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**DYNAMICS OF GROWTH AND DEVELOPMENT IN  
TROPICAL LOLIGINID SQUID *PHOTOLOLIGO*  
SPECIES**

by

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A thesis submitted for the degree of Doctor of Philosophy in the Department of  
Marine Biology at James Cook University of North Queensland, in May 1994.

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Natalie Moltschaniwskyj

## Abstract

Temporal and spatial abundance of juveniles of two *Photololigo* species on the continental shelf off Townsville, Australia was described using light-traps. The two species showed very distinct and separate spatial distribution patterns. *Photololigo* sp. A was found close to the coast and was the smaller and more abundant of the two species. This species was most abundant in surface waters, although larger individuals were generally caught deeper. There was no evidence of vertical movements during the night. The presence of small and large juvenile *Photololigo* sp. A during summer and winter months suggests that spawning and recruitment occurs throughout the year. In contrast, *Photololigo* sp. B was caught predominantly offshore. All sizes of *Photololigo* sp. B were caught both close to the benthos and in surface waters in the middle of the Great Barrier Reef lagoon, but juveniles were deeper and larger further offshore. This study demonstrated that light-traps are an effective way of sampling and catching small loliginid squid for research.

This study approached growth of squid by examining the dynamics of muscle tissue and changes in shape and size of body structures in juvenile and adult *Photololigo* sp. A. Animals change shape during growth because body structures increase in size at different relative rates. These changes are of particular interest because they are generally concomitant with changes in ecology. Length and mass measurements were taken from squid ranging in size (dorsal mantle length) from 2.77 mm to 117.03 mm. Small squid (<50 mm dorsal mantle length) had round bodies with large head and eyes, and poorly developed tentacles and arms. Larger squid (> 50 mm dorsal mantle length) were more elongate and narrow and the head was proportionally smaller. As squid reached a dorsal mantle length of 60 mm, the changes in shape with growth become slower and they reached a final shape. Small individuals allocated energy predominantly in the arms and tentacles during early stages of growth, while the viscera and head grew at a much slower rate. Once individuals began producing gametic tissue and gonad growth occurred, the mantle muscle tissue grew more slowly than the gonad.

Allocation of energy to somatic and gametic growth was investigated using information about the way muscle tissue grows. Growth of somatic tissue in *Photololigo* sp. was expressed in terms of muscle fibre recruitment and growth. Muscle blocks and muscle fibres were measured and the size frequency distributions were compared between different size-classes of squid. Muscle blocks increased in size as individuals grew. The size frequency distribution of the muscle fibres suggested that this increase resulted from the generation of new muscle fibres and an increase in the size of existing muscle fibres. The size frequency distribution of muscle fibres was very similar in all size-classes of squid examined and the presence of small muscle fibres in all individuals suggested that muscle fibre recruitment may be continuous. Growth of muscle tissue, by muscle fibre growth and recruitment, provides a mechanism to explain the continuous growth described for tropical squid. Two structural types of muscle fibres; mitochondria-poor and mitochondria-rich, are present in juvenile and adult squid. A poor relationship between the ratio of the muscle fibre types and dorsal mantle length suggests that generation of mitochondria-rich muscle fibres may not be influenced by growth. The presence of what, histologically, appears to be a breakdown in the organisation of circular muscle fibres was dependent upon the size of the individual and its reproductive status. However, there was no evidence to suggest that this is part of the senescence process.

An influx of immature individuals was detected in early September and this group of individuals was followed throughout reproductive maturation. From the time female *Photololigo* began producing primary oocytes to ovulation was less than two months. Four pieces of evidence supported the hypothesis that *Photololigo* sp. A has the potential to lay multiple, discrete batches of eggs. (1) The ovaries of maturing and mature females contained a large population of primary oocytes. (2) There was a poor correlation between the size of females and both the oviduct mass and the number of eggs per mass of oviduct. (3) Rapid increase in the oviduct mass of mature females indicated that the ovary released batches of ovulated eggs. (4) Gonad mass increased at a relatively slow rate compared to the increase in somatic tissue. There was no evidence, from an examination of the length-weight relationships and microscopic assessment of the mantle muscle tissue, of a cost of egg production.

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# CHAPTER I:

## GENERAL INTRODUCTION

### 1.1 OVERVIEW

Growth of cephalopods is continuous throughout their life. The form of the growth curve and its continuous expression results in relatively slow juvenile growth, an overlap of somatic and reproductive growth, and rapid senescence. The form of the growth curve will determine the length of the juvenile phase, the allocation of energy to reproduction and shape changes during ontogeny. In turn, variation in growth rates of individuals will determine their size at recruitment to the adult population, reproductive maturation and senescence (Forsythe 1993). Therefore, changes in growth rates and the form of the growth curve affect the maintenance of adult populations and the intensity of spawning events in the population (O'Dor & Coelho 1993). Cuttlefish, squid and octopus have been reared under different light, temperature and nutrition regimes to determine the cause of the observed growth patterns (Forsythe & Hanlon 1988 & 1989). However, these studies concentrate on the responses of growth to different conditions and examine variation in growth rates, but not the mechanisms responsible for the actual growth pattern. Descriptions of growth are obtained from size at age information for cephalopods but this does not provide information about the biology and mechanisms of growth. It is necessary to describe how growth occurs, but also to recognise that growth is the result of complex behavioural and physiological processes (Brett 1979).

A comprehensive study of growth in cephalopods requires a multi-level approach (see Fig. 1.1). Each level of organisation will interact with other levels and the information will not only be descriptive but also examine the processes responsible for the observed patterns. Changes in habitats used during the life-cycle will modify growth rates. Therefore, demographic information, especially spatial patterns of abundance of animals, during both adult and juvenile phases are needed. This information then needs to be complemented with a description of the structural and functional changes associated with increasing size and changes in habitats that will



affect the measured growth rates of the animal. An increase in somatic tissue is generally the currency of growth in animals and mechanisms of tissue increase will have major effects on the form of the growth curve (Weatherly 1990). The description of growth at the cellular level will provide an understanding of the mechanisms by which squid are able to continually grow throughout their life. However, somatic growth does not occur independently from the growth of other tissues. During the reproductive process the production of gametes will affect the allocation of energy to somatic tissue and will affect growth of somatic tissue. Our understanding of squid growth may be achieved by a more mechanistic approach. This dissertation examines information on four hierarchical levels to obtain a complete understanding how tropical squid are able to grow continuously and the consequences of this growth form.

## 1.2 GROWTH

Growth is the increase in body size, usually length or weight, of an individual. It is a rate process and descriptions of changes in size are used with reference to age or a period of time. Two features of growth are of interest to ecologists. The first is the rate of growth and the second the pattern or shape of the growth curve, i.e. how the rates of growth change between different periods of the life cycle. Variations in rates of growth at the individual level will determine the age at different stages of the life-cycle and when ecological changes occur. Rapid growth during the juvenile phase is likely to be associated with early maturation, small body size and possibly a short life-span while slow juvenile growth rates typically result in later maturation, larger body size and longer life times (Van Heukelem 1979). The interaction of morphology with the environment during growth will also influence ecological characteristics of a species (Werner & Gilliam 1984). It is unclear whether the changes in habitat undertaken by many species during ontogeny are a function of structural changes associated with growing bigger or whether habitat changes are required and structural changes must therefore occur. To understand the developmental ecology of an organism, accurate descriptions of growth patterns and variability in growth rates are required.

Table 1.1: Examples of different growth models used to describe growth in different cephalopod species.

GROWTH EQUATION	SPECIES	SOURCE
LINEAR	<i>Illex argentinus</i>	Rodhouse & Hatfield 1990b
	<i>Todarodes sagittatus</i>	Rosenberg <i>et al.</i> 1980
	<i>Gonatus fabricii</i>	Kristensen 1983
	<i>Loligo chinensis</i>	Jackson & Choat 1992
	<i>Sepioteuthis lessoniana</i>	Jackson & Choat 1992
	<i>Idiosepius pygmaeus</i>	Jackson & Choat 1992
ASYMPTOTIC	<i>Watasenia scintillas</i>	Hayashi 1993
EXPONENTIAL	<i>Loligo opalescens</i>	Yang <i>et al.</i> 1983
	<i>Ommastrephes bartrami</i>	Bigelow & Landgraf 1993
	<i>Berryteuthis magister</i>	Natsukari <i>et al.</i> 1993
	<i>Loligo gahi</i>	Hatfield 1991
DOUBLE EXPONENTIAL (EXPONENTIAL JUVENILE/ EXPONENTIAL ADULT)	<i>Loligo vulgaris</i>	Natsukari & Komine 1992
TWO PHASE GROWTH (EXPONENTIAL JUVENILE/ LOGARITHMIC ADULT)	<i>Loligo vulgaris</i>	Turk <i>et al.</i> 1986
	<i>Octopus joubini</i>	Forsythe 1984
	<i>Abralia trigonura</i>	Bigelow 1992
	<i>Sepia officinalis</i>	Boletzky 1983

A number of problems have prevented biologists from obtaining accurate descriptions of cephalopod growth. Size frequency data analyses have been used extensively because it was the only technique available and the information was easy to obtain (Squires 1967, Summers 1968, Mesnil 1977, Patterson 1988). Size-frequency analysis and the use of asymptotic growth models derived from fish studies suggested that asymptotic growth patterns were also typical for cephalopods. However, both size frequency analysis and the assumption of asymptotic growth are problematic when generating cephalopod growth models (Rodhouse *et al.* 1988). The observation of asymptotic growth described by tracking the size-frequency of a population of animals through time can be a function of the age structure of the population. Problems arise when faster growing individuals in a population attain maturity and die earlier than slow growing individuals because the larger individuals in the population are usually the older and slower growing component of the population (Lee's Phenomenon, Ricker 1975). The use of mean size-at-age information in the calculation of growth curves amplifies this problem (Alford & Jackson 1993). Both these problems overestimate the age of all large individuals in the population and produce an illusion of asymptotic growth for the species (Alford & Jackson 1993). The development of validated aging techniques using statoliths and gladii, particularly for squid, has generated growth curves using size at age information (see reviews by Lipinski 1981, Rodhouse & Hatfield 1990a, Jackson *in press*). Research over the past 10 years has produced a suite of growth patterns for cephalopods, using both wild caught and reared individuals (Table 1.1 and review by Forsythe & Van Heukelem 1987). It has become increasingly apparent that, in contrast with fish, non-asymptotic growth and fast growth rates may be more typical for most squid species (Jackson 1989, 1990a & b, Rodhouse & Hatfield 1990b). It has been argued that squid do not have the metabolic or physiological capacity to sustain the growth rates calculated from size at age information (Jarre *et al.* 1991). However, the mechanisms that enable squid to attain high growth rates and have continuous growth are unknown.

Descriptions of cephalopod growth during the juvenile phase are few and limited because it is difficult to catch and identify small cephalopods (Forsythe & Van Heukelem 1987). Growth curves from field caught juveniles are only available for *Abralia trianura* (Bigelow 1992) and *Illex* sp. (Balch *et al.* 1988). These growth

curves suggest that juveniles have an exponential rate of increase, ie. a constant specific growth rate. Extrapolating adult data (Jackson & Choat 1992) and rearing studies (Turk *et al.* 1986, Forsythe & Hanlon 1989) have also provided similar growth estimates. However, estimates of juvenile growth rates and the length of the juvenile phase cited in the literature have been contradictory. In some cases the calculated growth curves suggest that, during juvenile phase, growth is exponential but then slows during the adult stage becoming logarithmic (Forsythe & Van Heukelem 1987). Or growth is exponential throughout the life time of the individual, but the exponential growth is faster during the juvenile phase than the adult period (Natsukari & Komine 1992). In either case it is suggested that juveniles grow quickly to reduce the time spent as small individuals (Calow 1987). However, growth patterns calculated for *Loligo chinensis* demonstrate that a) growth of juveniles may be slower than, or equal to, adult growth rates, and b) that juveniles spend much of their life time as small individuals (Jackson & Choat 1992). It is therefore necessary to obtain juveniles of more cephalopod species to confirm these patterns of growth.

Descriptions of growth patterns and the experiments that examine how temperature and food levels change these growth patterns have studied cephalopod growth at the whole animal level (Hirtle *et al.* 1981, Forsythe 1993). An area that has received little attention by biologists studying cephalopod growth, is growth processes occurring at the tissue level of organisation. Patterns of growth observed at the whole animal level are expressions of growth at lower levels of organisation (Weatherly 1990), so that an increase in body length is the result of growth of organs. Likewise growth of an organ or element of the body is a result of cell multiplication. Therefore, changes in body size measured during the life time of an animal in response to temperature or food levels are expressions of changes underlying growth. As a result, it may not be possible to understand how environmental factors affect growth or why variability in growth rates occurs until the growth dynamics of the organs contributing to growth are understood. The study of growth dynamics of tissues and relative growth of organs will provide a valuable insight into overall growth and the allocation of energy (Weatherly & Gill 1985; Weatherly 1990, Hatfield *et al.* 1992, Rodhouse & Hatfield 1992).

Table 1.2: General areas where the juveniles of squid species have been found in relatively high numbers.

SPECIES	SIZE (mm)	HIGHEST ABUNDANCE	SOURCE
<i>Loligo pealei</i>	1.6-15	coastal - surface and subsurface	Vecchione 1981
<i>Loligo gahi</i>	8-44	coastal - near benthos <100m	Rodhouse <i>et al.</i> 1992
<i>Lolliguncula</i>	1.1-13.6	coastal - near benthos	Vecchione 1991
<i>Gonatus antarcticus</i>	2-32	shelf/off shelf - surface	Rodhouse <i>et al.</i> 1992
<i>Mesonchoteuthis hamiltoni</i>	4.8-26.5	oceanic - subsurface	Rodhouse & Clarke 1985
<i>Illex argentinus</i>	1.9-6	oceanic - subsurface	Brunetti & Ivanovic 1992
<i>Illex illecebrosus</i>	0.8-60	frontal zone - surface	Rowell & Trites 1985
	7-42	frontal zone & slope	Dawe & Beck 1985
<i>Abralia trigonura</i>	1-5	shelf - <70m	Young & Harman 1985
<i>Abraliopsis</i> sp. A	1.3-8.6	shelf - <95m	Young & Harman 1985
<i>Abraliopsis</i> sp. B	0.9-7.2	shelf - <70m	Young & Harman 1985
<i>Enoploteuthis reticulata</i>	1.2-6.8	shelf - <200m	Young & Harman 1985
<i>Enoploteuthis higginsi</i>	1.6-5.8	shelf - <125m	Young & Harman 1985
<i>Enoploteuthis jonesi</i>	1.1-4.4	shelf - <100m	Young & Harman 1985
<i>Nototodarus hawaiiensis</i>	1.7-9.2	shelf - >50m	Harman & Young 1985
<i>Hyaloteuthis pelagica</i>	2-6.5	shelf - <50m	Harman & Young 1985
<i>Sthenoteuthis oualaniensis</i>	1.4-7.1	shelf - <70m	Harman & Young 1985
		slope/shelf - surface	Piatkowski 1993
<i>Brachioteuthis</i> sp.	2-9.1	shelf - <150m	Young <i>et al.</i> 1985
Ommastrephids	0.5-4.0	slope & shelf - 50-2000m	Dunning 1985
Enploteuthids		slope/shelf - <100m	Piatkowski 1993
Cranchids		oceanic - <100m	Piatkowski 1993
<i>Martialia hyadesi</i>	9-101	shelf	Uozumi <i>et al.</i> 1991

### 1.3 CHANGES IN SHAPE

Few cephalopod studies have examined ontogenetic changes in the relative size of body components other than for taxonomic descriptions (Okutani 1987, Augustyn & Grant 1988). Ontogenetic changes in behaviour and ecology are often a function of changes in shape of an organism (Vecchione 1981, Segawa 1987). Mathematical descriptions of growth often ignore changes in the shape of the individual during growth. For instance, measurement of dorsal mantle length in studies of squid growth only assesses allocation of energy into muscle tissue along the longitudinal axis. If growth of body components occurs at different rates, then growth rates calculated from a single measurement such as body length are inadequate as an estimate of growth of the whole animal. Total body weight may provide a better estimate of growth, but the relative growth of somatic, visceral and gonadal material provides more information about the allocation of energy. The observed changes in shape of an organism occur either because a structure changes function or to ensure the continuing function of that structure. Allometric growth is responsible for changes in body shape will have a number of metabolic and physiological implications to the animal (Sebens 1987). Therefore, a study of ontogenetic changes in allometric growth indicates changes in habits and ecology that are difficult to detect by describing patterns of distribution and abundance alone. Recent efforts by a number of cephalopod biologists have provided preliminary information regarding the type of habitat used during the early phases of the life history (Table 1.2). Juvenile cephalopods often occupy a different part of the water column and sometimes a different water mass from the adults. Therefore extrapolations of adult growth to juvenile forms may be incorrect if juveniles encounter different environmental conditions.

Changes in body shape during development of juveniles will provide a better understanding of growth during the juvenile phase. Clearly there are changes in the allocation of energy during ontogeny producing differential growth in structures. Slower growth rates of somatic tissue during reproductive maturity may be the result of energy being committed to reproduction at the cost of somatic growth (Hatfield *et al.* 1992). Therefore an examination of the growth of different structures in adults

may provide an indication of how and where squid allocate energy during reproductive maturation (Hatfield *et al.* 1992, Rodhouse & Hatfield 1992).

#### 1.4 TROPICAL SQUID

Tropical squid have attracted less research interest than temperate species because they have yet to become a target species for the fishing industry, although they are a by-catch of the prawn trawl industry (Winstanley *et al.* 1983). During the last five years biological knowledge of tropical squid has increased considerably. Validation of daily ring deposition in the statoliths of the loliginid squid has allowed descriptions of growth and changes in growth rates (Jackson 1989, 1990a & b, 1991, Jackson & Choat 1992). There is no evidence that, like teleost fish, tropical squid attain an asymptotic size, although it is possible that large specimens of the species studied have eluded capture (Jackson & Choat 1992). Furthermore, exponential and linear growth models generated from size at age information suggest that growth of tropical squid does not slow dramatically as individuals get older and bigger.

Tropical squid are much smaller, shorter lived and faster growing than their temperate counterparts, suggesting that warmer temperatures may promote faster growth rates, but at the cost of earlier maturation and senescence (Jackson 1990a, Jackson & Choat 1992). It is not known whether terminal spawning occurs in tropical loliginid squid and further studies of growth and reproduction in tropical squid are necessary to determine this. There are four loliginid squids currently recognised in the Townsville region, Australia (Dr. C.C. Lu, Museum of Victoria Australia, pers. comm.): *Sepioteuthis lessoniana* Lesson 1830, *Loliolus noctiluca*, *Photololigo* sp. A (previously referred to as *Loligo chinensis*, Jackson 1991) and *Photololigo* sp. B. There are morphological descriptions of the adults of the two *Photololigo* species (Yeatman 1993) and allozyme electrophoretic techniques have been used to identify them (Yeatman & Benzie 1994). It appears that the juveniles of these species are morphologically very similar, but it is possible to identify them with allozyme electrophoresis techniques (Yeatman 1993). At some stage, both of these species have been referred to by the specific name *chinensis* (Jackson 1991, Jackson & Choat

1992, Yeatman & Benzie 1994), but genetically, neither correspond to *Photololigo chinensis* found in Thailand waters (Yeatman 1993).

This thesis examines tissue and organ growth in tropical squid, dynamics of muscle tissue growth and how energy is allocated and diverted to muscle and reproductive organs. The fast growth and short life cycle of the tropical squid *Photololigo* sp. A provides a suitable subject with which to examine these processes. Currently, ecological and growth information is available only from the adult phase of the life-history (Jackson 1991), largely because of problems catching juvenile (< 60 mm dorsal mantle length) individuals. As a result of this problem there is an incomplete knowledge of their life-history. The use of automated light-traps to catch juvenile, pelagic fish and squid will enable this phase of their life-history to be described (Doherty 1987, Thorrold 1992).

This thesis has four chapters that examine the ecology, growth and reproduction of two *Photololigo* species during juvenile and adult phases of their life-cycle:

### 1. Spatial and temporal distribution patterns of *Photololigo* juveniles

This chapter examines the juvenile phase of *Photololigo* sp. A and sp. B found in the Central Great Barrier Reef. Juvenile squid were caught using light-traps providing information about spatial distribution patterns across the continental shelf. Sampling during winter and summer months provided temporal comparisons of juvenile *Photololigo* sp. A distribution and abundance patterns. Size and age information were used to examine ontogenetic changes in spatial and temporal distribution patterns. An indication of the environmental conditions experienced by juvenile squid was provided by temperature and salinity data collected during the sampling program.

### 2. Morphometry of growth.

Changes in the relative size of body structures during growth result from either a change in the function of the body structure or preservation of an existing function. Newly hatched juveniles of nektonic squid are able to swim actively and have a



functional ink sac and chromatophores. Cephalopod juveniles are often described as 'miniature adults' but it appears that they undergo changes in form that can be related to their ecology. Descriptions of allometric growth throughout the life history of a species provide information about how squid allocate energy during growth. Both bivariate and multivariate statistical techniques were used to describe changes in shape and the rate of these changes in juvenile and adult *Photololigo* sp. A.

### 3. Growth and dynamics of mantle muscle tissue fibres.

Growth dynamics of the muscle fibres were examined to determine how squid are able to grow continuously. The increase in length and mass of the mantle muscle tissue was used as a measure of growth of the individual. Fibre size and number were examined in both juvenile and adult *Photololigo* sp. A to determine whether somatic growth was by fibres increasing in size or increasing in numbers or a combination of both.

### 4. Growth and reproduction.

Changes in somatic and growth tissue production were investigated together with growth dynamics on a weekly basis in short-lived species. Muscle tissue growth was examined in relation to energy partitioning with gametic tissue production. Reproductive activity of the Townsville *Photololigo* sp. A population occurs throughout the year because of their short life-time. A program sampling *Photololigo* sp. A was undertaken to determine if females are capable of several discrete spawning events or if terminal spawning occurs. By sampling the population intensively it was possible to determine if synchronised spawning events were occurring.

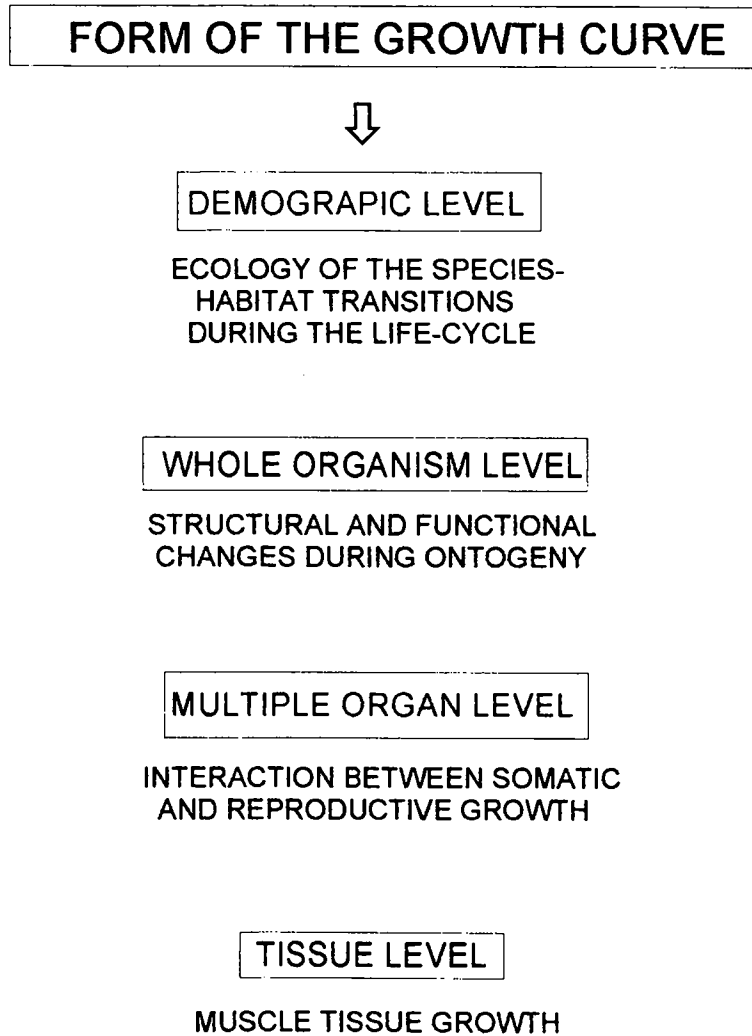


Figure 1.1: An understanding of the consequences of continuous growth and how it is achieved by deriving information on a number of organisational levels.

**CHAPTER II:**  
**DISTRIBUTION AND ABUNDANCE OF TWO JUVENILE**  
**TROPICAL *PHOTOLOLIGO* SPECIES**

**2.1 INTRODUCTION**

Life-history characteristics of squid populations have been derived from information about the adult phase resulting in a poor understanding of processes important in squid population dynamics (Voss 1983, Boyle 1990). Limited information about young squid is demonstrated when attempting to define the life-history phases (Young & Harman 1988). Jackson & Choat (1992) suggest, given the comparatively short life-time of tropical squid (<250 days), that a proportionally long period of the life-cycle is spent as small individuals. In the case of *Loligo chinensis*, with a summer life-time of 120 days, individuals less than 60 days old (<50 mm mantle length) have not been studied. Hence for almost half the life-history of most squid there is not even the most basic information. Temporal and spatial abundance patterns of juvenile squid will provide a basis for understanding the processes of mortality, growth and recruitment. However, such information has been difficult to obtain because of problems in capturing and identifying a sufficient size range of juvenile cephalopods (Vecchione 1987).

To examine the ecology of juvenile squid it is necessary to use techniques that catch a size range of individuals, hatchlings to juveniles, in good condition. Pelagic squid produce either benthic or pelagic eggs and have a planktonic juvenile phase (Boletzky 1977). Juvenile squid are alert, mobile organisms that easily avoid capture by towed nets (Vecchione 1987). The use of a combination of different towed nets to sample an area enables the collection of a wider size range of juvenile squid (Rodhouse *et al.* 1992). However, it is difficult to obtain sufficient replicates needed to provide density estimates using towed nets. In this study an alternative technique was employed based on light-attraction, this has been effective in sampling pelagic juvenile fishes. Automated light-traps (Doherty 1987) can overcome the problems of

net avoidance and enable sampling at discrete depths in the water column. The ability to sample concurrently within an area ensures that estimates of variability in abundance are not confounded by time. This technique also collects live material in good condition, which can facilitate taxonomic identification. However, sampling an unknown volume of water by individual light-traps requires cautious interpretation of abundance estimates (Choat *et al.* 1993).

Electrophoretic analysis of a subset of juveniles collected during three months of the program found that *Photololigo* sp. A were found less than 33 km offshore and 90% of the *Photololigo* sp. B were found 33 km or more offshore (Yeatman unpub. data). Since these species are morphologically identical as juveniles, it was assumed that all individuals found at stations less than 33 km offshore were *Photololigo* sp. A and that *Photololigo* collected more than 33 km offshore were *Photololigo* sp. B. *Photololigo* sp. A (previously known as *Loligo chinensis*) is a small short-lived neritic squid which has been the topic of recent growth studies using statolith aging techniques (Jackson & Choat 1992). Individuals are approximately 60 days old when they appear in the adult population and they can grow to 180 mm in 120 days. Little is known about the early life-history, juvenile distribution patterns or growth of either *Photololigo* species. Therefore, the objectives of this chapter were to:

1. Describe the spatial abundance patterns of juvenile *Photololigo* species across the continental shelf in the Townsville region of the Great Barrier Reef.
2. Examine temporal patterns of abundance during winter and summers months, and between years.
3. Describe the growth rates of juveniles of the two species.

## 2.2 MATERIALS AND METHODS

### 2.2.1 SAMPLING DESIGN

Two major habitat types are present on the continental shelf, offshore from Townsville, Australia. The inshore habitat is a 56 km wide soft bottom coastal lagoon ranging in depth from 15 m to 40 m. The offshore habitat is a complex reef matrix of similar extent, dissected by channels ranging from 40 - 75 m deep at the shelf break. To assess the cross-shelf distribution of juvenile squid, four automated light-traps (Doherty 1987) were deployed at fifteen sampling stations spanning the continental shelf and the western Coral Sea (Fig. 2.1). Abundance along this transect was assessed over four months, October to January, during two austral summers, 1990/91 and 1991/92. At each station, the abundance of juvenile squid was determined at two depths by deploying two pairs of light-traps. In each pair, one light-trap was suspended immediately below the surface while the other light-trap was set deeper. In 1990/91, all deep light-traps were suspended 20 m below the surface. In 1991/92, the deep light-traps were suspended within 5 m of the bottom to a maximum of 100 m in the Coral Sea.

In all deployments, the two pairs of light-traps were released approximately 300 m apart and allowed to drift for one hour. Allowing the traps to drift in the water column minimised potential problems with differential water movement among stations. Using drifting light-traps in open water, as opposed to anchored light-traps, has been shown to be a more effective way of catching pelagic organisms (Thorrold 1992). After one hour, the four light-traps were retrieved and the entire catch was fixed and preserved in 100% ethanol. Each evening the first light-trap was deployed after 1930 hrs (Eastern Standard Time) and the last light-trap retrieved before 0430 hrs. Travel time between each station allowed only five cross-shelf stations to be sampled per night. Thus, each night's activity concentrated on one of the two continental shelf habitats or the Coral Sea. Each monthly cruise consisted of nine nights during which time each of the 15 stations was sampled three times. However, sea conditions were not always favourable and sampling effort at each station is shown in Table 2.1. It was not logistically possible to sample all stations in each

habitat simultaneously. Therefore time of night is confounded with station position. Haphazard selection of the first station sampled each night ensured that no station was consistently sampled at the same time on all nights. Cruises were scheduled to include the new moon because this is the lunar phase when light attraction has proved most effective for fishes and various invertebrates (Milicich 1992). Temperature and salinity profiles of the water column were collected at each station using a Seabird Conductivity Temperature Device during the 1991/92 summer.

Concurrent with the summer cross-shelf sampling, light-traps were anchored within 100 m of the south easterly side (weather-side) of four reefs; Keeper, Helix, Faraday and Myrmidon, to sample near-reef waters (Fig. 2.1). Using drifting light-traps near the reefs was not possible. During the summer of 1990/91, four light-traps were anchored at each reef; three immediately below the surface and one at 20 m below the surface. In 1991/92, an extra light-trap was added at 20 m. The anchored light-traps had an automatic timer, enabling the lights to be switched on and off automatically at predetermined periods during the night. Each light-trap on the reef fished for a total of three hours per night; lights came on for one hour at 2200 hrs, 2400 hrs and 0300 hrs. Light-traps at all reefs were emptied the following day.

Squid were identified in the laboratory and dorsal mantle length recorded for each individual. Individuals were measured within 14 days of preservation in 100% ethanol. A comparison of measurements of individuals (ranging in size from 5.3 - 29.5 mm) before and 14 days after preservation found that shrinkage was on average 0.5 mm.

### 2.2.2 ANALYSES

Abundance patterns of the two *Photololigo* species during the two summers of sampling were examined using 'planned comparisons', where specific pre-generated hypotheses were examined (Day & Quinn 1989). The abundance of each species was compared between years, locations and depths.

To examine seasonality of juvenile *Photololigo* sp. A the inshore station (19 km) was sampled during the austral winter months of May, June, July and August 1991. Three sites at this station were sampled with four shallow and four deep (13 m) light-traps. Sites were sampled during the period of the new moon, on five nights in May and three nights in June, July and August. Densities in summer and winter months were compared using an unbalanced one-way analysis of variance (ANOVA), with month as the factor analysed. Values in each light-trap for nights and sites within a month were treated as replicates.

For all parametric analyses the data were examined for normality and homogeneity of variances. In the event that these assumptions were violated the data was  $\log_{10}+1$  transformed before analyses were carried out.

To determine whether vertical migration might influence horizontal distribution patterns the size-structure of *Photololigo* sp. A individuals was examined at two depths during the night. On at least one occasion in each month of the 1991/92 sampling period the 19 and 24 km stations were sampled both early (before 2400 hrs) and late in the night (after 2400 hrs. By combining data from stations, across nights and months, it was possible to compare size frequency distributions between depths and time of night. A frequency analysis was used to determine the effect of time of night and depth on the size frequency distribution.

Linear statistical techniques were not used to examine correlations between physical variables and abundance levels of juvenile squid because it was unlikely that a linear relationship exists. Instead bubble plots were used to look at the association between the variables. As the physical conditions were different and juveniles were present each month the relationship between physical variables and abundance levels was examined by month.

### 2.2.3 SIZE AT AGE

Statoliths were removed from a sub-sample of juveniles and stored in glycerol for several months before reading of the rings was attempted. The deposition of daily

rings has been validated for *Photololigo* sp. A (Jackson 1990b) and it was assumed that *Photololigo* sp. B also deposited daily rings in the statolith (Jackson 1991). Counts of daily rings were made from the natal ring to the edge of the statolith (Jackson 1991). Statoliths were, where possible, read whole using a light microscope with a polariser. When statoliths could not be read whole, they were mounted on a glass slide with Crystalbond™ posterior side up (using nomenclature from Clarke 1978). The statolith was then gently ground down using lapping film (9 µm) until the nucleus and edge could be clearly read. A final polish with 0.3 µm alumina polishing powder was used to remove scratches. When the hatching check could not be seen or three successive counts of rings were not within 10% of each other the statolith was rejected as being unreadable.



## 2.3 RESULTS

### 2.3.1 DISTRIBUTION PATTERNS

Juvenile *Photololigo* individuals were predominantly caught within 52 km of the mainland (Fig. 2.2). The few individuals found farther offshore were in the Magnetic Passage (five individuals) and on the reefs (six individuals). *Photololigo* species were not found in the Coral Sea. *Photololigo* sp. A was numerically the most abundant of the two species during both summers (Fig. 2.2), with 856 individuals caught in 181 hours of light-trapping, compared with 379 *Photololigo* sp. B in 348 hours of light-trapping. Overall, *Photololigo* sp. A juveniles were present in higher numbers at the 24 km station in surface waters (Table 2.2). This pattern was consistent in both years, but a greater number of this species were caught in 1991/92 (Table 2.2), as the result of very high catches in December 1991 (Fig. 2.2). In comparison, highest numbers of *Photololigo* sp. B were consistently found at the 33 km station and abundance levels tended to decrease further offshore (Fig. 2.2). Overall, *Photololigo* sp. B demonstrated no difference in abundance levels between the two years (Table 2.3). In contrast to *Photololigo* sp. A, juvenile *Photololigo* sp. B was more abundant deeper in the water column (Table 2.3). Further offshore, *Photololigo* sp. B juveniles were present in very low numbers and were only caught in the deep light-traps (Fig. 2.2).

Table 2.2: Planned comparisons of juvenile *Photololigo* sp. A densities between depths, years and sites.

Contrast	df	Contrast Sums of Squares	Mean Squares	F-value	Pr>F
Depths	1	9.8165	9.8165	12.20	0.0006
Years	1	3.7565	3.7565	4.67	0.0320
Sites	1	8.6892	8.6892	10.80	0.0012
Residual	177	142.3838	0.8044		

Table 2.3: Planned comparisons of juvenile *Photololigo* sp. B densities between depths and years.

Contrast	df	Contrast Sums of Squares	Mean Squares	F-value	Pr>F
Depths	1	17.0607	17.0607	37.85	0.0001
Years	1	0.0438	0.0438	0.10	0.7554
Residual	335	148.7448	0.4507		

*Photololigo* sp. A ranged in size from 2.6 - 47.9 mm. The size frequency distributions at the two depths were not significantly different between the 19 km and 24 km stations ( $\chi^2=12.28$  df 9 Pr=0.1979; Fig. 2.3). There was no systematic change in the size frequency distribution of *Photololigo* sp. A during either summer (Fig. 2.4). A modal shift to the right in the size frequency distribution in January 1992 suggested that fewer small individuals were available to be caught. However, catches were very low in this month. *Photololigo* sp. B ranged in size from 3.6 - 61.6 mm (Fig. 2.3). From the size frequency distributions it was clear that larger juveniles were found further offshore and deeper in the water column (Fig. 2.3). No modal shift in the size frequency distribution was apparent during the summers (Fig. 2.4). However, catches were low in most months.

The multiway frequency analysis established that the size frequency distribution of juvenile *Photololigo* sp. A at both depths changed as a function of time of night (Table 2.4). Small juveniles dominated in the surface waters and larger individuals were generally found closer to the benthos (Fig. 2.5). During the night, the relative abundance of small individuals decreased at both depths. Close to the benthos an increase in large individuals was evident. There was no striking pattern of vertical migration; although combining data across months to increase the number of juveniles in the analysis removed the possibility of detecting vertical migration in any one month.

Table 2.4: Results of the multiway frequency analysis to examine changes in the size distribution of *Photololigo* sp. A between depth and time of night.

Source	df	$\chi^2$	Probability
Depth	1	92.8	0.00
Time	1	25.57	0.00
Depth*Time	1	0.19	0.66

The number of *Photololigo* sp. A juveniles captured during the winter months was similar to most of the summer monthly catches (Fig. 2.6); although winter catches never reached levels such as those seen in December 1991 (Table 2.5). The large number of small juveniles captured over the winter (Fig. 2.6) indicates that spawning

and hatching occurred in both seasons. A similar size range was captured during both summer and winter months (Fig. 2.7).

Table 2.5: Analysis of variance examining differences between densities of *Photololigo* sp. A at the 19 km station between summer months of 1990/91 and 1991/92 and winter months of 1991.

Source	df	Contrast Sums of Squares	Mean Squares	F-value	Pr>F
Month	11	1118.200	101.654	9.55	0.0001
Residual	214	2277.910	10.644		

### 2.3.2 SIZE AT AGE RELATIONSHIPS

The age range of juveniles caught in the light-traps was similar for both *Photololigo* species. *Photololigo* sp. A ranged from 20 to 63 days, and *Photololigo* sp. B from 18 to 52 days. Considerable monthly variability in the size at age relationship was observed, therefore individuals from each month were examined separately (Fig. 2.8). Both linear and exponential growth equations could be used to provide an analytical description of growth (Table 2.6). Although in both cases an exponential fit was used, it was only marginally better than a linear. This may be a function of the age range of juveniles used for the analysis. When a narrow age range is used a linear equation is often a better fit. Increases in mantle length per day ranged from 1.42 to 0.037 mm for *Photololigo* sp. A and for *Photololigo* sp. B from 0.106 to 0.577 mm (Table 2.6). These values are averaged over the age range examined. There was a very similar age distribution of juveniles caught each month (Table 2.6), suggesting that new juveniles were entering the population continuously.

Table 2.6: Growth equations for the *Photololigo* two species by month. The age range (days) refers to individuals used in the equations. N is the number of juveniles aged.

MONTH	GROWTH	SLOPE	INTERCEPT	R <sup>2</sup>	N	AGE RANGE
Sp. A Oct '90	Linear	1.42	-35.70	0.896	22	29-44
Sp. A Dec '90	Exponential	0.037	1.17	0.932	21	15-55
Sp. A Jan '92	Linear	1.09	-10.51	0.944	18	20-48
Sp. B Nov '90	Exponential	0.106	0.293	0.952	14	18-29
Sp. B Jan '91	Linear	0.577	-3.74	0.483	51	21-44

## 2.3.3 PHYSICAL PARAMETERS

Both temperature and salinity decreased non-linearly across the lagoon, with discontinuities in both variables occurring midway across the Lagoon (Fig. 2.9). Temperature or salinity discontinuities were detected on at least six out of nine nights between the 33 km station and one or both of the neighbouring stations. This suggested that the water mass in the lagoon was heterogenous and may have influenced the distribution patterns of juvenile squid.

Salinity-temperature profiles of the water column at each station indicated thermoclines were present on some nights (Table 2.7). A thermocline was defined as a temperature change greater than 0.5°C between surface and bottom water; differences as great as 3°C were detected during January. However, these thermoclines were a temporally and spatially unstable feature of the water column, possibly because of changeable wind conditions and the shallow body of water sampled.

Table 2.7: Depth of the thermocline (m) at each station on every night of sampling during the three months of the 1991/92 summer. (- = no data collected).

		DEPTH OF THERMOCLINE		
		DAY 1	DAY 2	DAY 3
OCTOBER 1991	19km	14	11	ABSENT
	24km	13	14	ABSENT
	33km	10	13	ABSENT
	43km	10	ABSENT	ABSENT
	52km	25	ABSENT	ABSENT
	61km	31	ABSENT	-
NOVEMBER 1991	19km	ABSENT	ABSENT	ABSENT
	24km	ABSENT	ABSENT	ABSENT
	33km	ABSENT	ABSENT	20
	43km	ABSENT	ABSENT	22
	52km	ABSENT	ABSENT	25
	61km	ABSENT	ABSENT	29
JANUARY 1992	19km	7	7	9
	24km	9	9	9
	33km	11	13	12
	43km	15	15	18
	52km	28	24	27
	61km	31	47	-

There were no obvious patterns of association between the physical parameters measured and the number of juvenile squid in any of the months examined (Fig. 2.10). Each month high numbers of juveniles were present at different combinations of salinity and temperature. In October juveniles were present in high numbers at salinities of 35.4 ppm and at most temperatures. Whilst in November highest number were found at slightly higher salinities. Low numbers were found in January 1992, but is unclear whether this is a function of physical conditions, such as high water temperatures compared to other months, or variability in spawning and hatching events.

## 2.4 DISCUSSION

Light-traps provided a technique to describe the spatio-temporal distribution patterns of two *Photololigo* species. Identification of *Photololigo* species using allozyme electrophoresis suggests that the two species are separated geographically across the Great Barrier Reef lagoon (Yeatman & Benzie 1994). This separation occurs in a region of the coastal lagoon where temperature-salinity data indicate heterogeneity. High numbers of juvenile *Photololigo* sp. A at stations close to the mainland suggests that spawning grounds for this species may be close to the coast, a feature typical for loliginid squid (Mangold 1987). Furthermore the presence of small and large individuals during summer and winter months indicates that spawning, hatching and recruitment are not seasonal events. This characteristic may be more common for tropical species, which tend to have shorter lifespans than temperate species (Jackson & Choat 1992). Large numbers of small juveniles collected during the winter may be the result of slower growth during the winter (Jackson & Choat 1992). Little is known about the distribution of *Photololigo* sp. B adults, however the presence of juveniles in this region suggests that an adult population does occur in the Townsville region and that spawning occurs throughout the summer. It also appears that juveniles of this species undertake a distinct ontogenetic movement both further offshore and deeper in the water column. Identification of the juvenile *Photololigo* species was confirmed on a sub-sample of specimens captured during the summer. Therefore, conclusions drawn from this study are based upon the assumption that the offshore distribution pattern of the two species was consistent in all other months of sampling.

Juvenile squid are not easily sampled with towed nets (Vecchione 1979, Holme 1974). They have highly developed sensory and locomotor systems (Boletzky 1974) and it is likely that these animals are often under-sampled because of net avoidance. Choat *et al.* (1993) have shown that plankton nets select for small larval fish, but larger fish are captured from the same water column using light attraction. Squid biologists are seeking for new and novel techniques with which to observe juveniles. Submersible video cameras have been used to make behavioural observations and obtain population density estimates (Vecchione & Gaston 1985). Thorrold (1992), as

well as this study, have now demonstrated that light-traps are a useful technique for capturing juvenile squid. However, like most sampling techniques, light-traps have biases. One problem is that they sample an unknown volume of water. Nonetheless, they have been validated as useful devices for monitoring relative abundance patterns of pelagic juvenile fish at fixed locations (Milicich *et al.* 1992). Great care needs to be exercised when interpreting catch rates from different locations because changes in water transparency can bias light-trap efficiency. Similarly, it is not possible to compare catches from drifting and anchored light-traps quantitatively (Thorrold 1992). This is because the former act as lagrangian drifters and sample photopositive organisms from within a constant light pool. In contrast, moored light-traps experience a variable water flow that may greatly increase the volume of water swept in an hour of sampling. Despite more intensive sampling on the reefs, catches of *Photololigo* were low which suggests that spawning does not occur near the reefs and that juvenile *Photololigo* individuals are concentrated in the lagoon. In the present study, a gradient of turbidity across the shelf makes it possible that inshore catches would underestimate abundance if corrected for diminishing light-pools. However, if the error was significant it would only exaggerate, not diminish, the observation that juvenile squid were more abundant within the coastal lagoon.

High catches of juvenile squid in the coastal lagoon were at locations where discontinuities in surface temperature and salinity were observed. Hydrodynamic modelling of this region suggests that the coastal lagoon is often subject to velocity shear (King & Wolanski 1992). Water in the lagoon typically flows southward under the influence of the East Australian Current, which pushes water onto the outer shelf and through the reef matrix, especially through channels like the Magnetic Passage. Under typical south-easterly wind conditions, the shallow body of water trapped against the coast moves in the opposite direction, northwards. The result is a velocity shear between the two water masses and a zone of low residual displacement. Modelling studies suggest that the cross-shelf location of this feature, referred to as a separation front, will shift seawards as wind strength increases and vice versa (King & Wolanski 1992). Mobility of the frontal region is consistent with the daily and monthly variability of salinity and temperature at the surface indicated by the monitoring of physical parameters during the second summer.

