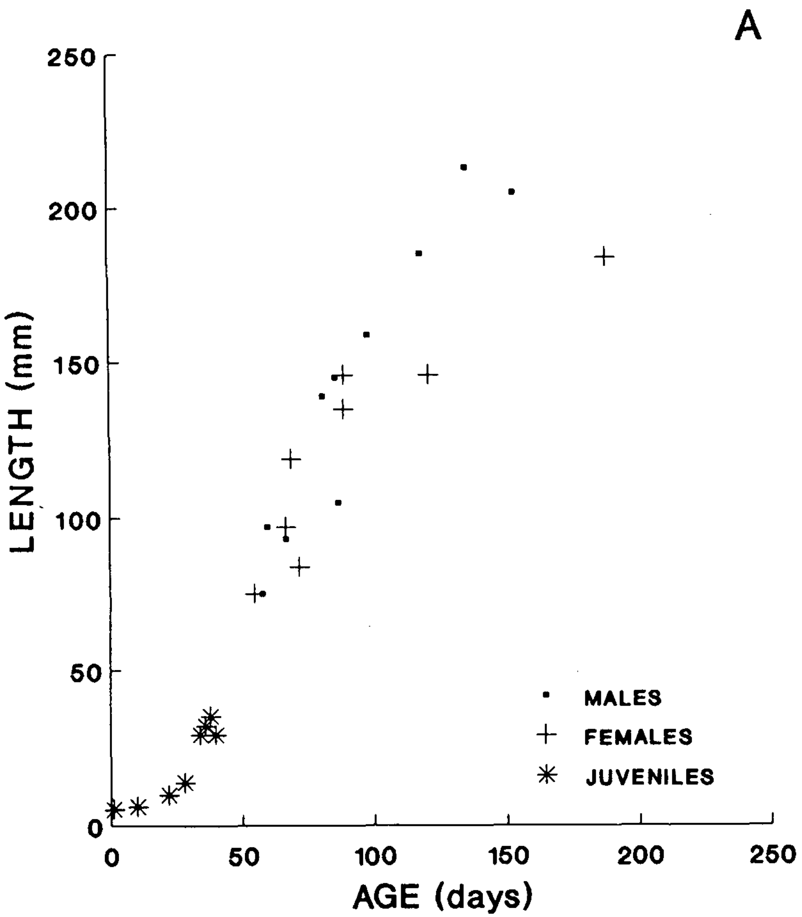

A
200
150
100
50
0
LENGTH (mm)
MALES
FEMALES
0
20
40
60
80
100
AGE (days)
B
100
80
60
40
20
0
WEIGHT (g)
MALES
FEMALES
0
20
40
60
80
100
AGE (days)

Figure 7.9

Relationship between (A) mantle length and age, and (B) weight and age, for *Sepioteuthis lessoniana* from hatchling to adult.


A
LENGTH (mm)
250
200
150
100
50
0
0
50
100
150
200
250
AGE (days)
MALES
FEMALES
JUVENILES

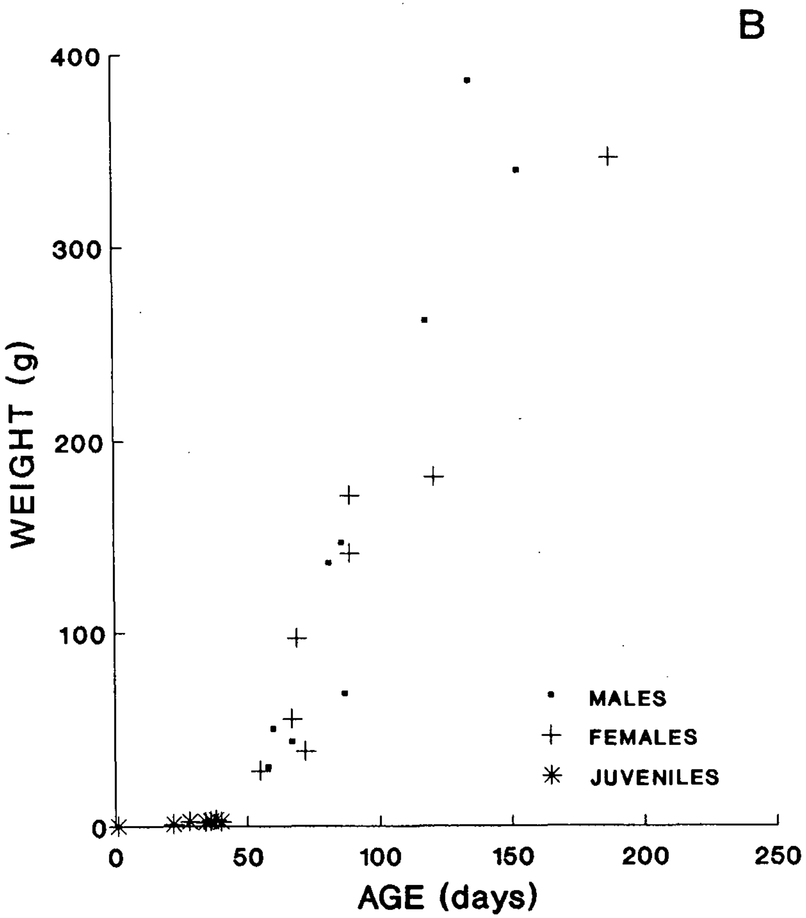

B
WEIGHT (g)
400
300
200
100
0
0
50
100
150
200
250
AGE (days)
MALES
FEMALES
JUVENILES

Figure 7.10

Relationship between (A) mantle length and age, and (B) weight and age, for *Sepioteuthis lessoniana* (same data as in fig. 7.9), displayed on a semi-log plot.