This low-shear zone is identified as a significant place for aggregation of planktonic organisms. Cross-shelf studies have shown highest abundances of larval reef fishes in the mid-lagoon area of the central region of the Great Barrier Reef (Thorrold in press). These catches included fish originating from reefs further offshore, as well as piscivorous larvae of various scombrids from inshore (Thorrold 1993). It is not clear whether aggregation of these stages is passive, due to hydrodynamics, or attraction to the coastal boundary area by enhanced secondary productivity in this frontal zone (Thorrold & McKinnon 1992). This physical discontinuity may be a mechanism separating the two *Photololigo* species geographically. Similar spatial separation of juvenile cephalopod species in the Gulf Stream east of New England is thought to be closely related to mesoscale hydrological features (Vecchione & Roper 1986). The importance of hydrological features in aggregating juvenile squid has been identified in a number of species (Rodhouse & Clarke 1985, Brunetti & Ivanovic 1992, Rodhouse *et al.* 1992), suggesting that these areas are ecologically important for these animals.

Shelf-scale hydrodynamics may also affect the stability of the water column by an intrusion of upwelled waters from the shelf-break driven onto the shelf by variations in speed and position of the East Australian Current. These cold intrusions can be tracked into the Great Barrier Reef lagoon (King & Wolanski 1992) and the strong thermal stratification observed in January 1992 was consistent with an intrusion of this type. A cold bottom layer at 33 km was evident on one night in November, but the inner stations were not stratified. The presence of juvenile *Photololigo* at most stations in all months, despite a range of physical conditions, suggests juvenile *Photololigo* can tolerate substantial environmental variation, especially salinity changes. This tolerance is consistent with a non seasonal reproductive strategy, which is essential for a species that lives for only four months.

During the night there was little evidence of a pronounced vertical migration such as the mass aggregations of juvenile *Loligo* spp. close to the sea-bed (Vecchione & Gaston 1985) or the general movement to the surface by juvenile *L. pealei* (Vecchione 1981). The absence of vertical movement during the night suggests that the observed ontogenetic shift of *Photololigo* sp. B further offshore and deeper is real and not a product of location confounded with time of night that the sample was



taken. However, as was noticed in the catch per unit effort values, both species are caught in relatively low numbers and hence conclusions based on small differences that are not significantly different are limited. There was also a problem with low numbers in all spatial and temporal trends described. More intensive sampling in boundary waters, both vertical and horizontal is needed to understand how juvenile squid react to the physical environment.

The considerable variability in age at size estimates for both species throughout the summer is possibly the result of variable physical and biological conditions. Variability in growth rates within a population has been documented for a number of squid species (Natsukari *et al.* 1988, Hatfield 1991, Rodhouse & Hatfield 1990b, Natsukari & Komine 1992, Jackson & Choat 1992, Jackson *et al.* 1993). Changes in growth rates in adults squid have been described between seasons (*Loligo chinensis* Jackson & Choat 1992) and between years (*Todarodes angloensis*, Villanueva 1992a). However, results presented here show that variation in growth is occurring on much shorter time scales. Potentially, relatively small changes in temperature can have considerable effects on growth and size of individuals by the time they reach sexual maturation (Forsythe 1993). During the second summer of sampling, surface water temperatures changed by 5°C during the four months, and between the surface and benthos by 3°C. Hence there was potential for variability in growth because of water temperature changes. Squid demonstrate considerable plasticity in many features of their life history and factors such as food availability, day length, light intensity and temperature play a role in this (Van Heuklem 1979). Long lived, large, late maturing species are more typical of temperate waters, whereas tropical species are generally smaller, short lived and early maturing (Jackson 1990, Jackson & Choat 1992). Consequently, a short-lived species must be able to reproduce throughout the year producing juveniles that can tolerate a range of conditions. However, some long-lived temperate species also demonstrate year-round recruitment (*Loligo forbesi*, Lum-Kong *et al.* 1992) or two spawning periods (*Loligo gahi*, Patterson 1988; *Loligo pealei*, Summers 1971) suggests that tolerance by juvenile cephalopods for a range of conditions may not be exclusive to tropical species. The population consequences of variable growth rates among juvenile squid are unknown (O'Dor & Coelho 1993) but

given the sensitivity of juveniles to different conditions, this variability will have the potential to alter the structure of the population.

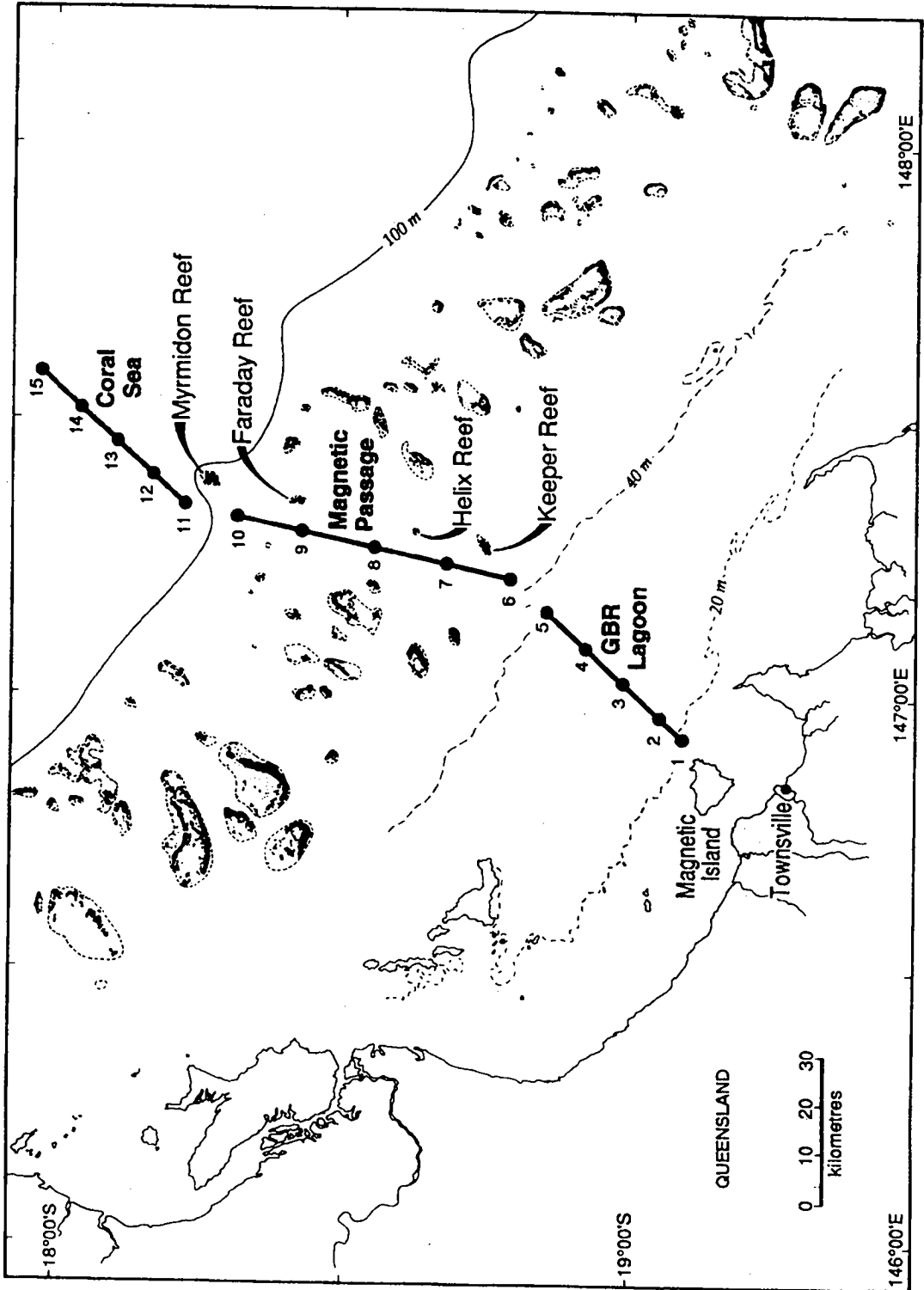


Figure 2.1: Map of the cross-shelf transect off Townsville, Australia, showing the position of each station along the transect. Station 1=19 km from Townsville, 2 = 24 km, 3 = 33 km, 4 = 43 km, 5 = 52 km, 6 = 61 km, 7 = 75 km, 8 = 92 km, 9 = 100 km, 10 = 115 km, 11 = 136 km, 12 = 145 km, 13 = 152 km, 14 = 163 km and 15 = 172 km.

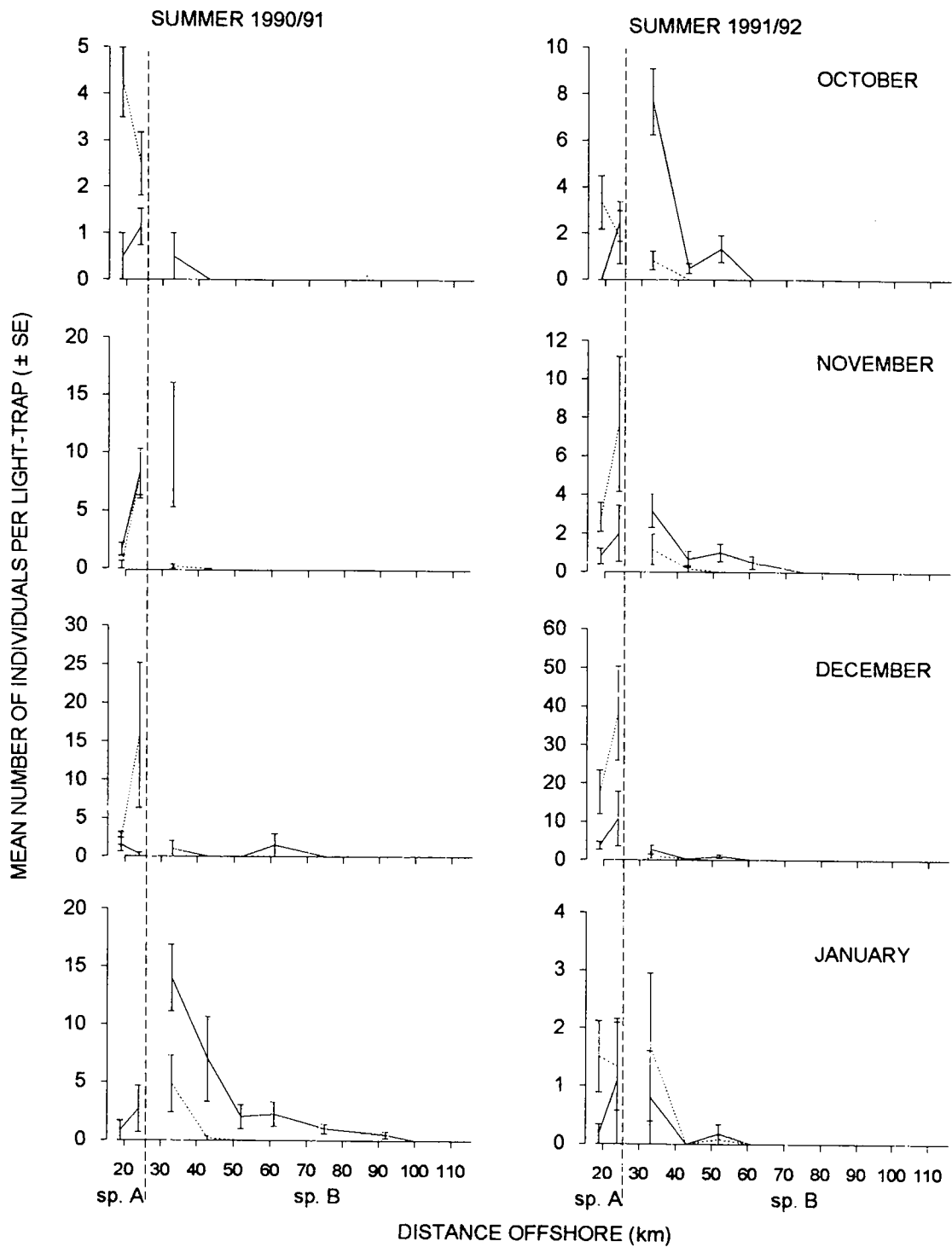


Figure 2.2: Catches of juvenile *Photololigo* sp. A (present 19 & 24 km) and *Photololigo* sp. B (present at 33 km and greater) with increasing distance from Townsville, Australia. Most values are average catches ( $\pm$  standard error) of six one-hour sets over three nights. See Table 1 for replicates at each station. (Solid lines, deep light-traps; dashed lines, shallow light-traps). Note the variable scale of the Y-axes.

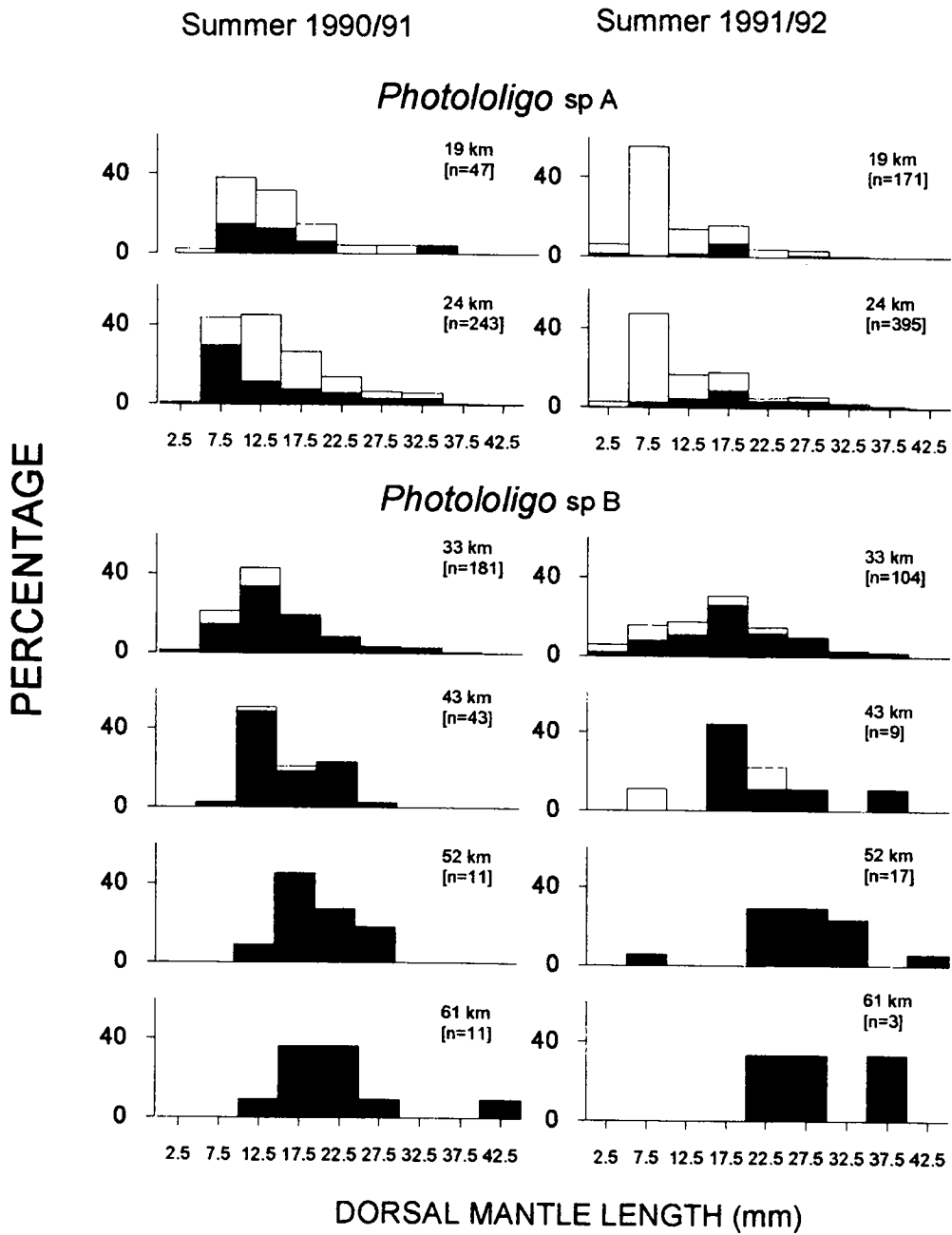


Figure 2.3: Size frequency distribution of *Photololigo* sp. A and *Photololigo* sp. B caught at each station (pooled across months) in deep (shaded) and shallow (unshaded) light-traps. Total number of juveniles indicated in brackets.

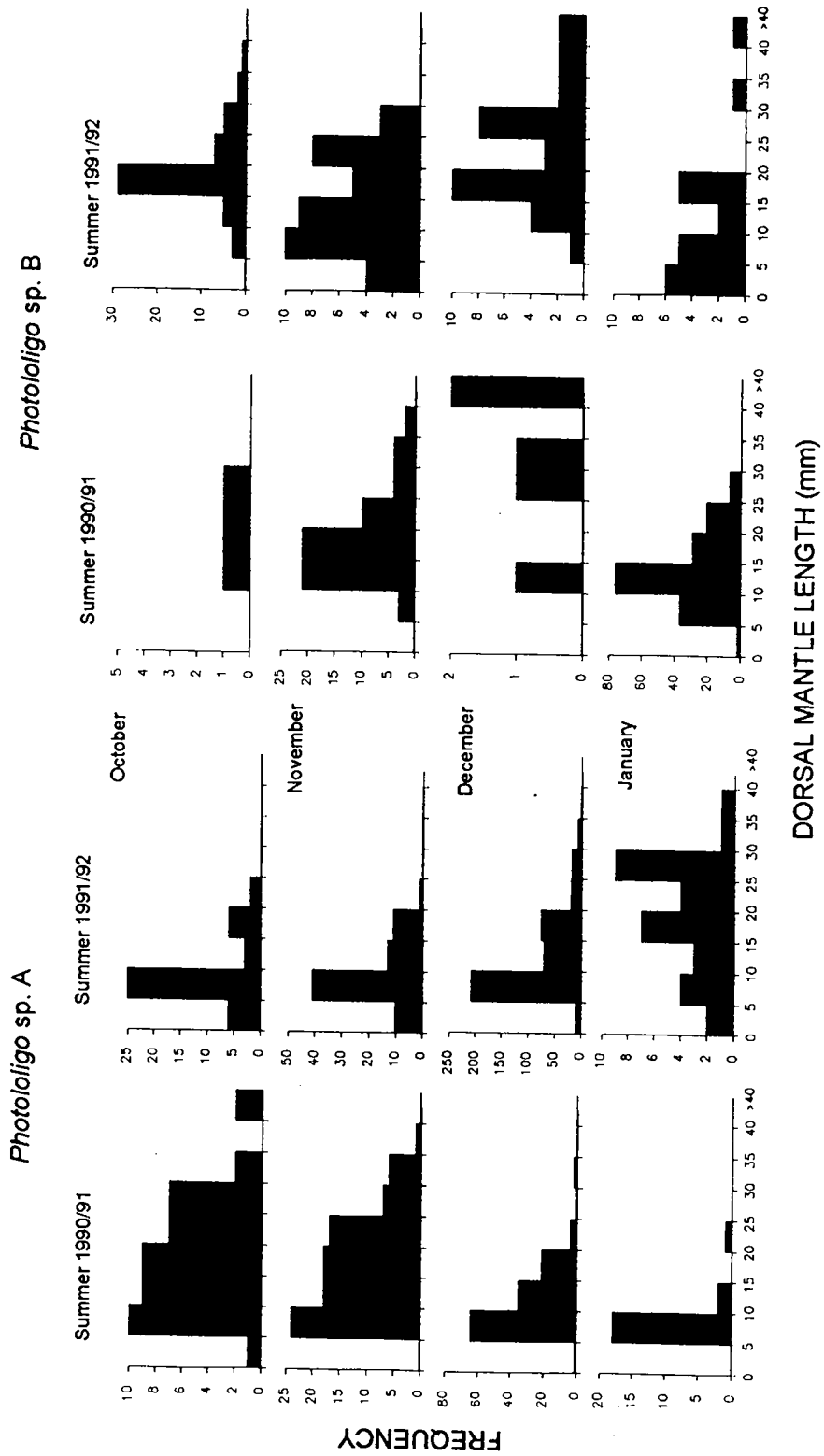


Figure 2.4: The size frequency distribution of juvenile *Photololigo* sp. A and *Photololigo* sp. B during eight months of summer sampling. Size classes are mid-points of each class. Data are pooled across depths and stations. Note the variable scale of the Y-axes.

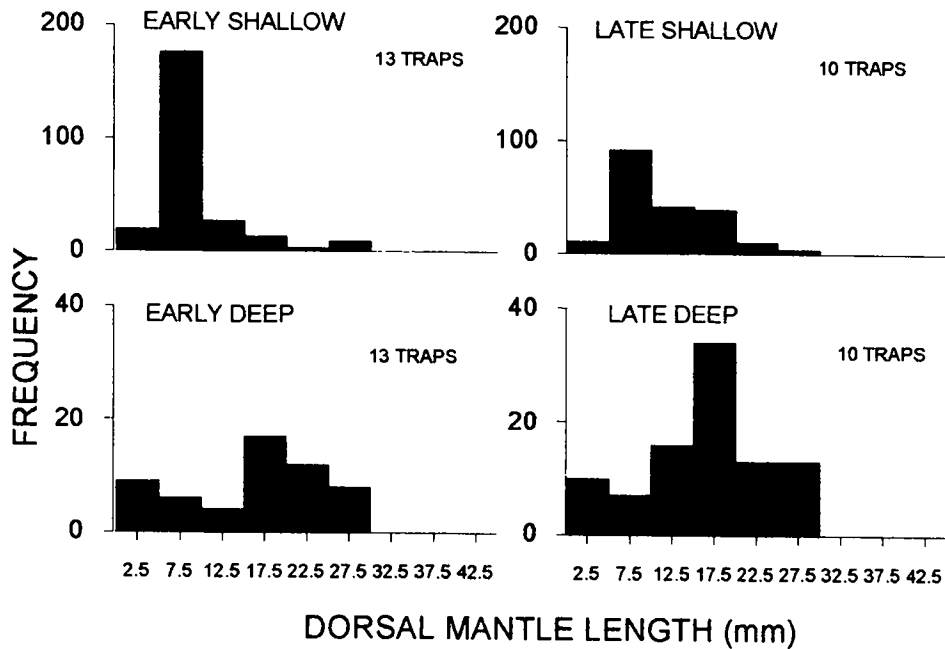


Figure 2.5: Size frequency distributions of juvenile *Photololigo* sp. A from the two inshore stations at two sampling depths (pooled across the summer months in 1991/92), captured early (before 2400 hrs) and late (after 2400 hrs) in the night. Note the variable scale of the Y-axes.

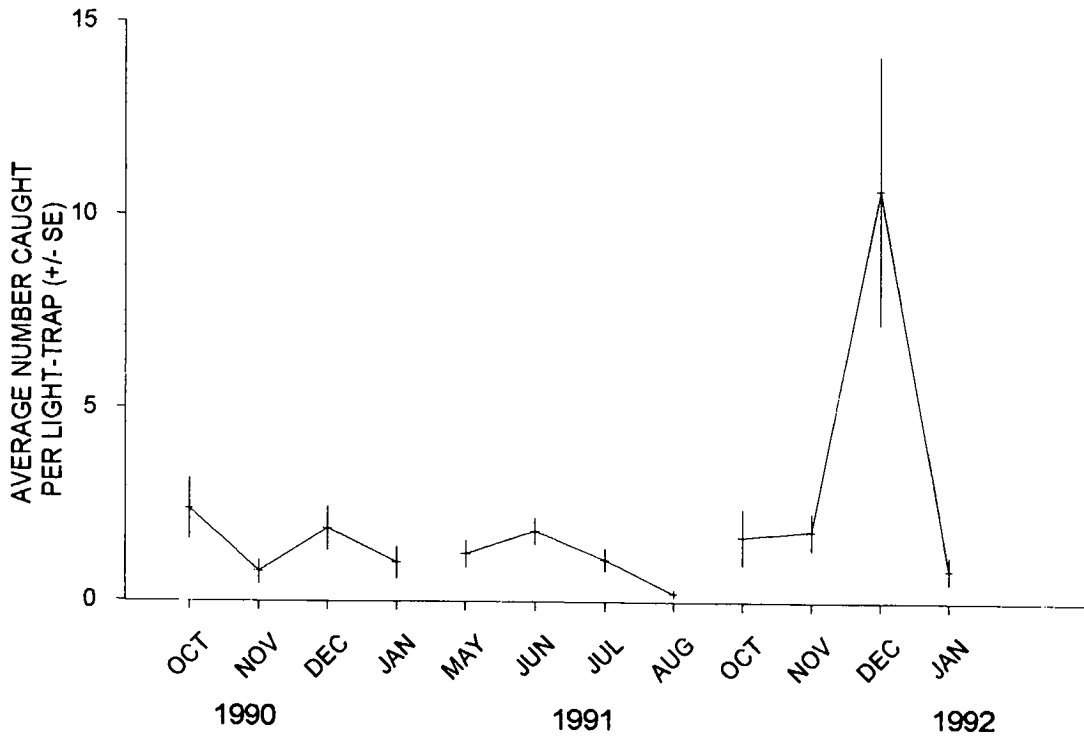


Figure 2.6: Catches of juvenile *Photololigo* sp. A at the 19 km station during twelve months; summer 1990/91, winter 1991 and summer 1991/92. (Data pooled across depth and nights).



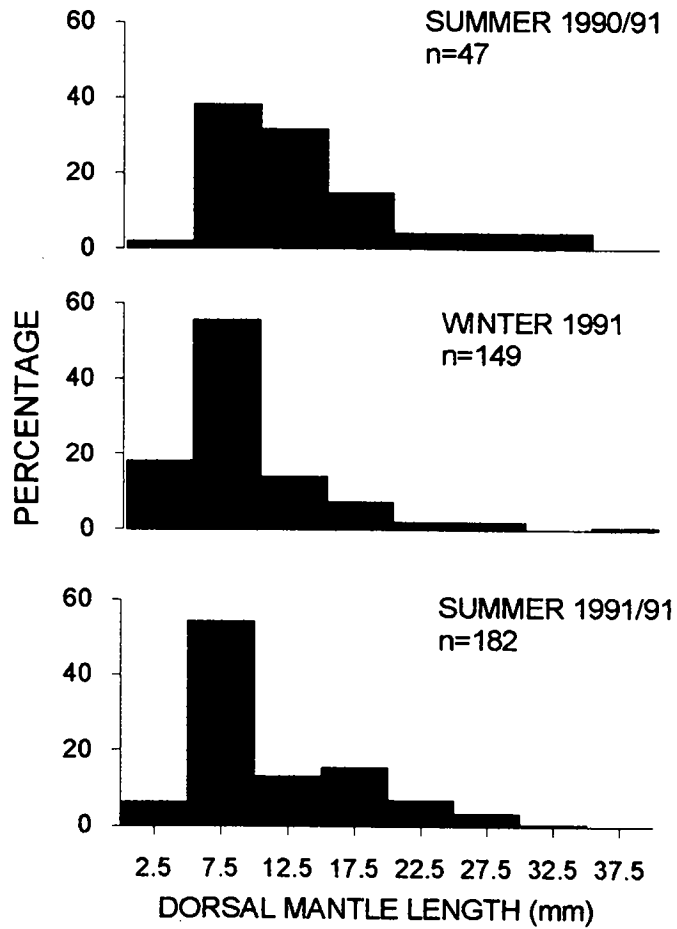


Figure 2.7: Size frequency distributions of juvenile *Photololigo* sp. A at the 19 km station during summer and winter months. (Numbers are pooled across months, depth and nights).

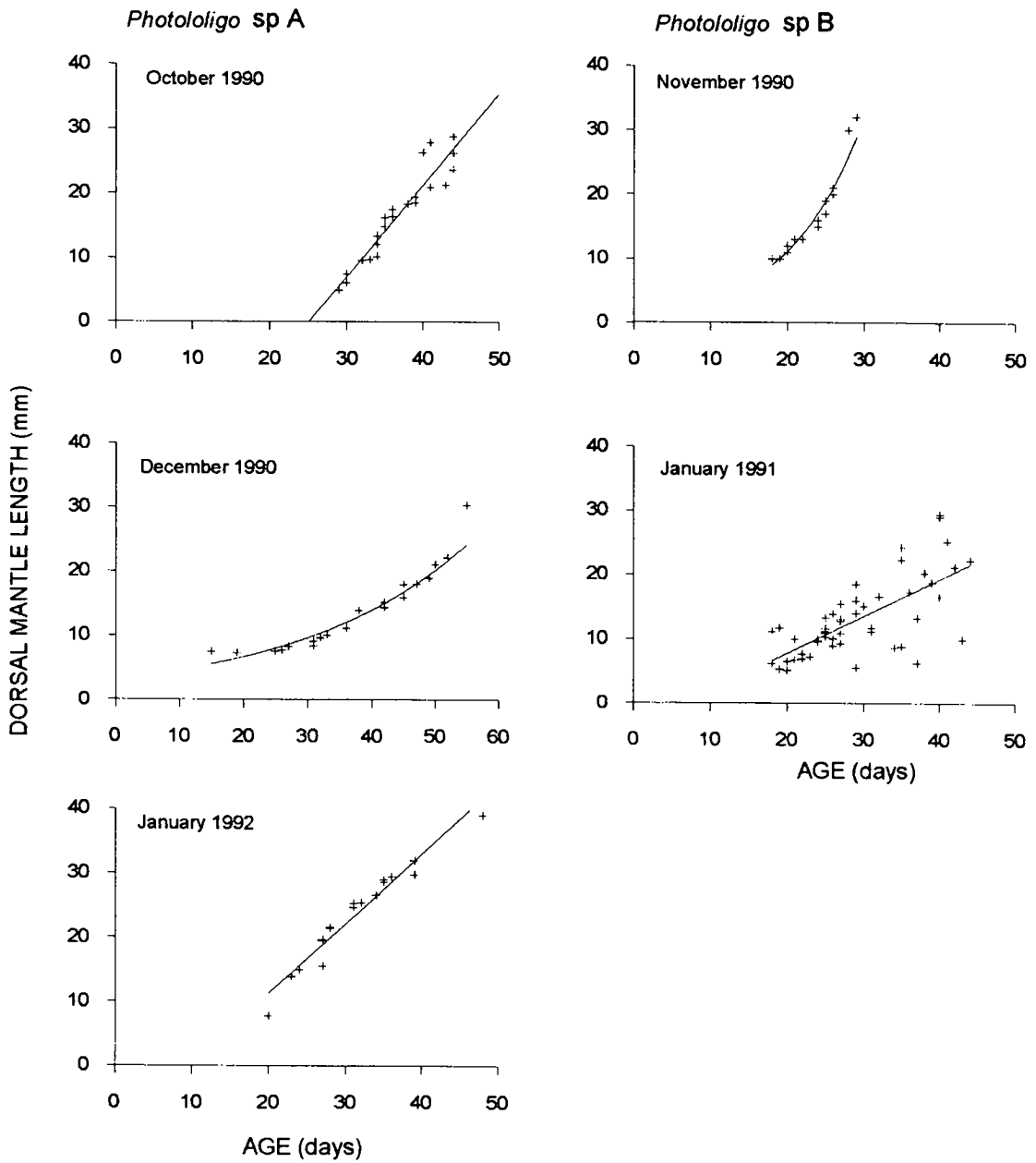


Figure 2.8: Size at age plots for the juveniles of two species of *Photololigo* during some of the summer months.

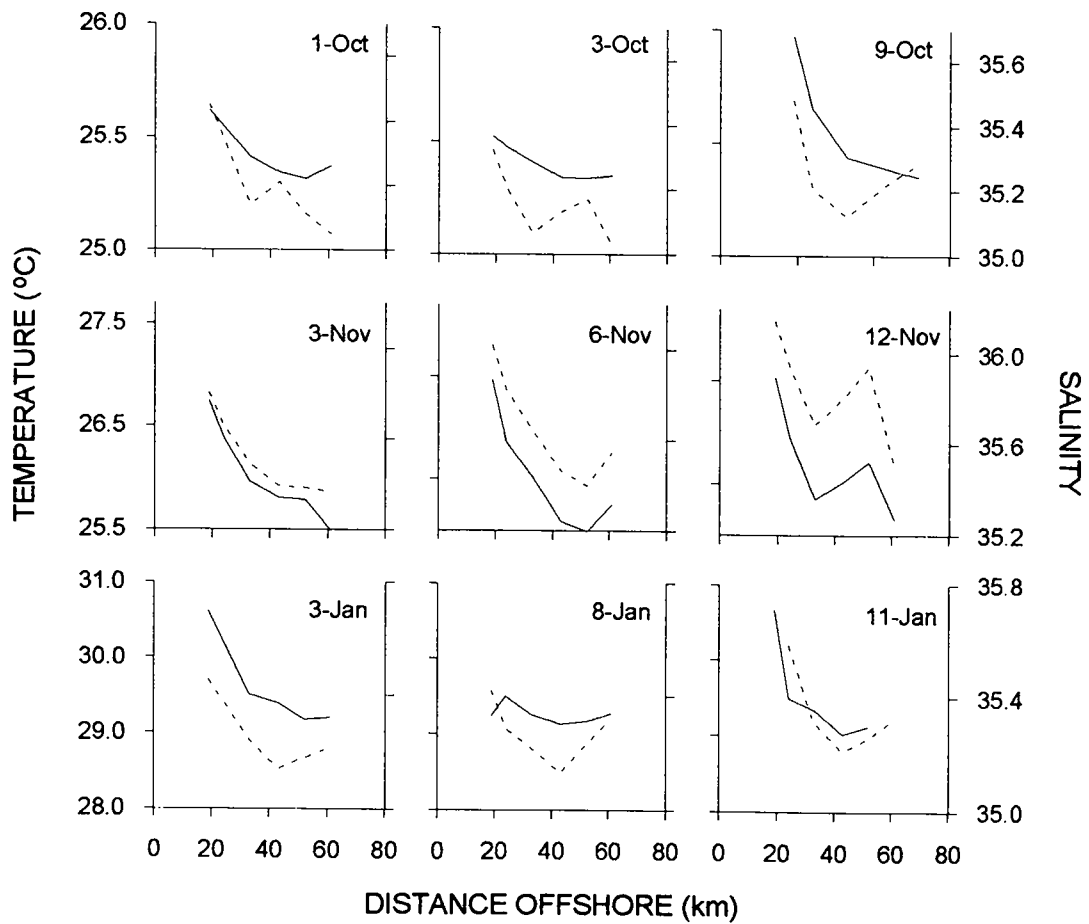


Figure 2.9: Surface temperature (dashed) and salinity (solid) profiles across the Great Barrier Reef Lagoon for each night of sampling in October and November 1991 and January 1992. The Conductivity Temperature Device failed during the December cruise. Note the variable scale of the Y-axes.

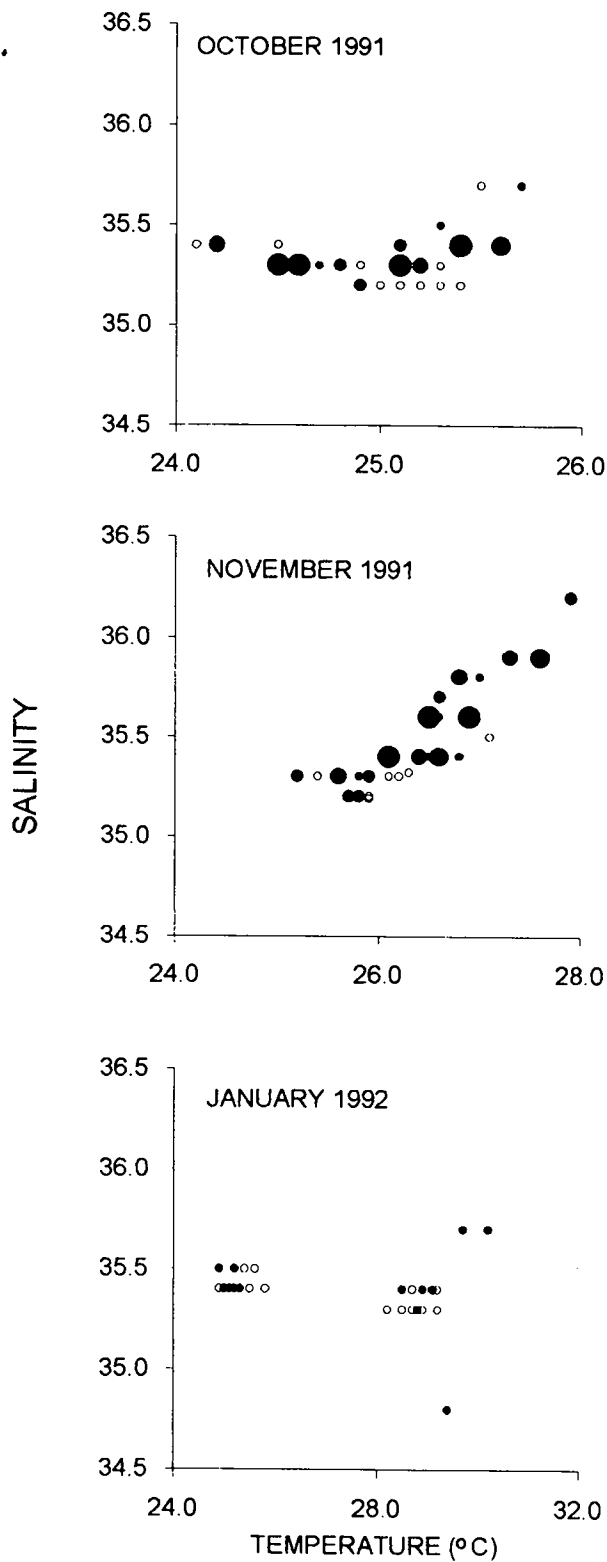


Figure 2.10: Temperature salinity plot showing the abundance of juvenile *Photololigo* for each combination of salinity and temperature by month. The size of the bubble represents the average number of juveniles per light trap (n=2):

o = no individuals, • = 0.5, ● = 1, ● = 2, ● = 3 and ● = >3.

# CHAPTER III:

## MORPHOLOGICAL CHANGES ASSOCIATED WITH ONTOGENY

### 3.1 INTRODUCTION

Few descriptions of ontogenetic changes in shape are available for squid. Most studies of squid growth have provided empirical descriptions that can be used to generate numerical models of population dynamics. As a result only body length and sometimes body weight have been used to quantify growth. However, these measurements are not always sufficient to provide a complete description of growth. A more detailed understanding of cephalopod growth requires knowledge of growth of different elements of the body during the development process. This study provides some of the first detailed information on the pattern of squid growth.

During growth few body structures increase in size at the same relative rate (isometric growth) and stay the same shape because of the geometric changes needed to maintain the function of a structure. Therefore, the body structures and organs must grow in a way that maintains their functional and physiological equivalence (Gould 1966). This is possible by differential growth of structural elements of the organism that results in a change in shape (allometric growth). To recognise the ecological and lifestyle consequences of these changes it is necessary to describe body shape through ontogeny. At the most extreme end of the scale some organisms undergo metamorphosis in which the body parts and organs are reorganised so that the juvenile form is not recognised as the same species as the adult. At the other end of the scale juveniles emerge from the egg or mother as miniature adults. However, usually the body structures of these juveniles will undergo allometric growth. This will enable complete growth and functioning of structures by the time the adult form is attained. In many cases mechanical and size restrictions of body parts in the juveniles force these individuals to have different life styles (Werner & Gilliam 1984). Many studies of allometric growth in squid have examined only part of the life history;

either juvenile or adult phases. A better understanding of growth and changes in lifestyle may be obtained by examining morphological changes throughout the entire life cycle.

Animals often undergo ontogenetic changes in their habitat and ecology. These differences are often associated with changes in shape, loss of structures and the development of new structures. Therefore information may be derived about the interaction of the organism with the environment from descriptions of ontogenetic shaping with growth (Tessier 1960). Cephalopods undergo direct development and in most species the basic adult form is present at the post-embryonic phase. Although juveniles do not assume an adult life-style immediately they do not undergo the extensive structural changes typically seen in other molluscs or teleosts during metamorphosis (Boletzky 1974). This implies that juveniles are functionally miniature adults. However, a superficial examination reveals that this may not be the case. Juvenile squid have small fins and arms and thin mantle muscle tissue that suggests the prey capture techniques and mobility of juveniles differs from adults (Okutani 1987). The pelagic habitat occupied by most juvenile squid is patchy and provides little shelter from predators. Characteristics such as mobility, visual acuity and colour changes may be important to ensure survival during this phase.

It is recognised that different species will differ morphologically because of subtle differences in habitat and resource use. Hence taxonomic studies use morphometric analyses to discriminate species (Kashiwada & Recksiek 1978, Kubodera & Okutani 1977, Augustyn & Grant 1988, Yeatman, 1993). Unfortunately these studies usually examine either juvenile or adult forms but rarely both. This has resulted in the use of interspecific juvenile and adult allometry as a static comparison of proportions of individuals of different sizes but similar life-history stage. As a result, taxonomic studies have also ignored changes in shape associated with growth of an organism. However, complete morphological descriptions during growth of cephalopods may be necessary to recognise structures useful for taxonomic description.

When examining and describing allometric growth, biologists have typically used bivariate regressions to describe the relative growth of one structure to another

structure. These relationships can then be compared between different size or age groups. Comparisons are possible when distinct forms are evident or different age groups occupy discrete habitats at specific stages during their life history. In that case, bivariate examinations of growth provide a detailed insight into changes in dimensions of certain body parts (Osse 1990). It is often difficult to assign an individual to a specific stage of development when ontogenetic changes in shape and life-style are gradual (Young & Harman 1988). For some species that undergo gradual and subtle changes, distinct 'growth stanzas' are not obvious. In such cases the transition from stanza to stanza is unclear and the use of multiple bivariate lines to describe growth of individuals in different 'stanzas' becomes arbitrary (Gould 1966).

Multivariate analyses of a number of measurements allows a complete description of growth, generating a comprehensive picture of the living organism (Jolicoeur 1963a). Multivariate techniques make it possible to obtain descriptions of relationships between the size and shape of a number of body structures simultaneously (Jolicoeur 1963a). Principal component analysis provides a way of breaking down the total variation of a biometrical character complex into size and shape components (Jolicoeur 1963b, Jolicoeur & Mosiman 1960). These more traditional multivariate approaches have an advantage over newer analytical techniques such as eigenshape analysis and landmark data because their long use has led to some biological understanding of the analysis (Jolicoeur & Mossiman 1960, Jolicoeur 1963, Brown & Davies 1972, Davies & Brown 1972, Shea 1981, 1985).

This study will use a combination of bivariate and multivariate approaches to examine and describe the relative growth of external structures and visceral elements during ontogeny of the loliginid squid *Photololigo* sp. A. Bivariate regressions will generate descriptions of allometric growth for comparisons with other studies, whilst multivariate morphometric techniques will provide a complete picture of ontogenetic growth using all measurements simultaneously.

### 3. 2 METHODS AND MATERIALS

#### 3.2.1 SPECIMEN HANDLING AND MORPHOMETRIC MEASUREMENTS

A wide size range of *Photololigo* sp. A individuals was collected from coastal waters of the central Great Barrier Reef using two techniques. Automated light-traps caught individuals less than 50 mm dorsal mantle length. Twin-otter bottom trawls captured larger specimens, greater than 50 mm dorsal mantle length. Although trawling may damage soft-bodied organisms, short trawl times (<10 minutes) minimised damage to squid. Allozyme electrophoresis was used to confirm that all adults used in the study were the same species.

All specimens were frozen on capture and measured later in the laboratory. Soft bodied organisms, like squid, are easily misshapen by handling during measuring. To minimise distortion and reduce handling of specimens, body measurements were obtained using computer aided image analysis. A video camera connected to an Apple Macintosh IICI computer, with a Rasterops 364 video colour card, displayed images of the squid onto a video screen. Images were stored with 'Framegrabber' software then measured and analysed with the 'NIH IMAGE' program (public domain software).

Specimens were defrosted at room temperature before being measured. Each individual was placed flat on a tray, with fins, arms and tentacles extended. Images of the dorsal and ventral surface were recorded before the mantle cavity was opened ventrally. Eleven length measures were taken from each individual (Fig. 3.1): dorsal mantle length (DML), maximum mantle width (MW), fin length (FL), maximum fin width (FW), head length (HL), eye diameter (ED), tentacle length (TL) and length of each of the four arms (A1-A4). Where possible, measurements were made on the right-hand side of the body. In addition five weight measures were recorded; total body weight (TWT), visceral weight (VWT) (excluding reproductive organs), head weight (HWT), gonad weight (GWT) (all reproductive organs) and mantle muscle weight (MWT).



### 3.2.2 SIZE AT AGE

Statoliths were removed from each specimen to obtain age information (Jackson 1990b, 1991). Statoliths of large squid were ground and polished to get a clear view of the nucleus and edge. Statoliths were ground along the dorso-ventral axis (Dawe & Beck 1993) with 1200  $\mu\text{m}$  grit wet and dry polishing paper and a final polish, to remove scratches, was done with 0.3  $\mu\text{m}$  alumina powder. For small statoliths the posterior side was lightly ground with 9  $\mu\text{m}$  lapping film and polished with 0.9  $\mu\text{m}$  alumina powder. Daily rings were counted under a light microscope with a polarised light source. Rings were counted from the natal ring (Jackson 1990b, 1991). Where the natal ring could not be seen, or three consecutive counts within 10% of each other could not be obtained, the statolith was discarded as unreadable.

### 3.2.3 BIVARIATE ANALYSES

Initial examination of the data entailed a series of regression analyses, with dorsal mantle length as the independent variable for length measures and total body weight for weight measures. Since all measurements were made with statistical error, and strictly speaking there is no independent variable, a 'reduced major axis' calculation is more correct than the 'ordinary least squares' calculation (McArdle 1987). However, when variables are closely correlated, the two calculations produce very similar results (Shea 1981), so the 'ordinary least squares' calculation was used. All variables were  $\log_{10}$  transformed so that specific growth rates of body structures were analysed in the equations of allometric growth (Shea 1985). Individuals were separated into two dorsal mantle length size groups for these descriptions: small (< 50 mm) and large (> 50 mm). This separation is based on the techniques used to capture the individuals, which may be related to differences in life style and ecology. This separation also allowed comparisons of relative growth rates between the two groups and with data from the literature.

### 3.2.4 PRINCIPAL COMPONENT ANALYSIS

Principal component analysis (PCA) is a multivariate ordination technique that enables observations in multidimensional space to be examined in fewer dimensions. It is useful because relationships between variables and among observations can be examined. The PCA identifies and summarises major patterns of variation in the observations and generates new axes to describe these patterns of variation. The new axes are combinations of the original variables so the relationship between the variables and the new axes is also described. The axes are orthogonal to one another, so the variation explained by each axis is unique. Furthermore, the analysis calculates the percentage of variation in the data set explained by each axis.

Each variable is related to the new axes and a 'eigenvector coefficient' is calculated. If a variable has a large coefficient, positive or negative, on a principal component this indicates a high correlation between the variable and that axis. Co-ordinates (principal component scores) on the new axes are calculated for each observation, allowing each observation to be plotted in the reduced space. Both negative and positive principal component scores and coefficients will be calculated because the origin of the new axes is in the centre of the data set. Using this analysis it is possible to describe the relationship between observations and variables and determine which variables are related to the patterns of variation among the observations. Principal component analyses were done separately on length and weight data, because body parts may differentially increase in bulk and not length.

### 3.2.5 TRANSFORMING AND STANDARDISING THE DATASET

The standardisations and transformations of the dataset used in the PCA has important implications for the interpretation of the analysis. This dataset was handled in three different ways to derive different information about the growth data. Centring of the data set involves calculating the difference between each observation and an average value. If column centred then the column average is used and if row centred then the row average is used. Where the data set is double centred row centring is followed by column centring. The use of column centring allows

relationships between the variables or columns to be examined, whereas row centring allows relationships between observations or rows to be examined. Double centring allows the relationships between columns and rows to be investigated. The use of correlation matrix results in standardising the data to unit variance.

i) Covariance matrix of log<sub>10</sub> transformed data. This is the usual data form to examine allometric relationships in multivariate morphometric data (Jolicouer 1963a). The use of the covariance matrix allows relationships between the variables and how they covary to be described.

ii) Correlation matrix of untransformed data. The data are standardised to unit variance allowing absolute changes in body structures as growth occurred to be examined. Therefore, variation among individuals will dominate the analysis because they differ in size. However, these differences can be examined as a function of the age of individuals.

iii) Double centring of log transformed data.

Row centred data sets allow relationships between observations to be examined without differences in absolute values affecting the interpretation. Both column and row centred of the data (double centring) has the potential to examine differences in shape that are not a function of size (Darrock & Mosiman 1985).

### 3.2.6 ALLOMETRIC AND ISOMETRIC GROWTH

The use of a covariance matrix of log<sub>10</sub> data expresses the variation along the first PCA axis resulting from growth (Shea 1985). The magnitude of the coefficients in the first eigenvector differs because of patterns of relative growth of the organism and changing body proportions. The magnitude of the coefficients can be used to determine the nature of allometric growth for each variable (Jolicouer 1963a). Variables that have coefficient scores on the first principal component vector equal to  $(1/p)^{0.5}$  ( $p$  = number of variables in the analysis) are isometric. Values less than and greater than the mean coefficient, demonstrate negative and positive allometry

respectively (Jolicoeur 1963a). Hence this axis reflects changes in the relative growth of structural elements of the organism (Shea 1985).

The re-sampling technique, jack-knifing, was used to calculate the mean and standard error of each coefficient (Marcus 1990)<sup>1</sup>. This technique involves sampling a random sub-sample of observations from the data set, in this case (n-1), multiple times to calculate a population of values. From this population of values a mean coefficient and standard error of the mean can be calculated. The probability that the mean coefficient is significantly different from the hypothesised value is calculated as follows (Marcus 1990):

$$\text{Pr} (|\text{mean coefficient} - \text{hypothesised coefficient}| > T * \text{SE} * \text{mean coefficient}) < 1/T^2$$

Where T = the number of standard errors for which one wants to make the probability statement. In this case T = 4.47 provides a probability of 0.05. In other words the difference between the mean coefficient and the hypothesised coefficient is greater than a critical difference (T\*SE\*calculated coefficient) with a probability < 1/T<sup>2</sup>.

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<sup>1</sup> The computer program used to calculate the jackknifed means and standard errors of the eigenvalues was written in SAS/IML by Leslie Marcus and provided with the Proceedings of the Michigan Morphometrics Workshop in 1988.

### 3.3 RESULTS

#### 3.3.1 SIZE AT AGE

Dorsal mantle length and total body weight increased exponentially through time (Fig. 3.2 a & b). These relationships indicate that the specific growth rates were constant throughout the life-history of the individuals, 4% per day increase in dorsal mantle length and 9% per day body weight (Table 3.1). The high coefficients of determination ( $r^2$ ) for both equations suggest that age explains much of the variation in body size. However there was approximately 15% of the variation in body size not explained by age.

Table 3.1: Growth relationships for dorsal mantle length and total wet weight size for *Photololigo* sp. A.

VARIABLE	GROWTH RATE	SE	$r^2$	n	RANGE
Mantle length	0.04	0.002	0.85	57	2.77-117.03 mm
Body weight	0.09	0.005	0.86	57	0.006-37.8 g

#### 3.3.2 BIVARIATE MORPHOMETRIC RELATIONSHIPS

All length measurements showed strong relationships with dorsal mantle length (Fig. 3.3). Rapid growth of most body structures relative to mantle length in small squid was evident (Table 3.2). Exceptions to this were mantle width and head length that increased at similar relative rates throughout growth of *Photololigo* sp. A. However, adult squid size was more variable than juveniles (Table 3.2).

Table 3.2: Growth coefficients describing the relative growth of the lengths of ten body structures. All length measures are log<sub>10</sub> transformed. Slope values for small squid in bold are significantly different (using a t-test) from the slopes calculated for large squid.  $\alpha=0.05$ .

Variable	Small (< 50 mm DML)				Large (> 50 mm DML)			
	Slope	SE	Intercept	r <sup>2</sup>	Slope	SE	Intercept	r <sup>2</sup>
Mantle width	0.65	0.03	0.03	0.94	0.64	0.06	0.22	0.70
Fin length	<b>1.29</b>	0.04	-0.90	0.95	1.11	0.08	-0.48	0.81
Fin width	<b>1.13</b>	0.04	-0.50	0.95	0.79	0.06	0.16	0.79
Head length	0.64	0.05	-0.26	0.78	0.59	0.08	0.09	0.55
Eye diameter	<b>0.77</b>	0.05	-0.58	0.84	0.50	0.10	0.06	0.36
Tentacle length	<b>1.46</b>	0.08	-0.77	0.90	0.62	0.07	0.76	0.61
Arm 1 length	<b>1.22</b>	0.05	-0.80	0.93	0.87	0.10	-0.38	0.59
Arm 2 length	<b>1.03</b>	0.06	-0.56	0.88	0.67	0.10	0.18	0.49
Arm 3 length	<b>1.08</b>	0.07	-0.59	0.85	0.88	0.08	-0.14	0.70
Arm 4 length	<b>1.06</b>	0.08	-0.82	0.81	0.69	0.08	0.19	0.61

Three of the four body components measured increased in mass faster in small squid than large squid (Fig. 3.4; Table 3.3). However, only mantle muscle mass of small squid grew proportionally faster than total body weight. Mass of all the structures increased more slowly than total body weight for the larger individuals (Table 3.3). Gonad weight and total body weight are poorly related because reproductive maturation was not a function of size in *Photololigo* sp. A.

Table 3.3: Growth coefficients to describe the relative growth of the mass of four organs. All weight measures are log<sub>10</sub> transformed. Slope values for small squid in bold are significantly different from the slopes calculated for large squid.  $\alpha=0.05$ .

Variable	Small (< 50 mm DML)				Large (> 50 mm DML)			
	Slope	SE	Intercept	r <sup>2</sup>	Slope	SE	Intercept	r <sup>2</sup>
Mantle muscle	<b>1.10</b>	0.02	-0.30	0.98	0.95	0.01	0.01	0.99
Visceral	<b>0.99</b>	0.03	-0.98	0.97	0.78	0.08	-0.82	0.67
Head	<b>0.97</b>	0.03	-0.41	0.96	0.81	0.02	-0.12	0.97
Gonad	-	-	-	-	0.75	0.12	-0.71	0.45

### 3.3.3 RELATIVE GROWTH

The biggest source of variation in the data was the size of the squid (Fig. 3.5a). This first principal component axis described 97% of the variation among individuals, which means that all variables were increasing in relative size (Table 3.4). The

coefficients for each variable in the first eigenvector describe the relative growth rates of the all the components simultaneously (Shea 1985). The shape of small squid changed rapidly before individuals attained a final shape at approximately 60 mm beyond which point changes in shape were slower (Fig. 3.5a). Therefore, most of the variation expressed along this axis was due to growth of smaller individuals (dorsal mantle length <60 mm). There was a correlation between the principal component scores on the first axis and age, but there was a weak relationship between the shape of an individual and its age (Fig. 3.5b).

The mantle length and fin length grew proportionally faster than mantle width and fin width (Table 3.4). Thus, there was a transformation from a rounded shape to a more elongate form. Tentacles and arm 4 grew quickly especially when compared to the other arms. The advanced development of the eyes and head were evident in their slow relative growth.

Table 3.4: Relative growth of length measures. Means and standard errors (SE) of the coefficients of the first eigenvector for log10 transformed data length data. Isometric growth occurs when the coefficient equals  $(1/p)^{0.5} = 0.316$ . The difference equals the coefficient minus 0.316, which must be greater than the critical difference if allometric growth has occurred.

Variable	Coefficient	SE	Critical difference	Difference	Growth
DML	0.269	0.003	±0.004	0.047	positive allometry
MW	0.221	0.004	±0.004	-0.095	negative allometry
FW	0.312	0.004	±0.006	0.004	isometry
FL	0.374	0.004	±0.009	0.058	positive allometry
HL	0.237	0.007	±0.008	-0.079	negative allometry
ED	0.235	0.007	±0.008	-0.081	negative allometry
TL	0.366	0.013	±0.017	0.050	positive allometry
A1	0.269	0.010	±0.017	-0.047	negative allometry
A2	0.276	0.011	±0.013	-0.040	negative allometry
A3	0.326	0.007	±0.011	0.010	isometry
A4	0.377	0.009	±0.017	0.061	positive allometry

It was possible to describe gross changes in the shape of small versus large individuals on the first axis. However, there was some additional variation described on the second and third PCA axes that was attributable to small individuals (Fig. 3.6a & b; Table 3.5). Growth of arms and tentacles was important as individuals approached their final size. Tentacles and arm 1 were proportionally larger in the

juveniles, and arms 4 and 3 were proportionally smaller immediately before individuals reached the final adult shape. Positive allometry was evident in dorsal mantle length, fin length, and length of tentacle and arm 4. During growth of *Photololigo* sp. A the mantle width, head length, eye diameter, and arms 1 and 2 displayed negative allometry. Fin width and arm 3 length were the only structures to grow isometrically. This suggests that during ontogeny there is considerable growth of the tentacles relative to arms 3 and 4.

Table 3.5: Relative growth of length measures. The coefficients for the first three components of the analysis on length variables. Data are log10 transformed and the covariance matrix was used.

VARIABLE	EIGENVECTOR		
	PCA1	PCA2	PCA3
DML	0.269	0.127	0.047
MW	0.221	-0.107	-0.041
FW	0.312	0.073	-0.090
FL	0.374	0.075	-0.140
HL	0.237	-0.267	0.193
ED	0.235	-0.132	0.282
TL	0.366	0.544	-0.694
A1	0.269	0.436	0.410
A2	0.276	0.165	0.765
A3	0.326	-0.371	0.072
A4	0.377	-0.546	-0.325

The analysis of the weights of structures suggested that most of the increase in weight is due to the mantle muscle tissue. The mantle muscle tissue increased in mass rapidly compared with the growth of the other structures (Table 3.6).

Table 3.6: Relative growth of weight measures. Mean coefficient and standard errors (SE) for log10 transformed data weight data. Isometric growth occurs when the coefficient equals  $(1/p)^{0.5} = 0.5$ . The difference equals the coefficient minus 0.500, which must be greater than the critical difference if allometric growth has occurred.

Variable	Coefficient	SE	Critical difference	Difference	Growth
TWT	0.486	0.002	±0.004	-0.014	negative allometric
VWT	0.460	0.007	±0.014	-0.040	negative allometric
MWT	0.559	0.009	±0.022	0.059	positive allometric
HWT	0.487	0.010	±0.022	-0.013	negative allometric
GWT	0.048	0.010	±0.002	-0.452	negative allometric



The trends displayed along the first axis resulted from the heavier weights of larger individuals (Fig. 3.7a). There was a trend for increasing mass of the body structures with growth for all individuals, except for gonad mass (Table 3.6). Variation among the large individuals (Fig. 3.7a) was due to differences in gonad mass as indicated by the large coefficient for gonad weight in the second eigenvector (Table 3.7). The viscera grew more slowly than the gonads therefore the viscera became proportionally smaller in reproductive adults. Allocation of energy to the viscera and mantle muscle tissue in the small squid changes during ontogeny, with the mantle muscle becoming proportionally larger (Fig. 3.7b).

Table 3.7: Relative growth of weight measures. The first three eigenvectors for principal component analysis on log<sub>10</sub> weight data.

VARIABLE	EIGENVECTOR		
	1	2	3
TWT	0.486	0.112	-0.163
VWT	0.460	-0.286	1.650
MWT	0.559	0.024	-1.513
HWT	0.487	0.034	0.309
GWT	0.048	0.959	0.535

The relationship between the mantle weight of an individual and the principal component score on the first axis was similar to that of the length measures (Fig. 3.8a). Most of the variation resulted from differences in the proportions of small individuals, with larger squid having similar scores, regardless of body weight. Hence, once the squid attained a final size the relative changes in mass were very small. There was a weak relationship between age of an individual and the proportional size of the body structures (Fig. 3.8b)

### 3.3.4 ABSOLUTE GROWTH

Most of the variation among the individuals was because all structures were larger in larger individuals (Fig. 3.9a). Given the linear relationship between principal component scores on the first axis and size of individuals, the major source of variation was a difference in the size of individuals (Fig. 3.9b). There was a delineation in the PCA scores for individuals of different sizes, at approximately

50 mm dorsal mantle length. This may result from the analysis of absolute size differences as opposed to relative differences. Differences between large values are greater on an absolute scale than differences between small values. Growth of arms was dominant in smaller squid. Arm 1 grew at a faster rate during ontogeny than the other arms, especially arm 4 (Fig. 3.9a). Absolute decreases in the head length of small individuals were also evident.

Increase in mass during growth was represented along the first axis. Large squid accounted for most of the variation because the effect of large values has not been removed by the log<sub>10</sub> transformation (Fig. 3.10a). Mantle muscle tissue was the main contribution to the increase of total mass. However this increase in mantle muscle tissue was slower than growth of gonad tissue in maturing individuals. The principal component scores were correlated with total body weight (Fig. 3.10b) largely because larger rather than the smaller squid.

### 3.3.6 REMOVING SIZE EFFECTS

The size trend along the first principal component axis was not because of the greater size of all variables in large individuals (Fig. 11a & b). Small individuals had larger mantle width, head length and eye diameter relative to their body size, suggesting that these structures grew very little during ontogeny. The larger squid had longer tentacles and fins and arm 4 was longer. The variation in small squid during growth is because of the increasing length of arms 3 and 4. Allometry of growth is not interpretable from the first eigenvectors of the variables because the double centred transformation does not describe relative changes.

The weights of the body structures showed trend along the first principal component axis that is attributable to the size of the individuals (Fig. 3.12a & b). This trend appeared to be because of the relative weights of the gonad and mantle muscle tissue. The differences in the relative mass between viscera and mantle muscle tissue was also important (Fig. 3.12a). Difference between the sexes of larger individuals was evident (Fig. 3.12b). The weights of mantle muscle tissue and gonad differed

### CHAPTER III: MORPHOMETRIC GROWTH

between males and females and reproductive females had scores similar to larger immature individuals.

### 3.4 DISCUSSION

Changes in the shape of squid occur very rapidly in small individuals, but as individuals approach an adult form, changes in shape happen more slowly. Small *Photololigo* sp. A are round with relatively little tentacular and arm development. In contrast the eyes and head are relatively larger. During ontogeny the body lengthens and narrows, as do the fins. As individuals attain the adult shape, the head and eyes become proportionally smaller. *Photololigo* sp. A allocates considerable energy to tentacle and arm development as small squid grow. At approximately 60 mm the relative growth of all the body structures slows and individuals acquire a final adult shape. A major source of the increase in mass of squid is in the mantle muscle tissue, with the head and visceral masses growing relatively slowly. Once gonad growth starts, the relative weight of body structures changes substantially. In particular the mantle tissue grows proportionally more slowly. During growth of small squid the biggest changes in mass distribution are between the viscera and mantle muscle tissue. Mantle muscle tissue becomes a greater proportion of the total body mass as individuals approach an adult shape.

Small *Photololigo* sp. A squid have a different life-style than larger individuals. Small individuals were caught in surface waters and the larger individuals were caught deeper in the water column. Adult *Photololigo* sp. A were never caught using jigs but they are captured by prawn fishermen using bottom nets suggesting that this species is predominantly demersal. Therefore, *Photololigo* sp. A occupies the neuston during the juvenile phase then descends closer to the bottom as adults. It becomes difficult to isolate those changes in morphology that must occur for juveniles to adopt an adult mode of life from changes that occur as a function of increasing size and require a change in life style. Fast relative growth in the length of the mantle, tentacles, fins and arms in small individuals may be in response to their rudimentary development at birth: in contrast structures such as eye diameter and head length grew at relatively slower rates. Newly hatched individuals need well-developed sensory systems and motor control for prey location and capture. It appears that growth in the egg is concentrated on development of neural structures in the growing embryo, resulting in newly hatched juveniles with relatively large heads and eyes (Vecchione 1982,

Segawa 1987, this study). Many of these differential changes in structures can be related to changes in the mode of prey capture and handling during ontogeny. Newly hatched squid have an internal yolk sac that provides nutrition for some days after hatching. However, once hunting and feeding begins juveniles have non-contractile tentacles and long arms for prey capture; consequently they must be very close to the prey to capture it (Vecchione 1981, Boletzky 1987a). The actual capture of the prey is made by pouncing on it; jet propulsion provides a burst of forward movement. Growth of the arms and tentacles enables capture of larger prey and the use of tentacle movements instead of movement of the whole body. The changes in relative growth rates of these structures during ontogeny may indicate changes in habitat and lifestyle (Kubodera & Okutani 1977, Vecchione 1981). The changes in relative growth of structures usually corresponds well to boundaries in growth stages. The nature of the change provides considerable information about the morphological requirements of juveniles and adults in their respective habitats.

Direct development in cephalopods means that major metamorphic changes do not occur during ontogeny. The only exception to this are ommastrephid paralarvae that have a different tentacular structure from the adults. Changes in relative growth of body elements are not unusual and inflections in growth trajectories during the juvenile phase of squid are known to occur (Vecchione 1981, Segawa 1987, Kubodera & Okutani 1977). However, *Photololigo* sp. A did undergo some very subtle but important changes in body shape and proportions. Juvenile *Photololigo* sp. A has a globular shape, but relative growth of the mantle length is greater than the mantle width resulting in a streamlined shape in the adult form. Size and shape have important implications for the hydrodynamics of marine organisms (DeMont & Hokkanen 1992). For small and slow moving organisms seawater is a viscous substance that prevents the persistence of motion. For larger and faster moving animals seawater becomes an inertia dominant system, where moving objects remain in motion. For species such as *Photololigo* sp. A, that do not use ammonia for buoyancy, fins become an efficient way of maintaining position in the water column (Wells 1990). As growth occurs and the animal moves from a viscosity dominant to an inertia dominant system development of the fin structure becomes important. Shape has further implications for friction and pressure drag on animals during

movement. Streamlining of shape has the effect of reducing pressure drag but an associated increase in the surface area increases friction drag on an animal. However, for small animals the friction drag is greater than the pressure drag regardless of shape hence streamlining has little effect. Instead, a more rounded globular shape reduces surface area and reduces friction drag. This change in shape with growth is very evident in *Photololigo* sp. A. Squid have a high drag coefficient despite their streamlined shape, but they have greater thrust than fish because of the volume of water ejected out of the mantle cavity during jet propulsion (Alexander 1977). Therefore, the change in shape in *Photololigo* sp. A during growth may be to increase mantle volume rather than to obtain a streamline outline. Jet propulsion is not very efficient in adult squid, especially when compared with the undulatory swimming mode of fish. However, jet propulsion is more efficient in small squid that have a proportionally larger mantle opening and use a high frequency jetting action (O'Dor & Webber 1986, O'Dor 1988a & b). Typically the cost of transport decreases with growth (Schmidt-Nielsen 1972), but it appears that the rate of decrease is slower in squid than in fish (O'Dor & Webber 1986). It may be that the changes in shape with growth described here are important in maintaining relatively high swimming efficiencies.

The relative growth rates of body elements of *Sepioteuthis lessoniana* also decrease once individuals reach 60 mm dorsal mantle length, but this slower rate of growth occurs at an earlier age in *S. lessoniana* than *Photololigo* sp. A. Descriptions of morphometric growth for small juvenile *Loligo pealei* (< 15 mm) showed a discontinuity at 4.5 mm (Vecchione 1981). There was no indication of a discontinuity this early in the life of *Photololigo* sp. A, and it is possible that *L. pealei* descends to deeper water earlier than *Photololigo* sp. A. For *Photololigo* sp. A, *L. pealei* and *S. lessoniana* a discontinuity resulted from decreasing relative growth of body structures during growth. This suggests that most of the body elements reached full size during the juvenile phase. However, a discontinuity in growth of *Gonatus madokai* (Kubodera & Okutani 1977) is because relative growth rates of body structures are faster in adults than juveniles. It appears that this species may undergo more dramatic changes in shape and form between juvenile and adult forms than those displayed by loliginid squid.

The exponential growth of *Photololigo* sp. A during juvenile and adult phases of the life history is the result of growth in length and mass of the mantle muscle tissue. The mantle muscle mass continued to grow relatively fast in contrast to other structures, particularly in larger individuals. The viscera, comprising of the stomach, caecum and digestive gland, grew relatively slowly throughout the life time of the squid and as a result became relatively small compared with other structures. The slowing of growth once adulthood is reached by many animals is hypothesised to be a function of slowing metabolic rates (Bertalanffy 1957). For cephalopod species that have a slowing of growth the slowing of metabolic rates may be due to the decreased relative size of the digestive tract resulting in less ability to process food and produce energy (O'Dor & Wells 1988). However, despite the viscera becoming relatively smaller, *Photololigo* sp. A was able to grow exponentially, suggesting the relative size of the digestive system did not decrease the efficiency of processing food. Similar metabolic rates during juvenile and adult phases (Hurley 1976, O'Dor 1982) suggest that energy levels are high enough to support constant growth rates. This is particularly true if no additional demands on energy, such as migrations to spawning grounds, are made during gametic production. The presence of juvenile and adult *Photololigo* sp. A in the same areas (Chapter II) suggests that this species may not undertake extensive migrations similar those described for other species (Nesis 1983). This means that energy is not being directed towards extended periods of activity. Furthermore individuals are remaining in areas with prey items instead of moving into spawning areas where food levels may be low (Sauer *et al.* 1992).

Several studies have provided interpretations of principal component analyses when used to describe changes in size and shape (Jolicoeur & Mossiman 1960, Jolicoeur 1963b, Davies & Brown 1972, Shea 1981, 1985). Much of the debate is about whether the first principal component axis describes only differences in size and not changes or differences in shape (Darrock & Mosiman 1985, Somers 1986, 1989, Rohlf & Bookstein 1987, Sunderberg 1989). As demonstrated with this study where an ontogenetic trend was evident, the PCA provided information about relative changes in growth and the major structural differences that change during ontogeny. More recently biologists interested in evolutionary taxonomy have proposed the use of homologous landmarks and outline information as alternatives to the techniques

used here (Bookstein 1990, Rolf 1990). This is mainly because conventional multivariate morphometrics and outline techniques have been criticised for not being supportive of biological explanations (Bookstein 1982, 1990). Unfortunately, the use of homologous landmarks and outline data are more successful on rigid structures with clearly defined points rather than soft-bodied specimens (Marcus 1990). This study and others (Shea 1985, Brown & Davies 1972) have successfully shown that clear biological descriptions of the changes in body shape that occur during ontogeny can be demonstrated using multivariate techniques.

Poorly developed features or structures in juveniles may show positive allometry as growth of the structure results in the generation of an adult form. Changes in shape and size during growth may be either to maintain the functional equivalence of an organ during growth (Gould 1966) or because changes in habitat and behaviour require specific structural changes (Werner & Gilliam 1984). Either way these changes will affect the locomotion, feeding and metabolism of an organism. Changes in structural elements during ontogeny of *Photololigo* sp. A suggests that both were occurring. Future work is needed to determine the function of organs at the cellular level to complement the macroscopic description made here. Further work providing descriptive analyses of ontogenetic changes in shape and form will provide valuable comparisons and insights into different life-history traits.



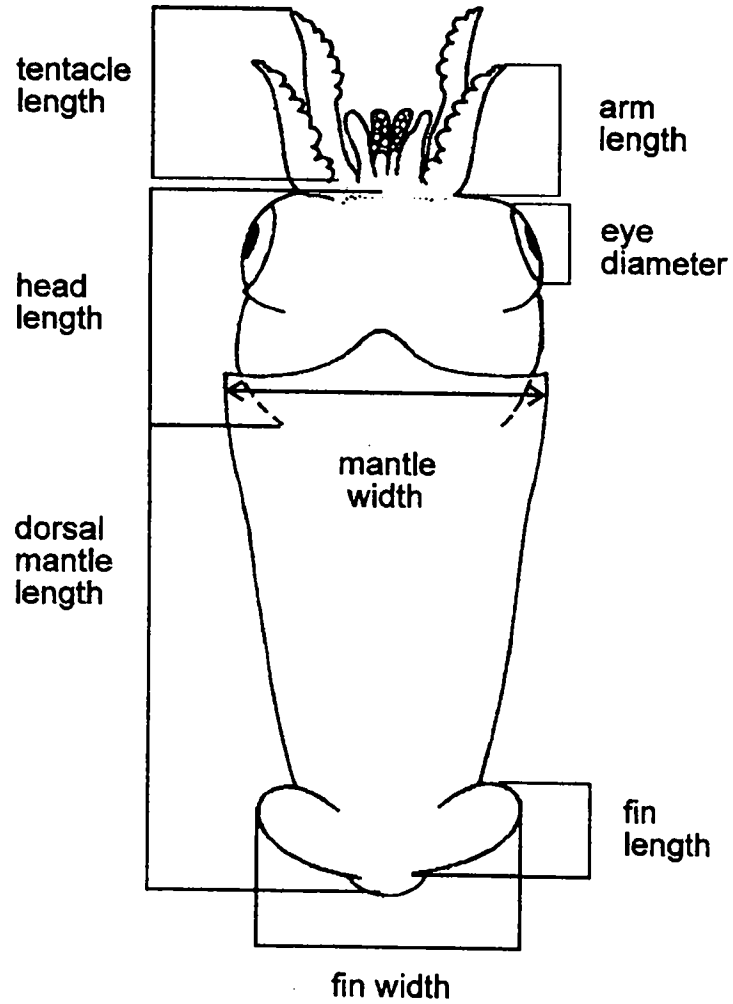


Figure 3.1: Diagram of a squid showing where length measurements were taken.

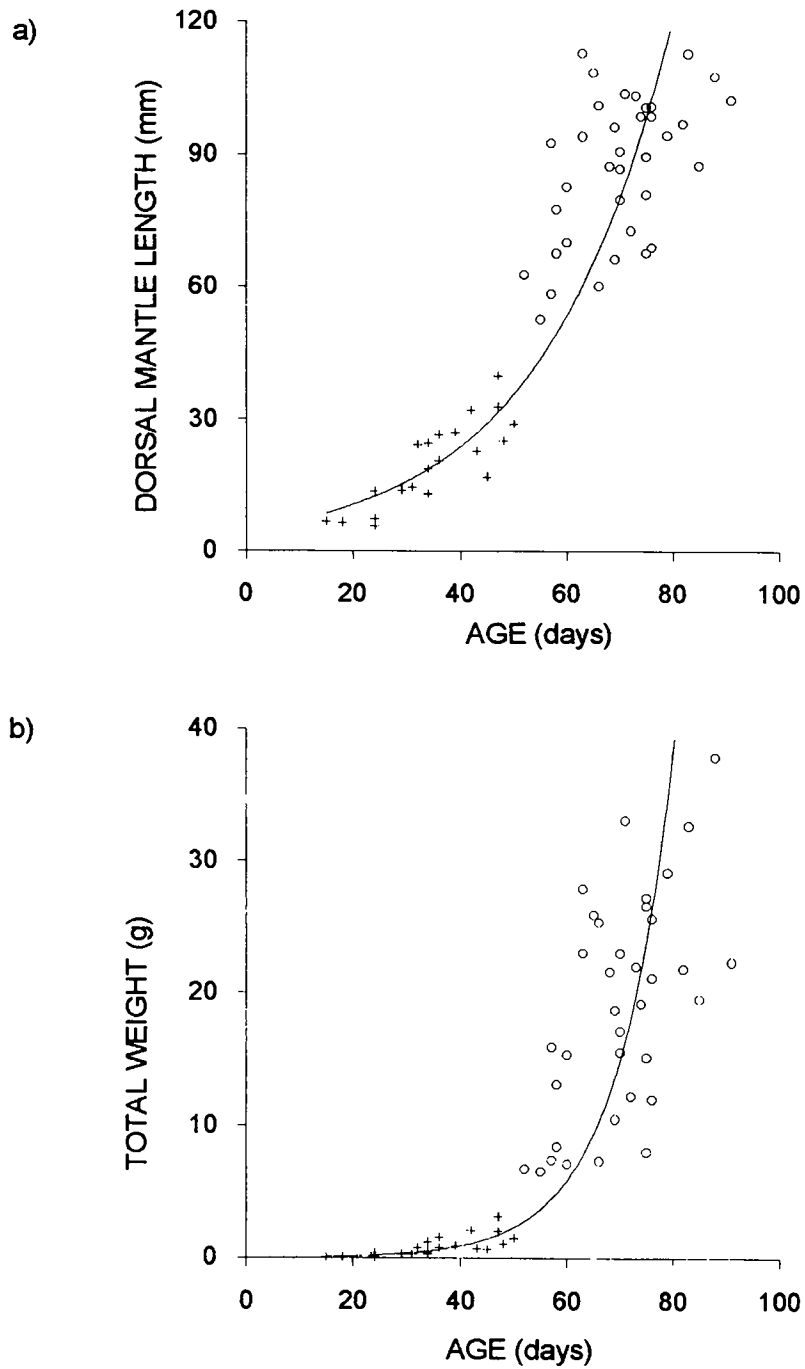


Figure 3.2: Age at size growth curves for adults and juveniles against a) mantle length and b) total wet weight. Exponential lines have been fitted to both graphs. Growth coefficients are presented in Table 3.2. Crosses are small individuals and circles large individuals.

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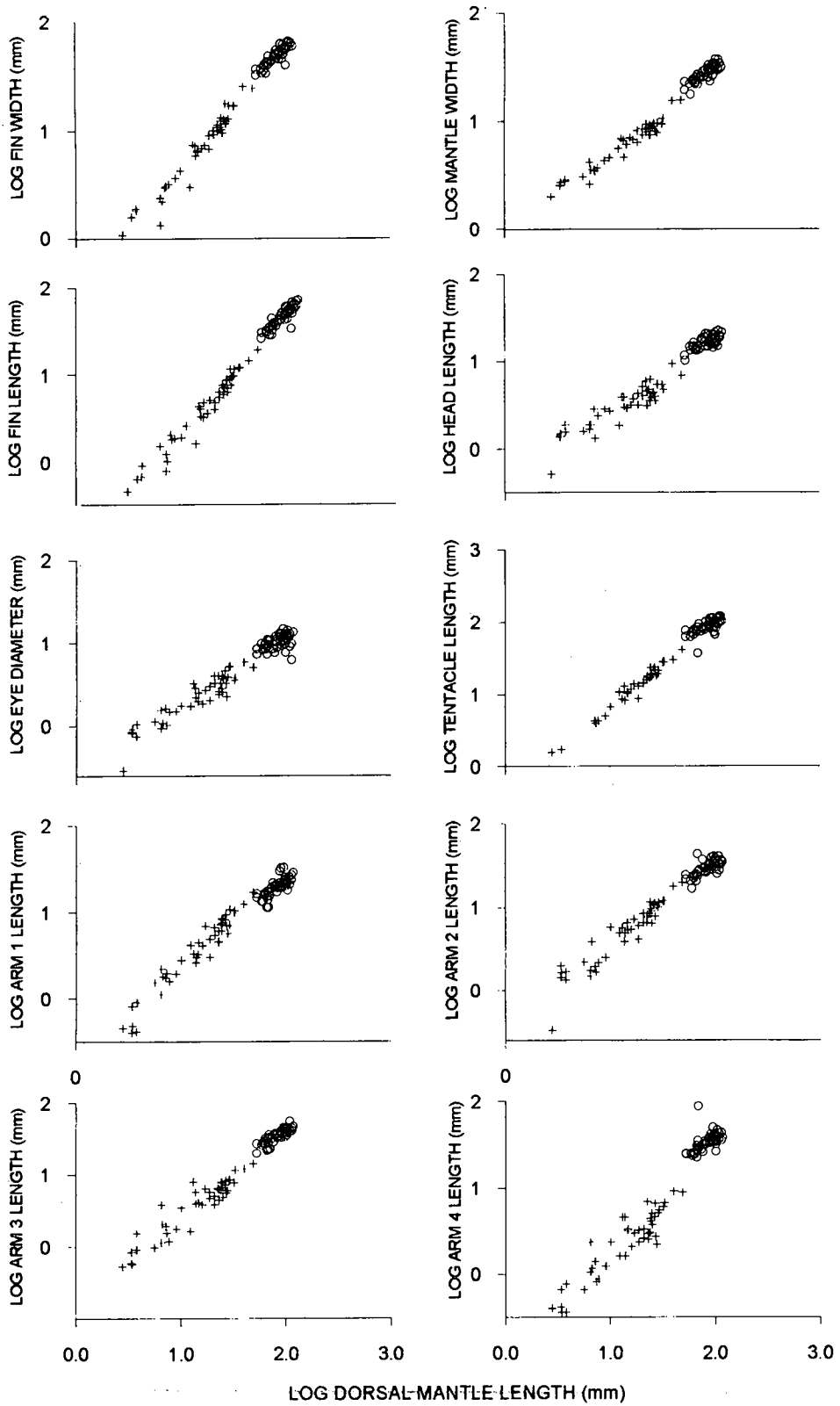


Figure 3.3. Bivariate regressions of length measures against dorsal mantle length showing the relative growth of eight body structures. Regression coefficients are presented in Table 3.2. Crosses are small individuals and circles large individuals.

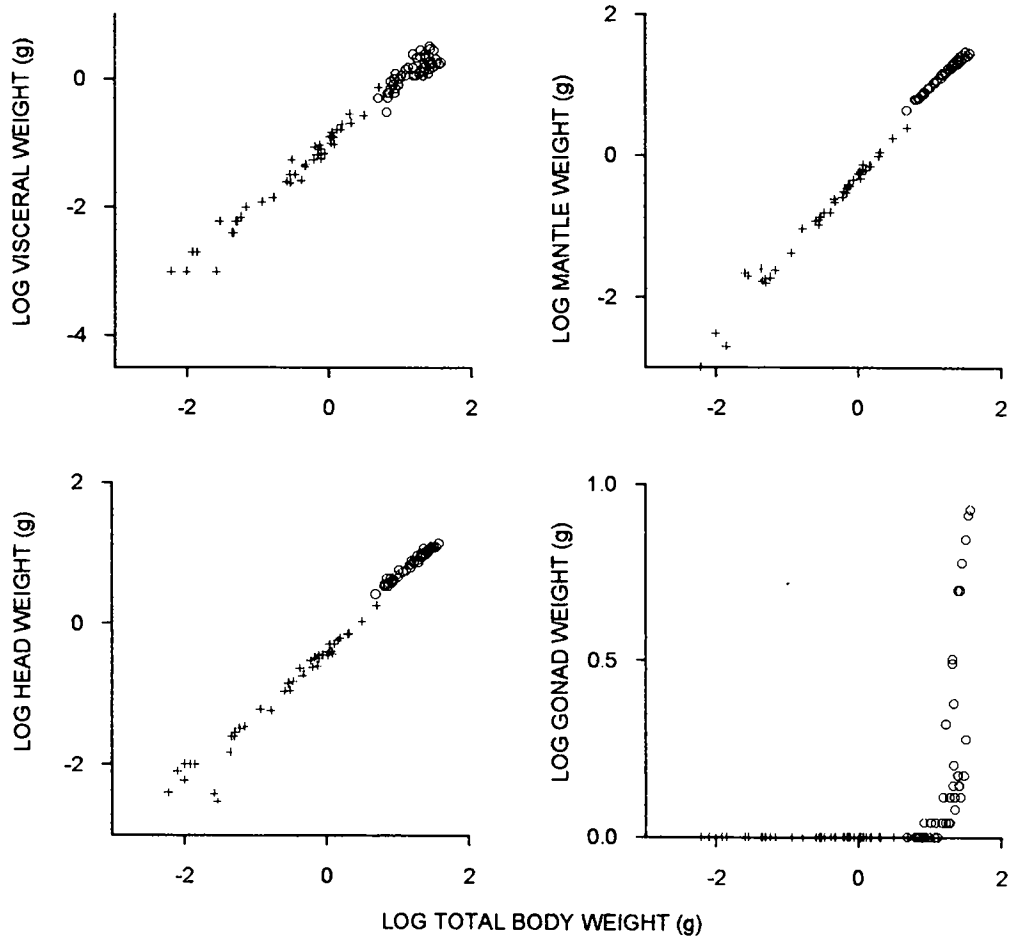


Figure 3.4: Bivariate regression plots showing the relative weight of four body structures against total body weight. Regression coefficients are presented in Table 3.3. Crosses indicate small individuals and circles large individuals.

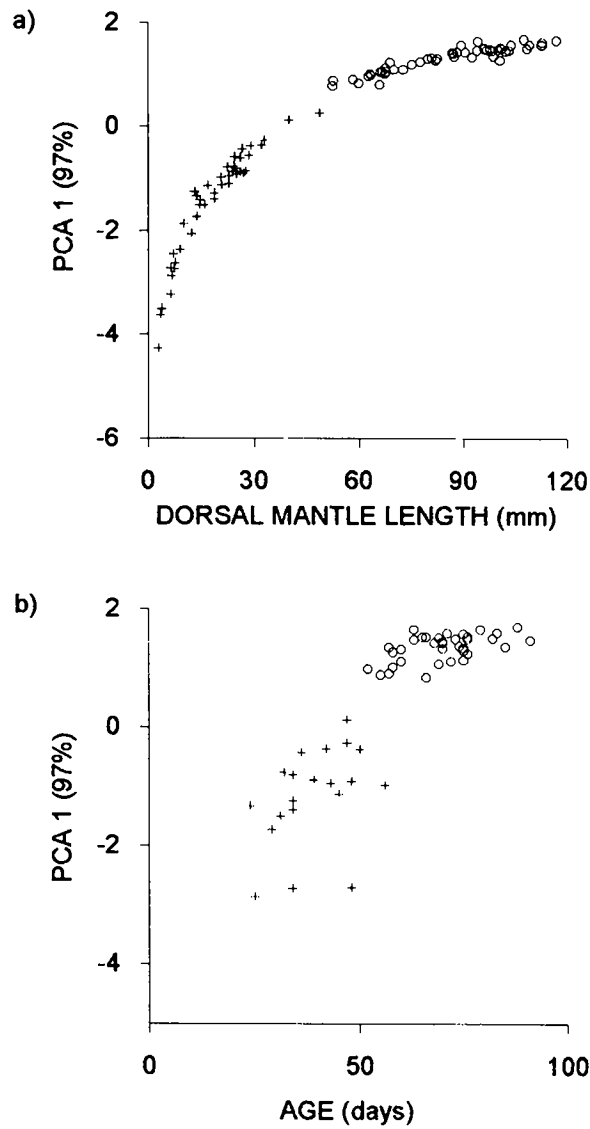


Figure 3.5: Relative growth of the length of body structures. The relationship between the principal component score of each individuals squid against a) its dorsal mantle length and b) its age. Crosses are small individuals and circles large individuals.

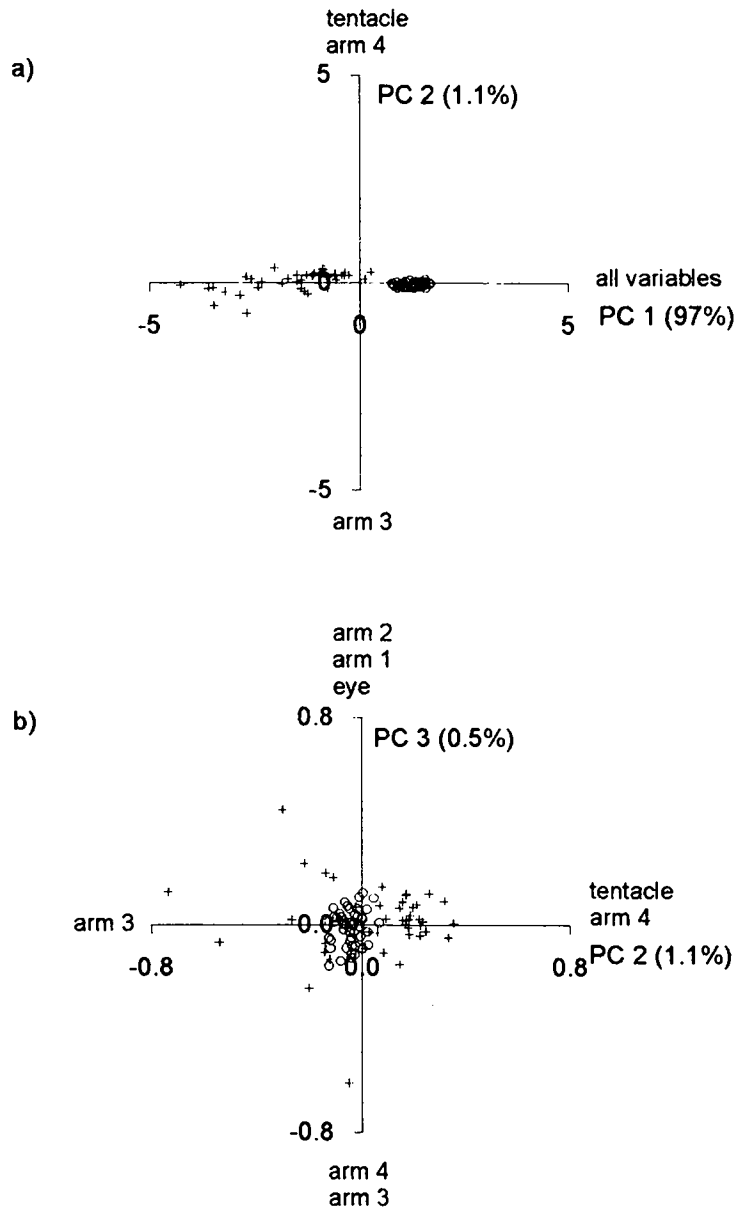


Figure 3.6: Relative growth of the length of body structures. Data are log<sub>10</sub> transformed and column centred. Percentages indicate how much variation in the data set has been described by each principal component axis. Crosses are small individuals and circles large individuals.