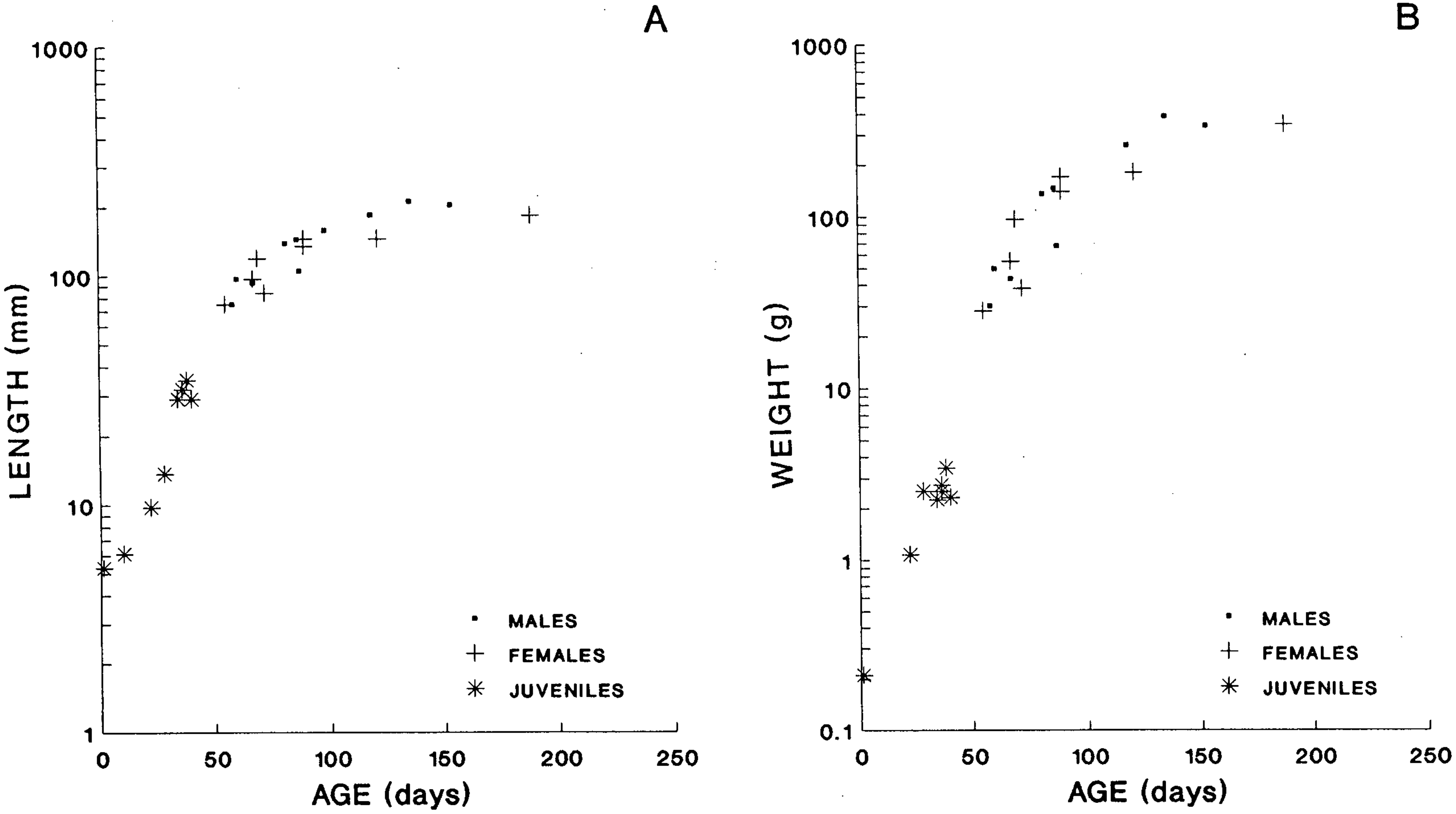

A
LENGTH (mm)
1000
100
10
1
0
50
100
150
200
250
AGE (days)
MALES
FEMALES
JUVENILES
B
WEIGHT (g)
1000
100
10
1
0.1
0
50
100
150
200
250
AGE (days)
MALES
FEMALES
JUVENILES

Figure 7.11

Exponential/linear growth curves developed for *Sepioteuthis lessoniana* (males and juveniles only) for both (A) growth in mantle length, and (B) growth in weight.

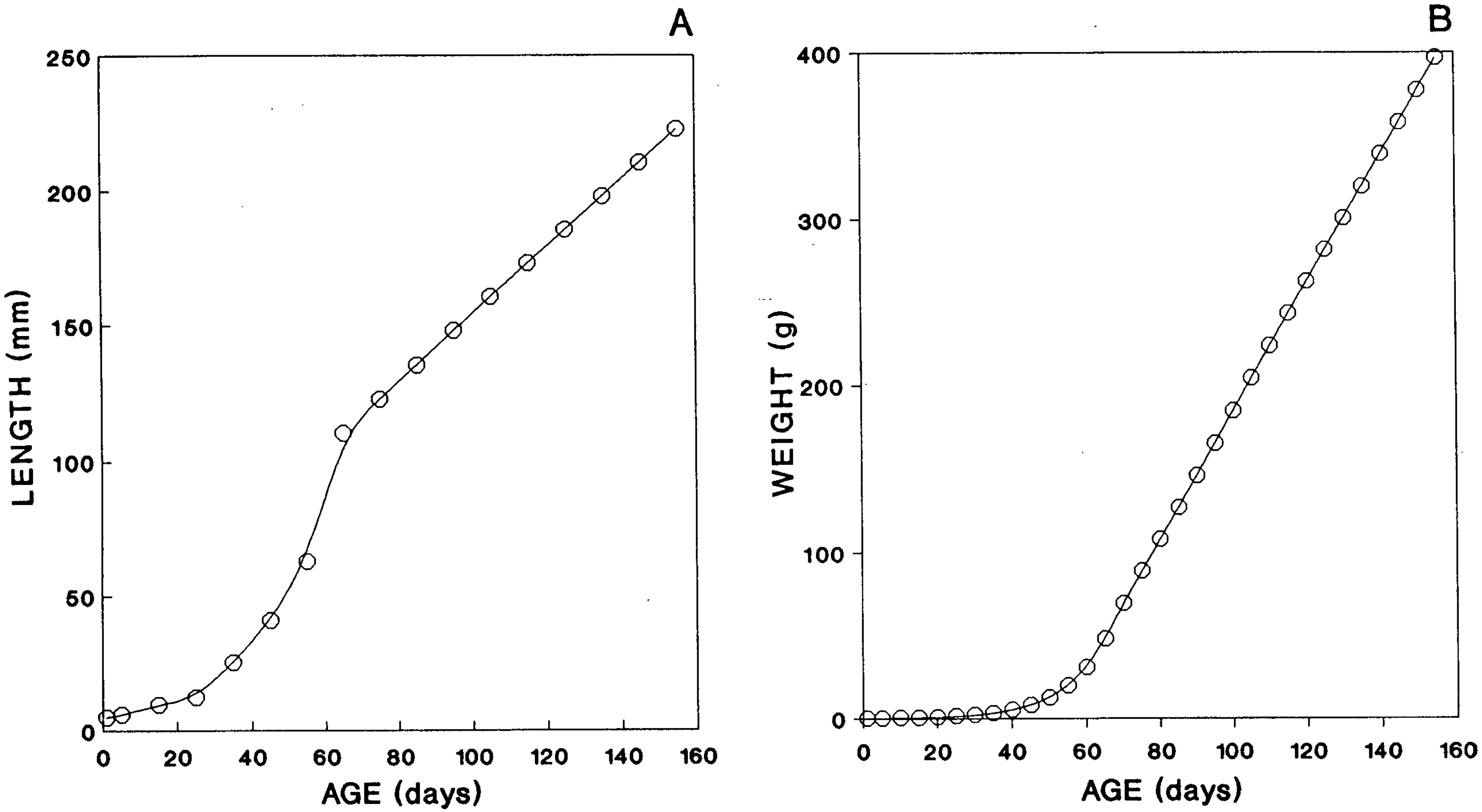

A
LENGTH (mm)
250
200
150
100
50
0
0
20
40
60
80
100
120
140
160
AGE (days)
B
WEIGHT (g)
400
300
200
100
0
0
20
40
60
80
100
120
140
160
AGE (days)

Figure 7.12

Growth curve developed for *Loligo chinensis* compared to growth curves determined for *Loligo opalescens* and *Loligo forbesi* (based on data given in Forsythe & Van Heukelem, 1987).

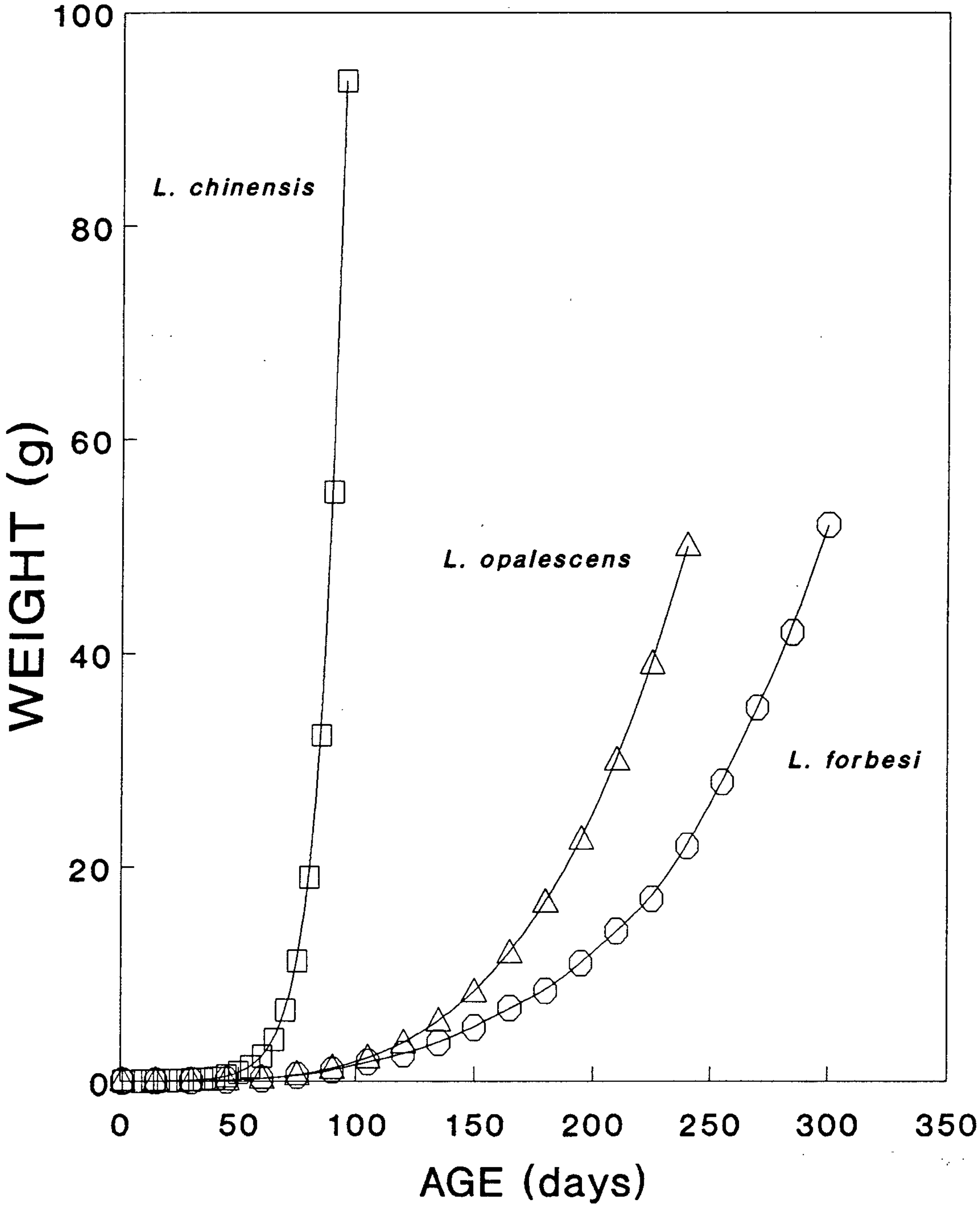

L. chinensis
L. opalescens
L. forbesi
WEIGHT (g)
0
20
40
60
80
100
AGE (days)
0
50
100
150
200
250
300
350

Figure 7.13

Length-at-age data for *Loligo opalescens* captured in the vicinity of Monterey Bay, California, displayed with length-at-age data for this species determined from culture experiments (from Yang *et al.*, 1986). Crosses 'X' represent means while bars represent the range.