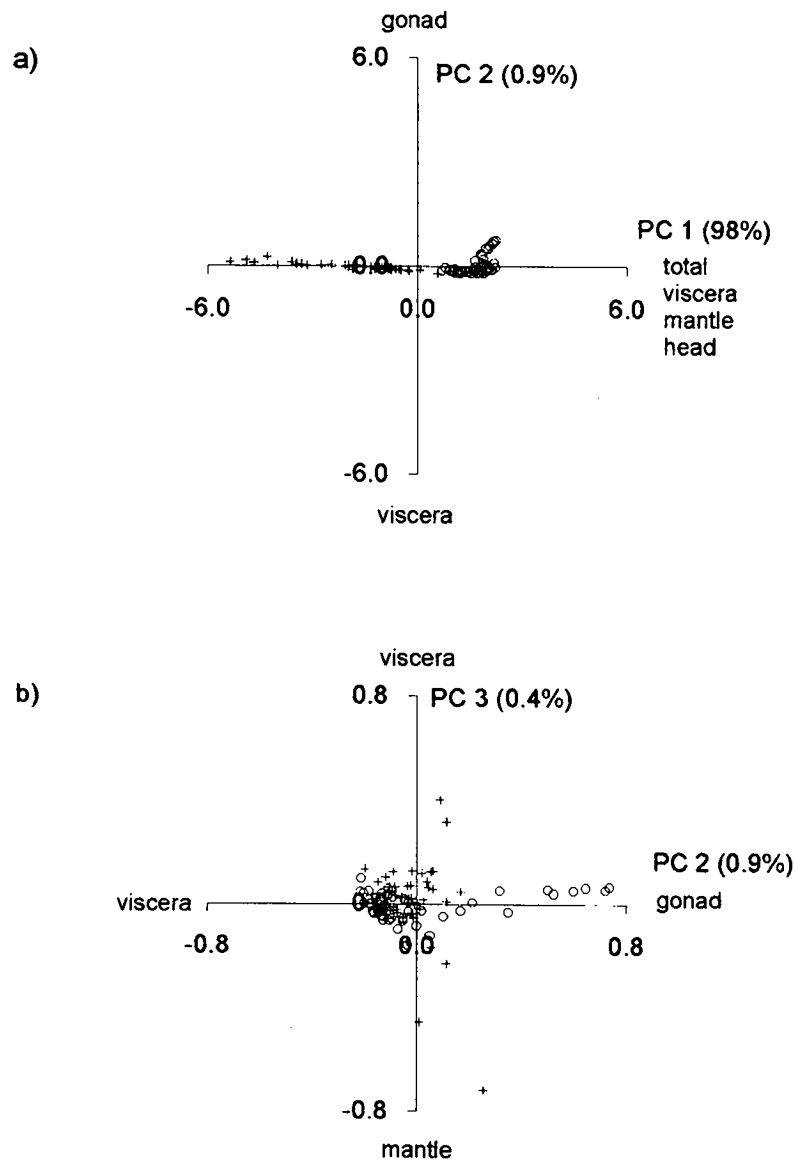


Figure 3.7: Relative growth of the organ weights. Principal component scores for the weights of five body structures on the first three axes. Data are log<sub>10</sub> and column centred. Crosses are small individuals and circles large individuals.

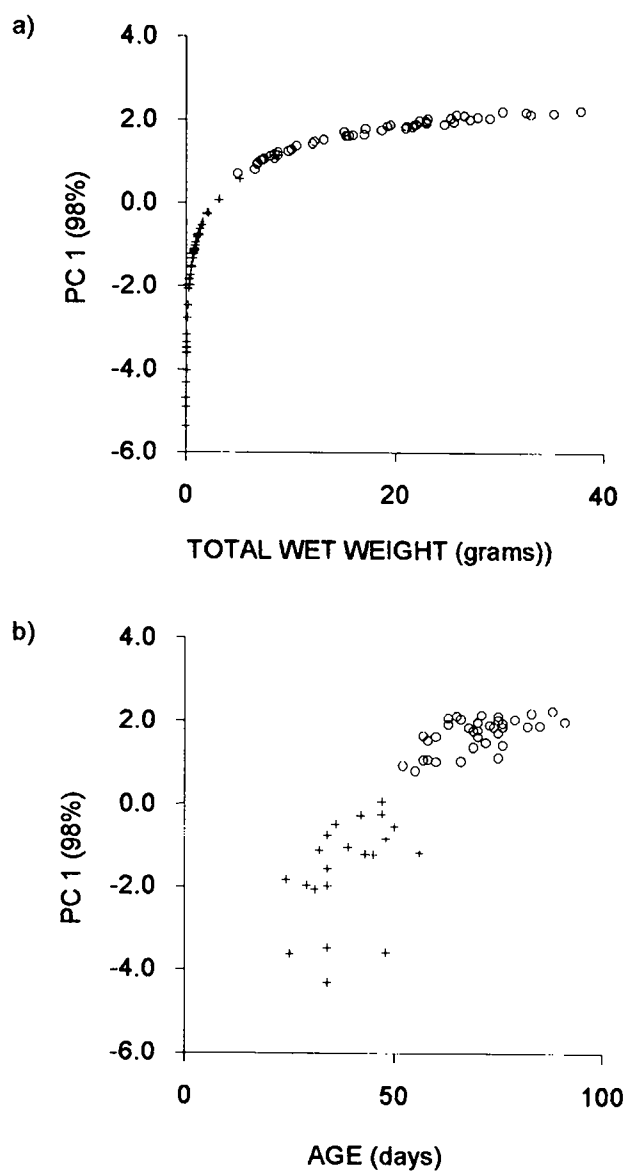


Figure 3.8: Relative growth of the mass of five body component. Plots of the principal component score on the first axis for each individuals squid against a) total body weight and b) age. Data are log<sub>10</sub> transformed and column centred. Crosses are small individuals and circles large individuals.



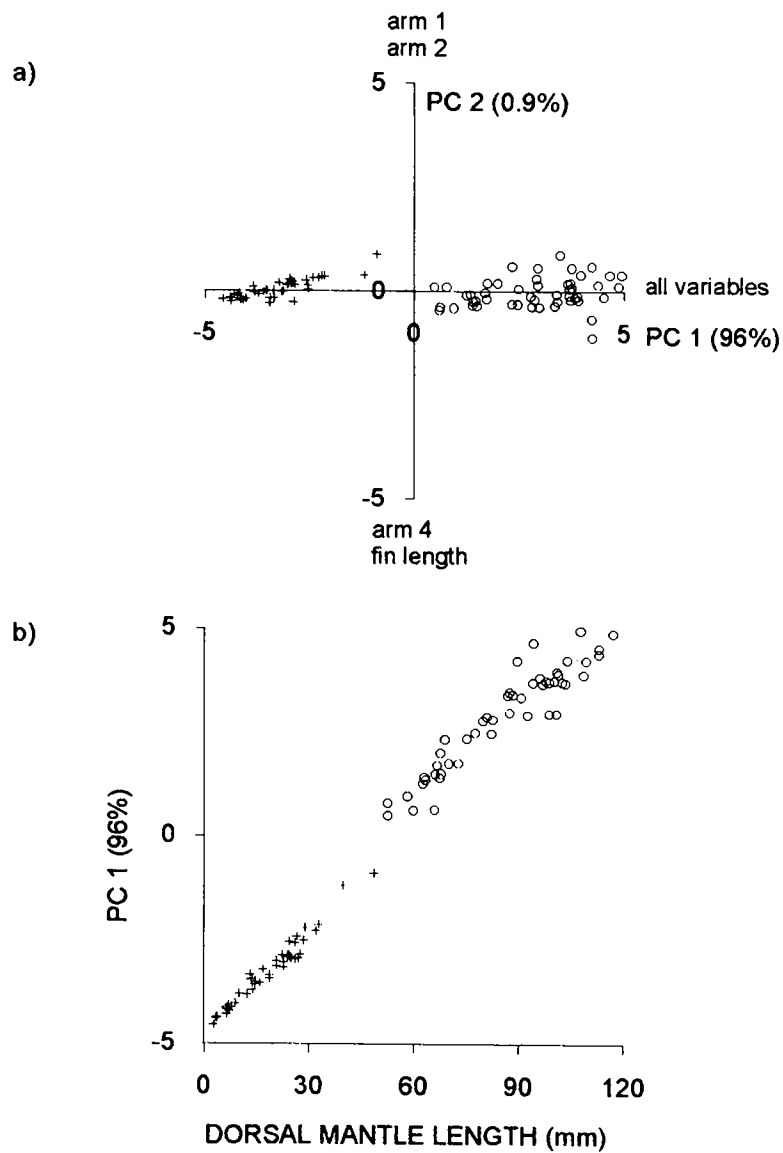


Figure 3.9: Absolute growth of the structure lengths. Crosses are small individuals and circles large individuals.

a) Principal component scores for the first two axis for the eleven raw length measurements.

b) Principal component scores for the raw length data for each individual squid against dorsal mantle length.

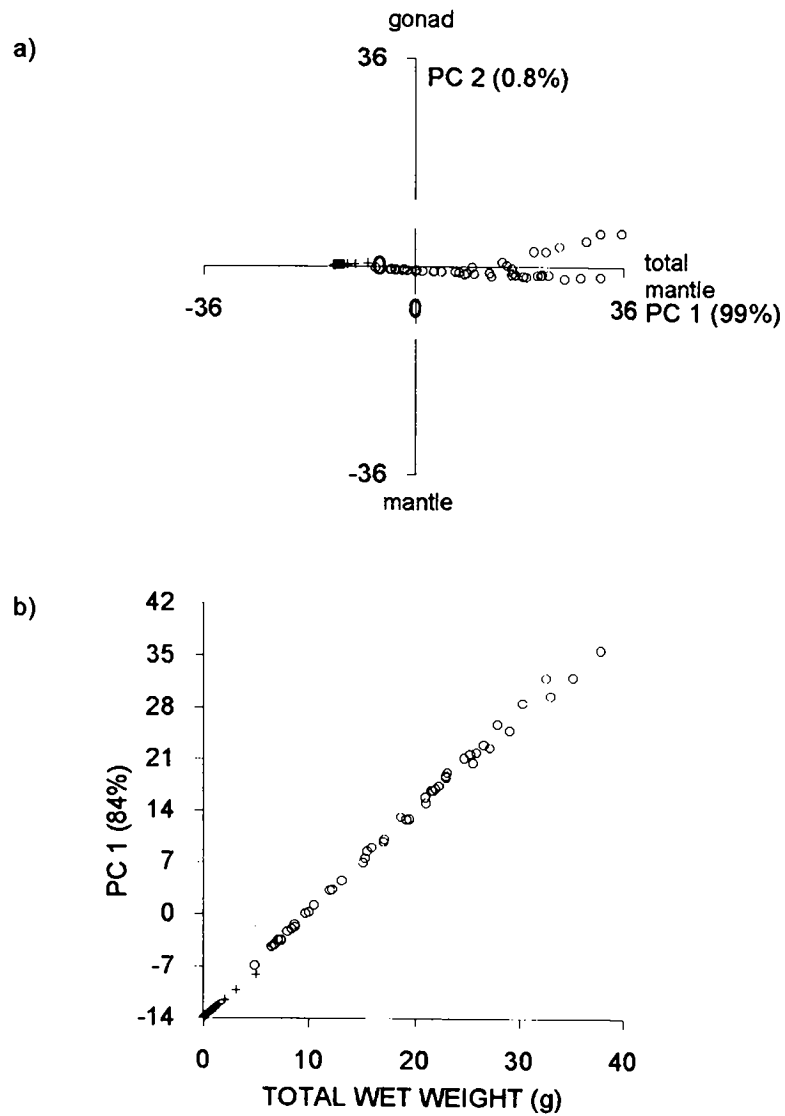


Figure 3.10: Absolute growth of mass. Crosses are small individuals and circles large individuals.

a) Principal component scores for the first two axis for the raw weight measurements.

b) Principal component scores for the raw weight data for each individual squid against total wet weight.

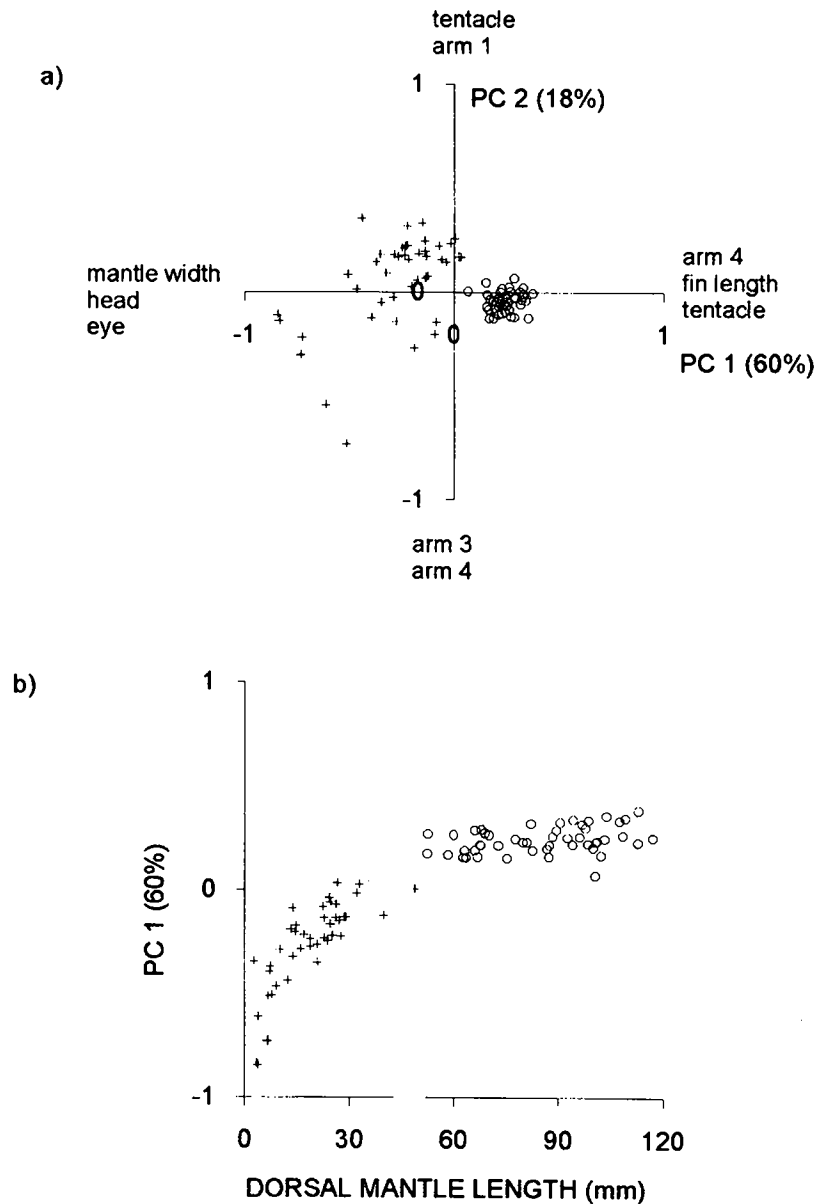


Figure 3.11: Ordination of the lengths of body structures for which effect of size has been removed by double centring. Data are log<sub>10</sub> transformed. Crosses are small individuals and circles large individuals.

- a) Scores on the first two principal component axes.
- b) Principal component scores on the first component axis for each individual squid against dorsal mantle length.

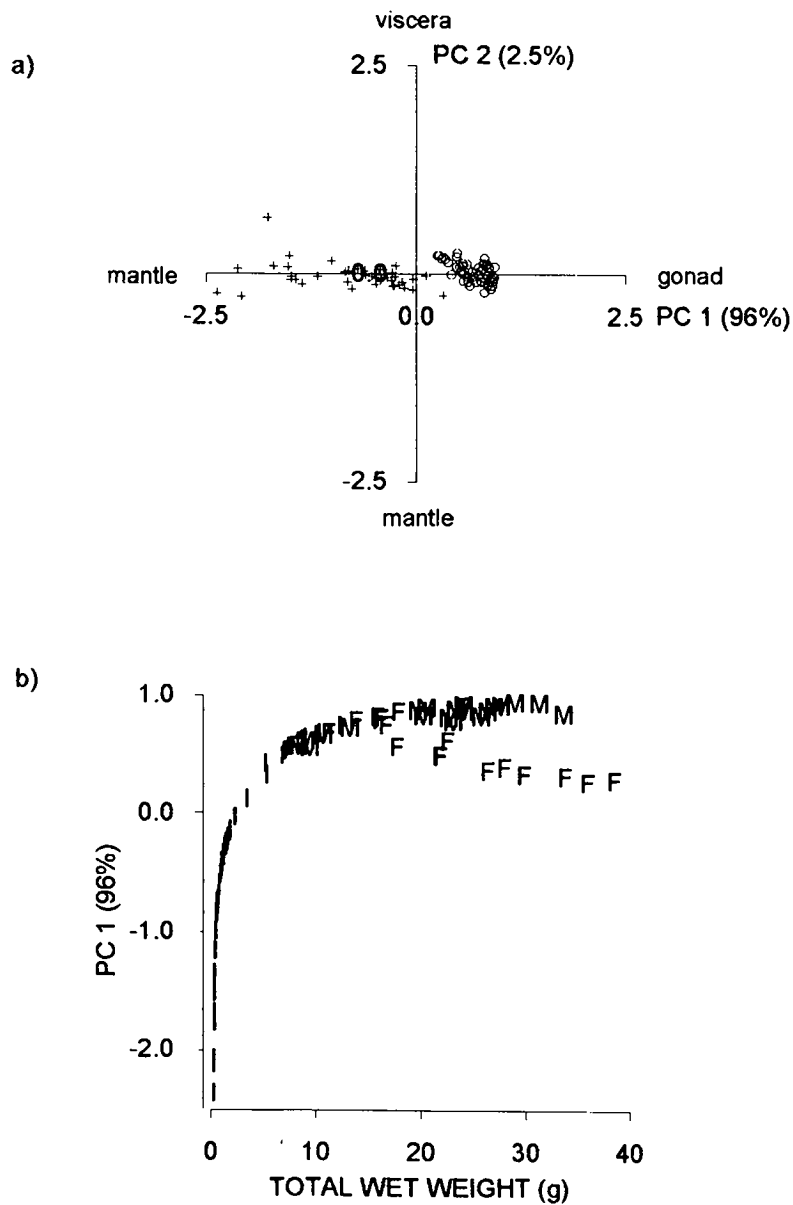


Figure 3.12: Removing the effect of size from weights of body organs. Principal component analysis on weight data that has been log<sub>10</sub> transformed and double centred. Percentage values refer to the percentage of variance in the data set described by each axis. Crosses are small individuals and circles large individuals.

- a) Principal component scores on the first two axes.
- b) The first principal component score for each individual squid against total wet weight. I = immature individuals, F = females and M = males.

## CHAPTER IV:

### MUSCLE TISSUE GROWTH AND MUSCLE FIBRE DYNAMICS

#### 4.1 INTRODUCTION

The live-fast, die-young lifestyle of many squid species has attracted considerable attention, with the result that a suite of growth curves has been produced (Forsythe & Van Heukelem 1987). The literature suggests that squid growth may comprise of two growth stanzas; a period of exponential growth followed by a logarithmic growth phase (Forsythe & Van Heukelem 1987). However, there is evidence that such mathematical models may have methodological problems (Alford & Jackson 1993). Exponential or linear growth rates throughout the life of squid may be a more typical growth pattern. Both these growth equations have been described for ommastrephid squids (Rodhouse & Hatfield 1990b, Arkhipkin & Mikheev 1992) and tropical loliginids (Jackson & Choat 1992). Overall growth rates of squid exceed those of fish because squid do not appear to cease growing. The capability of squid to achieve such high growth rates has been met with some scepticism, because biologists do not understand the mechanisms that allow cephalopods to grow rapidly (Jarre *et al.* 1991). The underlying assumption is that the mechanics of somatic growth are comparable between squid and fish.

The description of growth as an increase in somatic tissue, usually weight or length, is common throughout the literature (e.g., Ricker 1979, Weatherley & Gill 1987, Forsythe & Van Heukelem 1987). Such increases in mass and length are a function of muscle tissue growth, a process that occurs at the cellular level. For both fish and squid the muscle tissue comprises approximately 90% of the body mass. Therefore, an understanding of muscle fibre dynamics will provide information about how growth actually occurs. Muscle growth in teleosts is by two mechanisms. Initially the recruitment of new muscle fibres (hyperplasia) causes rapid growth, followed by an increase in muscle fibre size (hypertrophy) as the capability of generating new muscle fibres diminishes, resulting in slower growth (Weatherley &

Gill 1985). The physiological constraints of surface area to volume ratios will restrict the maximum size of a muscle fibre. The final, or asymptotic, size fish may attain will be determined by the number of muscle fibres present once hyperplasia ceases (Weatherley & Gill 1985). Therefore, an individual that has more muscle fibres has a greater capacity to reach a larger size. By examining the muscle fibre dynamics in squid species an insight of how growth is occurring and the limiting factors of growth rates and ultimate size is possible.

For squid there is currently little known about changes in the dynamics of muscle fibre recruitment and growth during somatic growth. Descriptions of squid mantle muscle tissues have largely contributed to an understanding of locomotor function and operation (Ward & Wainwright 1972, Packard & Trueman 1974, Moon & Hurlbert 1975, Bone *et al.* 1981, Gosline *et al.* 1983). Measures of muscle fibre size and density have received attention in only a few cases (*Alloteuthis*, *Loligo* and *Sepia* Bone *et al.* 1981; *Illex argentinus* Hatfield *et al.* 1992). However, there has been no examination of variation in muscle fibre arrangement and size throughout the mantle muscle.

Growth in *Photololigo* sp. A does not slow down (Jackson & Choat 1992) and it is possible that exponential growth may result from continuous muscle fibre generation. This chapter examines how the dynamics of muscle fibres determine growth in juvenile and adult *Photololigo* sp. A. This will be done by examining juveniles and adults over a full spectrum of body sizes to allow the description of how growth relates to changes in muscle fibre size, number and structural state.

## 4.2 MATERIALS AND METHODS

### 4.2.1 MUSCLE TISSUE COLLECTION AND PREPARATION

Juvenile *Photololigo* sp. A (2.5 - 40 mm dorsal mantle length) were obtained using automated light-traps (Doherty 1987). Capture of juvenile squid was in the Cleveland Bay region between October and January of the 1991/92 austral summer. Adult *Photololigo* sp. A (40 - 150 mm dorsal mantle length) were caught in the same area using pair otter trawls (4 cm mesh) between August and November 1991 and in March 1992.

Measurement of the dorsal mantle length of each individual was taken before tissue was fixed. Juveniles were fixed whole and a sample of muscle tissue was removed later. From each adult a sample of muscle tissue was taken before fixation. All muscle tissue was fixed in FAACC (a formalin - acetic acid - calcium chloride solution: 10 ml 37% formaldehyde, 5 ml glacial acetic acid, 1.3 g calcium chloride (dihydrate); distilled water to 100 ml). Fixed material was transferred to 70 % ethanol 48 hrs before processing tissue for paraffin wax. Muscle tissue was dehydrated through an ascending isopropanol series, cleared in chloroform and infiltrated with paraffin wax (Paramat). Tissue blocks were sectioned at 7  $\mu\text{m}$ , decerated in xylene and hydrated through a descending ethanol series. Histological sections were stained with trichrome Mallory-Heidenhain stain.

Squid mantle tissue is predominantly made up of circular smooth muscle fibres separated into blocks by thin regions of radial muscle tissue (Ward & Wainwright 1972). These smooth muscle fibres have helical myofibrils and are typically small, <10  $\mu\text{m}$  diameter (Hanson & Lowy 1957). In many cephalopods circular muscle fibres are present in two structural states; mitochondria-rich and mitochondria-poor, analogous to fast and slow twitch muscle fibres in vertebrates (Mommensen *et al.* 1981). Muscle tissue was sectioned longitudinally, so that circular muscle fibres, ie. those muscle fibres that encircle the muscle mass, were sectioned radially and radial muscle fibres were cut longitudinally (Fig. 4.1). The bulk of the mantle muscle tissue is made up of circular, mitochondria-poor muscle fibres. Therefore, these muscle fibres were used

to assess muscle fibre dynamics and mantle muscle growth. The dynamics of muscle fibre recruitment and growth were described by examining size frequency distributions of circular muscle blocks and muscle fibres.

Sections of muscle tissue were examined at 160x and 400x (oil immersion). An Olympus CH-2 high power microscope connected to an Apple Macintosh IICI computer displayed images using a Rastorops 364 video colour card. Images were stored using 'Framegrabber' software and analysed using 'NIH IMAGE' (public domain software).

#### 4.2.2 PILOT STUDY

Since little is known about the dynamics of muscle growth in squid it was necessary to determine if and how muscle fibre dynamics and growth differed between areas of the mantle muscle tissue. This would enable the region of the mantle muscle tissue from each individual to be standardised, allowing comparisons between individuals. Five individuals (5.9 - 16.7 mm dorsal mantle length) were sectioned longitudinally through the entire body cavity. A starting section was selected and designated distance zero. Points 70, 140, 210 and 280  $\mu\text{m}$  around the body from this starting point were selected for measurement (these will be referred to as 'distances' around the body). The longitudinal axis of individuals was divided into three 'areas': anterior, mid and posterior. From each area and each of the five distances in every individual, 20 measurements of circular muscle block width were taken (Fig. 4.2). In this way it was possible to determine variations in muscle block width between individuals, among distances around the body and among areas. These results were then used to make a decision of where measurements of muscle block widths should be taken for comparison of different sized individuals.

#### 4.2.3 MUSCLE BLOCK AND FIBRE DYNAMICS

Results from the pilot study indicated considerable variability in the size of muscle fibres and muscle blocks within an individual. There was also no apparent trend of difference either around the body or along the longitudinal axis (see Results). It was



therefore decided to standardise the location from where tissue was removed. The cartilaginous mantle locking mechanism was used as a standard location on the mantle muscle. Tissue samples were removed from the dorsal side of the mantle, at a point level with the locking mechanism. Given the variability within an individual and time required it was calculated that measurements of 42 circular muscle blocks would provide the sample size necessary to detect differences between individuals. From the muscle tissue sample from each individual, seven histological sections were prepared. From each histological section seven randomly chosen circular muscle blocks were measured.

To obtain adequate numbers of muscle fibre diameters for size frequency analysis 60 muscle fibres were measured from each individual. As the muscle fibres tend to be fusiform in shape and are oval shaped in cross-section, the longest diameter of each muscle fibre was measured. Twenty muscle fibres were randomly selected for measurement from three different circular muscle blocks for each squid.

#### 4.2.4 MITOCHONDRIA-RICH AND MITOCHONDRIA-POOR FIBRES

Circular muscle tissue is present in mitochondria-rich and mitochondria-poor states. Changes in the ratios of the two muscle fibre types were examined during growth. Mitochondria-rich muscle fibres are present at two distinct locations in the mantle muscle tissue, along the inner and the outer surface of the mantle tissue. Ten measurements of the width of the mitochondria-rich muscle fibre region were taken on each of the inner and outer surfaces for each individual. In addition, ten measurements of the total width of the mantle muscle were obtained. The width of the mitochondria-poor muscle fibre region could then be calculated. Values were averaged for each individual. Changes in the relative proportion of the two muscle fibre types were examined as a function of dorsal mantle length.

## 4.2.5 ANALYSES

The pilot study, to determine differences in muscle block width, was analysed using a three factor orthogonal analysis of variance. The three factors: along the body, around the body and between individuals, were all treated as fixed.

In order to determine how the size frequency distribution of the muscle blocks and muscle fibres changed with increasing dorsal mantle length, individuals were placed into eight dorsal mantle length size categories. Circular muscle block and muscle fibre diameter size frequencies were compared between dorsal mantle length size-classes using a multiway frequency analysis. This analysis allows the hypothesis that the size frequency distribution of muscle fibres and muscle blocks is independent of dorsal mantle length to be examined.

If the multiway frequency analysis was significant the data was further examined using a correspondence analysis. Correspondence analysis enables the frequency of observations in different classes (dorsal mantle length class) to be examined with respect to the sizes of muscle block or muscle fibres. The relationship between the size of the individuals and the size frequency of the muscle blocks or muscle fibres can then be presented graphically. This allows differences between the size-classes of individuals to be determined and which muscle block or muscle fibre size-class contribute to that difference. The axes used to display the correspondence analysis maximise the difference between the size groups of squid, partitioning the total chi-square distance between the new axes. By plotting the size-classes of squid on one set of axes and the size-classes of muscle blocks or muscle fibre diameters on the same set of axes it is possible to understand the relationship between dorsal mantle length and the variable of interest.

## 4.3 RESULTS

### 4.3.1 PILOT STUDY

A significant interaction in the analysis of variance among the three factors (individual squid, distance around the body and areas along the body) indicated that there were differences in muscle block widths within individuals (Table 4.1). Therefore, the differences in muscle block widths with distance around the body or areas along the longitudinal axis were not the same for the five individuals (Fig. 4.3). Hence in order to obtain adequate comparisons between individuals of different sizes it was necessary to sample tissue from one location consistently on all individuals. If the muscle block widths had been the same at all distances and areas, then tissue samples could have been removed from any region of the mantle muscle tissue. The analysis provided estimates of the variability in muscle block widths within individual squid, enabling enough circular muscle blocks to be measured, so that a description of each individual could be obtained.

Table 4.1: ANOVA table for the pilot study examining variation in circular muscle block widths between individuals, distance around the individual and areas along the longitudinal axis.

Source	Df	Sum Of Squares	Mean Squares	F	Probability
Individual	4	0.3509	0.0877	1476.00	0.0010
Distance around the body	4	0.0007	0.0002	2.92	0.0204
Area along the body	2	0.0616	0.0308	518.27	0.0001
Individual*Distance	16	0.0066	0.0004	6.98	0.0001
Individual*Area	8	0.0454	0.0057	95.64	0.0001
Distance*Area	8	0.0047	0.0006	9.84	0.0001
Individual*Distance*Area	32	0.0244	0.0008	12.85	0.0001
Error	1347	0.5829	0.00006		

### 4.3.2 MUSCLE FIBRE BLOCKS

Muscle block width varied from 7.5  $\mu\text{m}$  in the smallest juveniles, to 1187  $\mu\text{m}$  in the larger adults. A significant difference was evident in the size distribution of muscle blocks ( $\chi^2 = 4766.4$ ,  $df = 8$ ,  $Pr < 0.05$ ) with muscle block widths growing with increasing dorsal mantle length (Fig. 4.4). Most of the Chi-squared distance was

explained by Axis 1 of the correspondence analysis, clearly showing a difference between the juveniles (<20 - 80 mm dorsal mantle length) and the adults (80 - >140 mm dorsal mantle length) (Fig. 4.5a). This pattern of points is similar to the pattern for muscle block sizes (Fig. 4.5b), suggesting that in the smaller individuals there is a predominance of narrow muscle blocks, with new muscle blocks appearing in individuals less than 80 mm dorsal mantle length. Individuals 60 - 80 mm dorsal mantle length clearly had a mixture of muscle block widths, but as growth continues circular muscle blocks increase in width with no new muscle blocks being added. Individuals greater than 100 mm dorsal mantle length had similar size frequency distributions of muscle block widths.

#### 4.3.3 MUSCLE FIBRE DIAMETERS

Muscle fibre diameters ranged from 0.49 - 6.17  $\mu\text{m}$  (Fig. 4.6). There was a significant difference in the size frequency distribution of muscle fibre between the size-classes of squid ( $\chi^2 = 143.41$ ,  $df = 7$ ,  $Pr < 0.05$ ). Fig. 4.7a shows how the dorsal mantle length size-classes of the squid separated along Axis 1. The difference between the small and large squid was the predominance of very small muscle fibre sizes in the small squid (4.9 mm to 80 mm dorsal mantle length) (Fig. 7 a & b). Larger individuals (>80 mm) had a prevalence of medium sized muscle fibres. The presence of muscle fibres less than 1  $\mu\text{m}$  in all sizes of squid (Fig. 4.6) suggests that new muscle fibres were still being generated in bigger squid, but they were in relatively lower numbers than in smaller squid.

#### 4.3.4 MITOCHONDRIA-RICH AND MITOCHONDRIA-POOR MUSCLE FIBRES

In all individuals the mitochondria-rich muscle fibre region was relatively small, comprising less than 1% of the overall mantle tissue width. The ratio of the two muscle fibre types changed as a function growth in these squid (Fig. 4.8). The amount of mitochondria-rich tissue decreased relative to the mitochondria-poor tissue with increasing dorsal mantle length. Considerable variability in the width of mitochondria-rich muscle fibre region, between individuals of similar sizes, suggests that the amount of mitochondria-rich muscle fibres may not be a function of increasing mantle length.

## 4.3.5 STRUCTURAL CHANGES

In some of the adult specimens examined there was evidence of a breakdown in the organisation of both the circular and radial mitochondria-poor muscle fibres. The disorganisation tended to occur in nodes or discrete areas in the mantle muscle tissue (Fig. 4.9). When disorganisation was present in low amounts it tended to occur near the internal or external edge of the mantle muscle. The amount of disorganisation ranged from a few scattered nodes (Fig. 4.9b) to the complete muscle section having no organisation, such that it was not possible to measure either blocks of muscle or fibre diameters. The state of disorganisation was rated on a qualitative scale from 0 (not present) to 4 (complete). There were no nodes of disorganised muscle fibres in the juvenile squid muscle tissue so that only adult muscle tissue was examined and analysed. A multiway frequency analysis examined the relationship between the size, reproductive status and muscle state of the squid. There was no interaction between size and reproductive status of individuals, but muscle status was dependent on size and reproductive status (Table 4.2).

Very little disorganisation was evident until the squid reach 70 mm dorsal mantle length, an effect that would have been inflated if juvenile squid had been included in the analysis (Fig. 4.10). In the larger size class over 60% of the individuals have some of the nodes present, but there was no evidence that larger individuals had more extensive disorganisation. As with the size, muscle status was dependent on reproductive status because immature individuals rarely had nodes of disorganised tissue. However, once individuals were maturing there was no clear indication that reproductive maturation was affecting the muscle tissue state (Fig. 4.11).

Table 4.2: Results of a multiway frequency analysis examining the muscle state of each individual and the relationship between size and reproductive status. The non-significant Likelihood Ratio indicates that the main effects provide a good fit to the data and no interaction is present.

Source	df	Chi-square	Probability
Reproductive state	2	13.69	0.0011
Mantle length	3	34.97	0.0000
Likelihood Ratio	3	6.15	0.1047

#### 4.4 DISCUSSION

This examination of muscle fibre dynamics in squid suggests that growth occurs by a combination of hyperplasia and hypertrophy. Growth by these two mechanisms is typical of teleost species (Weatherley & Gill 1985). However, this study highlights a major difference between squid and fish: squid have the capacity to continue hyperplastic growth throughout their entire lifetime. This will have a net result of continuous growth of an individual with no maximum size imposed by the physiological constraints of fibre size. Accordingly, squid growth cannot be described using mathematical growth models generated for teleost species where the assumption of asymptotic growth is often implicit.

The striated muscle fibres of the mantle tissue are elongate and fusiform, hence the presence of small muscle fibre diameters maybe result from cutting the terminal and narrower portion of the muscle fibre. It was not possible to differentiate between small muscle fibres and the ends of muscle fibres. However, two pieces of evidence support the idea that some of the small muscle fibres were new fibres. Firstly, as muscle fibres grow they will increase both in diameter and length, so the probability of cutting the terminal portion of a muscle fibre becomes reduced and fewer small diameters, due to muscle fibre ends, will be recorded. Secondly, the oblique striated circular muscle reaches a maximum diameter of 10  $\mu\text{m}$  (Hanson & Lowy 1957, Bone *et al.* 1981), in this study it was 6.17  $\mu\text{m}$ . Therefore, if muscle fibre recruitment ceased and existing muscle fibres reached maximum size the muscle blocks, and therefore the individual, would not continue to increase in size. Yet the circular muscle blocks continued to increase in size as the squid grew larger. The evidence collected in this study suggests that muscle fibre recruitment must still be occurring within existing muscle blocks. Hence growth is accomplished by an increase in muscle fibre number and muscle fibre size in existing muscle blocks throughout the life of *Photololigo* sp. A

Recruitment occurs within existing muscle blocks as evidenced by the increase in muscle block width with increasing mantle length. The absence of small muscle blocks in large squid suggests that new circular muscle blocks do not arise within the

region of the tissue examined. Circular muscle blocks at the anterior end of the animal tended to be smaller and better defined when compared with muscle blocks in the posterior region. This suggested that muscle blocks are created at the anterior end. Hence growth is predominantly by an increase in muscle fibre number and the creation of new muscle blocks. There is a need to examine whether new muscle blocks are being created in larger squid and to what extent their creation contributes to growth.

Rates of metabolism are linked to the efficiency of respiration, assimilation and excretion at the cellular level. Therefore, the larger the surface area the more efficient the metabolism. In the case of muscle fibres that are responsible for oxygen uptake for movement and growth, the small diameters result in a greater surface area and faster metabolism. Compared with fish, squid have relatively faster metabolic rates (O'Dor & Webber 1986). The small diameter of the muscle fibres and the constant production of small muscle fibres would enable fast growth and metabolism throughout the life of squid. Fish stop producing small muscle fibres and their muscle fibres attain a relatively large size, which would effectively slow metabolism and growth (Weatherly 1990). Therefore small muscle fibres of squid enable high metabolic activity for the production of energy to continue growth.

The phenomenon of sustained muscle fibre recruitment and minimal muscle fibre size increase has been observed in juveniles of large fast-growing teleost species (Weatherley *et al.* 1988). It appears that exponential growth rates described for *Photololigo* spp. (Jackson and Choat 1992) may be explained by continuous muscle fibre recruitment. Weatherley *et al.* (1988) attributed the maximal size of fish species to the relative length of time during which hypertrophic and hyperplastic growth of muscle fibres occurs. It may be possible to examine differences in squid growth in the same light. *Photololigo* sp. A is a relatively small squid when compared to its large muscular oceanic relatives. It would be interesting to ascertain the relative contribution of hyperplastic and hypertrophic growth in such species. It is possible that larger squid species are using hyperplastic growth to a greater extent to attain larger sizes. The location of the tissue sample removed from the mantle muscle mass of the squid and the techniques of fixing and processing of squid muscle tissue can influence muscle fibre size. As there are no standard techniques for collection and

preservation, comparisons of muscle fibre dynamics in squid mantle muscle tissues are currently limited.

With little in the way of lipid and glycogen reserves, the muscle tissue of squid also serves as an energy reservoir (O'Dor & Webber 1986). Hence changes in muscle tissue may provide a tool to assess the effect of nutrition on growth. Weatherley *et al.* (1980) examined the muscle tissue of juvenile fish reared under differing temperature and feeding regimes. They recorded little difference in the size frequency structure of the muscle fibres, until hyperplasia ceased. From these results they postulated that in juvenile fish the rates of hyperplasia are dictated by growth conditions, but hypertrophy remains constant. In other words it is not possible to assess rates of somatic growth by examining muscle fibre size distributions during a period of muscle fibre recruitment. If the relative rates of hyperplasia and hypertrophy are constant in squid, then the only way to detect the effects of differing growth regimes will be by examining the size frequency distribution of muscle blocks. Clearly there is the need to rear squid in a variety of temperature and ration regimes and examine the resulting consequences on muscle fibre dynamics, muscle block dynamics and growth.

The structural makeup of the muscle tissue changed little during growth. Both mitochondria-rich and mitochondria-poor muscle fibre types were present throughout the life of *Photololigo* sp. A. The decreasing proportion of mitochondria-rich tissue with increasing dorsal mantle length indicates that increasing muscle bulk is predominantly a function of mitochondria-poor muscle tissue growth. Mitochondria-rich muscle fibres are restricted to the inner and outer surfaces of the mantle muscle and are predominantly involved in slow, steady state swimming and respiratory movements (Mommensen *et al.* 1981). It is possible that a significant portion of oxygen required by an individual is taken up across the mantle surfaces and the localisation of mitochondria-rich muscle fibres near the surface may be related to oxygen up-take (O'Dor *et al.* 1990). The number of mitochondria-rich muscle fibres and the width of zone of these muscle fibres maybe limited by diffusion of oxygen across the surface and not related to linear growth of the mantle.



The presence of what appears to be disorganised circular muscle fibres in the adult squid has not been recorded before. It is unclear where this is because past studies have examined relatively few individuals or it was considered an artefact of the tissue processing. Examination of the fibres with the light microscope indicated that the fibres were intact and not in the process of cellular breakdown. Handling of all muscle tissue samples was identical and at no stage was muscle tissue frozen. All specimens were dead when tissue was removed and fixed so there is no suggestion that dead material is fixing differently from live tissue. The very patchy occurrence of disorganisation in some of the individuals further suggests that this is not a fixation effect, such as fixative failing to penetrate the tissue. Moreover if the fixative had failed to penetrate the tissue then any effect on the muscle fibres would have been in the central region, but early stages of disorganisation were evident along the margins. If the fixative affected the fibres near the margin then the muscle tissue of juvenile squid would have been similarly affected. Although the presence of disorganised fibre arrangement cannot be definitively separated from fixative and processing artefacts, the very obvious absence from juvenile muscle tissue indicates that it is a real phenomenon. All the individuals used in this study appeared to be healthy, externally, with no lesions. No obviously senescent squid were ever captured, so it is unclear what changes the muscle tissue undergoes during senescence. Dying squid undergo very dramatic changes in muscle tissue in which the muscle fibres are completely absent and just the collagen structure remains (Jackson & Mladenov 1994). Likewise, the muscle fibres of senescent *Octopus vulgaris* breakdown leaving spaces in the tissue (Tait 1986). This phenomenon was not present in any of the *Photololigo* tissue samples examined. Furthermore, the poor relationship between size, reproductive status and muscle status suggests that this phenomenon was not due to senescence.

How new muscle fibres arise is poorly understood, Bone *et al.* (1981) observed 'small crescentric profiles containing myofilaments' and suggested that these are early muscle fibre precursors. However, this has neither been studied nor observed in subsequent studies. Understanding how new muscle fibres arise will assist in understanding the mechanics of muscle growth and factors affecting muscle fibre recruitment. By understanding the mechanics of growth at the muscle fibre level it is possible to begin examining how factors such as nutrition levels, temperature and age

are mirrored in the somatic status of an individual. Examination of muscle fibre variation would allow analysis of muscle tissue dynamics during times of stress, migration, starvation and reproductive activity.

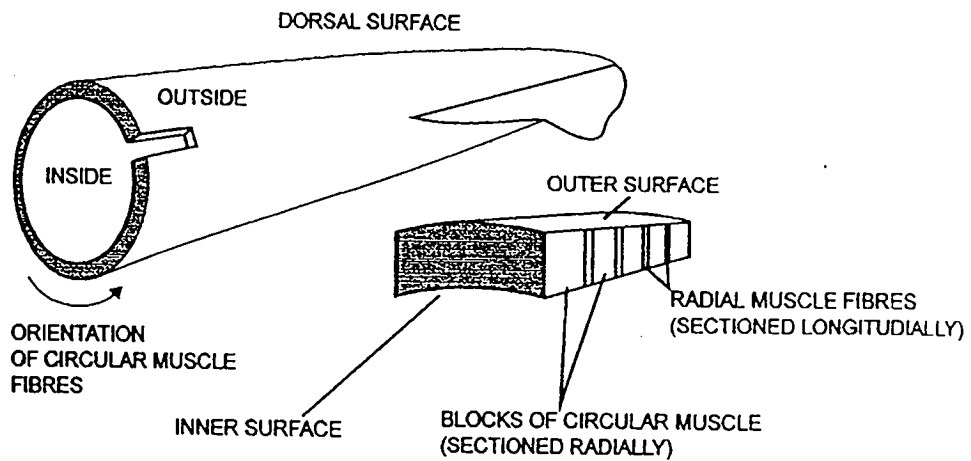


Figure 4.1. Schematic diagram of a squid showing where the muscle block was sampled and the orientation of the muscle tissue that was examined.