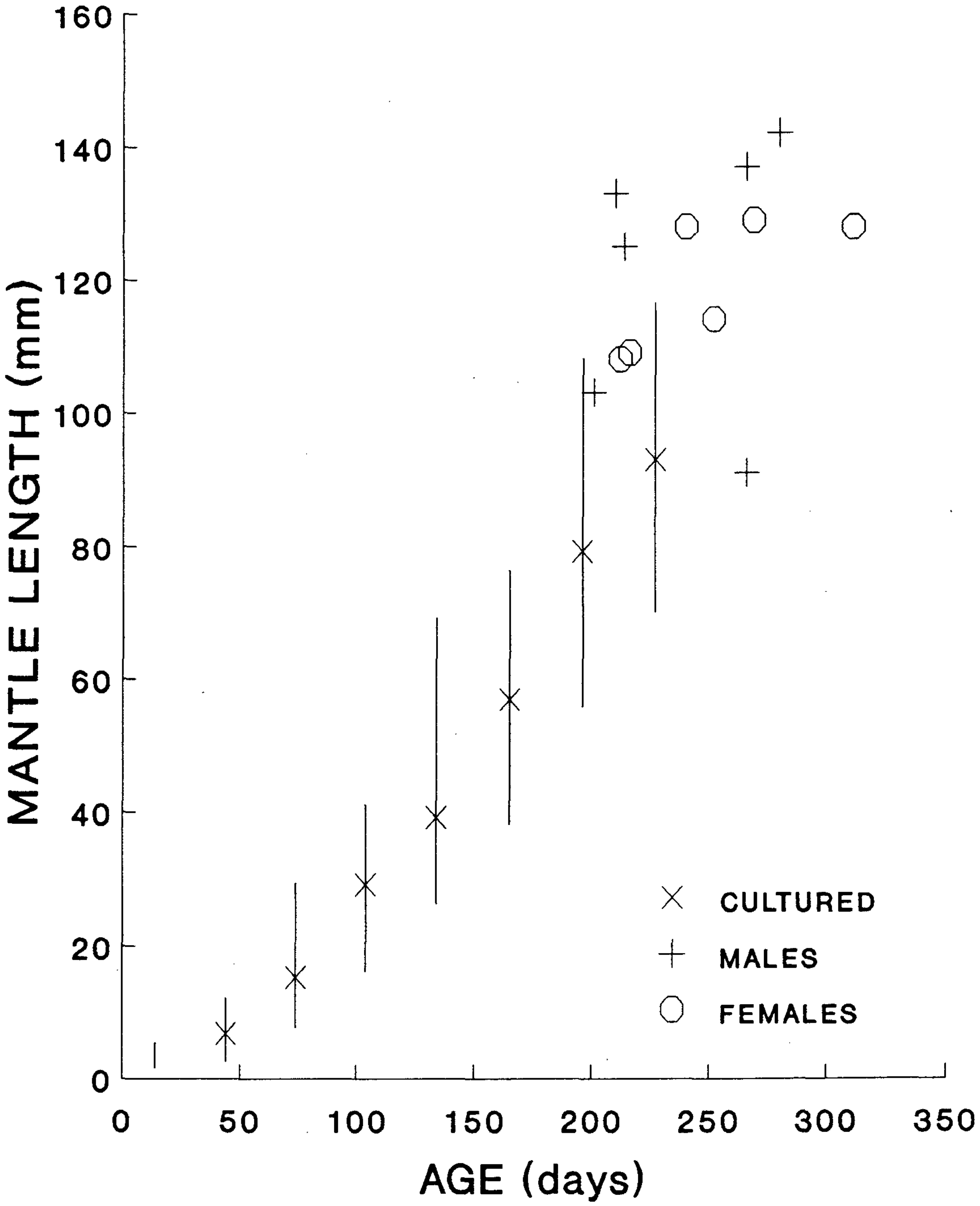
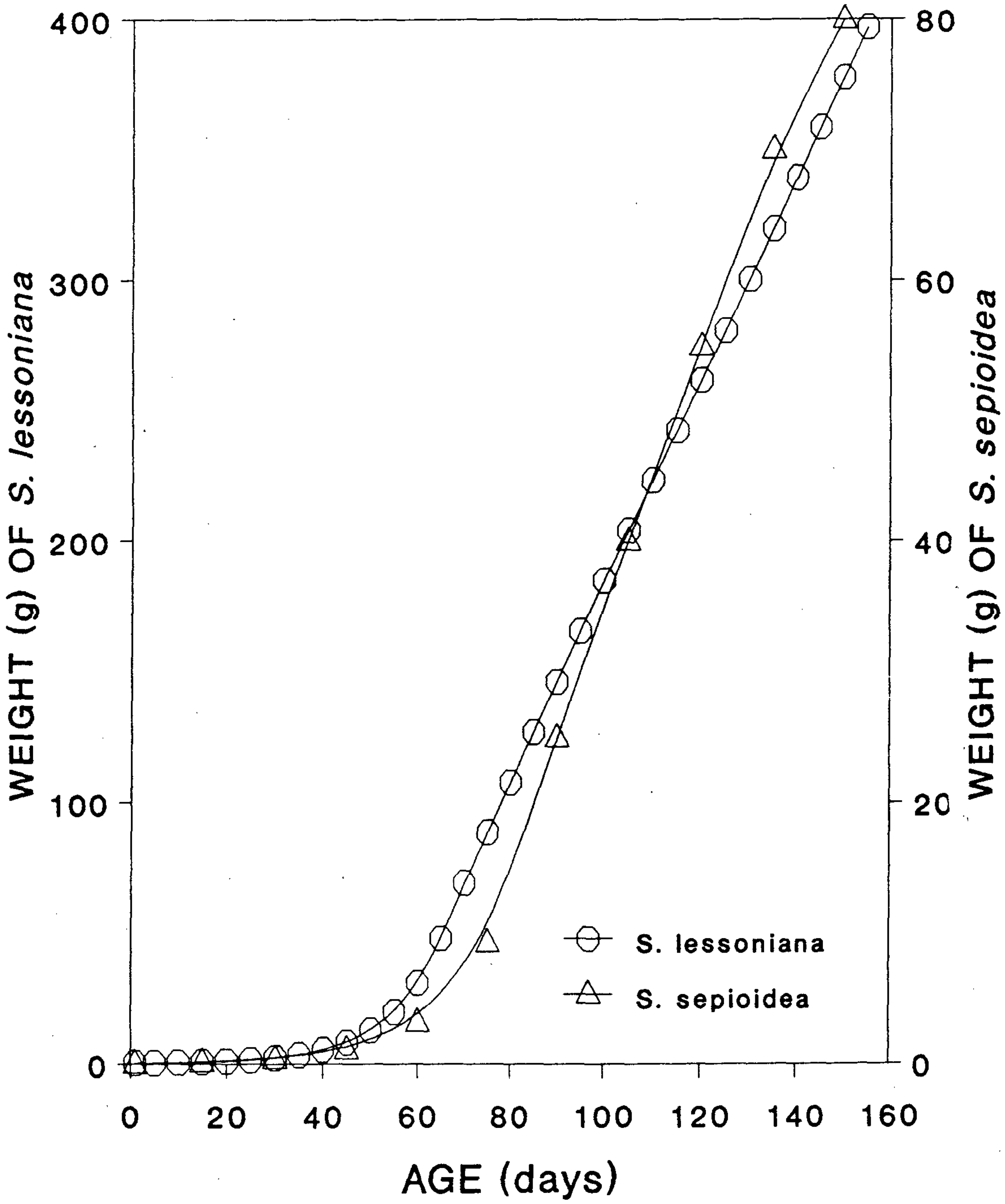

MANTLE LENGTH (mm)
160
140
120
100
80
60
40
20
0
0
50
100
150
200
250
300
350
AGE (days)
CULTURED
MALES
FEMALES

Figure 7.14

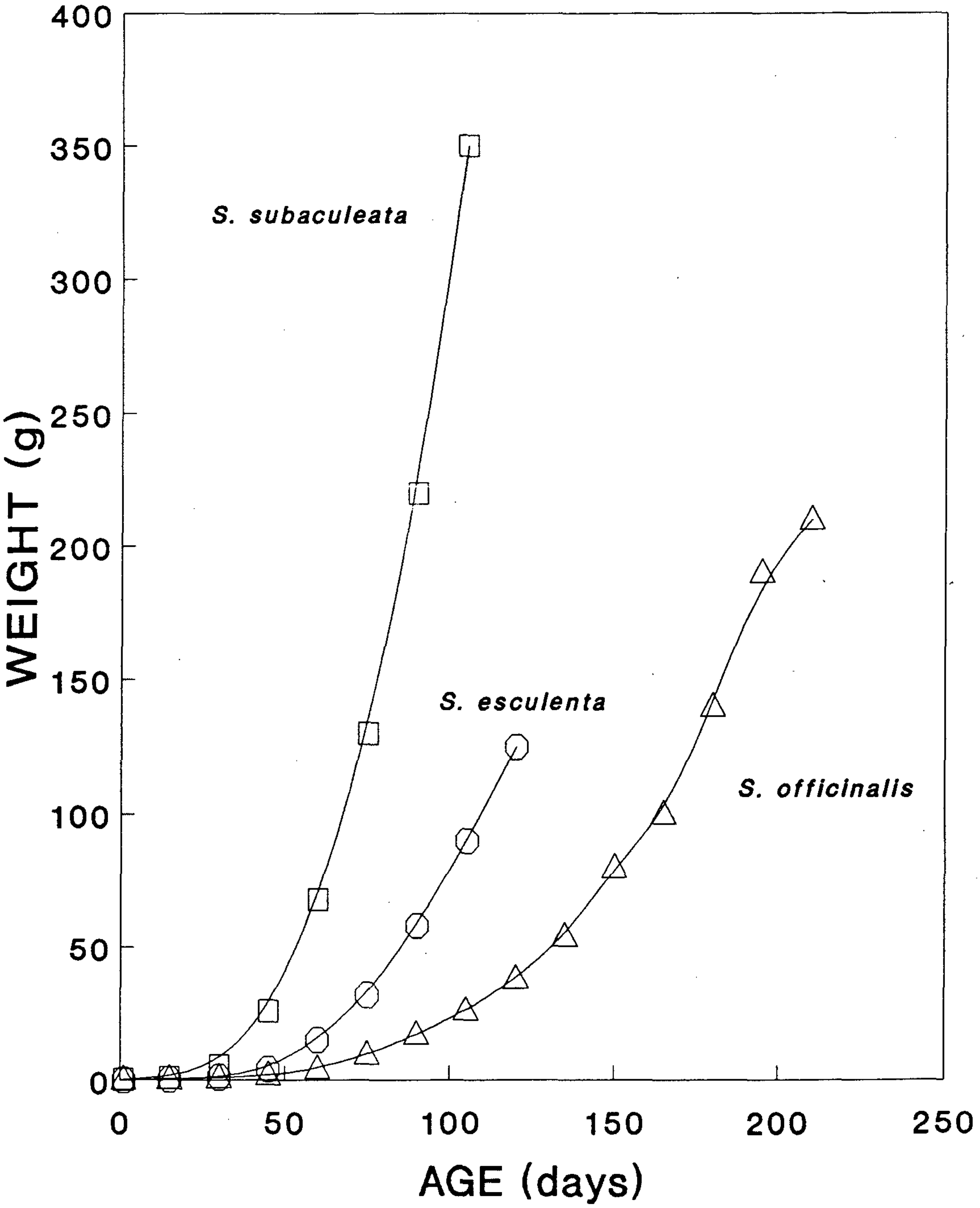
Growth curve developed for males and juveniles of *Sepioteuthis lessoniana* from this study, shown with growth curve developed for *Sepioteuthis sepioidea* based on culture experiments (curve constructed from data given in Forsythe & Van Heukelem, 1987). Note different scales in each 'Y' axis.



WEIGHT (g) OF *S. lessoniana*
400
300
200
100
0
WEIGHT (g) OF *S. sepioidea*
80
60
40
20
0
AGE (days)
0
20
40
60
80
100
120
140
160
S. lessoniana
S. sepioidea

Figure 7.15

Growth curves developed for three species of cuttlefish. Data given in Forsythe & Van Heukelem (1987).



400
350
300
250
200
150
100
50
0
WEIGHT (g)
0
50
100
150
200
250
AGE (days)
S. subaculeata
S. esculenta
S. officinalis

Figure 7.16

Growth rings within the statolith microstructure of the juvenile *Architeuthis* specimen from this study. (A) Dorsal dome region of statolith. Scale bar = 80 μm. (B) Close up of ring structure within the dorsal dome region showing the clear ring sequence to the statolith margin. Note the unusual discontinuity within the ring structure approximately 17 rings from the margin. Scale bar = 40 μm.