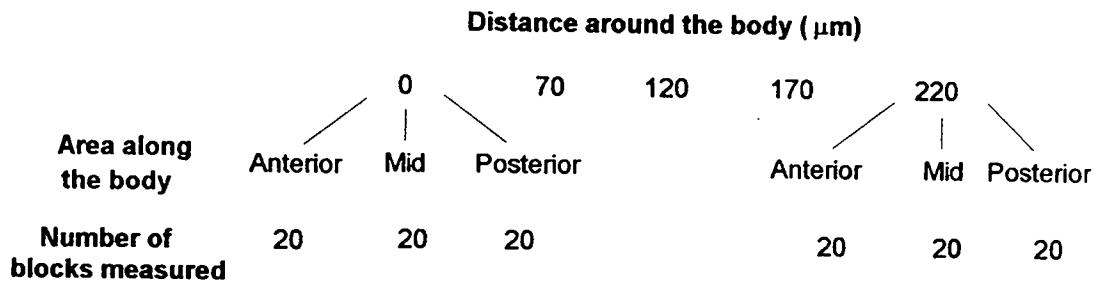


Figure 4.2. The sampling design for the pilot study to examine variability of circular muscle block widths at different areas along the longitudinal axis and distances around body.

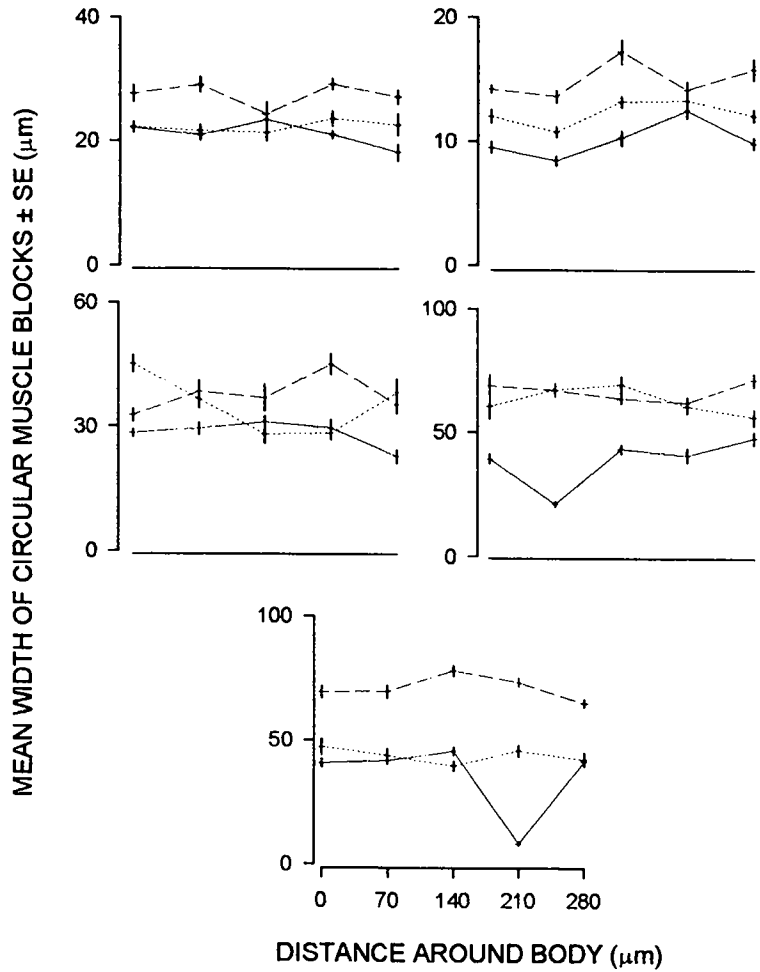


Figure 4.3. Circular muscle block widths for each of five individuals in the pilot study, showing how the average width changed with distance around the body and area along the body. Solid line = posterior, dotted line = mid and dashed line = anterior. Each point is the mean width of 20 circular muscle blocks. The five graphs represent each individual used in the pilot study. Note the different y-axis scales.

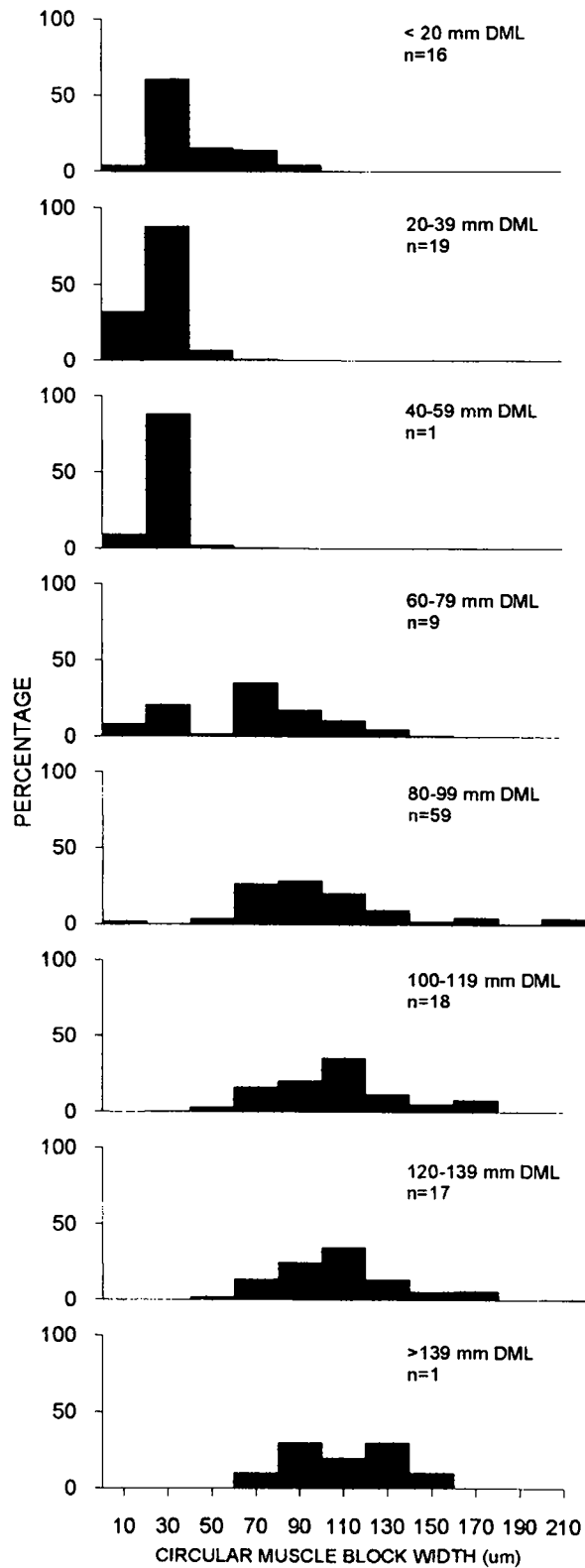


Figure 4.4. Size frequency distribution of circular muscle blocks in individuals of different dorsal mantle length (DML) size-classes. Forty-two circular muscle blocks were measured from each squid. Midpoints of muscle block size-classes are shown on the x-axis. n = number of individuals.

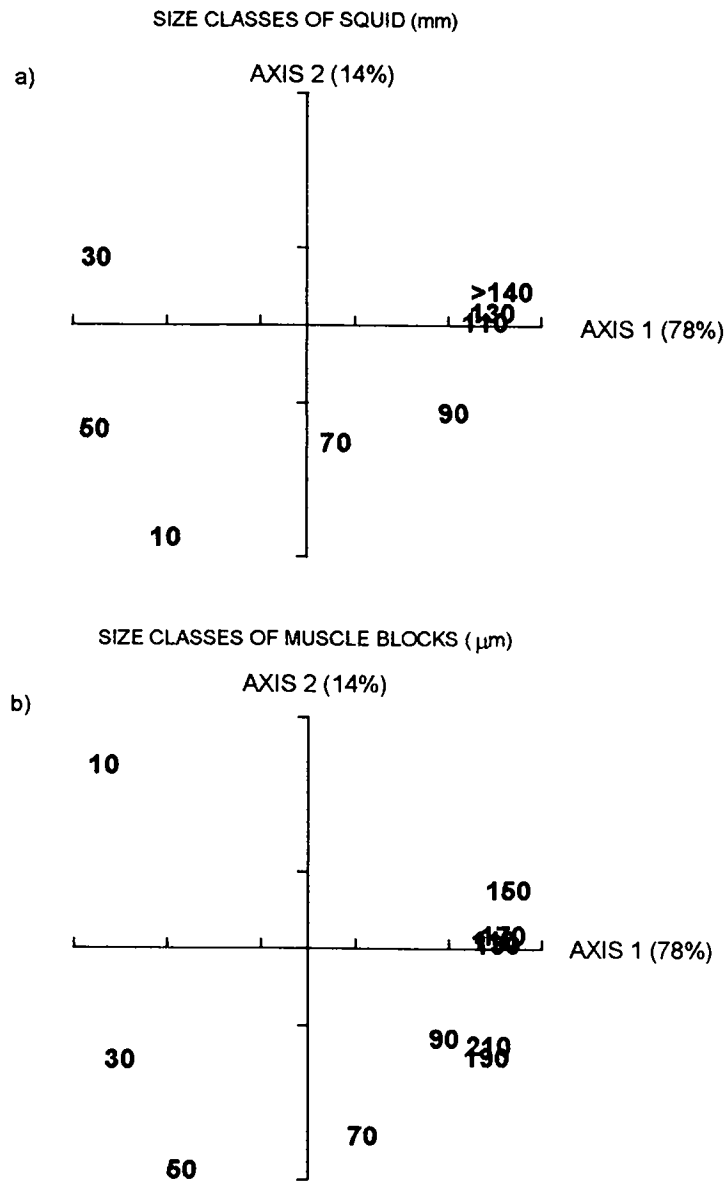


Figure 4.5. Results of the correspondence analysis, showing (a) the separation of different size-classes of individuals and (b) the separation of different size-classes of circular muscle blocks. Note that midpoints of size-classes are shown

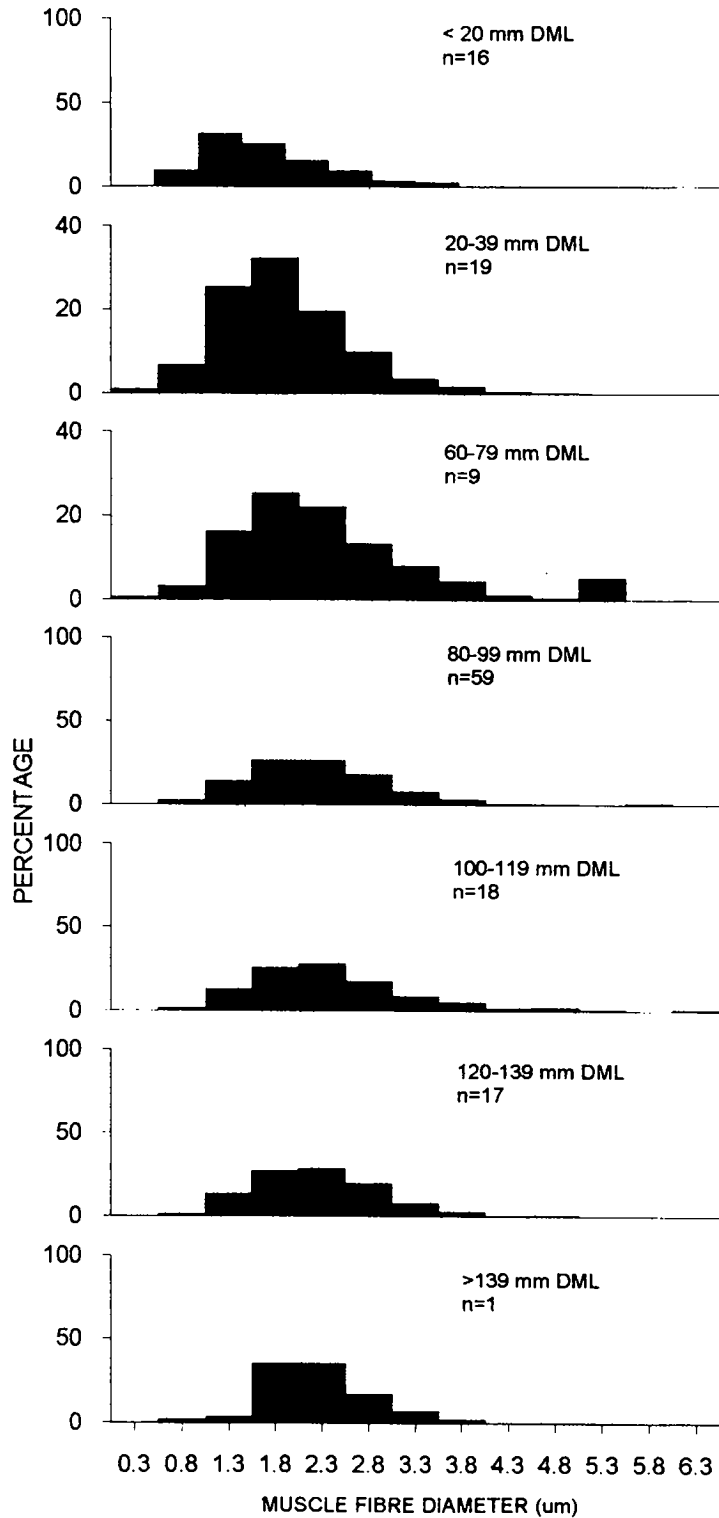


Figure 4.6. Size frequency distribution of circular muscle fibres in different dorsal mantle length (DML) size-classes of individuals. No squid in the 40 - 60 mm class were sampled. Sixty muscle fibres were measured from each individual. Midpoints of muscle fibre size-classes are shown on the x-axis. n = number of individuals.



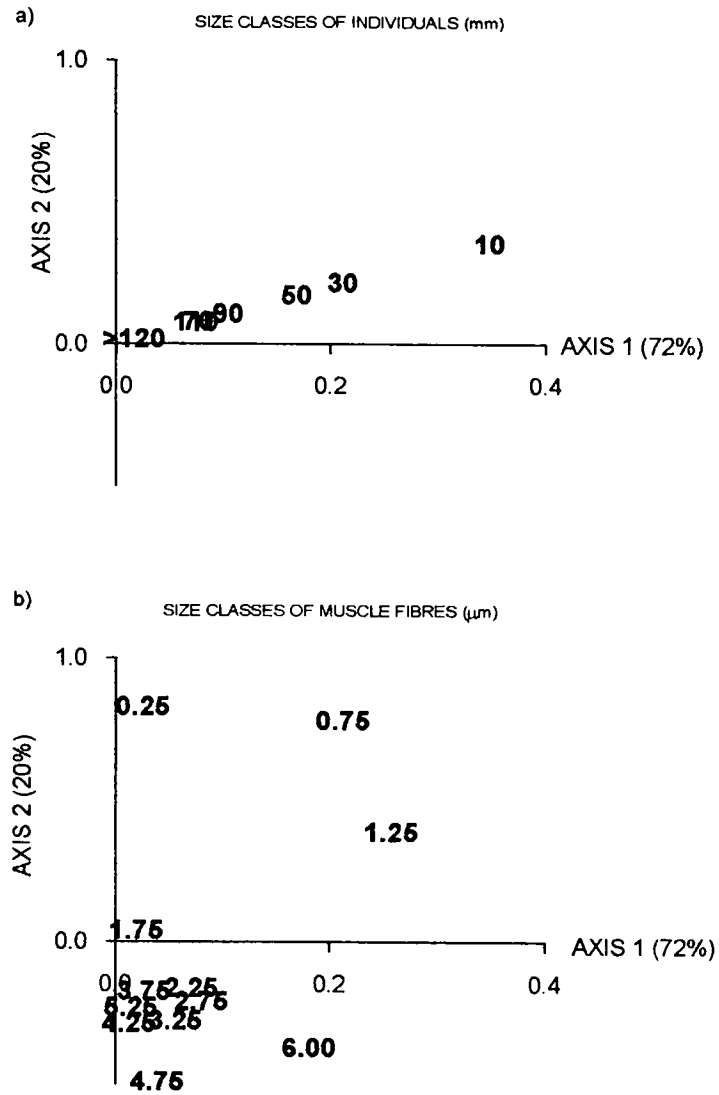


Figure 4.7. Results of the correspondence analysis, showing the separation of (a) different dorsal mantle length (DML) size-classes of individuals and (b) the separation of different size-classes of circular muscle fibres. Note that midpoints of size-classes are shown

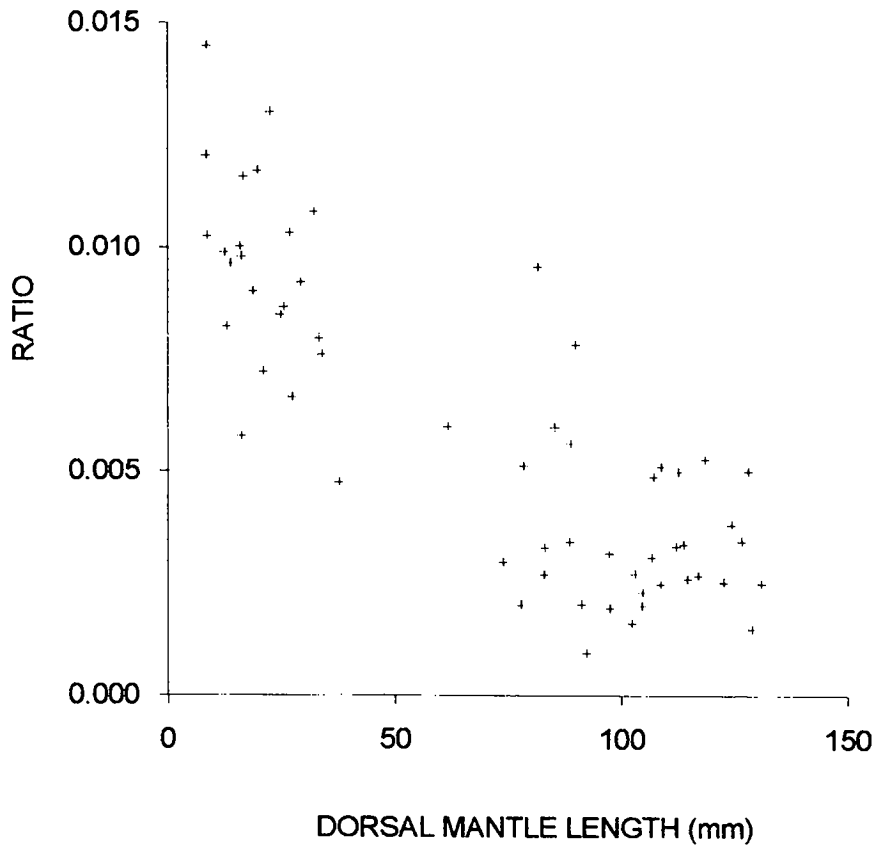


Figure 4.8. The proportion of the width of mitochondria-rich and mitochondria-poor muscle fibre areas as a function of dorsal mantle length.

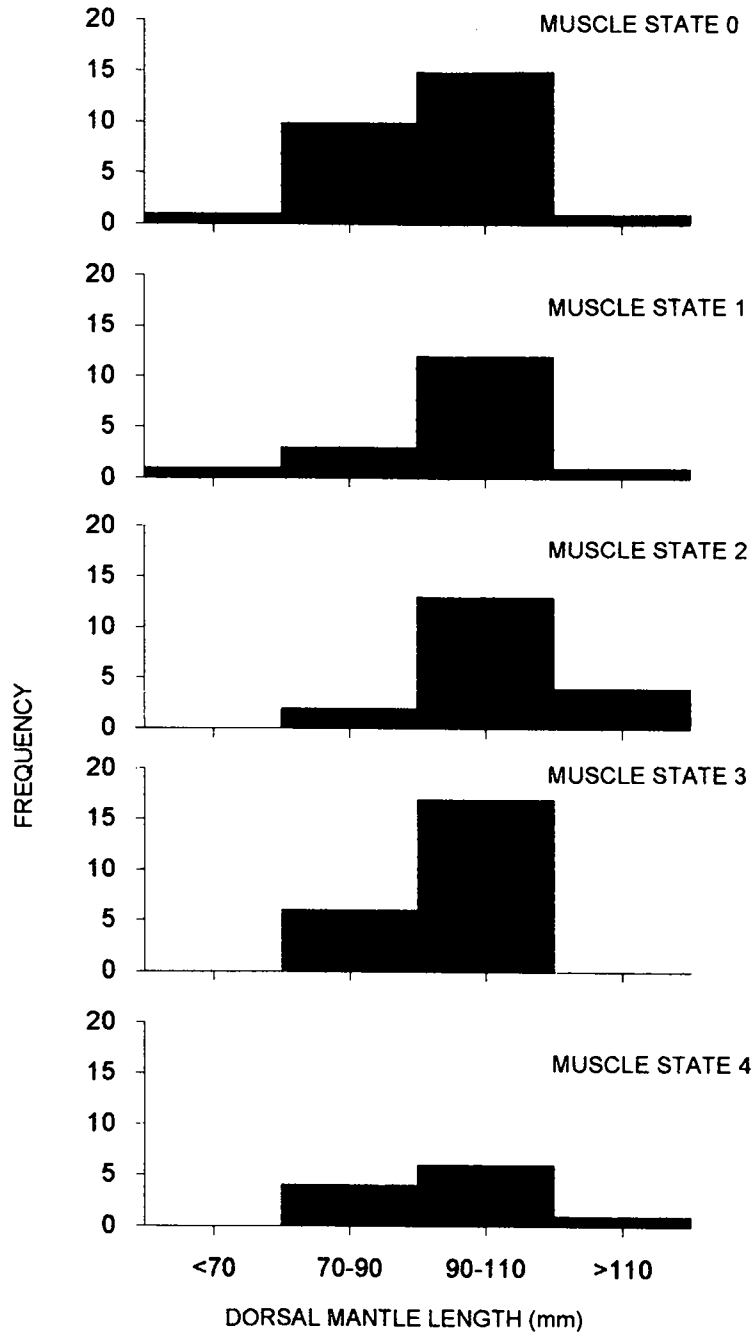


Figure 4.10: The size frequency distribution of adults in each of the mantle muscle state classes. 0 = 0%, 1 = <10%, 2 = 10-30%, 3 = 40-80% and 4 = 80-100% of muscle occupied by muscle fibre disorganisation.

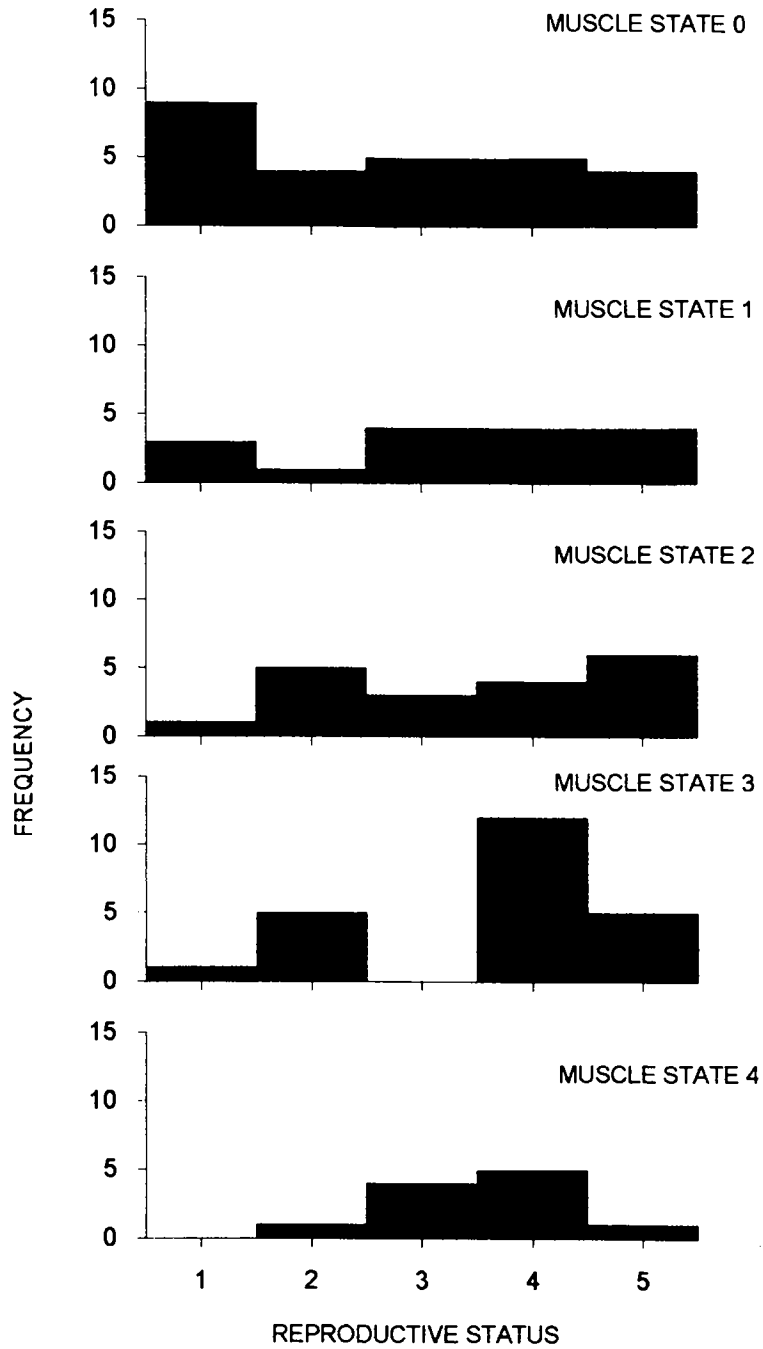


Figure 4.11: The number of individuals in each reproductive status class in each classification of muscle status. 0 = 0%, 1 = <10%, 2 = 10-30%, 3 = 40-80% and 4 = 80-100% of muscle occupied by muscle fibre disorganisation.

## CHAPTER V: GROWTH AND REPRODUCTION

### 5.1 INTRODUCTION

Biologists have generalised the life-history characteristics of cephalopods as fast growth, attainment of an asymptotic size and death occurring shortly after spawning (Callow 1987). With research extending to more species a diversity of life history characteristics are evident and generalisations are difficult. Many cephalopod species have non-asymptotic growth and there is evidence, both direct (Lewis & Choat 1993) and indirect (eg. Harman *et al.* 1989, Villanueva 1992b, Sauer & Lipinski 1990), that indicates protracted spawning activity may occur. Some of the most reliable information about spawning and growth can only be obtained by maintaining females in captivity and obtaining repeated measurements of the same females (eg. *Idiosepius pygmaeus*, Lewis and Choat 1993). Many squid species are difficult to hold in captivity and indirect evidence, such as sampling females from a population through time, must be used.

Indirect evidence for serial spawning events can be obtain from four pieces of information. Immature oocytes in the ovary of mature females and no correlation between oviduct fullness and female size have provided evidence of the potential for multiple spawning events (Harman *et al.* 1989, Sauer & Lipinski 1990). In contrast, cephalopods that are terminal spawners will often have a single batch of eggs maturing in the ovary and no primary oocytes present in mature females (Knipe & Beeman 1978, Perez & Haimovici 1991). Positive allometric growth of reproductive organs, where the gonad tissue increases in size at a faster rate than the body weight, is a phenomenon typically expected in terminal spawning animals (Rodhouse *et al.* 1988). This is because energy is allocated to gonad growth and away from somatic growth, this effect is also demonstrated by slowing of growth rates. Squid have a number of well defined organs that make up the gonadal tissue. There are correlations between the relative size of the organs and maturity stage, but

the relative growth of the organs in terminal versus serial spawners is likely to differ. Females that lay eggs in batches may not hold ovulated eggs in the oviduct for long. Instead batches of eggs will be released from the ovary and then laid. In contrast terminal spawners that gradually produce eggs during the adult phase are likely to slowly release ovulated eggs into the oviduct. Conclusive evidence for serial or multiple spawning events during which time somatic growth continues can only be obtained by holding animals in tanks or following tagged animals. However, the evidence outlined can provide a strong case for the potential for serial spawning to occur.

The diversity of spawning modes in cephalopods may be related to growth patterns (Mangold *et al.* 1993). The allocation of energy to somatic and reproductive growth will provide information about modes of spawning and longevity of the organism. Growth rates of many animals slow or cease once production of eggs or young starts. Energy used in egg production often occurs at the cost of muscle tissue production. For some cephalopod species the muscle tissue breaks down resulting in thin flaccid muscle tissue during egg production and spawning (eg. *Loligo vulgaris reynaudii* Sauer & Lipinski 1990, *Loligo opalescens* Fields 1965, Karpov & Cailliet 1978 *Moroteuthis ingens* Jackson & Mladenov 1994). However, muscle breakdown has not always been detected in species suspected to be terminal spawners (eg. *Illex argentinus* Hatfield *et al.* 1992). It may be expected that species that spawn serially will continue growing during most their life and that there will be no dramatic effect on muscle growth during reproductive maturation. The loss of muscle tissue weight and changes in texture could occur because the mantle muscle tissue may be a major energy reservoir (Packard 1972, O'Dor & Webber 1986). Proteins may be stored in the arm and mantle muscle tissue and then used to produce eggs (O'Dor & Wells 1979). This use of protein from body tissues is thought to contribute to the death of females shortly after spawning (Tait 1986, Pollero & Iribarne 1988). Breakdown of muscle tissue during egg production can be detected at the microscopic level. Histological examination of mantle muscle tissue is available for some species of terminally spawning cephalopods (Hatfield *et al.* 1992, Jackson & Mladenov 1994) but no information of this type has been undertaken for species that are known to be serial spawners.

The rate at which eggs mature is not well documented but there is some evidence to suggest that maturation may occur rapidly. For example *Todarodes pacificus* were shown to be ready to spawn within a month of primary oocytes being produced (Ikeda *et al.* 1993). Again because of the difficulty in holding females of pelagic squid for long periods of time this data may have to be derived from population level information. However, temporally intensive sampling of squid populations will be needed to obtain this type of information for short lived species. Examination of spawning patterns on small (eg. weekly) temporal scales may assist in the determining whether females do undergo multiple spawning events and in obtaining more accurate descriptions of population spawning patterns.

Limited is known about the reproductive biology of *Photololigo* sp. A. Given that there is a poor correlation between reproductive maturity and the age or size of a female it is likely that somatic growth continues during maturation and spawning and therefore females can spawn multiple times (Jackson 1991). Samples of *Photololigo* sp. A taken in the Townsville region during a 14 month period indicate that high numbers of reproductive females were present in early summer (Jackson 1993). *Photololigo* sp. A lives for approximately four months and an annual peak in population reproductive activity is unusual. It may be expected that high levels of spawning activity should occur on a regular basis throughout the year. The presence of juvenile squid and mature females in coastal waters of the Townsville region suggests that a spawning population is present in this area. The aims of this study were to:

1. Determine the potential ability of females to spawn repeatedly throughout the adult life-span.
2. Examine variability in size and age at maturation of females and growth rates.
3. Investigate how egg production affects the condition of somatic tissue.
4. Estimate rates of maturation from population level information.

## 5.2 MATERIALS AND METHODS

### 5.2.1 COLLECTION AND PROCESSING

Sampling collections were made over a sixteen week period. Twelve days of trawling were conducted from August 21 1991 to November 27 1991. This period was selected because previous studies have indicated that high numbers of mature females were present during spring in the Townsville region (Jackson 1991, 1993).

Squid were captured during daylight hours (between 0800 hrs and 1500 hrs Eastern Standard Time) in Cleveland Bay, Townsville, using twin otter trawls. Fifteen, 10 minute, trawls were taken each sampling day. Each pair of nets was considered to be a single trawl and catches for each net were combined. Squid were stored at 4°C for processing in the laboratory on the same day. The dorsal mantle length (mm), and weights (g) of mantle muscle (minus head, tentacles, viscera and skin), ovary, nidamental gland, oviducal gland and oviduct was measured for every individual that could be positively identified as female by the presence of the ovary. Dorsal mantle length, all gonad tissue (testis, spermatophoric complex and penis) and mantle muscle mass was recorded for male squid. The weight of the mantle muscle, not total body weight, was used to calculate gonosomatic indices (Eq. 1) because stomach fullness varies and damage to squid in the trawl nets often resulted in the loss of heads and tentacles affecting total weight.

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Eq. 1:

Gonosomatic Index Females =  $(\text{ovary} + \text{oviduct weight} / \text{mantle muscle weight}) \times 100$

Males =  $(\text{all gonad tissue} / \text{mantle muscle weight}) \times 100$

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Statoliths were removed from each specimen to obtain age information (Jackson 1990b, 1991). Statoliths of large squid were ground and polished to get a clear view of the nucleus and edge (methods described in Section 3.2.2).



Table 5.1: Descriptions of the macroscopic reproductive stage scale for males and females. The scale is based on Lipinski's "Universal Scale" (Juanico 1983).

STAGE	CATEGORY	FEMALES	MALES
I	Juvenile	No gonad tissue visible	No gonad tissue visible
II	Immature	Traces of ovary and oviduct	Spermatophoric complex just present
III	Preparatory	Oviduct just visible, ovary and nidamental gland obvious	Spermatophoric complex clear visible
IV	Maturing	Ovary and nidamental gland large	Testis present, white streaks in Needhams sac
V	Mature	Ovulated oocytes, red accessory nidamental glands	Spermatophores in penis and Needhams sac
VI	Spent	Unknown	Unknown

### 5.2.2 EXAMINATION OF GONADS

Individuals were opened along the ventral mid line allowing inspection of gonads. The state of reproductive maturity was assessed macroscopically based on the size and colour of the reproductive organs (Table 5.1) Squid that could not be reliably sexed because the gonad was too small were classified as juveniles. To determine the size at maturity the dorsal mantle length at which 50% of squid were maturing and mature (ie. Stages III, IV and V) was calculated. The relationship between the mass of the ovary, oviduct, oviducal gland and nidamental gland was examined for each maturity stage using Canonical Discriminant Analysis (SAS 1987).

For microscopic inspection of the oocytes, ovary tissue from females was fixed in FAACC. Fixed ovary material was transferred to 70% alcohol for 48 hrs then dehydrated through an alcohol series and impregnated with wax. Sections (6  $\mu\text{m}$ ) were cut and stained with Mallory-Heidenhain Trichrome stain. Stage frequency distributions of eggs from females of different reproductive maturity stages were obtained by counting and assigning approximately 100 eggs to a maturity stage. The developing oocytes (Fig. 5.1) were assigned to one of five maturity stages on a scale the same as the one used by Sauer & Lipinski (1990).

To obtain estimates of egg batch sizes the oviducal material from Stage V females was removed and stored in FAACC. All eggs in a 20 mg sample of oviduct were counted and measured using an ocular micrometer in a stereo microscope. Estimates of the total numbers of oocytes in the oviduct were made by scaling the number of oocytes in the subsample by the total weight of the oviduct. Batch size was then calculated as the number of oocytes per gram of oviduct.

### 5.2.3 MANTLE TISSUE AND SOMATIC GROWTH

The condition of female squid from each maturity stage was assessed by the allometric relationship between wet weight of the mantle muscle and dorsal mantle length. The relative change of mass with length was described using the linear

relationship between log<sub>10</sub> transformed variables. A comparison of the slopes of these regression lines was made using an extension of the analysis of covariance, with dorsal mantle length as the covariate. This technique reduced the effect of comparing length-weight relationships between individuals of very different sizes.

Growth of the mantle muscle tissue occurs predominantly by an increase in muscle fibre number and not by increasing muscle fibre size (Chapter III). This growth is recorded as an increase in the width of the circular muscle blocks. Therefore, it may be expected that reproductive growth will affect the width of the circular muscle blocks. Muscle tissue was removed, processed and analysed according to methods outlined in Chapter III. Size frequency distributions of the muscle blocks were generated for reproductive females from stage I to stage V.

### 5.2.4 RATES OF OOCYTE MATURATION

Stages of reproductive maturity were combined to increase the numbers in the different categories; Stages I and II were immature individuals, Stage III was maturing and Stage IV and V were mature. On three occasions, low numbers of squid were caught so information for consecutive weeks was combined. To examine the temporal changes in population reproductive status, a log-linear multi-way frequency analysis was used. This analysis generated the expected frequencies of individuals in each maturity stage through time, given that the number of individuals in each maturity stage and time are independent of one another. The difference between the observed frequency of individuals in the maturity stage and the expected calculated from the model was calculated (residuals). It is usual to standardise the residuals to allow interpretation of the results (Tabachnick & Fidell 1989). The standardisation used to present the data was the difference between the expected and observed values divided by the square root of the expected frequency. These standardised residuals were then plotted through time to determine when frequencies of females in each reproductive stage were greater or less than expected.

## 5.3 RESULTS

### 5.3.1 SIZE AND AGE AT MATURATION

There was some evidence of size specific maturation in female *Photololigo* sp. A as no females less than 80 mm were found in final stages of reproductive development (Fig. 5.2). However, some large females, 100 mm dorsal mantle length, were immature. Female squid mature at 85 mm, approximately 20 mm larger than the males during the summer months (Fig. 5.3). Reproductive maturation, indicated by an increase in mass of the nidamental and oviducal glands, had begun once females reached a mantle muscle mass of 10 g (Fig. 5.4) The average ages of females in each maturity stage were very similar and there was considerable overlap in the ranges of ages (Fig. 5.5). This suggested that age was not a good determinant of sexual maturity.

### 5.3.2 MULTIPLE SPAWNING

Reproductive maturity involved growth of all the reproductive organs, but the relative size of the ovary and oviduct gland were important discriminators (Fig. 5.6). The weight of the ovary was the major organ discriminating between the stages of maturation. Discrimination of Stage IV and V females was also possible by the presence of ovulated oocytes in the oviduct, which was a feature initially used as a macroscopic discriminator to initially identify Stage IV and V females. As a spawning event is likely to result in a depletion of oocytes in the oviduct and therefore a decrease in mass of the oviduct, it is possible that Stage IV females included individuals that had previously spawned and individuals that have yet to spawn.

Oocyte development was similar to that described for other loliginid species. Females with small developing ovaries had a predominance of primary and secondary oogonia. Oocytes completed maturation in the ovary before ovulating and moving into the oviduct. In mature females, the oviduct weight never exceeded 15% of the

wet mantle weight. As maturation of oocytes progressed, the ovary continued to produce new primary oocytes and fully mature (Stage IV and V) females had a range of oocyte types present in the ovary (Fig. 5.7). Asynchronous oocyte production, with no definite peaks of oocyte type present in the ovary, indicated that females continually produce and release oocytes into the oviduct. Unfortunately, no time scale is available for the period over which females can hold fully mature eggs in the oviduct. The failure to observe eggs in the process of resorption suggests that vitellogenic eggs are likely to survive to laying.

There was a linear relationship between the mass of the components of the reproductive system in the females and dorsal mantle length (Table 5.2). All the elements of the reproductive system grew relatively slower than the mantle length, as indicated by slope values of less than 1. The correlation coefficients for all components were small, especially for oviduct. This provides further support to the suggestion that size is a poor predictor of sexual maturity.

Table 5.2: Allometric growth of elements of the female reproductive system against dorsal mantle length. Allometric equations calculated using the least squares analysis. All variables have been log<sub>10</sub> transformed. n=162. SEb = standard error of the slope. Critical r (df 161, α=0.05)=0.1549.

Variable	Slope	SEb	Intercept	r
Ovary	0.322	0.040	-0.687	0.534
Oviduct	0.169	0.028	-0.389	0.434
Oviducal gland	0.214	0.023	-0.498	0.588
Nidamental Gland	0.266	0.031	-0.578	0.558

The number of eggs in the oviduct bore little relation to the size of individuals (Fig. 5.8a). The number of eggs per gram of oviduct ranged from 131 to 420. Often oviducts with few oocytes had more connective tissue between the oocytes. The greatest number of eggs calculated in the oviduct was 983 in a 107.4 mm dorsal mantle length individual, but on average females had 268 eggs per gram of oviduct mass. Ovulated oocytes ranged in size from 0.96 to 2.00 mm long and on average were 1.58 mm long. The size of the oocytes was not dependent upon the volume of oocytes in the oviduct (Fig. 5.8b).

## 5.3.3 MANTLE MUSCLE CONDITION

Macroscopic examination of the muscle tissue indicated no loss of condition, even in females who had obviously expended considerable energy in producing reproductive tissue (Fig. 5.9). The slopes of the linear relationships of wet weight of the muscle mantle tissue against with dorsal mantle length for each reproductive stage were not different (Table 5.3). This suggests that there is little evidence of maturation causing loss of condition by slowing the increase in mass of the mantle muscle tissue.

Some loss of condition by females is sometimes associated with damage done by males while transferring spermatophores to the female. The presence of spermatophores in the buccal sac indicated that approximately 20% of the females had mated, but there were no external signs of lesions or sores on the head or mantle muscle associated with damage during mating.

Table 5.3: Length-weight relationships for females in each of the five reproductive categories.

STAGE	RELATIONSHIP	SE OF THE SLOPE	$r^2$	n
I	$LWT=2.26*LML-3.27$	0.06	0.95	75
II	$LWT=2.10*LML-2.93$	0.08	0.94	43
III	$LWT=2.41*LML-3.53$	0.11	0.96	23
IV	$LWT=2.26*LML-3.24$	0.28	0.82	16
V	$LWT=2.29*LML-3.31$	0.10	0.92	49

Ho: All slopes are equal.

Source	SS	df	Mean SS	F	Prob>F
Stage	0.0087	4	0.0022	1.05	0.3831
DML	2.3476	1	2.3476	1138.45	0.0001
Slopes	0.0081	4	0.0020	0.98	0.4177
Residual	0.4051	196	0.0021		

The size frequency distributions of circular muscle blocks showed no consistent trend that could be related to reproductive maturity (Fig. 5.10). Stage III females in the 80-100 mm size class had some very wide mantle muscle blocks, but this was not consistent for females in that reproductive stage or the size class, so it is unlikely to be a function of spawning activity.

## 5.3.5 POPULATION DYNAMICS

Catches of *Photololigo* sp. A during the sampling period were variable on a weekly basis (Fig. 5.11). Highest numbers of squid caught in this area was during November ( $F=4.56$   $df$  12, 174  $Pr<0.05$ ). It is unclear whether the variation in numbers is because schools of squid moving in and out of Cleveland Bay or squid moving around the Bay making it difficult to sample the population. The percentage of males and females remained similar over this period (the exception was August 21). Higher proportions of juveniles in the population were evident at the end of August, mid September and end of November (Table 5.4).

Table 5.4: Percentage of individuals in each category. Juveniles were individuals that were too immature to sex.

DATE	JUVENILES	FEMALES	MALES	N
21 Aug	48.5	13.6	37.9	66
28 Aug	12.5	43.8	43.8	16
3 Sep	29.4	38.2	32.4	68
17 Sep	64.6	19.2	16.2	99
24 & 29 Sep	36.3	26.4	37.4	91
29 Sep & 15 Oct	24.1	34.5	41.4	58
22 & 30 Oct	29.1	34.5	36.4	55
6 Nov	28.2	40.8	31.0	71
11 Nov	48.4	30.8	20.9	91
27 Nov	65.6	16.7	17.7	96

There were always reproductively mature males and females in the population during the fourteen week period (Fig. 5.12). The number of females in each maturity class was dependent on the sampling date ( $\chi^2 = 40.40$ ,  $df = 18$ ,  $Pr = 0.002$ ). Immature females were present in higher numbers than were expected during late August and early September (Fig. 5.13). Maturing females were also caught during September in higher numbers than expected. In late October and during November slightly higher numbers of mature females than expected were caught. It appeared that the sampling program detected a peak of immature females and it was possible to track this pulse of individuals during the period that they matured and eventually until they were mature. This group of females matured during September and October and were ready to spawn during November. Based on this series of samples, from the

time oocytes were produced until the ovulated oocytes were present in the oviduct is less than two months. Immature individuals arrived over a short time, but higher numbers than expected of maturing and mature individuals occurred for relatively longer periods, suggesting variability in maturation times and extended spawning periods. Relatively higher numbers of mature females in late August may be the tail end of the last peak in maturing females. There was a decrease in the average size of female squid at the point when large numbers of immature individuals were entering the population (Fig. 5.14). After this time there seemed to be groups of females in the population; the group that was evident as a pulse in early September and continuous presence of smaller individuals. Increasing mass of the components of the reproductive system of the females in the population occurred during late September and early October then decreased during November (Fig. 5.15). There also appeared to be a peak in mass at some point in August.

Proportions of males in different stages of reproductive maturity was also dependent on time ( $\chi^2 = 39.48$ ,  $df = 18$ ,  $Pr = 0.002$ ). Numbers of immature males were higher than expected in late August, a week before the females (Fig. 5.13). During early September males were maturing with highest numbers of mature male seen in late September and early October. The picture during late October and November is not clear at all. Maturation rates were faster, less than two weeks, and possibly less variable for males than females (Fig. 5.13). Since female squid can store spermatophores, the changes in gonosomatic indices of the males through time provide a poor indicator of temporal patterns of spawning (Fig. 5.16). There were no clear patterns of gonad development evident for male *Photololigo* sp. A suggesting that continuous production of spermatophores occurs once males are mature and spawning activities may not reduce gonad mass to the same extent as females. There was a decrease in average gonosomatic index during November suggesting that a new influx of immature squid occurred, but this was not as obvious as the decrease seen in the females.



## 5.4 DISCUSSION

This study produced four pieces of evidence suggesting that female *Photololigo* sp. A are able to intermittently spawn. (1) Female *Photololigo* sp. A had asynchronous gamete production and females with ovulated eggs in the oviduct still had primary oocytes in the ovary. A positive correlation between ovary and oviduct weight suggested that females continued to produce oocytes in the ovary whilst ovulated eggs were released into the oviduct. (2) A poor correlation between size of females and oviduct mass suggests that the oocytes are not resident in the oviduct for long. (3) Rapid increase in oviduct mass suggests that the ovary releases batches of eggs into the oviduct. (4) Very slow increase in gonad mass during growth of the mantle muscle indicating that large amount of energy is allocated to reproductive tissue at the cost of the somatic tissue.

Ovaries of all mature females examined had a large population of primary and pre-vitellogenic eggs. The presence of a range of oocyte maturity stages in the ovary may not be sufficient evidence in itself that a species is a repeating spawner since groups of eggs may be 'batched' into the oviducts for a single release. However, asynchronous production of oocytes, with no distinct peaks of oocyte size in the ovary indicates multiple spawning events in teleosts (de Vlaming 1983). For multiple spawning events to occur, new oocytes must be generated whilst ovulated eggs are laid. It was based upon this argument that Harman *et al.* (1989) suggested that *Stenoteuthis oualaniensis* is capable of multiple spawning events. Support for this is provided from squid that are repeat spawners that always have immature oocytes in the ovary (eg. *Idiosepius pygmaeus* Lewis & Choat 1993). The ovaries of spent *Loligo opalescens* females that spawn once have no primordial cells (Knipe & Beeman 1978). Interestingly, Stage IV and V female *Loligo vulgaris reynaudii* had no primordial oocytes present in the ovaries (Sauer & Lipinski 1990). Either *L. v. reynaudii* stops producing gametes, suggesting a limited capacity for extended spawning, or they produce temporally discrete batches of gametes.

Ovulated egg sizes did not vary much suggesting that females of all sizes produce the same size eggs and no further growth of the egg occurs in the oviduct. The poor correlation between oviduct mass and body size in *Photololigo* sp. A in this study suggested that females periodically spawn and empty the oviduct of eggs. A strong relationship between oviduct mass and body size should be evident if females hold ovulated oocytes in the oviduct until all oocytes complete maturation (Harman *et al.* 1989). This is further supported by the linear relationship between ovary and oviduct weight, suggesting that the ovary continues to produce more oocytes even when the oviduct is very full. If *Photololigo* sp. A is a multiple spawner the number of eggs estimated per gram of oviduct represents a single batch of eggs and not the total fecundity for the life time of the female.

The rapid increase in oviduct mass suggested that mature *Photololigo* sp. A females release batches of ovulated eggs into the oviduct. Clearly these eggs are not accumulated in the oviduct (see previous point), therefore the eggs must be laid in batches. Once that batch is laid, there will be a period in which there will be no eggs in the oviduct until the next batch mature. Therefore, it may be expected that immediately after a female has laid a batch of eggs the oviduct would be very small and individuals may appear to be Stage IV females. It is not possible to distinguish between females approaching their first spawning event and those that may already have spawned. Unfortunately no histological description of a recently spawned *Photololigo* sp. A female is available.

Negative allometric growth of reproductive organs in *Photololigo* sp. A supports the idea that species is an intermittent spawner. This is in direct contrast to the growth rates of reproductive organs in *Illex argentimus* (Rodhouse & Hatfield 1990b, Hatfield *et al.* 1992) and *Alloteuthis subulata* (Rodhouse *et al.* 1988). Both these species demonstrated very strong positive allometric growth of reproductive organs, a phenomenon typically expected in terminal spawning animals (Rodhouse *et al.* 1988). This suggests that the allocation of energy to somatic and gametic growth differs between terminal and intermittent spawning animals.

There was no evidence of muscle tissue degradation during egg production in *Photololigo* sp. A. These data lend support to the possibility that growth patterns may be linked to mode of spawning in cephalopods (Mangold *et al.* 1993). It is not clear whether senescence in squid is due to the metabolism of body protein so that egg production can occur, or a hormonal change that occurs after spawning. An examination of *Illex argentinus* muscle tissue suggest that although this species spawns only once there is no evidence that production of gametic tissue is at the cost somatic tissue (Hatfield *et al.* 1992, Rodhouse & Hatfield 1992). Therefore, it is possible that some species cease feeding shortly after spawning begins and the mantle muscle tissue is used as an energy source (Laptikhovsky & Nigmatullin 1993). Given that both the work done by Hatfield *et al.* (1992) on a terminal spawner and this study on a serial spawner conclude no effect on somatic tissue during egg production the interaction between somatic and gametic growth is still unclear. It is possible that our understanding of this may be achieved at the biochemical level. Research that has focussed on sources of amino acids for egg production and biochemical changes during egg production has examined terminal spawning species (O'Dor & Wells 1987, Pollero & Iribarne 1988). A useful, and necessary, comparison will be to examine biochemistry in cephalopods that spawn intermittently.

It is possible that the trigger for reproductive maturity and the allocation of energy to gametic production is provided at the population level. There was evidence of an influx of immature individuals in early September, with subsequently higher numbers of mature females than were expected if the frequency of females in each reproductive class was independent of time. This suggests that either spawning activities of the population may be co-ordinated, which would explain the range of sizes and ages at maturity. Alternatively it is possible that the sampling program detected an influx of immature individuals in the adult population. Juvenile squid tend to associate with hydrodynamic features of the water mass in the Townsville region (Chapter II, Appendix I) and it is possible that a large number of juveniles aggregated in this area and recruited to the adult population at the same time. However, a longer term sampling program covering several years with weekly sampling is needed to confirm that this pattern is representative. Many squid species do spawn throughout the year often showing annual or biannual peaks (eg. *Loligo vulgaris reynaudii* Augustyn

1990, *Loligo pealei* Summers 1971, *Loligo forbesi* Lum-Kong *et al.* 1992, *Illex illecebrosus* Coelho & O'Dor 1993, *Loligo sanpaulensis* Costa & Fernandes 1993). Similarly some tropical fish have very definite peaks of spawning despite having extended spawning activities during warmer months (Johannes 1978).

This study suggests that during the austral summer it takes less than two months for immature *Photololigo* sp. A females to mature. Once females began producing primary oocytes the ovulated eggs were released into the oviduct in usually less than 30 days. Clearly these calculations of the rate of maturation of oocytes were based on an indirect method of population level information. The only other squid, *Todarodes pacificus*, for which rates of oocyte maturation have been generated is based on a more reliable method of following a population of females in the laboratory (Ikeda *et al.* 1993). In this closed population the movement of females and reproductive maturation cannot be confused. The temporal relationship between ovulation and spawning in squid is an important issue to enable biologists to determine when spawning events occur. If spawning occurs shortly after ovulation then observations of mature females in the field will indicate that spawning events are taking place. If ovulated eggs are held in the oviduct for weeks then observations of the frequency of females in reproductive stages will not provide information about the time of spawning. In fish, the oocytes are ovulated shortly before spawning and fertilisation rates decrease if ovulated eggs spend longer than eight hours in the oviduct (Scott *et al.* 1993). Given the fast metabolic rates of squid and the relatively fast growth rates it is likely that rates of reproductive development are also rapid. The maturation process of eggs from primary oocytes to spawning occurred within a month for female *Todarodes pacificus*. Individuals that had begun maturing ovulated in less than two weeks and spawning occurred within two days of ovulation (Ikeda *et al.* 1993). *Sepia officinalis* has a similar time scale for egg maturation (Boletzky 1987b). Although conditions in captivity will be different from those in the wild, rapid maturation can occur. There is no reason to doubt that such rates may occur in the field. Females that have released ovulated oocytes into the oviduct will lay them shortly afterward.

Only female *Photololigo* sp. A that had ovulated oocytes into the oviduct had mated. This suggests there is a close temporal relationship between the release of ovulated oocytes into the oviduct and spawning. This has also been observed in *Loligo forbesi* (Boyle & Ngoile 1993a). The presence of unmated mature females indicates that males only mate with mature females and rapid fertilisation of mature eggs may be important. Therefore, storage of spermatophores may not occur for long in *Photololigo* sp. A. For species where males mate with immature females and storage of spermatophores does occur for a long time, a specialised apical filament mechanism fertilises ovulated oocytes as they enter the oviduct (*Eledone massyae* Perez *et al.* 1990). The specialised apical filament and mating with only mature females may be mechanisms to ensure that fertilisation of oocytes occurs shortly after ovulated oocytes are released into the oviduct. If rapid egg maturation occurs and ovulated eggs need fertilising quickly, then differences in temporal patterns of maturation between females and males may be an artefact of the sampling programs (eg. Jackson 1993). Clearer pictures of population dynamics and reproductive activities may require more temporally intensive sampling.

Descriptions of population dynamics and processes of pelagic populations pose problems when immigration and emigration cannot be measured. This becomes further compounded when the geographic extent of the population is not and cannot be described. This description of population reproductive patterns is based upon several assumptions. The first is that the sample collected each day was representative of the population. On several days sample sizes were small and were combined to obtain more information. It is recognised that many of the patterns described were based on small sample sizes, however, they provide some biological hypotheses upon which future work can be built. The second assumption is that the patterns described through time are not a function of immigration and emigration but biological processes. Clearly the effects of movement are difficult to isolate and will always be a problem when describing population processes of pelagic species. However, obtaining weekly samples may have reduced the effect of individuals moving in and out of the population and allowed some picture of patterns of reproductive maturation in the population. This is supported by the temporal sequence in the presence of immature, maturing and mature females observed during

this study. Furthermore the presence of juvenile *Photololigo* sp. A suggests the spawning grounds for this species does exist in the Townsville region and mature females caught in the Townsville region may spawn at these grounds. It was assumed that females with ovulated eggs in the oviduct were likely to spawn within hours or days, rather than retain these eggs for weeks. The presence of juveniles of a range of ages throughout winter and summer months suggests that this is correct.

One problem that arose during the study was the variability in numbers of squid within the sampling area. This made assessments of the reproductive status of the population difficult and the interpretation of temporal patterns speculative. However this study has provided confirmation that female *Photololigo* sp. A are capable of multiple spawning events during their adult life time. The presence of large females still in the early phases of maturation indicates that female may be able to control when maturation occurs. It is unclear whether factors affecting maturation result from complex interactions between growth and reproduction or external factors such as food supply and interactions within the population.

CHAPTER V: GROWTH AND REPRODUCTION

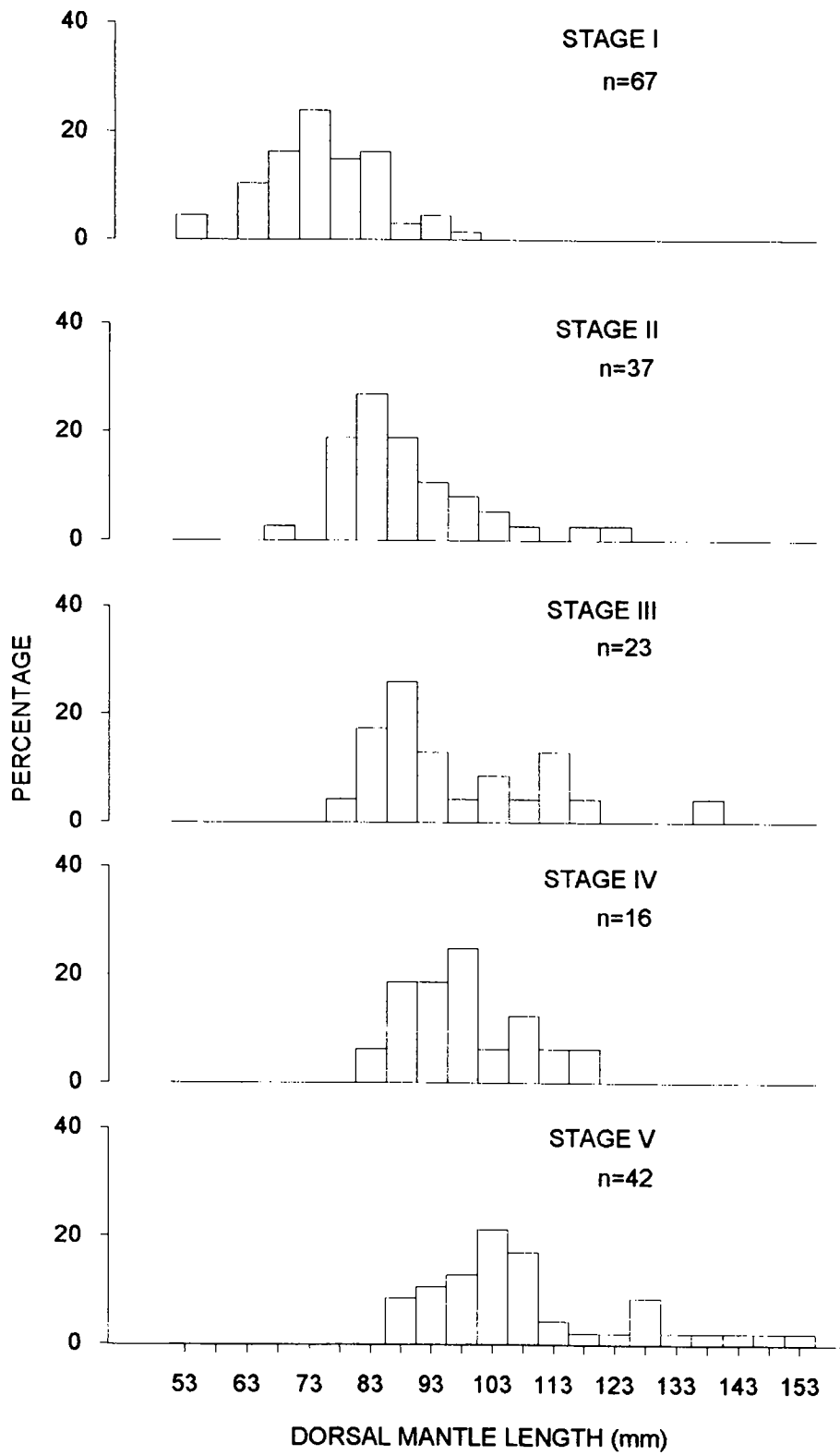


Figure 5.2: Size frequency distribution of females in each of the five reproductive stages. n = the number of females measured.

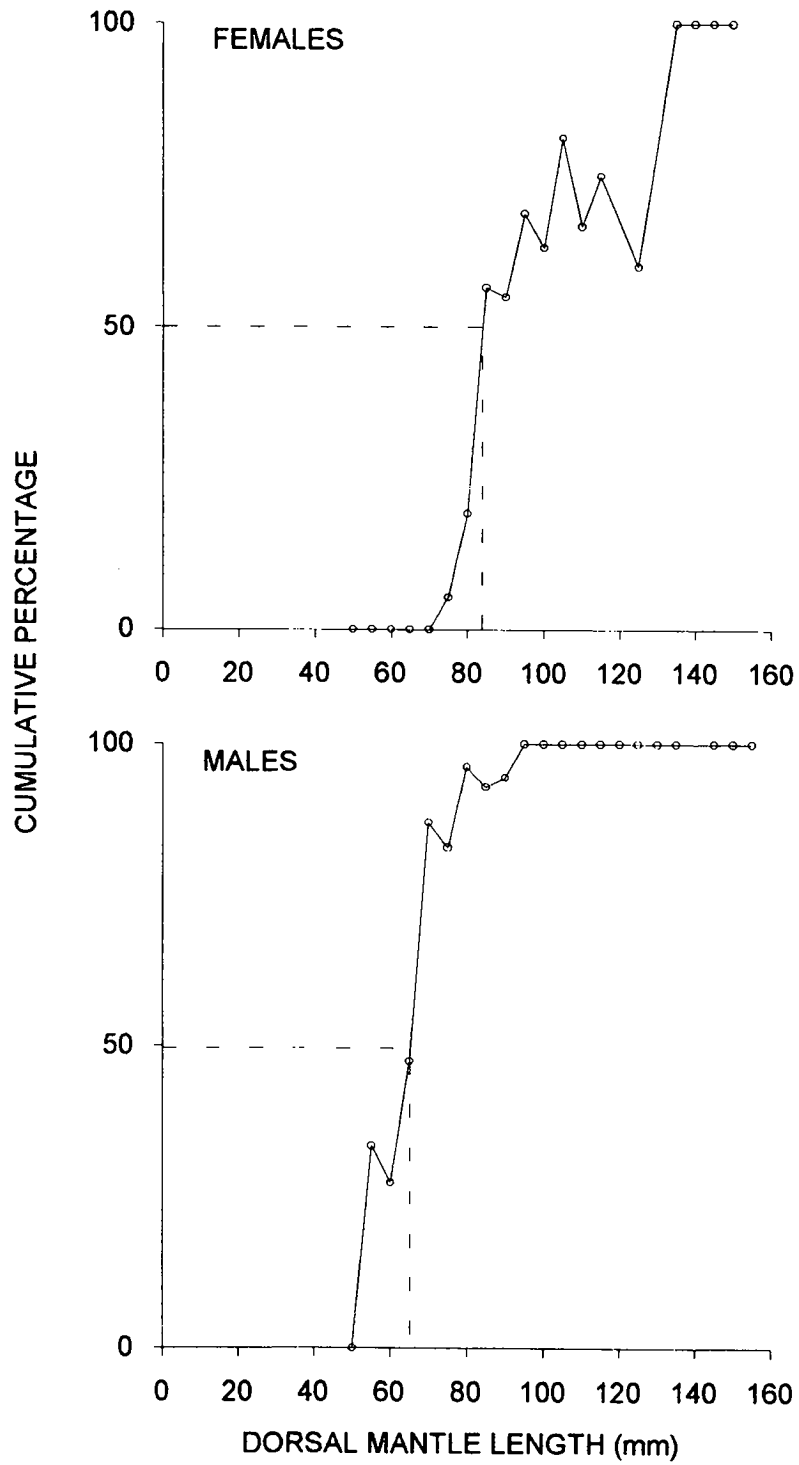


Figure 5.3: Size at maturity is calculated by ranking maturing and mature females by size. The dorsal mantle length at the 50% quartile is nominally declared as the size at maturity.



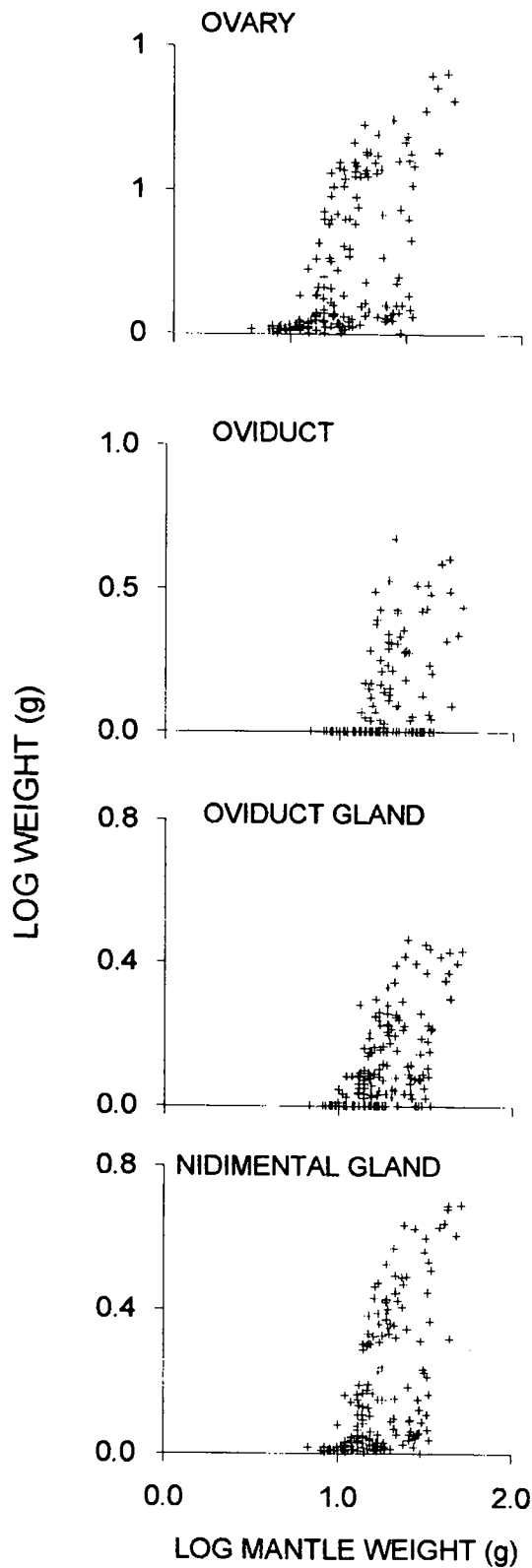


Figure 5.4: The relationship between growth of the mantle muscle mass and the four organs of the female squid reproductive system. Data are log<sub>10</sub> transformed. Linear equations and correlation coefficients are presented in Table 5.2.

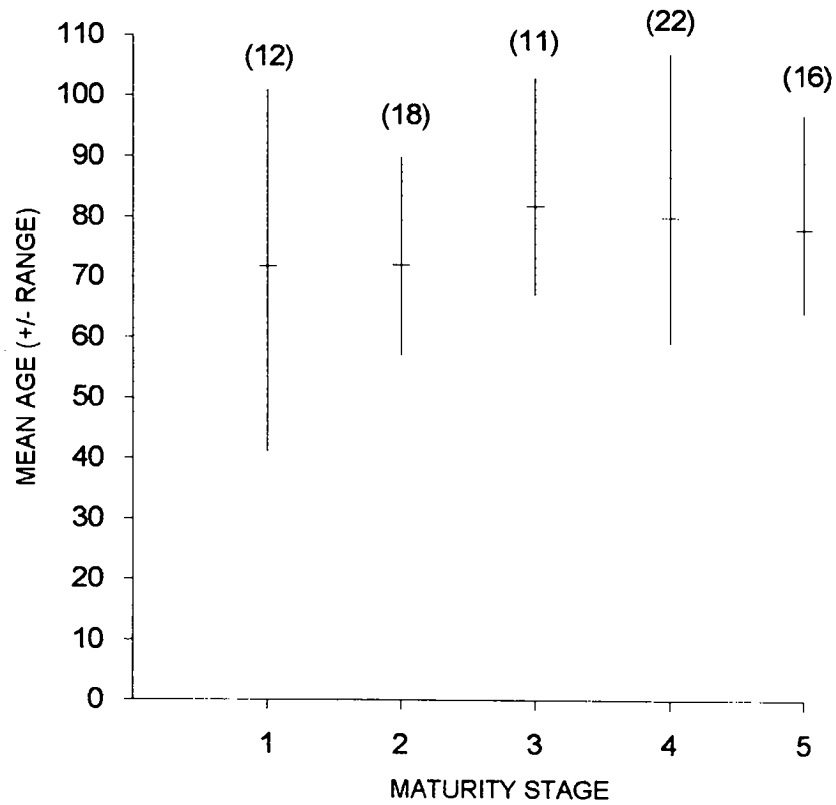


Figure 5.5: The average age and the range of ages of females allocated to the five reproductive stages. The number of females used to calculate the average is provided above each point.

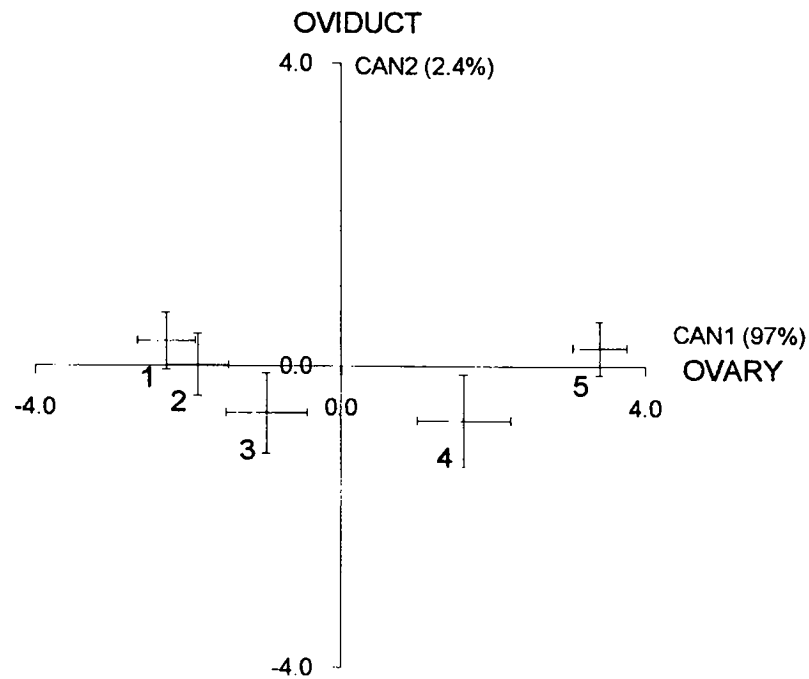


Figure 5.6: The results of the canonical discriminant analysis for the five reproductive maturity stages. The mean and 95% confidence limits for each centroid on the first canonical axes are plotted. The percentages indicate the amount of variation between the reproductive stages that is described by each axis.

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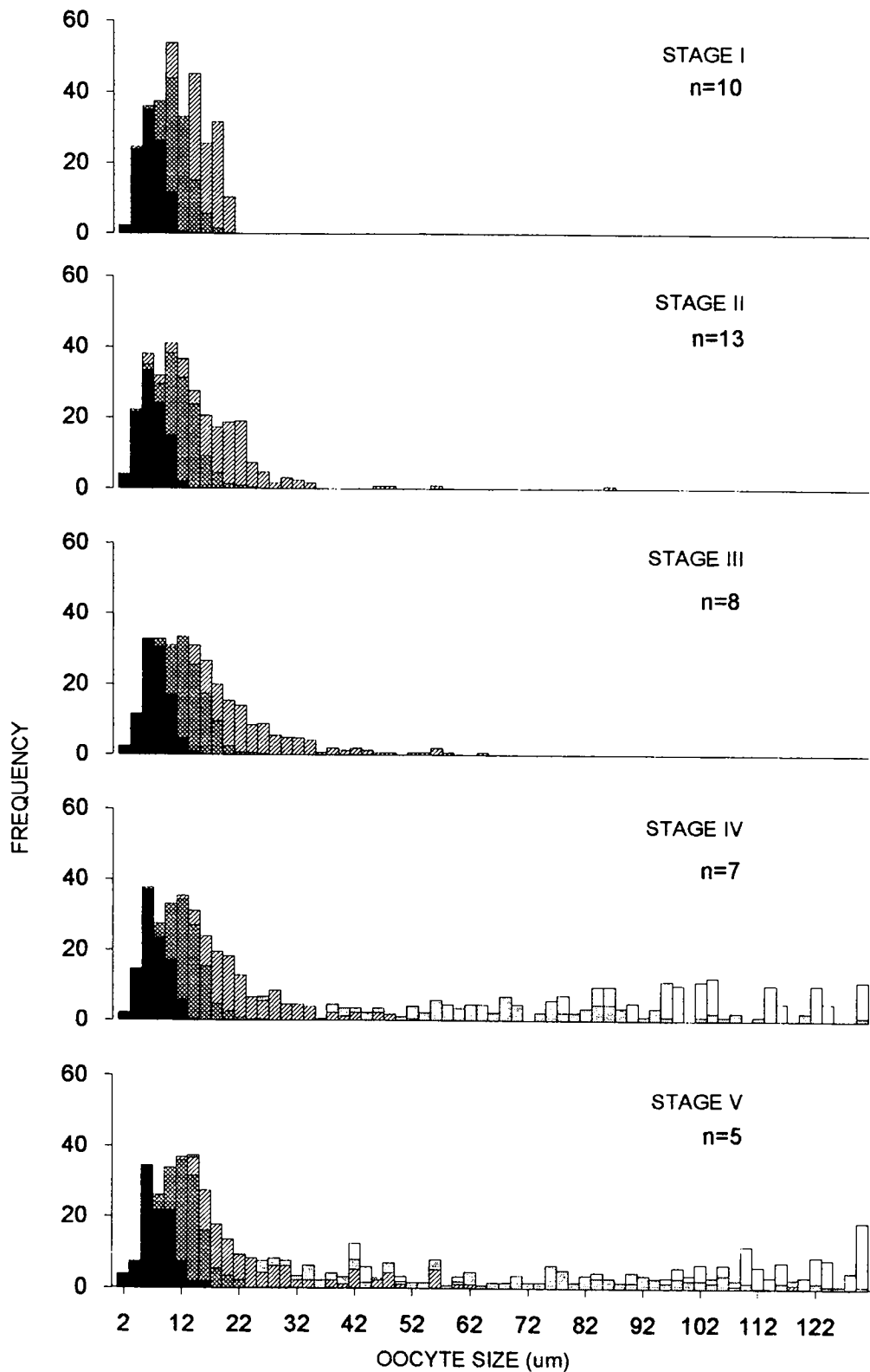


Figure 5.7 Oocyte size and maturity stage in each of the five reproductive stages of females. Solid bars = primary oogonia, cross-hatched = secondary oogonia, crossed = follicular oocytes, stippled = vitellogenic oocytes and open = mature oocytes. n = number of individuals for which oocytes were sized and staged.

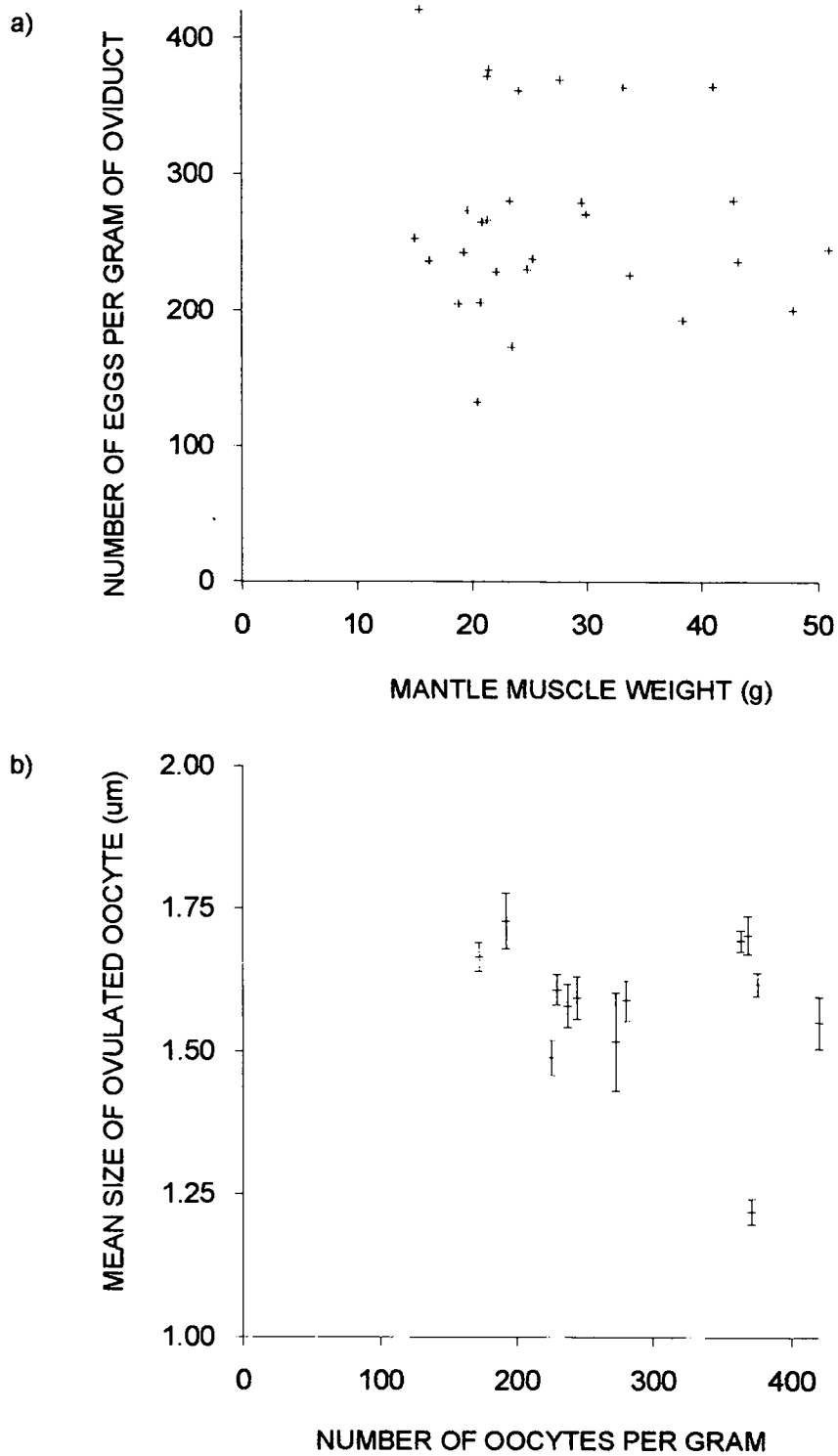


Figure 5.8: a) Batch size (number of eggs per gram of oviduct) of females as a function of the mantle muscle mass.

b) The relationship between the number of ovulated oocytes in the oviduct and the size of the oocyte. Error bars are the standard error.

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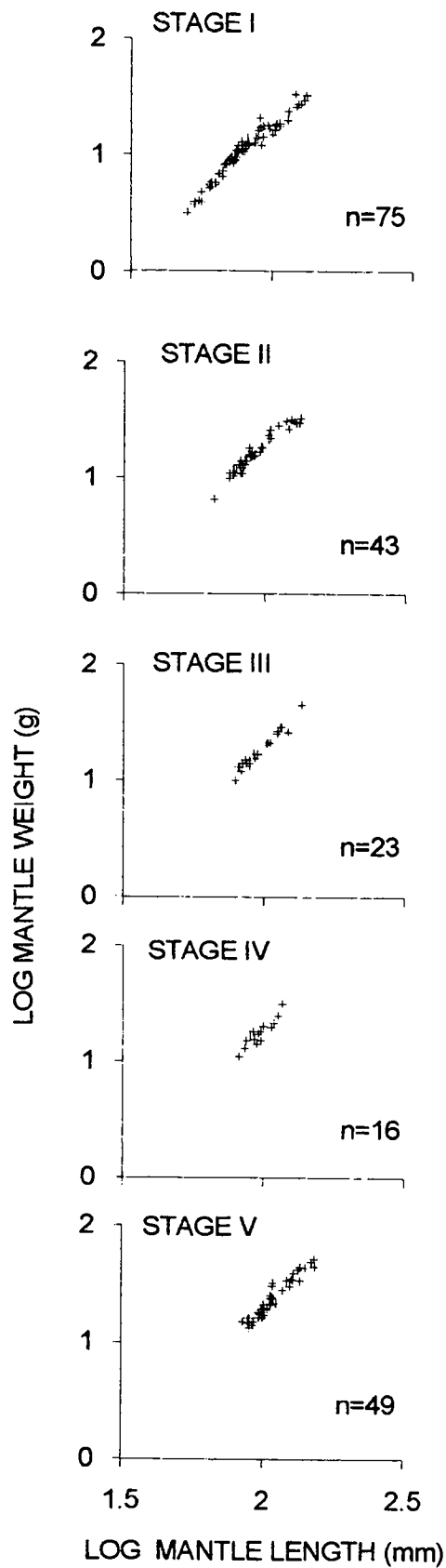


Figure 5.9: The length-weight relationship for females in each of the five reproductive maturity stages. The linear regression equations are provided in Table 5.3.

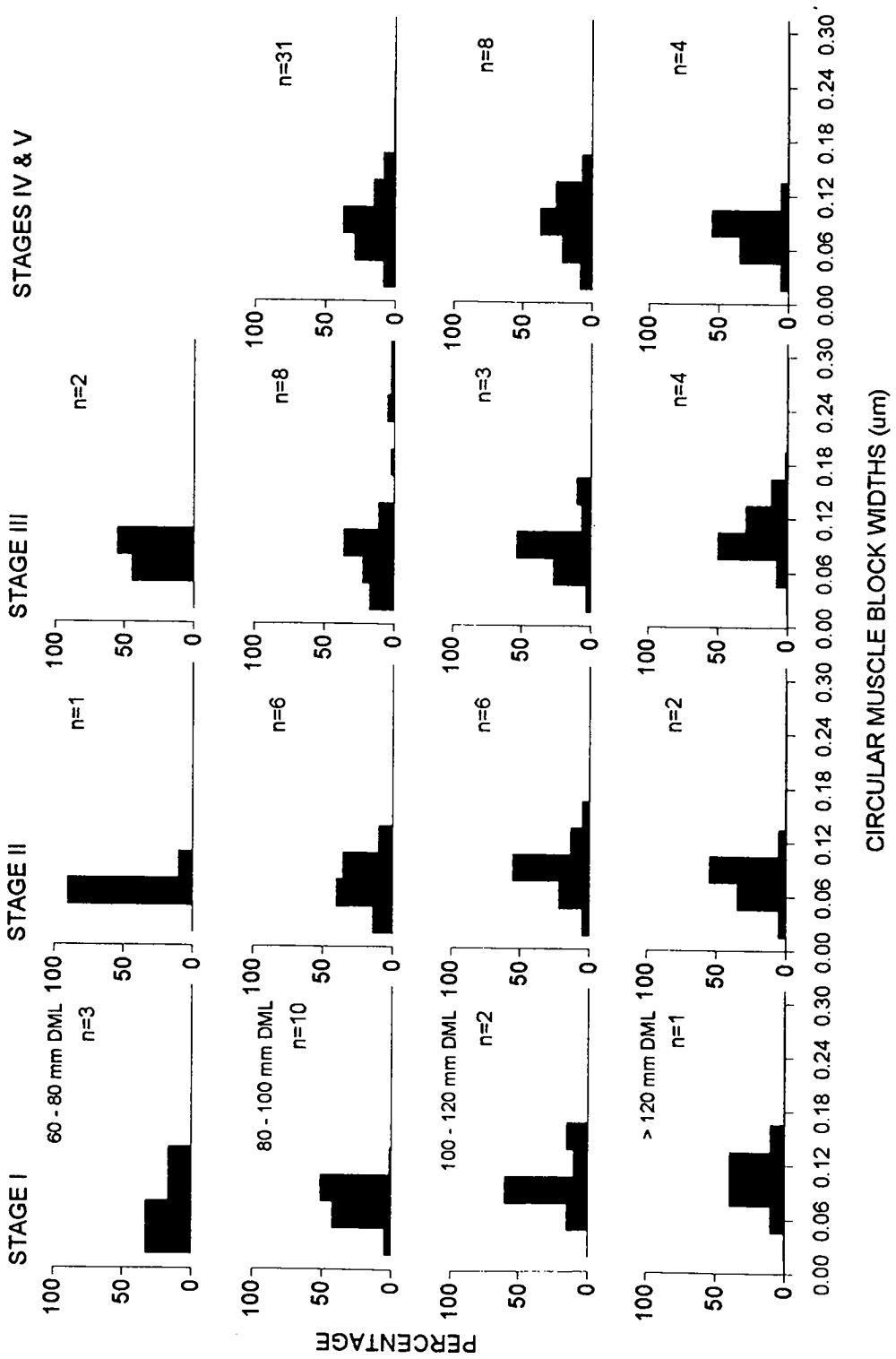


Figure 5.10: Size frequency of mantle muscle blocks in each combination of dorsal mantle length size class and reproductive maturity stage.

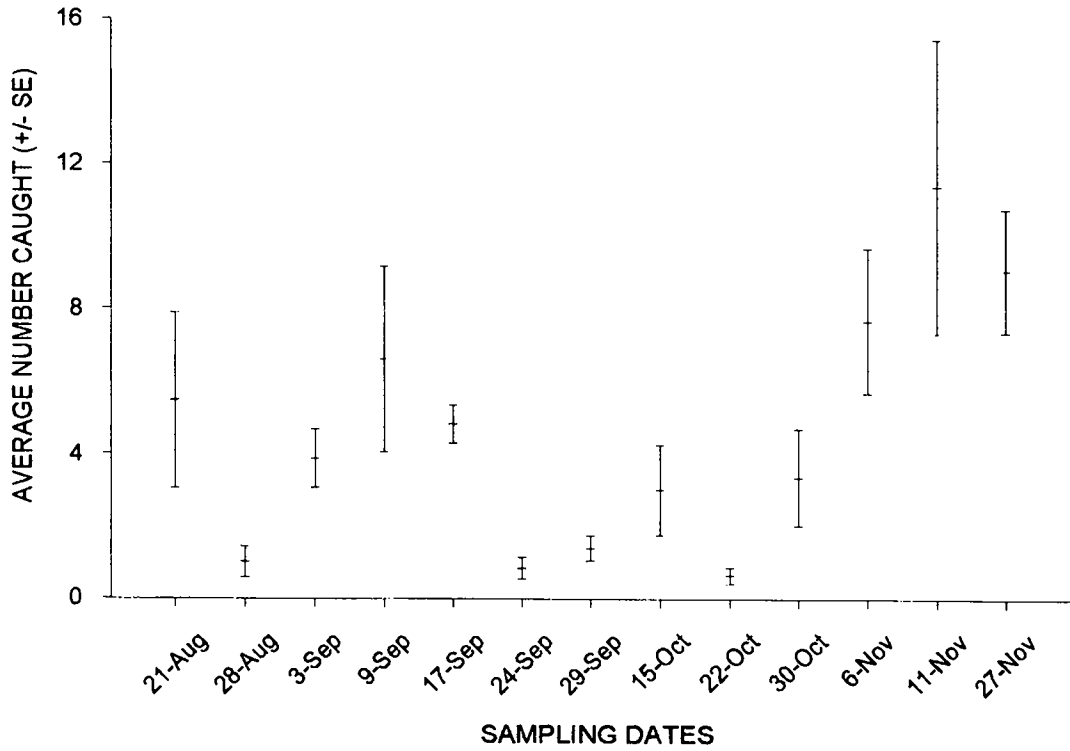


Figure 5.11: Number of squid caught per trawl on each day of sampling.



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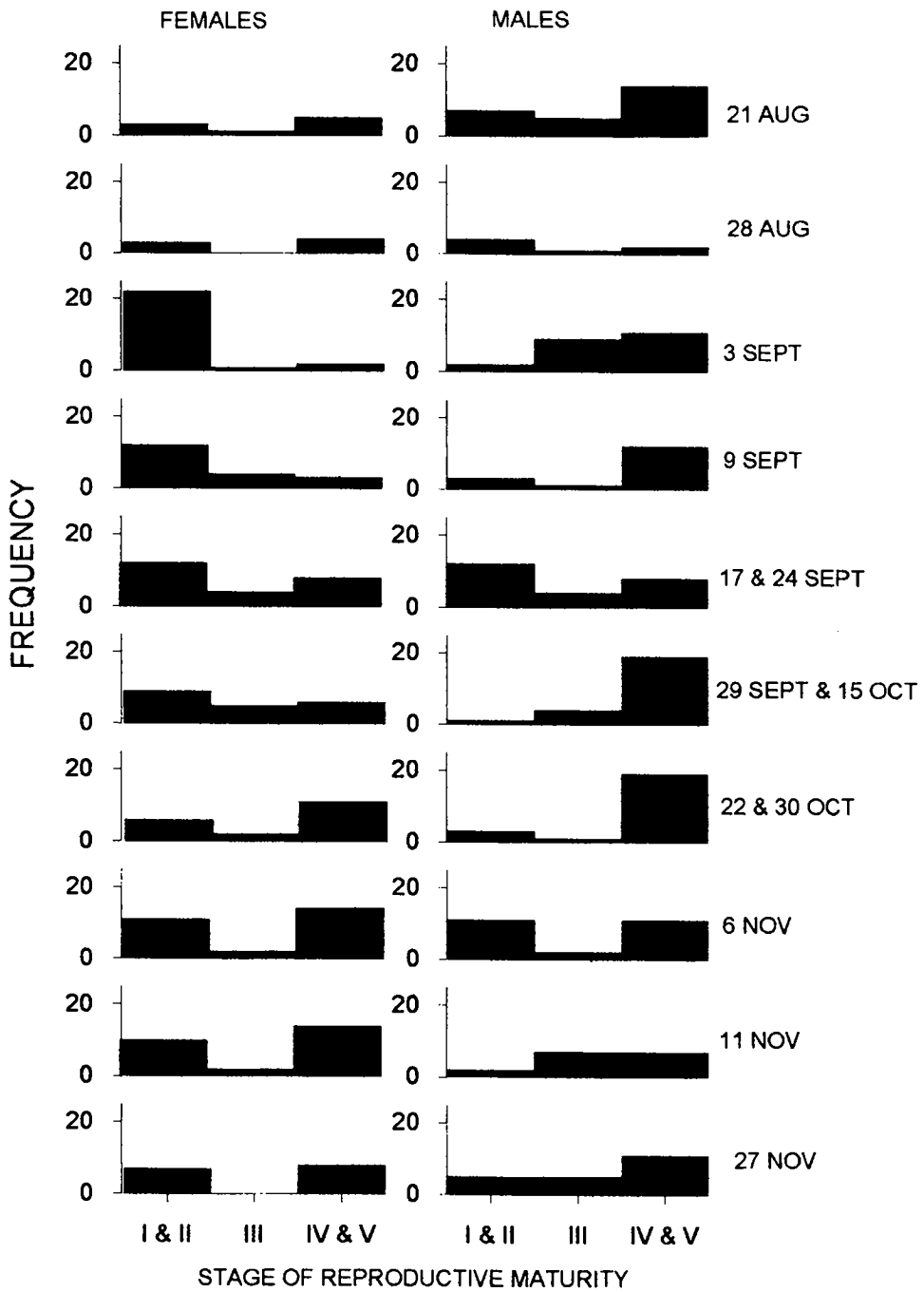


Figure 5.12: Frequency of females and males in three reproductive maturity groups through time. Stages I & II: immature, Stage III: maturing and Stages IV & V: mature. Some of the weeks have been combined to increase the sample size.