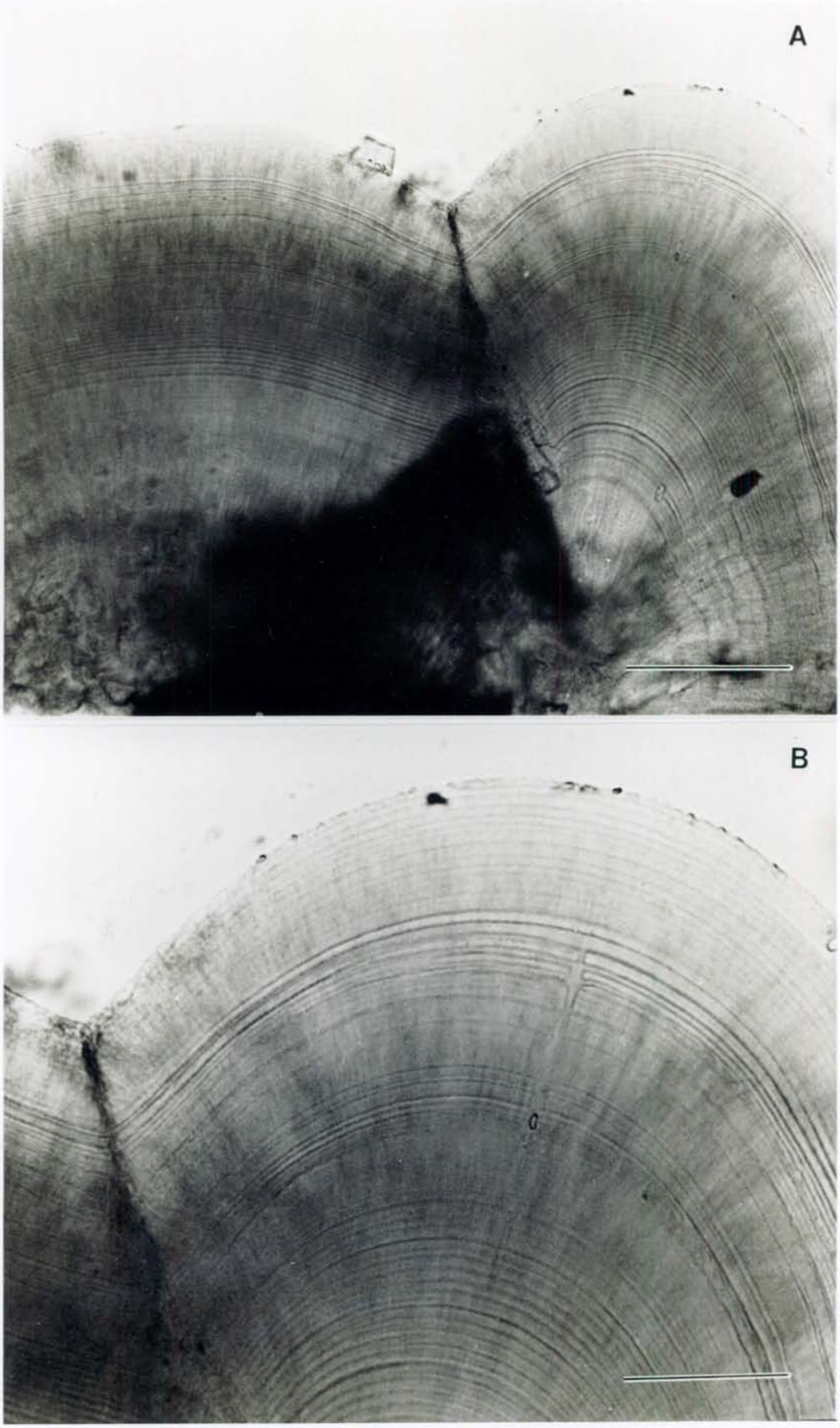
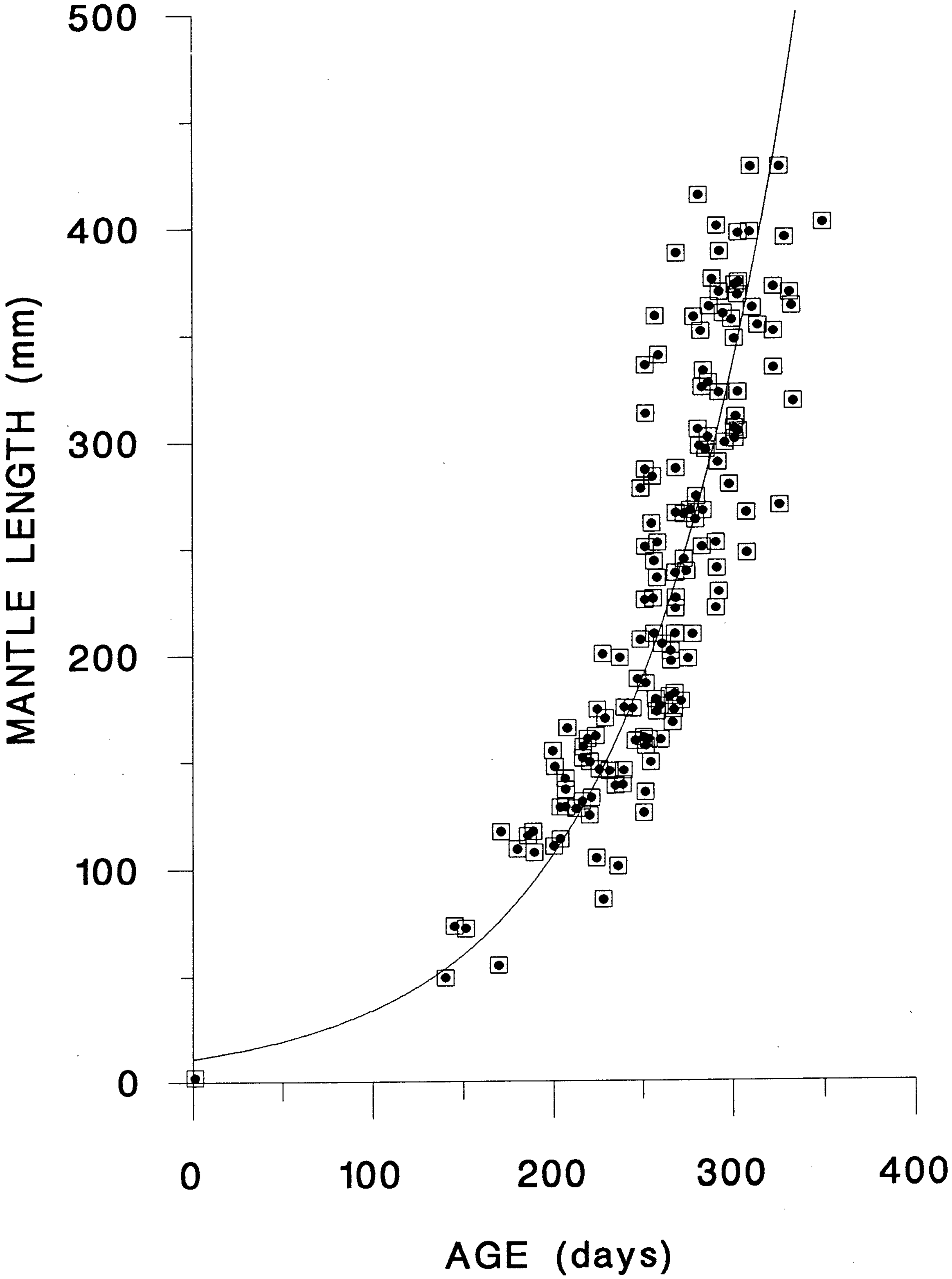

A
B

Figure 7.17

Length-at-age data for *Photololigo edulis* (data for warm-season brood, from Natsukari *et al.*, 1988) with fitted exponential growth curve.