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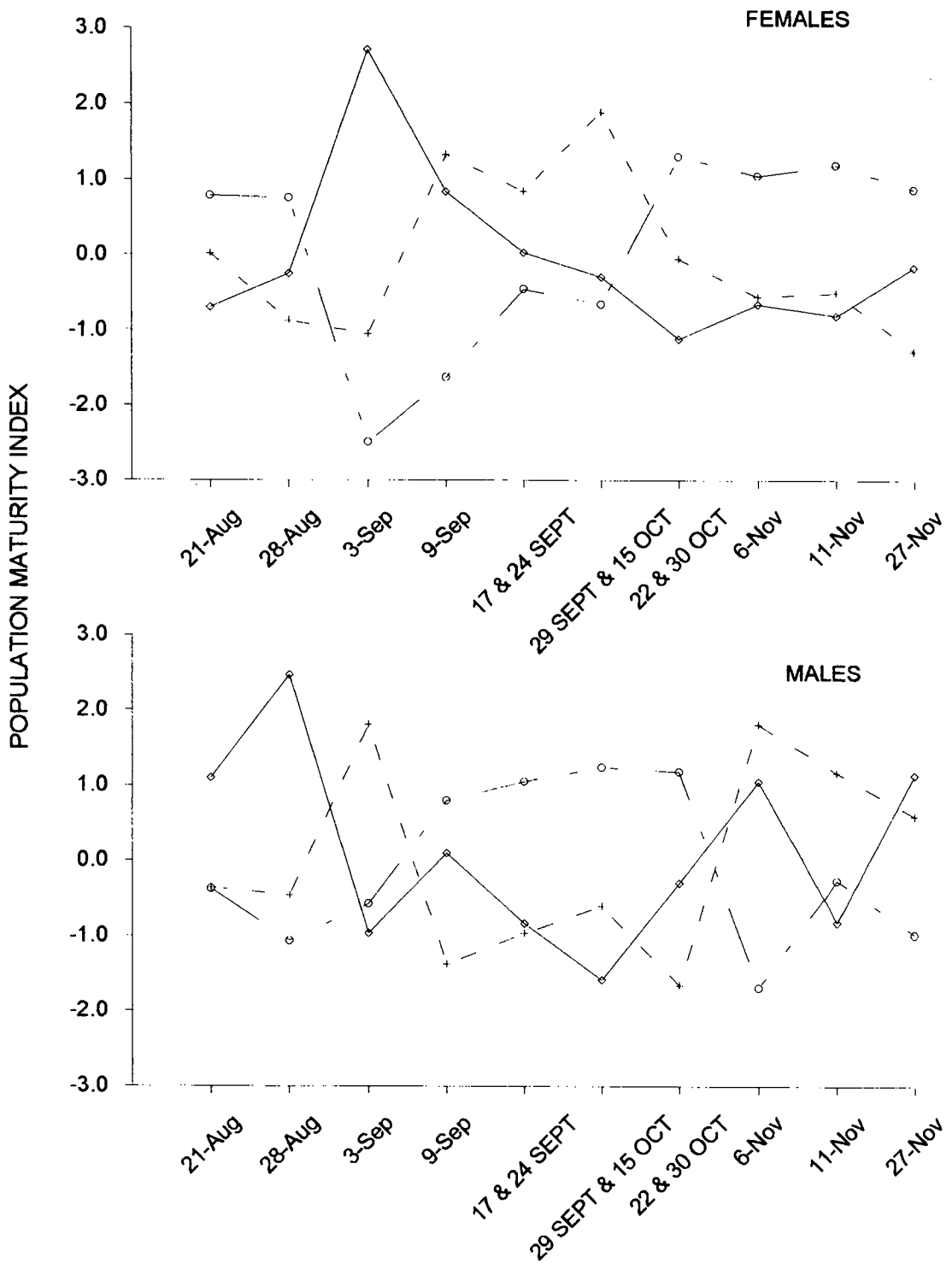


Figure 5.13: The population maturity index provides an indication of whether a maturity stage is present in higher (>0) or lower (<0) frequencies than the expected frequencies. Blue line is immature individuals, green is maturing and red the mature individuals.

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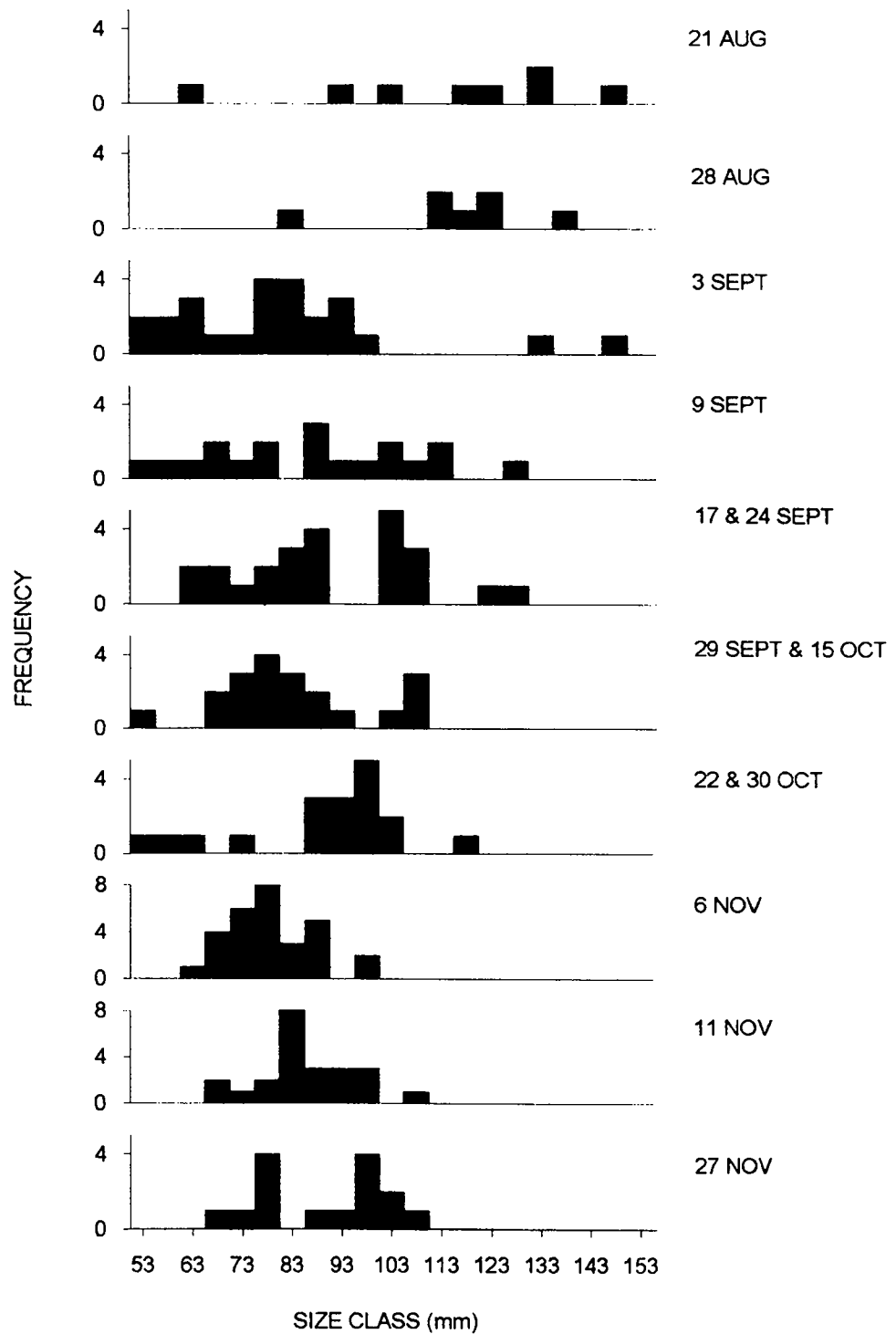


Figure 5.14: Size frequency of females in the population through time. Sizes are dorsal mantle length.

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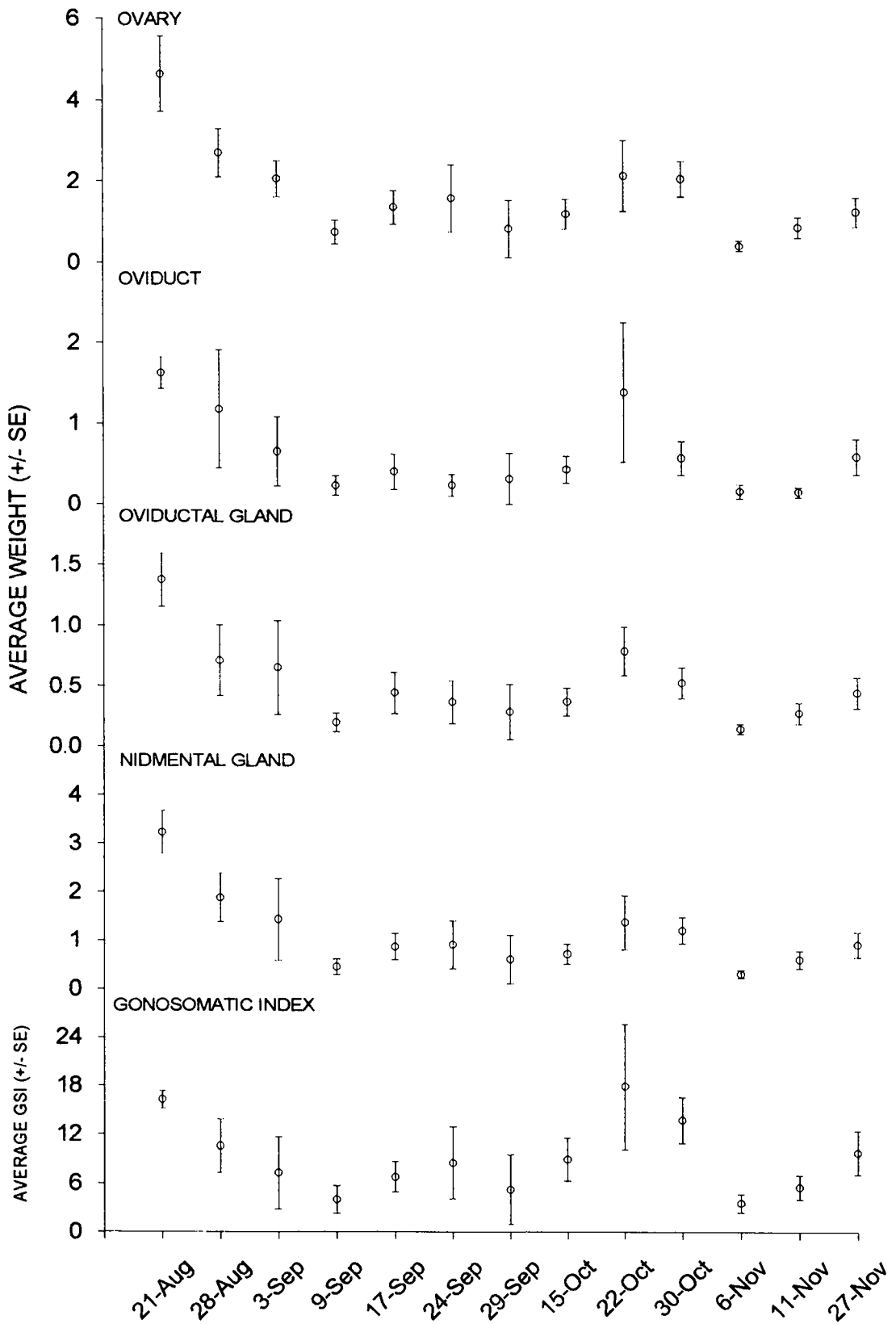


Figure 5.15: The average gonosomatic index and mass of components of the female reproductive system during the thirteen week sampling program.

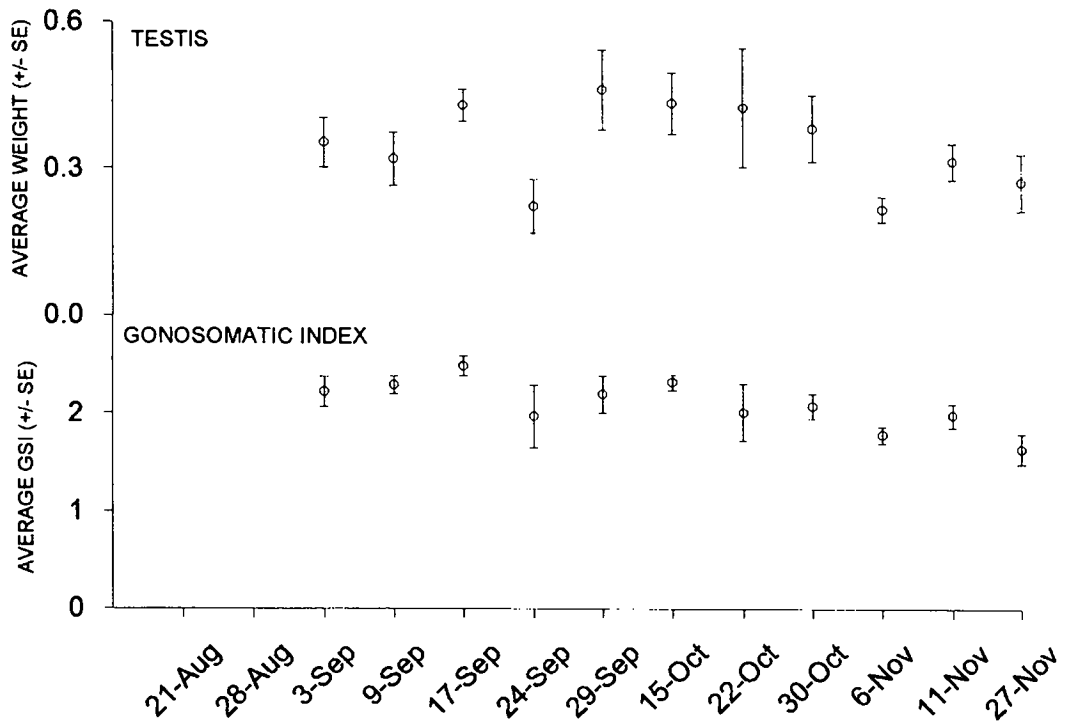


Figure 5.16: The average gonosomatic index for males and average testis weight during the thirteen week sampling program.

## CHAPTER VI:

### GENERAL DISCUSSION

#### 6.1 HOW DO CEPHALOPODS GROW?

The axiom 'live fast, die young' does not only refer to short life spans and rapid somatic growth but also to rapid metabolic rates, fast production of gametic tissue and rapid senescence. Growth estimates suggest squid grow to a large size in a short time. As a result some squid attain growth rates similar to those of fast growing fish (Jarre *et al.* 1991). Squid do not achieve these sizes by growing fast during an extended juvenile phase, but by growing at a constant rate during the entire life time. This is possible because of continual production of new muscle fibres and the loss of an external molluscan shell. The loss of the shell by modern cephalopods may have allowed several major changes in life style and life history characteristics to occur. Without the constraints of a shell, faster growth rates are possible and therefore earlier reproductive maturity and senescence (Heller 1990).

Simplified models of growth, reproduction and senescence have been proposed for cephalopod species that spawn once and then die (O'Dor & Wells 1973, Mangold 1987). These models propose that a one-way switch, possibly hormonal, controls growth and reproduction. The regulation of growth, reproduction and senescence for intermittent spawners will be more complex and may rely on feedbacks among the three processes (Mangold *et al.* 1993). A more likely explanation is a balance in all aspects of growth, including gonad growth, that depends upon the conditions experienced by individual squid. Life history theory predicts that once reproductive maturity begins, animals allocate energy to gametic growth at the cost of somatic growth (Stearns 1989). The partitioning of energy between somatic and gametic growth will cause somatic growth to slow. There was no evidence from an examination of mantle muscle tissue that a trade-off was occurring in *Photololigo* sp. A. Instead continuous production of eggs throughout the adult life span resulted in multiple spawning events and continuous somatic growth. The production of

germinal cells occurred in the ovaries of small squid, but the maturation of the germinal cells did not appear to be age or size related. It is possible that the rate of growth and reproduction was dependent on outside factors, probably food or temperature. Once maturation began the number of eggs produced and the frequency of spawning events may have been controlled by energy available for both somatic growth and reproductive output.

*Photololigo* sp. A juveniles are neustonic and their presence in coastal waters throughout the year indicated that juveniles can tolerate a range of environmental conditions. The juveniles moved deeper in the water column as ontogenetic changes in shape and size enabled greater mobility. Although juvenile *Photololigo* sp. A individuals did not metamorphose, more gradual changes in shape occurred. Changes in shape and size will affect many of the physical properties associated with living in a liquid environment. It is likely that many of the life style characteristics of juvenile squid are a function of the mechanical constraints of being small in high viscosity medium. Limited information about rates of digestion and energy requirements makes currently impossible to assess why juvenile squid grow at a relatively slower rate than the adults

## **6.2 INDIVIDUAL-BASED COMPARED WITH POPULATION-BASED APPROACHES TO GROWTH.**

It is unclear whether squid demonstrate deterministic or indeterministic growth. The major difference between the two growth types is at the point during the life the pattern of growth is determined (Sebens 1987). Growth is determinate when conditions during the juvenile phase only determine the pattern of growth. However, if the organism is able to respond to environmental conditions throughout the life span, then growth is indeterminate. This second condition is more common amongst soft-bodied invertebrates, both marine and freshwater (Sebens 1987). Given the plasticity in growth and the poor correlation between reproductive maturity and age or size it is likely that squid growth is indeterminate. Growth and metabolic rates of squid are temperature dependent (O'Dor 1982, Forsythe 1993). Given the

exponential growth of squid changes in water temperature associated with vertical movement and movement between water masses may change the pattern of growth on very short time scales. Hence growth curves generated from individuals that have experienced different environmental conditions will be an average of the growth history for all individuals. As a result, many growth models are over simplified.

This poses some real problems when describing generalised growth patterns for a population because information derived at one hierarchical level is used to explain processes on another level (Pepin 1993). The recently argued individual-based approach to population biology may provide valuable age/size response curves (Van Winkle *et al.* 1993). There is the potential to derive environmental information about individuals that have experienced different growth histories from the microstructure of statoliths. It may be possible to derive information about the environmental history of individuals from statolith chemistry or crystal structure. Changes in isotopic ratios in fish otoliths have been suggested to be associated with changes in metabolic and growth rates of the individual (Kalish 1991, Gallaher & Kingsford 1992). If information about growth and environmental conditions experienced by each individual can be derived it may be possible to obtain a clearer picture of the mechanics of growth and the feedback mechanisms involved in growth and reproduction.

The possible consequences on the population dynamics as a result of variability in reproductive and growth rates in an annual squid species have been explored (O'Dor & Coelho 1993). The population dynamics of short lived species that spawn throughout the year have not been investigated extensively (Pauly 1985). *Photololigo* sp. A individuals have slower growth rates and possibly lower levels of reproductive activity during the winter (Jackson & Choat 1992, Jackson 1993). It is possible that increased reproductive activity during the summer (Jackson 1993) may be a function of a change in the population structure between summer and winter populations because of seasonally variable growth rates. The model generated by O'Dor and Coelho (1993) suggests that variability in growth rates **within** a cohort may allow extensive movement of some juveniles and result in geographically distinct sub-populations being generated. In contrast, variation in growth rates **between** cohorts



born in different seasons may also result in sub-populations being generated. These transient sub-populations may use different geographic regions throughout the year, depending on the growth rates of the individuals and local hydrodynamics. The location of these populations may be dependent upon growth and movement of juveniles and not migration of the adults.

Descriptions of population processes are often based upon samples taken at one point of time and space with collection techniques that may be biased to catching individuals of a particular age, size or reproductive state. The dangers of approaching biological descriptions from a population approach have been exemplified in Lee's Phenomenon where individuals that are outliers to the population have considerable weight in descriptions. Likewise selecting a different temporal or spatial scale can provide a very different picture of population dynamics. There is the problem of identifying life history characteristics such as age and size at reproductive maturity, fecundity, growth and mortality if these processes are examined at too gross a spatial or temporal level. The seasonal variability in reproductive activity of *Photololigo* sp. A (Jackson 1993) may be unimportant by comparison with the variability detected weekly or even daily. Sampling the population should be on a temporal scale appropriate to biological processes driving the population dynamics. A better picture of rate processes could be obtained by following individuals from birth to death, a feasible option given the short life-span of coleoid squid.

### **6.3 GROWTH AND METABOLISM**

A requirement of growth is to maintain the functional and physiological equivalence of some of the organs. The relationship between body mass and metabolic rate has been established for a number of taxa. However, there is relatively little information on the metabolic consequences and changes during growth of a single species. An increase in size of any structure has a resulting decrease in surface area a phenomenon that has physiological consequences for the animal. Isometric growth of length with mass will only maintain these ratios for a limited period, then major structural changes will have to occur (Sebens 1987). Therefore unless a change

in physiology accompanies growth, changes in shape will need to occur to maintain volume to surface area ratios (Sweet 1980). It becomes important to recognise the nature of these changes regarding energy demands, feeding regimes and oxygen requirements. Clearly allometric growth of body elements relates to maintaining or avoiding physiological constraints associated with growing bigger. Unlike their nautiloid ancestors coleoid squid are not constrained by a hard external surface or like fish are they constrained internally by a skeleton. The exposure of organ surfaces to oxygenated water and small size of muscle fibres means that high metabolic activities can be maintained. The elongate streamline shape of the adults will assist in increasing the surface area of the mantle muscle for oxygen uptake. There is little evidence for oxygen limitation given the elaborate counter current exchange systems on the gill surface, movement of oxygenated water associated with locomotion, and oxygen uptake across the mantle muscle surface (O'Dor *et al.* 1990). Temperature inversely affects levels of soluble oxygen in water and affects oxygen uptake by squid that will in turn modify growth rates. This effect of water temperature on oxygen uptake may be responsible for geographic differences in shape detected among populations of *Loligo opalescens* (Kashiwada & Recksiek 1978). Certainly the small size and short life span of tropical squid may be related to higher metabolic rates and lower oxygen availability in warmer waters. The development of techniques to determine the oxygen requirement of squid (O'Dor *et al.* 1990) needs to be extended to assess how these requirements and efficiencies change during ontogeny. We do not clearly understand the physiological requirements of juvenile squid and how changes in morphometry are related to these requirements are not clearly understood.

Squid are metabolically very efficient especially in their abilities to extract and transport oxygen around the body, fast digestion, and the ability to use protein as an energy source (Packard 1972, O'Dor *et al.* 1990, Mangold 1983, Houlihan *et al.* 1990, Boyle 1990). It appears that senescence as a direct result of reproductive activities via either cessation of feeding or optic gland activity is not a ubiquitous explanation for cephalopods (Guerra 1993). Several other options need investigating. It is possible that death is a product of metabolic demands for oxygen no longer being met by the size and shape of the adult. For *Photololigo* the power relationship with a slope of less than one for surface area against mass suggests that the surface area of

Table 6.1: Summary of life history characteristics of some temperate and tropical loliginid squids.

Species	Life-span	Spawning Mode	Reproductive Season	Growth	Size at Maturity
<i>Loligo forbesi</i> <sup>1</sup>	≈1-2 yrs	unknown	all year	N/A	F 45% M 25%
<i>Photololigo sp. A</i> <sup>2</sup>	≈4 mo	multiple	all year	5%/day	F 71% M 36%
<i>Loligo vulgaris</i> <sup>3</sup>	≈1 yr	unknown	all year	3%/day	F 15%
<i>Sepioteuthis lessoniana</i> <sup>4</sup>	≈6 mo	terminal	spring	1 mm/day	F 42%
<i>Loligo vulgaris reynaudii</i> <sup>5</sup>	≈2-3 yrs	multiple	all year	N/A	F 80% M 72%
<i>Loligo pealei</i> <sup>6</sup>	≈1 yr	terminal	6 mo peaks	10 mm/day	F 48%
<i>Loligo opalescens</i> <sup>7</sup>	≈1-2 yrs	terminal	spring/summer	1.7%/day	F 48%
<i>Loligo gahi</i> <sup>8</sup>	≈1 yr	terminal	two peaks	1%/day	F 59% M 53%

1 Lum-Kong *et al.* 1992; Boyle & Ngoile 1993a & b.

2 Jackson 1990b, 1991, 1993; Jackson & Choat 1992; this study.

3 Worms 1983; Turk *et al.* 1986; Jackson 1991; Natsukari & Komine 1992.

4 Segawa 1987; Jackson 1990b; Jackson 1991; Jackson *et al.* 1993.

5 Augustyn 1990; Saur & Lipinski 1992; Saur *et al.* 1992.

6 Hixon 1980; Vecchione 1981; Summers 1971 & 1983.

7 McGowan 1954; Fields 1965; Hurley 1976; Hanlon *et al.* 1979; Hixon 1983; Spratt 1978.

8 Patterson 1988; Hatfield *et al.* 1990; Hatfield 1991; Hatfield & Rodhouse 1991.

the mantle muscle is unable to maintain a constant relationship with the mass. This becomes important for oxygen uptake especially since oxygen transport across the mantle muscle occurs (O'Dor *et al.* 1990). Alternatively, it is not the uptake of oxygen that is limited but the transport of oxygenated blood around the body (O'Dor 1988b) hence as the body mass gets larger the circulatory system is unable to meet the demands of metabolism.

#### 6.4 REPRODUCTION AND GROWTH

There is indirect evidence that females of *Photololigo* sp. A and *L. vulgaris reynaudii* do not have a terminal spawning strategy. Instead multiple spawning events are likely to occur throughout adult life. Although the sample size is small it appears that multiple spawning does not only occur in tropical species. A comparison at the point at which the oocytes began maturing in relation to body size demonstrated a difference between loliginid intermittent and terminal spawner. Oocyte maturation did not begin until individuals were over 70% of their final body size (Table 6.1). In comparison species that are terminal spawners were closer to 50% of their final body size when reproductive maturation began. Most of the terminal spawning species have the familiar adult logarithmic growth pattern suggesting a cost to somatic growth whilst egg production occurs. This suggests that for extended periods of gametic production and multiple spawning events to occur a certain amount of energy or body mass must be available. Furthermore terminal spawners spend a longer period of their life time allocating energy to somatic and gametic growth. Therefore rates of egg maturation may differ enormously between these two groups of spawning types. It is unclear where energy for gametic production is sourced, a feature that may determine the rate at which egg growth can occur. The digestive gland appears to have limited energy storage function in loliginids (Bidder 1950) but there is evidence that muscle tissue is used as an energy source during reproduction (O'Dor & Wells 1987, Pollero & Iribarne 1988, Jackson & Mledenov 1994). However, this may not be a universal source of energy for gametic production (Hatfield *et al.* 1992, this study).

Reproduction occurs throughout the year in both tropical and temperate systems, either continuously or during discrete periods (Table 6.1). Furthermore, extended spawning by females appears to be a feature common to both tropical and temperate loliginids (*Loligo vulgaris reynaudii* Sauer & Lipinski 1990). It may be misleading to interpret the short life span and year long spawning activities as a product of aseasonality in tropical systems. In the central region of the Great Barrier Reef surface sea temperatures range from 20 - 30 °C (Walker 1981). Although these temperatures are reasonably benign compared with water temperatures found in Southern Ocean waters tropical squid must still be able to cope with a range of water temperatures. Tropical waters do have very distinct summer and winter sea conditions that also affect zooplankton and larval fish numbers (Jenkins *et al.* 1985, Milward & Hartwick 1986). Furthermore, seasonal wind patterns affect hydrodynamics and water movement within the Great Barrier Reef, so the direction of transport and dispersal of juvenile squid may change between summer and winter.

## 6.5 FUTURE DIRECTIONS

Experiments that describe changes in growth rates resulting from changes in temperature and nutrition provide a whole animal approach to growth. This study attempted to understand growth by examining growth dynamics of muscle tissue and organs. Unfortunately this work was limited to describing growth in wild caught individuals for which no history of growth conditions is available. This study provided information about where juvenile *Photololigo* sp. A were found and Jackson (1991) provided information about the adults. This gives some information about environmental conditions likely to be encountered. However detailed temperature and nutritional histories for these individuals are needed. The next step is to rear squid in controlled conditions and examine relative growth of body structures, muscle fibre growth and reproductive output. It is possible that more subtle changes are taking place that are difficult to detect using changes in mass. Therefore, further understanding of the allocation of energy during growth and in particular the mobilisation of resources during reproduction may be provided by biochemical analyses of tissue.

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# **APPENDIX 1**

MANUSCRIPT SUBMITTED TO AUSTRALIAN JOURNAL OF  
MARINE AND FRESHWATER RESEARCH (17/1/94).

**Cross-Shelf Distribution Patterns Of Tropical Juvenile Cephalopod  
Sampled Using Light Attraction.**

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**Abstract**

This study investigated the association of cephalopod genera with location and depth in the waters of the central Great Barrier Reef. Stations along short (40 km) transects were sampled using light-traps at four locations across the continental shelf and slope: coastal Great Barrier Reef Lagoon, inter-reef passages (Magnetic and Palm), near reef environments ranging from mid to outer-shelf locations and the Coral Sea. A total of 13 cephalopod genera was caught from monthly cruises, conducted from October to January of 1990/91 and 1991/92. *Octopus*, the most abundant juvenile cephalopod was present in relatively high numbers at all shelf locations, with a few caught in the Coral Sea. The myopsid squid *Photololigo* was the most abundant squid in the collections but was rarely caught outside the Great Barrier Reef Lagoon. In contrast, the second most abundant squid, the oceanic *Sthenoteuthis* was uniformly distributed among all the habitats. Cephalopod assemblages at both depths in the Great Barrier Reef Lagoon were significantly different from those of the three other areas. This location supported highest abundances of *Octopus*, *Photololigo* and *Abralia*. Assemblages deeper in the water column were dominated by *Octopus*, and *Abralia* was always found near the benthos in the lagoon. In contrast *Euprymna*, the fourth most abundant genus was collected only at the surface. Reef passages and near-reef sites shared similar assemblages, with the squid component dominated by *Sthenoteuthis*. Very low numbers of cephalopods were caught in the Coral Sea using light attraction. High concentrations of cephalopods detected in the middle of the Great Barrier Reef Lagoon are consistent with present knowledge about oceanographic processes over this shelf.

## Introduction

Juvenile cephalopods are a diverse and important component of the nektonic community found in pelagic environments, being both predators and prey within the pelagic food chains and providing important sources of food for commercial fisheries. Despite this importance early life-histories of most cephalopods are poorly described and fundamental information is lacking. Australian waters have a rich diversity of cephalopod species (Lu & Phillips 1985), that extends into tropical waters (Roper & Hochberg 1987). High biological diversity and limited taxonomic base increases the difficulty of describing this fauna and juveniles of these species have received little attention. Historically, sampling has been limited by the effectiveness of towed nets as sampling devices. Juvenile squid are agile and effective swimmers capable of evading towed net designs (Vecchione 1987) and a size range of juveniles can only be obtained by using multiple gear types (Rodhouse *et al.* 1992). Logistically it is difficult to sample more than one location at a time using towed nets and hence synoptic views of spatial distribution usually ignore the temporal component in the data collection. This is no problem when distribution and abundance patterns are static, but juvenile squid distributions are often determined by current systems (eg. *Illex illecebrosus* Dawe & Beck 1985). Furthermore net damage suffered by small soft-bodied specimens hinders identification of specimens (Vecchione 1987). Automated light-traps (Doherty 1987) provide an alternative solution to both of these problems and allow juvenile cephalopods in good condition to be sampled through time at multiple locations (Thorrold 1992).

The aim of this study was (1) to investigate the usefulness of light-traps as tools for sampling a range of juvenile cephalopod genera and (2) to describe the distribution and abundance of the juvenile cephalopod fauna sampled by light attraction. Our sampling was based on regular sampling of a cross-shelf transect from turbid coastal to clear oceanic environment, both close to and far from reefs to include maximum contrast. Here we describe the cross-shelf and vertical patterns to provide the spatio-temporal framework for designing further work into the local dynamics or regional patterns of specific taxa.



## Materials and Methods

Sampling was based on repeated replicate trapping within four major cross-shelf locations near Townsville (Fig. 1):

a) Great Barrier Reef (GBR) Lagoon. This is a 56 km wide stretch of open water dividing the mainland from the nearest coral reefs. It is a shallow (15-40 m) gently sloping soft bottom habitat. A number of factors combine to influence the hydrodynamics of the GBR Lagoon: the East Australian Current in the Coral Sea and the outer half of the continental shelf, wind stress on the shallowing water column near the coast, water depth and fresh-water discharge from rivers (Wolanski 1981, King & Wolanski 1992). When winds oppose the poleward influence of the East Australian Current, a coastal trapped layer is formed and velocity shear occurs across the GBR Lagoon (Wolanski & Ridd 1990). The cross-shelf extent of the coastal boundary layer is controlled by wind stress and is unstable over time.

b) Reef Passages. Two broad relatively deep passages (Magnetic and Palm) dissect the reef matrix in the Townsville region of the GBR. Both provide major conduits for semi-diurnal tidal waves that oscillate perpendicular to the coast (Dight *et al.* 1990a). When the East Australian Current meanders close to the shelf-break, upwelling can occur and cold intrusions can be forced along the bottom of the passages, occasionally extending as far inshore as the coastal boundary in the GBR Lagoon (Andrews & Gentien 1982).

c) Coral Reefs. Four reefs (Keeper, Helix, Faraday and Myrmidon) of similar size, but different cross-shelf locations, were selected to represent shallow near-reef environs. All four reefs are located on the southern side of the Magnetic Passage and should have experienced the same dominant water flow.

d) Coral Sea. Waters beyond the shelf break, where depths exceed 1000m, were sampled to determine which cephalopod taxa are associated with oceanic waters and to monitor exchange between coastal and oceanic habitats.

TROPICAL JUVENILE CEPHALOPOD ASSEMBLAGES

Sampling this range of locations required that each was sampled in a way appropriate to its physical nature. The greatest difference in sampling strategy was that the three open water locations (GBR Lagoon, reef passages and Coral Sea) were sampled by drifting light-traps, whilst waters near the coral reefs were sampled by anchored light-traps. The important difference is that water around anchored traps can be exchanged by local current patterns leading to larger swept volumes per hour of operation compared to the drifting light-traps that should act as lagrangian drifters and fish the same body of water. Table 1 provides a summary of the sampling strategies employed at each location. To determine the vertical distribution of juvenile cephalopods in the water column light-traps were at two depths; at the surface and deep. In 1990/91 the deep light-traps fished at 20 m at all stations. During 1991/92 the deep light-traps were set within three meters of the benthos, except in the open sea where the maximum depth was 100 m.

Table 1. Sampling program in each location.

Location	# of Stations	Traps	1990/91		1991/92	
			Depth(m)	# of traps	Depth(m)	# of traps
Lagoon	5	Drifting	0	2	0	2
			20	2	Near benthos	2
Passage	5 <sup>a</sup> (10 <sup>b</sup> )	Drifting	0	2	0	2
			20	2	Near benthos	2
Reef	4	Anchored	0	2	0	3
			20	1	20	2
Open Sea	5	Drifting	0	2	0	2
			20	2	100	2

<sup>a</sup> 1990/91

<sup>b</sup> 1991/92 (Palm Passage was not sampled in 1990/91).

All sampling was carried out during ten day periods centred on the new moons of October, November, December and January of 1990/91 and 1991/92. Stations in the GBR Lagoon, passages and Coral Sea were sampled a maximum of three nights each period. Water masses near the reefs were sampled for a maximum of nine nights, during each of those months. At the end of the first summer of sampling, it was clear that cephalopod catches in the Coral Sea were very low and that other locations

warranted more sampling effort. Hence sampling effort was reduced offshore but increased elsewhere, notably by adding Palm Passage (in 1991/92 only). In addition to these changes bad weather resulted in occasional abandonment of stations and/or transects, which reduced effort equally in deep and shallow water (Table 2).

Table 2. Total fishing effort (in light-trap hours) in each location and depth, pooled across the two summers.

Location	Deep	Shallow
Lagoon	220	219
Passage	225	226
Reef	311	613
Open Sea	114	113

Replication at each station was provided by simultaneously deploying two light-traps at each depth several hundreds of meters apart. Each night the light-traps were fishing at the first station by 1930 hrs (Eastern Standard Time). Light-traps were retrieved after one hour of fishing and the catch was processed while the ship was moving to the next station on the transect. The last trap was recovered by 0530 hrs, which meant that it was only possible to sample five stations per night in this manner due to the distances between the stations. As stations in each location were sampled sequentially each night, time of night is confounded with station within a location. Likewise only one location could be sampled in a night, hence location is confounded with night. These effects were minimised, to some degree, by haphazardly selecting the location sampled on a night and the direction along the transect was sampled in each location on a particular night. Replication in the near reef waters consisted of shallow and deep light-traps anchored on the southern reef slope to standardise position with respect to water flow. All anchored light-traps at each reef fished for a total of three hours per night (between the hours of 2100 and 2200 hrs, 2400 and 0100 hrs, and 0300 and 0400 hrs) to reduce the confounding effects of tide and time.

Temperature and salinity profiles adjacent to drifting light-traps were obtained using a Seabird conductivity-temperature device during the cruises in October, November and January 1991/92. These data were used to determine the position of

the boundary layer in the Great Barrier Lagoon on the assumption that there would be a temperature differential across the front. To standardise for monthly changes in these parameters, deviations of temperature and salinity for each station were calculated from the pooled average for each month and the deviations averaged over time.

Specimens were fixed and preserved in 100% ethanol and identification of the cephalopods was undertaken in the laboratory. Given taxonomic problems associated with juvenile cephalopods specimens were identified to the genus level. Dr. C.C. Lu (Victoria Museum, Australia) kindly identified sample specimens for a reference collection that was used for all subsequent identifications. Terminology describing the pre-adult phase of cephalopods has recently been defined (Young & Harman 1989) and we have used the term 'juvenile' to describe the stage between hatching and sub-adult.

Multivariate techniques were used to analyse the relationships between the cephalopod genera and locations. These techniques are useful to examine relative abundances of a suite of species. Multivariate analysis of variance (MANOVA) determined the effect of location, depth and their interaction on the density of the cephalopod assemblages. The data were examined for multivariate normality and homogeneity of variances (Multivariate Levene's Test). The data were  $\log_{10}+1$  transformed before analysis. The MANOVA was followed by a canonical discriminant analysis (CDA) to determine which cephalopod genera were associated with different locations and depths.

## Results

A total of 3862 juvenile cephalopods representing 13 genera, including sepioids, myopsids, oegopsids and octopods, were caught using light-traps during the two summers of sampling. The two most abundant genera were *Octopus* and *Photololigo* (Table 3). Most of the genera were very rarely caught, especially *Sepia*, *Pyroteuthis*, *Abraliopsis*, *Argonauta* and *Pterygioteuthis*.

The diversity of juvenile cephalopods was similar in the GBR Lagoon, the passages and the coral reefs. In the Coral Sea, very few juvenile cephalopods were captured and the diversity was very low. *Sthenoteuthis* and *Octopus* were both caught but in very low numbers. Numbers of juvenile cephalopods varied as a function of an interaction between location and depth (Table 4). Highest catches of juveniles were taken in the GBR Lagoon, especially from deep light-traps (Fig. 2). Catches from the reefs and passages were lower than those in the GBR Lagoon, but the relative proportion of cephalopods between depths remained the same with highest catches deeper in the water column (Fig. 2).

Table 4. Analysis of variance table, examining the catch of juvenile cephalopods as a function of depth and location. Data for both summers of sampling have been combined.

Source	df	Sums of Squares	Mean Square Estimates	F-value	Probability
Location	3	51.729	17.243	173.89	0.0001
Depth	1	1.703	1.703	17.17	0.0001
Location*Depth	3	1.237	0.412	4.16	0.0060
Error	2030	201.592	0.0992		

Catches among stations in the GBR Lagoon were significantly different (Table 5). Low numbers of cephalopods were caught on the edges of the GBR Lagoon, with elevated abundances at two stations (24 and 33 km) in the middle (Fig. 3). Clear temperature and salinity gradients are evident across the GBR Lagoon (Fig. 4). Surface water temperature and salinity at the two stations closest to the coast were

consistently higher than the average values for the GBR Lagoon. Further offshore surface water parameters were lower than the average.

Table 5. Analysis of variance table, examining number of juvenile cephalopods caught at the four different stations within the GBR Lagoon. Data for both summers are combined.

Source	df	Sums of Squares	Mean Square Estimates	F-value	Probability
Distance	4	10.713	2.678	12.24	0.0001
Error	434	94.940	0.219		

*Octopus*, the most abundant of all the cephalopod juveniles, was present at all locations, although rare in the Coral Sea. Juvenile *Octopus* were considerably more abundant in deep traps within each location and highest catches were taken in the GBR Lagoon (Table 3). Due to its numerical abundance, this genus greatly influenced the aggregate patterns shown in Figs. 2 and 3. Among the squid, *Photololigo* was also very abundant in the GBR Lagoon, but low numbers were caught around the reefs and in the passages. In contrast to *Octopus*, *Photololigo* was more common in surface waters (Table 3). The other nine genera were caught in relatively low numbers. *Sthenoteuthis*, the second most abundant squid, was ubiquitous throughout the areas sampled and was the only species caught with any consistency in the Coral Sea. The sepioid *Euprymna*, the fourth most abundant genera, was predominantly caught in the surface light-traps in the GBR Lagoon, passages and reefs.

Multivariate analyses were carried out using the most abundant genera: *Octopus*, *Photololigo*, *Sthenoteuthis*, *Euprymna*, *Sepioteuthis* and *Abralia*. It was evident that the distribution of juvenile cephalopods was depth and location specific as indicated by the significant location-depth interaction (Pillai's Trace 0.466 F=24.4786 df 42, 12198 Pr=0.0001). Most of the differences in juvenile cephalopod composition occurred between the GBR Lagoon and the other three locations (Fig. 4a). In particular the GBR Lagoon was clearly discriminated due to high numbers of *Octopus* and *Photololigo*. Without this dominance, the other locations appeared to be very similar (Fig 4a). An examination of the variation described by the second and third

axes clearly showed the differences between the depths (Fig. 4b). In particular, the GBR Lagoon exhibited different assemblages of cephalopods at the two depths. This major difference was largely due to *Abralia* (Table 3). This genus was only caught in relatively high numbers during the 1991/92 summer when light-traps were deployed close to the benthos. *Abralia* and *Octopus* were very dominant in samples caught in the deep traps in the GBR Lagoon, passages and reefs. *Octopus* was also caught more commonly in deep light-traps whereas the sepioid *Euprymna* was caught predominantly in the surface light-traps (Table 3). *Photololigo*, *Sepioteuthis* and *Sthenoteuthis* were more common in surface light-traps, but these genera were also relatively abundant in catches from deep light-traps (Table 3). The depth distribution of *Sepioteuthis* was dependent upon the location; they were present in deep samples at the reefs and surface traps in the GBR Lagoon.

## Discussion

This study demonstrates that submersible light-traps can be useful alternative sampling devices to assess the relative abundance of some cephalopod species. Twelve genera of juvenile cephalopods were caught using active attraction instead of passive collection. Although no independent assessment is available to show that we sampled all the available diversity, clearly a wide range of taxa show responses to light that can be exploited to determine their relative abundance levels. Both *Octopus* and *Photololigo* were caught in high enough numbers to allow the examination of temporal and spatial distributions in more detail (Moltschaniwskyj & Doherty 1994). The live state of all material collected by this method also demonstrates the usefulness of light-traps to provide material for physiological and behavioural investigations not previously possible.

By deploying light-traps in drifting and anchored modes, we were able to sample a wide a range of habitats from coastal to oceanic conditions, near and far from reefs. However, there must be caution when interpreting light-trap catch rates. With little known yet about the sampling efficiency of light-traps, comparisons of catch rates can only provide an index of relative abundance. While this is adequate for many questions about recruitment and juvenile supply, such comparisons depend on unchanging efficiency. This is less of a problem when sampling the same place over time (eg. Milicich *et al.* 1992), but it can become a problem when sampling a wide range of environments as in this study. This assessment has not been tested for cephalopods, but the following arguments suggest that the patterns shown here were not caused by differential catching efficiency of the light-traps.