500
400
300
200
100
0
MANTLE LENGTH (mm)
0
100
200
300
400
AGE (days)

8.0 GENERAL DISCUSSION AND CONCLUSIONS

The application of statolith ageing techniques in this study has helped to elucidate the specific aspects of growth in nektonic cephalopods. The ability to obtain accurate size-at-age data has enabled the description of growth, development and reproduction in age specific terms. The ageing studies and growth modelling has revealed that tropical squid and sepioid lifespans are short and that the form of growth is very different to that of teleost fishes. These results contradicted previous work with squid length frequency analysis which have indicated that squids have longer lifespans and asymptotic growth (eg. Rao, 1954; Pauly, 1985; Supongpan, 1988). Regular sampling for two of the study species, *I. pygmaeus* and *L. chinensis*, provided a way to compare more traditional length frequency analysis with new size-at-age data (chapter 5). This comparison demonstrated that applying different techniques to determine age and lifespan produces considerably different conclusions with regards to the form of growth in squids (eg. rapid non-asymptotic growth versus long lifespan conforming to the von Bertalanffy growth function).

Combining the results of squid culture experiments in the literature with data from this study suggests that statolith size-at-age data is providing a more accurate description of squid growth than is length frequency analysis. This study provides further evidence that using the von Bertalanffy growth function to describe squid growth is probably inappropriate, a sentiment already expressed by other cephalopod workers (Forsythe & Van Heukelem, 1987; Saville, 1987; Forsythe & Hanlon, 1989).

8.1 Why can't squid growth be modelled by teleost models?

This study has confirmed that teleost growth models are inappropriate for describing squid growth, despite the fact that squid growth data has been incorporated into finfish models in the past. An analysis of some of the biochemical and metabolic differences between squids and fish should help to clarify why these two groups of organisms display such different features in their growth.

Unfortunately we do not have the physiological and biochemical knowledge for cephalopods that we have for fish with which to construct biologically meaningful growth models. Asymptotic growth as described by the von Bertalanffy growth equation, which is based on rates of anabolism and catabolism has been successfully applied to finfish growth models (Wetherley & Gill, 1987). The physiological and biochemical understanding of teleost growth has provided the theoretical reasons for supporting such a model of growth. Further cephalopod research is necessary, including physiological and biochemical studies, to enable the construction of relevant growth models based on meaningful biological principles. However, enough research has been undertaken with cephalopods to at least be able to understand the biochemical and physiological differences between teleosts and coleoid cephalopods. These differences should help elucidate why the form of growth is so different between these two groups of organisms.

A major difference between cephalopods and teleosts lies within the energy sources and metabolic products utilised. Fish have been considered to be lipid specialists, and many species concentrate oils in their bodies. Some fish species have been so successful in accumulating lipids that dried fish were used as torches by the American Indians (Pitcher & Hart, 1982). Teleosts are thus well adapted to utilise lipids both as an energy source and as a storage product. Cephalopods on the other hand, have a limited capacity to store and catabolise lipids. They utilise protein predominantly for both energy and as a storage product (O'Dor *et al.*, 1984; Boucher-Rodoni & Mangold, 1985). Dried cephalopods would probably make poor torches. As a result of these metabolic constraints, cephalopods do not possess the ability that fish do to store energy rich reserves to 'carry them over' lean times. It has been estimated that the lipid reserves in a squid would only last 13 days at rest and 4 days at full activity, while a herring could survive 600 days at rest and nearly 80 days during activity (O'Dor & Webber, 1986). Cephalopods are thus adapted to convert their energy intake into growth while fish allocate a considerable amount of energy into reserves.

There are also important structural differences between squids and fish. Fish need to devote a considerable amount of energy into skeletal material and hard body parts (ie. they need to lay down a bony template for the attachment of muscular material). In contrast, growth in cephalopods can proceed rapidly without the need to allocate energy into skeletal material. The form of growth in teleosts may be due to an initial rapid phase of growth in which the skeletal template is laid down, followed by the asymptotic phase in which bone size increases and muscle mass is increased around this template. This is an area which deserves further research.

A further clue to the rapid growth in cephalopods may also be in the total percentage of the body tissue composed of white muscle fibres. Houlihan *et al.* (1990) have pointed out that the white muscle is the major site of protein accretion. Therefore, the greater the proportion of white muscle within the organism the greater the proportion of protein synthesis that is retained as growth. Houlihan and co-workers have shown that while white muscle in fish accounts for 40 to 60% of total protein content, in *Octopus vulgaris* white muscle accounts for greater than 76% of total protein content. Houlihan *et al.* (1990) also attributed rapid growth in *O. vulgaris* to the combination of high rates of protein synthesis along with a high degree of efficiency in the retention of synthesised protein.

Another major difference between cephalopods and teleosts is in the ingestion and assimilation of food. Fish are constrained because food ingestion is restricted to the gape of the mouth. Furthermore, growth inhibition has been related to prey size, with a positive correlation being shown between prey size and growth rate (Pitcher & Hart, 1982). Moreover, since fish predominantly swallow their prey whole, this necessitates the need for the digestive system to deal with large amounts of indigestible material such as bones and chiton. Cephalopods on the other hand are more efficient than fish with regards to both feeding and digestion. Squids are not constrained by gape size. The raptorial mode of feeding enables squids to feed on prey of similar size to themselves (Dawe, 1988). In addition, squids employ a great amount of extra alimentary processing of their food with the beak and radula. As a result, only food material composed of predominantly digestible material of high calorific value is ingested. Moreover, the stomach-caecum arrangement of cephalopods allows for food in different stages of digestion to be processed simultaneously which is more efficient than the 'in-line' digestive system in fish (Karpov & Calliet, 1978; O'Dor & Webber, 1986).

The ecological, physiological and biochemical constraints acting on coleoid cephalopods, selects for rapid growth, with allocation of energy into growth rather than into storage products. The inability of cephalopods to allocate much energy into storage products and their lack of efficiency in catabolising lipids selects for short lifespan and early maturity (ie. grow fast and reproduce before periods of low food availability). The lack of an internal skeleton and the efficient means of feeding and digestion promotes rapid growth in cephalopods. These factors set the coleoid cephalopods apart from the teleost fishes which share their environment, and should emphasise the differences between these two groups of organisms. There is thus an invalid basis for attempting to model squid growth based of teleost growth models. There is a considerable need to continue to develop cephalopod-specific growth models to better describe growth in these organisms.

8.1.1 The reliability of cephalopod length frequency data

The study raises the question of the reliability of length frequency data for obtaining growth parameters for pelagic cephalopods. The rapid growth and short lifespans shown in these five study species suggested that there were a number of cohorts in any size frequency sample. This factor was probably the most significant in hindering the determination of growth from modal progression analysis. This phenomenon appears to be a common feature of cephalopod length frequency data. The question could be justifiably raised as to whether obtaining a larger sample size for *L. chinensis* and *I. pygmaeus* would have provided a clearer distinction of cohorts within the length frequency data. However, a confusing 'mix' of modal groups has also been encountered with length frequency analysis of oceanic squids. Roberts (1983) also found no clear modal progression with length frequency data of *Nototodarus* sp. off New Zealand, despite a sample size of about 150,000 individuals.

It appears that cephalopod length frequency data needs to be considered cautiously. As pointed out in chapter five, some squids display age-specific or size-specific migrations out of populations. Application of length frequency analysis to these squid populations would tend to suggest misleading patterns of growth. Sampling methodologies for cephalopods generally, need to be carefully considered. Because it is now understood that these organisms grow very rapidly, a greater frequency in sampling would help to elucidate short term changes in population size structure or in maturation events.

8.2 Age data and reproductive events

The timing and duration of maturity in pelagic cephalopods has been difficult to determine in the past. The study of the maturation process is one area of study in which will be greatly benefited by the application of ageing techniques. Although analysis of reproduction for *L. chinensis* and *I. pygmaeus* was only preliminary in scope, by obtaining an understanding of the age of an individual, important age-specific trends in reproduction could be detected. Ageing research provided valuable information on seasonal trends in age at maturity and on how individual age influences reproductive investment. These patterns would have remained undetected without an ability to age individuals.

There is a distinct need to clarify terms such as semelparity and iteroparity in reference to the Cephalopoda. The greater understanding of the extremely short lifespans of cephalopods should now pave the way for the establishment of more accurate terms to describe modes of reproduction in the future. The evidence of this study along with recent reports in the literature (Boletzky, 1987; Harman *et al.*, 1989) suggests that repeated spawning is probably typical for many cephalopods.

Combining ageing research with analysis of maturation and gonad growth will enable a time scale to be placed on the period of the life cycle devoted to reproduction. This should also lead to a more accurate estimate of fecundity for squids.

8.3 Coleoid growth versus nautiloid growth

It is interesting to note that the pattern of rapid growth and short lifespan is not displayed by all cephalopods. Growth of the nautiloids is strikingly different to that of the coleoids. Although many coleoids have been shown to be able to achieve rapid non-asymptotic growth with the possibility of reaching maturity in a matter of months, this is not the situation with the nautiloids. Estimates of both mark-recapture (Saunders, 1983) and radiometric determination of chamber formation (Cochran & Landman, 1984) in field specimens along with the monitoring of chamber formation in captive *Nautilus* (Greenwald & Magnier, 1981; Ward & Chamberlain, 1983; Ward, 1985) have revealed that *Nautilus* is long lived and probably reproduces over a number of years. Lifespan estimates range from 10 years (Cochran & Landman, 1984) to as great as 20 years (Saunders, 1983). Ward (1985) emphasised the differences in growth between the nautiloid and coleoid cephalopods, suggesting that while squids and cuttlefish can reach large sizes (up to 10 kg) in less than two years, *Nautilus* takes 10 to 20 years to reach a body size of a mere 0.5 kg. Ward (1985) attributed this difference in growth rates to the considerable penalty of producing an external calcareous chambered shell.

8.4 The paradox of the extended juvenile growth phase

The rapid increase in growth coincident with growth into the adult phase could be related to the fact that the young coleoid cephalopods are reaching a threshold in size in which they can capture and ingest suitable nutrient-rich food sources (eg. fish). The growth and feeding dynamics of very young cephalopods will probably reveal the constraints encountered during the paralarval/juvenile phases of growth.

Despite the many reasons discussed above which promote rapid growth and short lifespans in the coleoid cephalopods, analysis of the form of growth clearly demonstrates a post-hatchling/juvenile phase in which growth is negligible. This has been ignored when applying ecological theory to the Cephalopoda (eg. Calow, 1987) in which young cephalopods are incorrectly thought to grow rapidly to 'speed them through' a vulnerable juvenile period. Furthermore, very small squids are thought to be metabolically more efficient than larger individuals, since jet propulsion is more efficient at smaller sizes (O'Dor & Webber, 1986). The reason for this lag in growth (which is directly opposite to the form of growth in teleosts) is unknown and deserves further research. The paradox of extended juvenile growth is vexing since juvenile squids should be more energetically competitive, relative to fish, based on the arguments of O'Dor & Webber (1986).

Slow initial growth may be due to a feeding constraint. Squid paralarvae and juveniles inhabit the plankton environment and may have a limited feeding ability. Many of the potential planktonic prey items would possess extensive spiny exoskeletons and therefore would probably have a proportionally low level of digestible tissue. The raptorial mode of feeding in young cephalopods may be inefficient at very small sizes, and energy rich prey sources might be difficult to capture.

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APPENDICES

1) The use of statolith mictrostructures to analyze life-history events in the small tropical cephalopod *Idiosepius pygmaeus*.

2) Age and growth of the tropical near-shore loliginid squid *Sepioteuthis lessoniana* determined from statolith growth ring analysis.

3) The use of tetracycline staining techniques to determine statolith growth ring periodicity in the tropical loliginid squids *Loliolus noctiluca* and *Loligo chinensis*.

THE USE OF STATOLITH MICROSTRUCTURES TO ANALYZE LIFE-HISTORY EVENTS IN THE SMALL TROPICAL CEPHALOPOD *IDIOSEPIUS PYGMAEUS*

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Age and Growth of the Tropical Nearshore Loliginid Squid *Sepioteuthis lessoniana* Determined from Statolith Growth-Ring Analysis

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The Use of Tetracycline Staining Techniques to Determine Statolith Growth Ring Periodicity in the Tropical Loliginid Squids *Loliolus noctiluca* and *Loligo chinensis*

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