By including pelagic and near-reef habitats this study deployed light-traps in water conditions ranging from shallow coastal turbid water to deep oceanic transparent water . Therefore biases in light-trap efficiency due to water clarity will result in better performance in clearer water. Thus any species demonstrating rising abundance away from the mainland could provide an ambiguous case. None of the species sampled in this study showed this pattern and highest catches were from lagoonal



stations close to the coast. While these may be biased estimates of true abundance it is likely that the inshore samples have underestimated densities, therefore resulting in a greater difference between inshore and offshore patterns than identified in our study. On this basis we do not believe that variable light-trap efficiency among different water masses contributed to the qualitative patterns of abundance observed.

As different modes of deployment were necessary to sample habitats near and far from reefs caution is needed when comparing catch rates from drifting and anchored traps. Thorrold (1992) showed that drifting traps catch higher numbers of fish in open water than anchored ones implying that current speed past the light-traps affected capture efficiency. If this was true, real abundances may have been underestimated in near-reef habitats when sampling was deliberately spread over three sampling periods each night to include periods of tidal flow and slack. The extent to which this was offset by the longer period of sampling by light-traps each night in the near-reef habitat and the exposure to greater volumes of water is unknown and is unlikely to be simple. However, we have emphasised relative abundance levels of cephalopod genera rather than absolute comparisons. At this level light-traps captured similar cephalopod genera on the reefs and in the adjacent passages and few differences were detected the relative abundance of cephalopod genera.

The greatest differences detected by this study were those related to cross-shelf location and depth. The Coral Sea yielded surprisingly sparse catches of cephalopods with only the oceanic genus *Sthenoteuthis* being caught with any consistency. Apart from this one genus that was ubiquitous to all locations and obviously able to tolerate a wide range of conditions, the Coral Sea appears not to provide suitable nursery conditions for any shelf taxa. This may be due to the oligotrophic status of the East Australian Current that dominates this habitat or the selective disadvantage imposed by rigid southward advection in this strong boundary current. Most genera sampled by this study complete their early life history on the continental shelf where there was no evidence that the coral reef habitat or passages contained any unique assemblages.

Using towed nets, Dunning (1985) was able to obtain reasonable numbers of juvenile ommastrephid squid in deep oceanic water off the east Australian coast, but far to the south. It appeared that the two techniques, towed nets and light-traps were catching different sized individuals. Ommastrephids caught in towed nets ranged in size from 0.8 mm to 4.4 mm (Dunning 1985) compared with 2.4 mm to 59.0 mm caught using the light-traps (unpub. data). This difference in sizes may be due to the abilities of the two techniques to target different ontogenetic stages. Or the two studies were sampling different locations and the larger ommastrephid juveniles are undergoing a shift into shallower water as they grow. Other studies have demonstrated that the light-traps do show size selectivity, capturing larger fish larvae and juveniles than towed plankton nets (Choat *et al.* 1993, Thorrold 1993)

Although the highest diversity occurred in near reef waters, the GBR Lagoon was not that much different and yielded the highest catch rates for the six most abundant genera. High numbers of juvenile cephalopods in a region of the GBR Lagoon 24 to 33 km offshore suggest that juveniles in this area either have higher probabilities of surviving or are aggregating, actively or passively, in this area. High numbers of juvenile cephalopods have also been caught in this area with towed nets (Jackson 1986). There is a frontal system in this region of the GBR Lagoon, produced by the interaction of a coastal boundary water mass and the East Australian current (Wolanski 1981, Wolanski & Ridd 1990). Differences in the surface water temperatures and salinities across the GBR Lagoon indicate that this interaction of the two water masses is occurring midway across the GBR Lagoon. High secondary productivity (Sammarco & Crenshaw 1984, Thorrold & McKinnon 1992) and high densities of juvenile and larval fish (Thorrold in press) suggest this area is important biologically and hydrodynamically. Given that juvenile squid are able to exogenously feed within hours of hatching (Boucher-Rodoni *et al.* 1987), the higher secondary production of the GBR Lagoon would provide suitable feeding grounds for rapidly growing predators. Boundary regions have been identified as areas in which juvenile cephalopods are an important component of the nektonic community (Reid *et al.* 1991, Rodhouse *et al.* 1992). The interactions of cephalopods in this community are

not recognised and these areas may determine growth and survivorship of juvenile squid.

The presence of a juvenile cephalopod assemblage characteristic of specific locations and depths has interesting implications on the dispersal of the juveniles to and away from adult populations. The location and depth occupied by juveniles will modify the extent and rate of dispersal, thereby determining growth rates and recruitment patterns (O'Dor & Coelho 1993). During the summer the longshore current is predominantly southward, however, closer to the coast in shallower water (<40 m) water movement is more restricted than on the outer shelf (Williams *et al.* 1984). Dispersal rates and extent will also be affected by the depth in the water column, closer to the benthos dispersal will be more restricted than at the surface (Williams *et al.* 1984, Dight *et al.* 1990b). Given the complex nature of hydrology interacting with topography it is difficult to speculate on the source of juveniles. Generally the trend is for movement southward and inshore according to modelling of the dispersion of passive particles (Dight *et al.* 1990a). So it is likely that adult populations to the north and offshore may be responsible for the juvenile cephalopods caught in the GBR Lagoon. Since water movement is restricted across the shelf the dispersal of juvenile cephalopods across the shelf will be limited (Williams *et al.* 1984, Dight *et al.* 1990). Therefore, the observed cross-shelf patterns of abundance of different genera, may be a function of species specific spawning areas across the shelf. Such distinct cross-shelf patterns of species have been described for larval flathead (Andrews 1982) and juvenile *Photololigo* (Moltschaniwskyj and Doherty 1994).

This is the first study that has used automated light-traps for a quantitative examination of juvenile cephalopod assemblages. It indicates that cephalopods may be an important component of a nektonic community that has been described in the GBR Lagoon (Sammarco & Crenshaw 1984). The use of light-traps to describe spatial and temporal abundance of pelagic organisms is still relatively new (Doherty 1987). Ecological investigations of the juvenile phase of both pelagic and benthic cephalopods require the capture of juveniles over a variety of locations and in different water conditions. Catches of cephalopods were often very low making

generalisations about spatial patterns difficult to make. However, a sampling program concentrating on regions of importance, such as the GBR Lagoon, is now possible in the future. Light-traps are a successful and useful technique to capture juvenile cephalopods that have eluded other methods used in this region (Jackson 1986). Furthermore identification of areas where high densities of zooplankton, teleosts and cephalopods occur provides an exciting opportunity to investigate community interactions involving juvenile cephalopods, particularly from the perspective of squid-predator-prey interactions.

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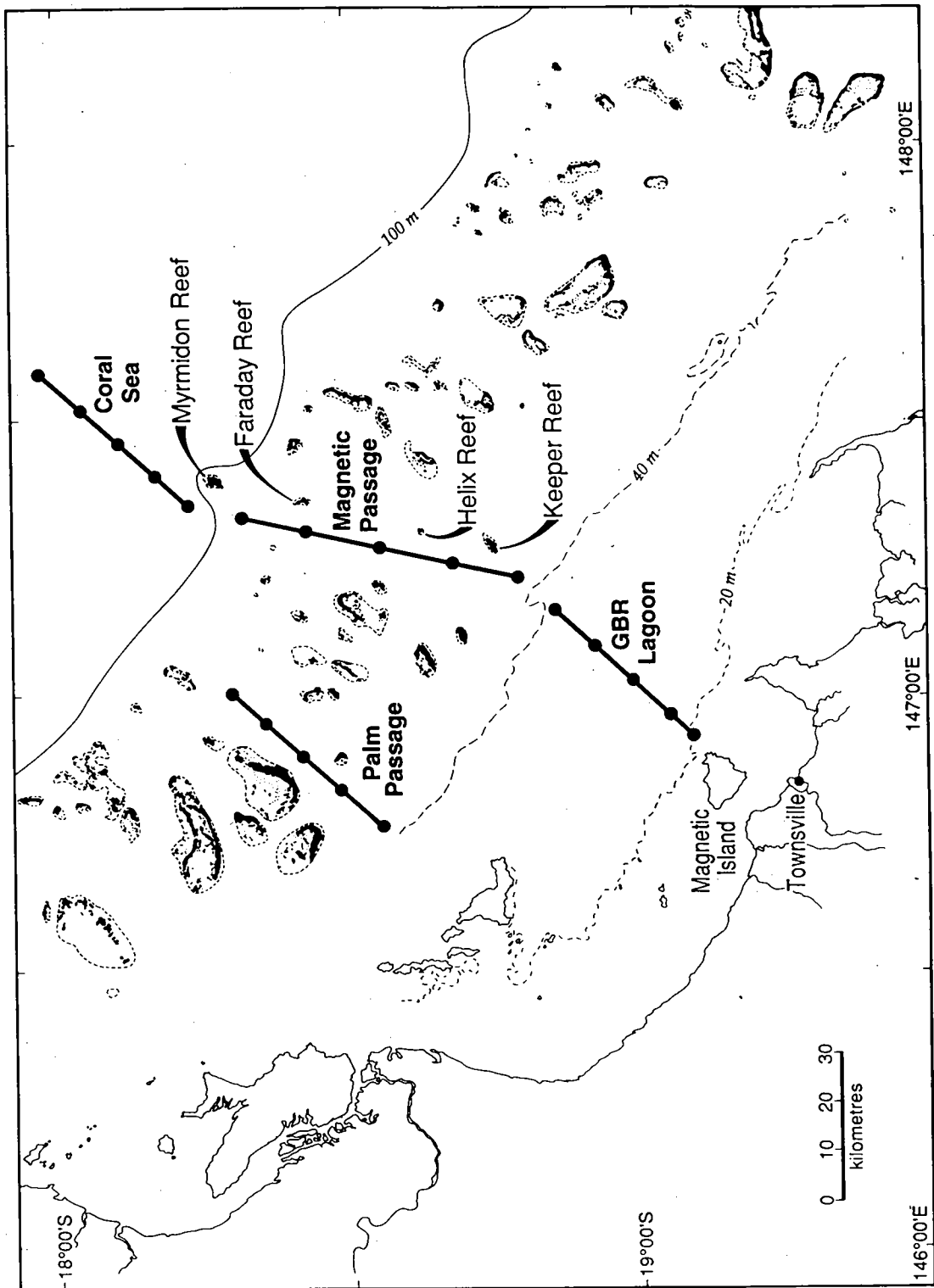


Figure 1. A map of the continental shelf and slope of the coast of Townsville, Queensland, Australia. The locations and stations sampled are indicated.



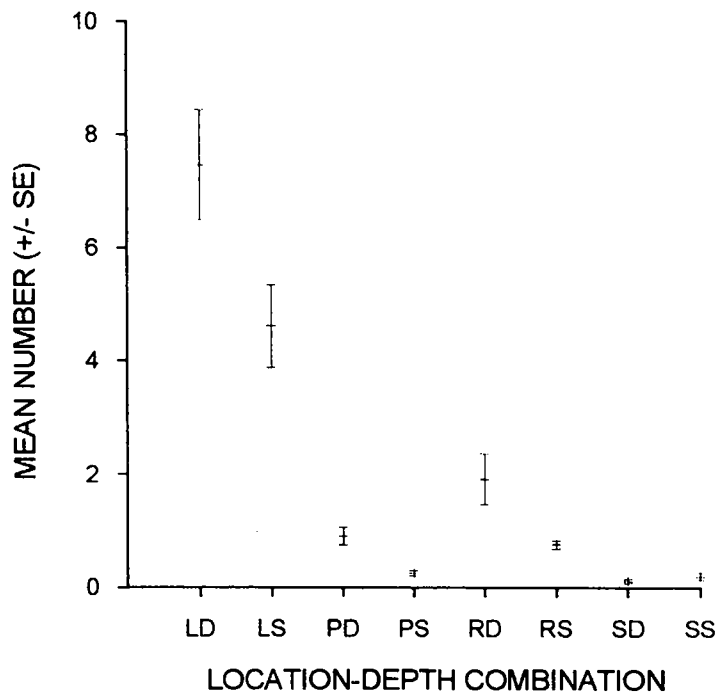


Figure 2. A comparison of the average number of cephalopods captured per hour of light-trapping between the four locations sampled in the GBR. Values are the average per light-trap hour  $\pm$  standard error. LS - GBR Lagoon shallow, LD - GBR Lagoon deep, PS - passage shallow, PD - passage deep, RS - reef shallow, RD - reef deep, SS - open sea shallow, SD - open sea deep.

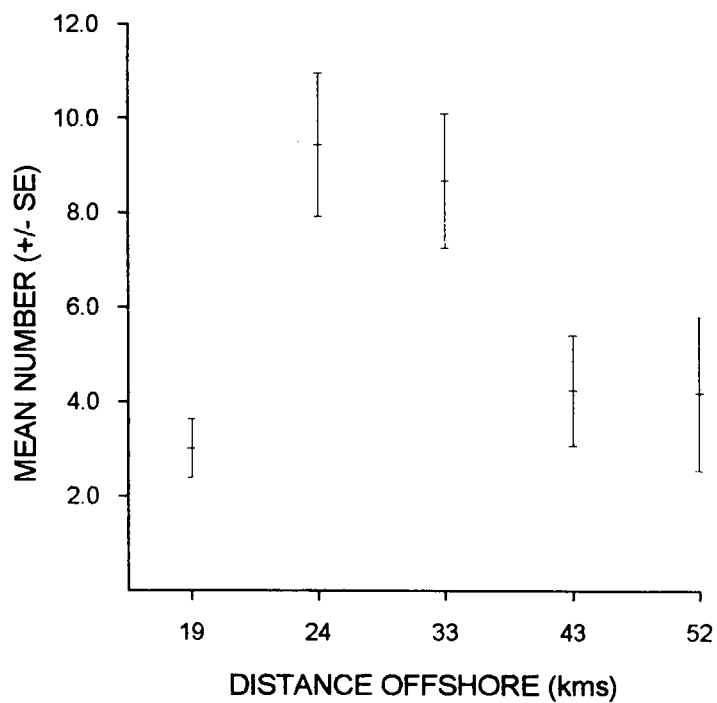


Figure 3. The distribution of cephalopods sampled using light-traps across the GBR Lagoon.

# TROPICAL JUVENILE CEPHALOPOD ASSEMBLAGES

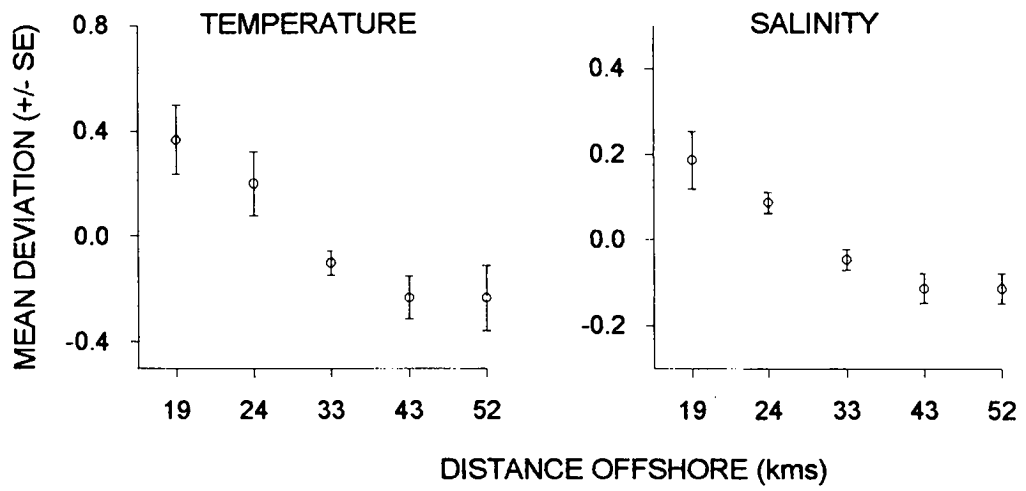


Figure 4. For each station in the GBR Lagoon the average deviation from the mean temperature and salinity for each month has been calculated. Standard errors for the average deviation over three months are shown.

TROPICAL JUVENILE CEPHALOPOD ASSEMBLAGES

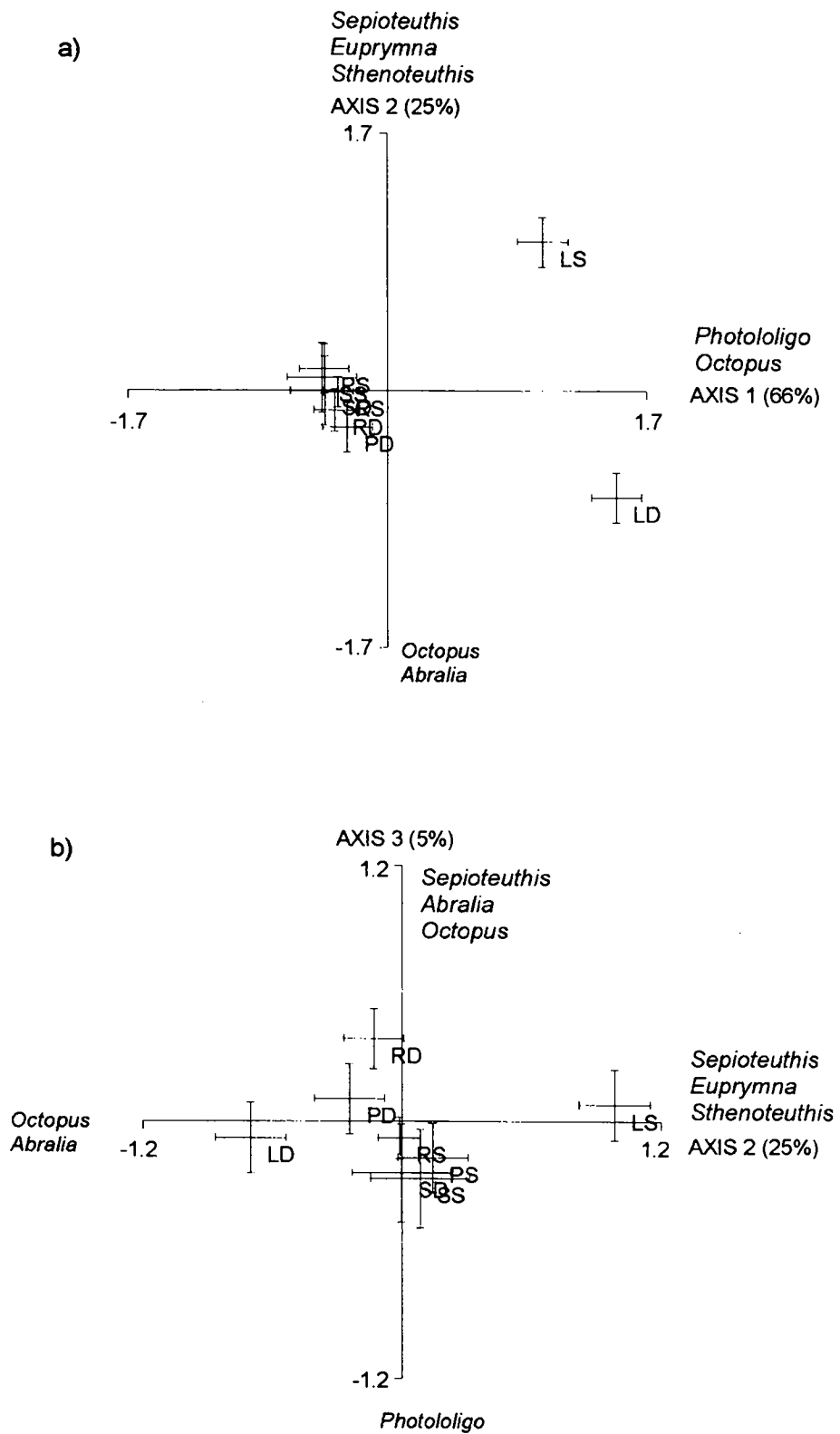


Figure 5. Canonical discriminant analysis results, showing the relationship of each area-depth combination on the first two discriminant axes. Values plotted are means and standard errors of canonical scores for each location-depth combination. Location symbols as in Figure 2.

Table 3. The number of cephalopods caught per light-trap hour at each depth location combination. Data have been combined across years. Catch less than 0.01 individuals per light-trap hour are indicated by \*. - indicates that no cephalopods were present.

GENERA	TOTAL NUMBER	LAGOON		REEF		PASSAGES		SEA	
		SHALLOW	DEEP	SHALLOW	DEEP	SHALLOW	DEEP	SHALLOW	DEEP
<i>Octopus</i>	2066	0.57	4.57	0.51	1.49	0.05	0.64	0.02	0.07
<i>Sepiola</i>	27	0.01	0.01	*	0.05	*	0.02	-	-
<i>Euprymna</i>	117	0.18	*	0.09	0.03	0.04	-	0.03	-
<i>Photololigo</i>	1314	3.16	2.69	*	0.01	-	0.10	-	-
<i>Sepioteuthis</i>	83	0.18	-	0.03	0.07	0.02	-	-	-
<i>Abralia</i>	57	-	0.12	*	0.07	*	0.02	-	-
<i>Abraliopsis</i>	2	-	-	-	0.01	-	-	-	-
<i>Pyroteuthis</i>	1	-	-	-	-	-	-	*	-
<i>Pterygioteuthis</i>	1	-	*	-	-	-	-	-	-
<i>Sthenoteuthis</i>	182	0.17	0.02	0.09	0.11	0.10	0.05	0.10	0.02
<i>Onkyia</i>	6	*	-	*	*	-	-	*	-
<i>Sepia</i>	1	-	-	*	-	-	-	-	-
<i>Argonauta</i>	5	-	-	*	-	-	-	-	-
TOTAL	3862	936	1630	458	573	50	187	18	10

## **APPENDIX 2**

### **PUBLISHED PAPERS PRODUCED FROM RESEARCH IN THIS THESIS**

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# Muscle Tissue Growth and Muscle Fibre Dynamics in the Tropical Loliginid Squid *Photololigo* sp. (Cephalopoda: Loliginidae)

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Moltschaniwskyj, N.A. 1994. Muscle tissue growth and muscle fibre dynamics in the tropical loliginid squid *Photololigo* sp. (Cephalopoda: Loliginidae). *Can. J. Fish. Aquat. Sci.* 51: 000-000

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**Abstract.**—This study quantified the temporal and spatial abundance of juveniles of two *Photololigo* species on the continental shelf off Townsville, Australia with the use of light-traps. The two *Photololigo* species (A and B) showed very distinct and separate spatial distribution patterns. *Photololigo* sp. A was found close to the coast and was the smaller and more abundant of the two species. This species was most abundant in surface waters, although larger individuals were generally caught deeper. There was no evidence of vertical movements during the night. The presence of small and large juvenile *Photololigo* sp. A during summer and winter months suggests spawning and recruitment occur throughout the year. In contrast, *Photololigo* sp. B was caught predominantly offshore. All sizes of *Photololigo* sp. B were caught both near the benthos and at the surface in the mid-lagoon, but farther offshore juveniles were deeper and larger. The presence of small juvenile squid of both species throughout the summer suggests that these species spawn for an extended period during the summer. This study demonstrates that light-traps are an effective way of sampling small cephalopods.

## Distribution and abundance of two juvenile tropical *Photololigo* species (Cephalopoda: Loliginidae) in the central Great Barrier Reef Lagoon

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The current poor state of knowledge about processes important in squid population dynamics is mainly due to limited information about the juvenile phase (Voss, 1983; Boyle, 1990). Life-history characteristics have largely been derived from information about the adult phase. Our limited information about young squid is demonstrated in attempting to define the life-history phases (Young and Harman, 1988). Jackson and Choat (1992) suggest, given the comparatively short life time of tropical squid (<250 days), that a proportionally long period of the life cycle is spent as small individuals. In the case of *Loligo chinensis*, with a summer life time of 120 days, individuals less than 60 days old (<50 mm mantle length) have not been studied. Hence, for almost half the life history of most squid there is not even the most basic information. Temporal and spatial abundance patterns of juvenile squid will provide a basis for understanding the processes of mortality, growth, and recruitment. However, such information has traditionally been difficult to obtain because of problems in capturing and identifying a sufficient size range of juvenile cephalopods (Vecchione, 1987).

To examine the ecology of juvenile squid it is necessary to use techniques that catch a size range of individuals, hatchlings to juveniles, in good condition. Pelagic squid produce either benthic or pelagic eggs and have a planktonic juvenile phase (Boletzky, 1977). Juvenile squid are alert, mobile organisms that easily avoid capture by towed nets (Vecchione, 1987). The use of a combination of different towed nets to sample an area enables the collection of a wider size range of juvenile squid (Rodhouse et al., 1992). However, it is difficult to obtain replicates needed to provide density estimates from towed nets. In this study we have employed an alternative technique based on light-attraction that is effective in sampling pelagic juvenile fishes. Automated light-traps (Doherty, 1987) can overcome the problems of net avoidance and enable sampling at discrete depths in the water column. The ability to sample concurrently within an area ensures that estimates of variability in abundance are not confounded by time. This technique also collects live material in good condition, which can facilitate taxonomic identification. However, sampling an unknown volume of

water by individual traps requires cautious interpretation of abundance estimates (Choat et al., 1993).

There are four species of loliginid squid currently recognized in the Townsville region: *Sepioteuthis lessoniana*, *Loliolus noctiluca*, *Photololigo* sp. B, and *Photololigo* sp. A.<sup>1</sup> There are currently no morphological descriptions of the two *Photololigo* species, but they can be readily identified using allozyme electrophoretic techniques (Yeatman and Benzie, in press). Previously both of these species have been referred to as *Photololigo* (*Loligo*) *chinensis* (Jackson and Choat, 1992; Yeatman and Benzie, in press), but neither correspond to *P. chinensis* from Thailand.<sup>2</sup> Electrophoretic analysis of a subset of juveniles collected during three months of the program found that all *Photololigo* sp. A were found less than 33 km offshore and 90% of the *Photololigo* sp. B were found 33 km or more offshore.<sup>2</sup> Because these species are morphologically identical as juveniles, we assumed that all individuals found at stations less than 33 km offshore were *Photololigo* sp. A and that *Photololigo* collected more than 33 km offshore were *Photololigo* sp. B. *Photololigo* sp. A (previously known as *Loligo chinensis*) has been the topic of recent growth studies using statolith aging techniques (Jackson and Choat, 1992). This species is a small short-lived neritic squid. Individuals are approximately 60 days old when they appear in the adult population and they can grow to 180 mm in 120 days. Little is known about the early life-history and juvenile distribution patterns of either *Photololigo* species. The objectives of this study were to describe the spatial and temporal distribution patterns of juvenile *Photololigo* species across the continental shelf in the Townsville region of the Great Barrier Reef.

## Materials and methods

### Sampling design

Two major habitat types are found on the continental shelf, off Townsville, Australia. The inshore habitat is a 56 km wide soft bottom coastal lagoon ranging in depth from 15 m to 40 m. The offshore habitat is a complex reef matrix of similar extent, dissected by channels ranging from 40 m to 75 m deep at the shelf break. To assess the cross-shelf distribution of juvenile squid, four automated light-traps (Doherty, 1987) were deployed at fifteen sampling stations spanning the continental shelf and the

western Coral Sea (Fig. 1). Abundance along this transect was assessed over four months, October to January, during two austral summers, 1990/91 and 1991/92. At each station, the abundance of juvenile squid was determined at two depths by deploying two pairs of light-traps. In each pair, one light-trap was suspended immediately below the surface while the other light-trap was set deeper. In 1990/91, all deep light-traps were suspended 20 m below the surface. In 1991/92, the deep light-traps were suspended within 5 m of the bottom to a maximum of 100 m in the Coral Sea.

In all deployments, the two pairs of light-traps were released approximately 300 m apart and allowed to drift for one hour. Allowing the traps to drift in the water minimized potential problems with differential water movement among stations. The use of drifting light-traps has been shown to be a more effective way of catching pelagic organisms than anchored light-traps in open water (Thorrold, 1992). After one hour, the four light-traps were retrieved and the entire catch was fixed and preserved in 100% ethanol. Each evening the first light-trap was deployed after 1930 hours (Eastern Standard Time) and the last light-trap retrieved before 0430 hours. Travel time between each station allowed only five cross-shelf stations to be sampled per night. Thus, each night's activity concentrated on one of the two continental shelf habitats or the Coral Sea. Each monthly cruise consisted of nine nights during which time each of the 15 stations was sampled three times. However, sea conditions were not always favorable. Sampling effort at each station is shown in Table 1.

It was not logistically possible to sample all stations in each habitat simultaneously. Therefore, time of night is confounded with station position. Haphazard selection of the first station sampled each night ensured that no station was consistently sampled at the same time on all nights. Cruises were scheduled to include the new moon because this is the lunar phase when light attraction has proved most effective for fishes and various invertebrates (Milicich, 1992). Temperature and salinity profiles of the water column were collected at each station by using a Seabird Conductivity Temperature Device during the 1991/92 summer.

Concurrent with the summer cross-shelf sampling, light-traps were anchored within 100 m of the south easterly side (weather-side) of four reefs; Keeper, Helix, Faraday, and Myrmidon, to sample near-reef water (Fig. 1). The use of drifting light-traps near the reefs was not possible. During the summer of 1990/91, four light-traps were anchored at each reef; three immediately below the surface

<sup>1</sup> C. C. Lu, Museum of Victoria, Australia, pers. commun.

<sup>2</sup> J. Yeatman, James Cook Univ., Australia, unpubl. data.

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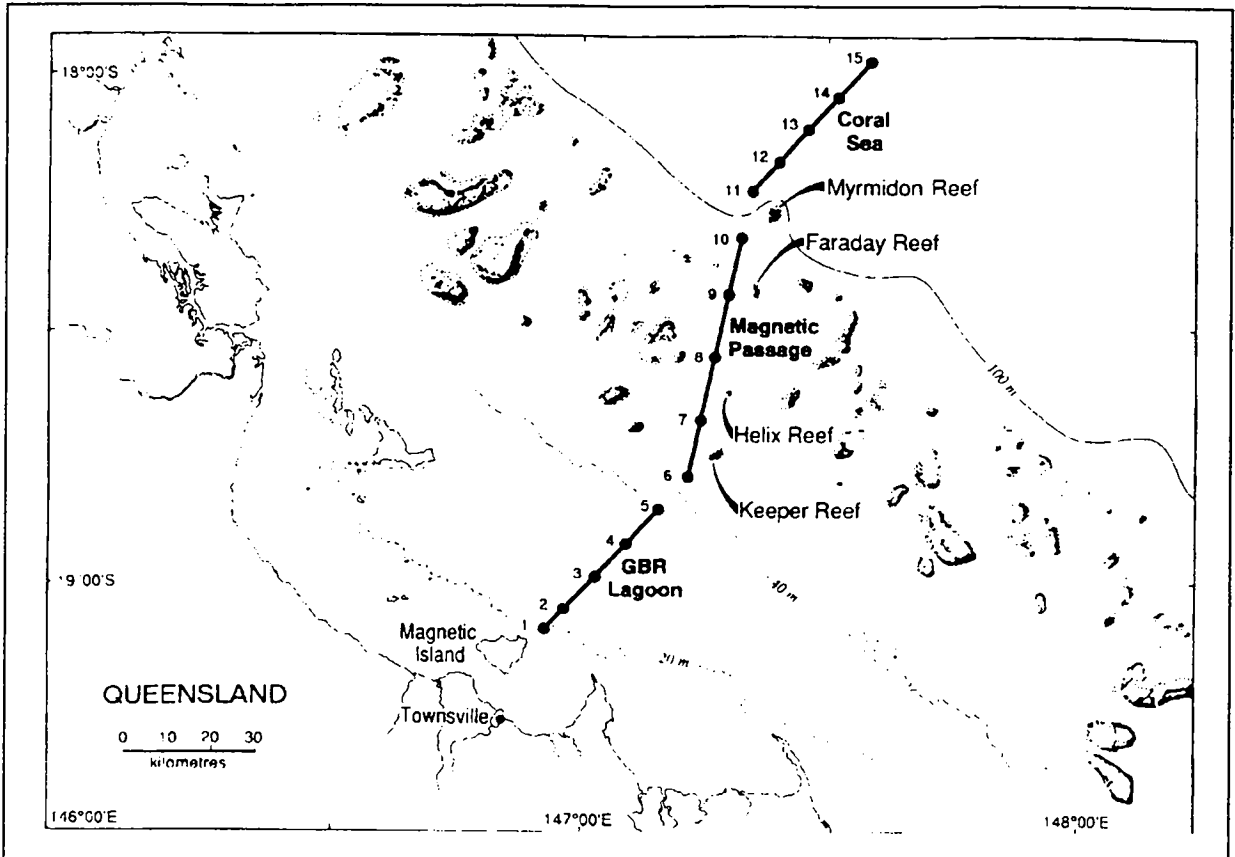


Figure 1

Map of the cross-shelf transect off Townsville, Australia, showing the position of each station along the transect. Station 1 = 19 km from Townsville, 2 = 24 km, 3 = 33 km, 4 = 43 km, 5 = 52 km, 6 = 61 km, 7 = 75 km, 8 = 92 km, 9 = 100 km, 10 = 115 km, 11 = ~~145~~ <sup>136</sup> km, 12 = ~~152~~ <sup>145</sup> km, 13 = ~~163~~ <sup>152</sup> km, and 14 = ~~172~~ <sup>163</sup> km, and 15 = 172

and one at 20 m below the surface. In 1991/92, an extra light-trap was added at 20 m. The anchored light-traps had an automatic timer, enabling the lights to be switched on and off automatically at predetermined periods during the night. Each light-trap on the reef fished for a total of three hours per night; lights came on for one hour at 2200 hours, 2400, and 0300 hours. Light-traps at all reefs were emptied the following day.

Squid were identified in the laboratory and the dorsal mantle length recorded for each individual. Individuals were measured within 14 days of preservation in 100% ethanol. A comparison of measurements of individuals (ranging in size from 5.3 mm to 29.5 mm) before and 14 days after preservation found that shrinkage was on average 0.5 mm.

Abundance patterns of the two *Photololigo* species during the two summers of sampling were examined by using 'planned comparisons,' where specific pregenerated hypotheses were examined (Day and

Quinn, 1989). For each species we were interested in differences in abundance between years, locations, and depths.

To examine seasonality of juvenile *Photololigo* sp. A, the inshore station (19 km) was sampled during the austral winter months of May, June, July, and August 1991. Three sites at this station were sampled with four shallow and four deep (13/1m) light-traps. Sites were sampled during the period of the new moon, on five nights in May and three nights in June, July, and August. Densities in summer and winter months were compared by using an unbalanced one-way analysis of variance (ANOVA), with month as the factor analyzed. Values in each light-trap for nights and sites within a month were treated as replicates.

To determine whether vertical migration might influence horizontal distribution patterns we examined the size structure of *Photololigo* sp. A at two depths during the night. On at least one occasion

Table 1

Total sampling effort for *Photololigo* sp. in each month in light-trap hours (and number of nights sampled) at each station during the two summers of sampling.

Year and month	Distance (km) from Townsville															TOTAL
	19	24	33	43	52	61	75	92	100	115	136	145	152	163	172	
<b>1990</b>																
OCT	8(2)	15(4)	16(4)	12(3)	16(4)	15(4)	4(1)	4(1)	4(1)	4(1)	12(3)	10(3)	10(3)	10(3)	10(3)	150(40)
NOV	12(3)	12(3)	12(3)	0(0)	16(3)	12(3)	12(3)	12(3)	12(3)	12(3)	8(2)	8(2)	8(2)	4(1)	4(1)	144(35)
DEC	8(2)	8(2)	4(1)	4(1)	4(1)	4(1)	4(1)	3(1)	4(1)	4(1)	4(1)	4(1)	4(1)	4(1)	4(1)	67(17)
<b>1991</b>																
JAN	12(3)	12(3)	12(2)	12(2)	12(2)	8(2)	8(2)	8(2)	8(2)	8(2)	8(2)	8(2)	4(2)	8(2)	8(2)	136(32)
OCT	12(3)	12(3)	12(3)	12(3)	12(3)	12(3)	8(2)	8(2)	8(2)	8(2)	4(1)	4(1)	4(1)	4(1)	4(1)	124(31)
NOV	12(3)	12(3)	11(3)	12(3)	12(3)	12(3)	12(3)	12(3)	12(3)	12(3)	4(1)	4(1)	4(1)	4(1)	4(1)	139(35)
DEC	12(3)	10(3)	11(3)	12(3)	12(3)	12(3)	8(2)	8(2)	8(2)	8(2)	4(1)	4(1)	4(1)	4(1)	4(1)	121(31)
<b>1992</b>																
JAN	12(3)	12(3)	12(3)	11(3)	12(3)	12(3)	8(2)	8(2)	8(2)	8(2)	4(1)	4(1)	4(1)	4(1)	4(1)	123(31)
TOTAL	88(22)	93(24)	90(22)	75(18)	96(22)	87(22)	64(16)	63(16)	64(16)	64(16)	48(12)	46(12)	42(12)	42(11)	42(11)	1004(254)

99 in each month of the 1991/92 sampling period the 19/ and 24/km stations were sampled both early and late in the night. The samples were separated into early (captured before 2400 hrs) and late (captured after 2400 hrs). By combining data from stations, across nights and months, it was possible to compare the size distributions between depths and time of night. A multiway-frequency analysis was used to determine the effect of time of night and depth on the size-frequency distribution.

## Results

### Distribution patterns

Juvenile *Photololigo* individuals were predominantly caught within 52 km of the mainland (Fig. 2). The few individuals found farther offshore were in the Magnetic Passage (five individuals) and on the reefs (six individuals). *Photololigo* species were not found in the Coral Sea. *Photololigo* sp. A was numerically the most abundant of the two species during both summers (Fig. 2); 856 individuals were caught in 181 hours of light-trapping (4.73 individuals caught per hour), compared with 379 *Photololigo* sp. B caught in 348 hours of light-trapping (1.09 individuals per hour). Catch per hour of light-trapping was greatest for *Photololigo* sp. A, especially at the 24/km station. The catch per unit of effort for *Photololigo* sp. B was greater at the 33/km station (Table

2). Overall, *Photololigo* sp. A juveniles were present in higher numbers at the 24/km station in the surface waters (Table 3). This pattern was consistent in both years, but higher numbers were caught in 1991/92 (Table 3), largely because of very high catches in December 1991 (Fig. 2). In comparison, highest numbers of *Photololigo* sp. B were consistently found at the 33/km station and abundance levels tended to decrease farther offshore (Fig. 2). Overall, *Photololigo* sp. B demonstrated no difference in abundance levels between the two years (Table 4). In contrast to *Photololigo* sp. A, juvenile *Photololigo* sp. B was more abundant deeper in the water column (Table 4). Farther offshore, *Photololigo* sp. B juveniles were present in very low numbers and were caught only in the deep light-traps (Fig. 2).

*Photololigo* sp. A ranged in size from 2.6 to 47.9 mm. The size-frequency distributions at the two depths were not significantly different between the 19/km and 24/km stations ( $\chi^2=12.28$ ; df 9;  $P=0.1979$ ) (Fig. 3). There was no systematic change in the size-frequency distribution of *Photololigo* sp. A during either summer (Fig. 4). A modal shift in the size-frequency distribution in January 1992 suggested that fewer small individuals were available to be caught. However, catches were very low in this month.

*Photololigo* sp. B ranged in size from 3.6 to 61.6 mm (Fig. 3). From the size-frequency distributions it was clear that larger juveniles were found farther offshore and deeper in the water column (Fig. 3). No



modal shift in the size-frequency distribution during the summers was apparent (Fig. 4). However, catches were low in most months.

The multiway-frequency analysis established that the size-frequency distribution of juvenile *Photololigo* sp. A at both depths changed as a function of time of night (Table 5). Small juveniles dominated in the surface waters, but larger individuals were generally found closer to the benthos (Fig. 5). During the night, the relative abundance of small individuals decreased at both depths. Close to the

benthos an increase in large individuals was evident. There was no discernible pattern of vertical migration; however, combining data across months to increase the number of juveniles in the analysis removed the possibility of detecting vertical migration in any one month.

The number of *Photololigo* sp. A juveniles captured during the winter months was similar to most of the summer monthly catches (Fig. 6); although winter catches never reached levels such as those seen in December 1991 (Table 6). The large number

of small juveniles captured over the winter (Fig. 6) indicates that *Photololigo* sp. A spawns and hatches in both seasons. A similar size range was captured at each sampling during the summer months (Fig. 7).

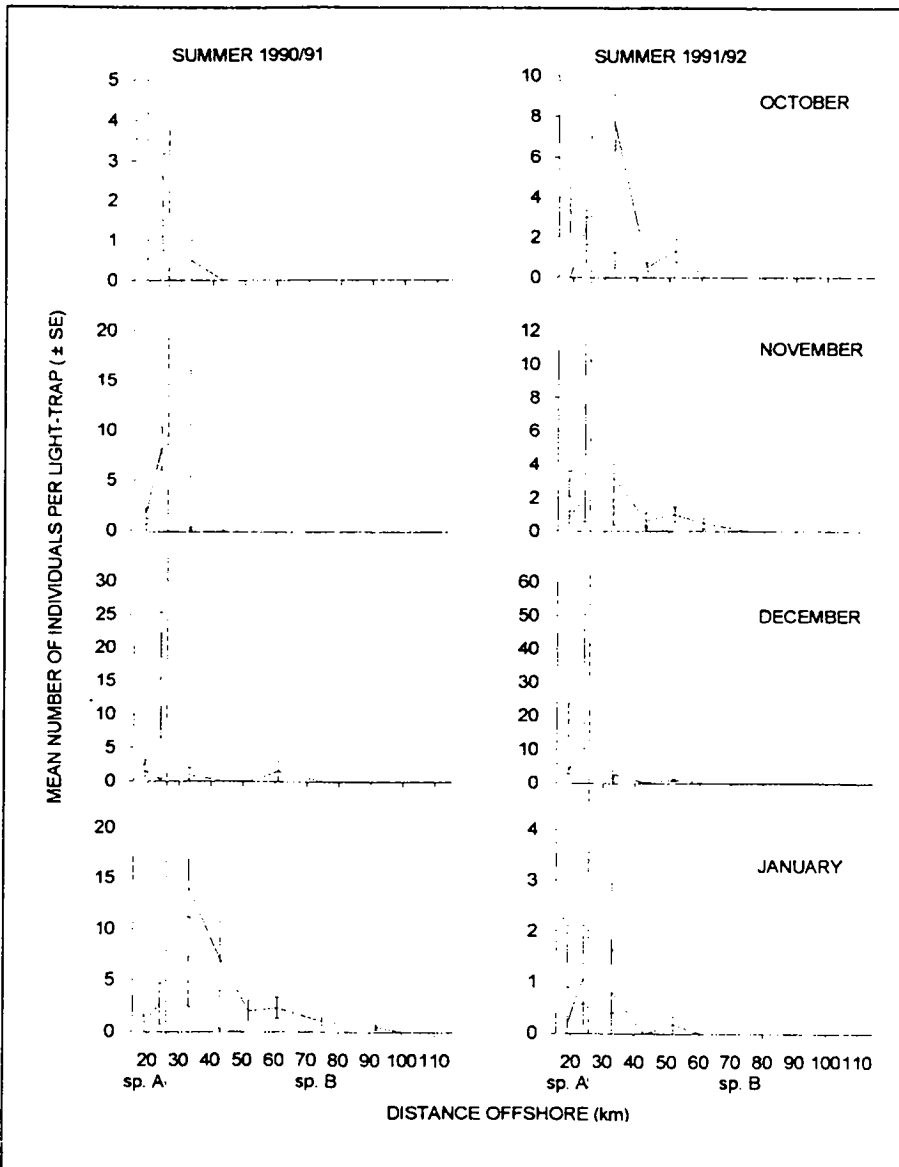
### Physical parameters

Both temperature and salinity decreased nonlinearly across the lagoon; discontinuities in both variables occurred midway across the Lagoon (Fig. 8). Temperature or salinity discontinuities were detected on at least six out of nine nights between the 33 km station and one or both of the neighbouring stations. This suggested that in the lagoon the water mass was heterogenous and may have influenced the distribution patterns of juvenile squid.

Salinity-temperature profiles of the water column at each station indicated thermoclines were present on some nights (Table 7). A thermocline was defined as a temperature change greater than 0.5°C between surface and bottom water; differences as great as 3°C were detected during January. However, these thermoclines were a temporally and spatially unstable feature of the water column, possibly due to variable wind conditions and the shallow body of water being sampled.

### Discussion

Light-traps have provided a technique by which spatio-temporal distribution patterns of two *Pho-*



**Figure 2**

Catches of juvenile *Photololigo* sp. A (found at 19 and 24 km) and *Photololigo* sp. B (found at 33 km and greater) from Townsville, Australia. Most values are averages ( $\pm$  standard error) of six one-hour sets over three nights. See Table 1 for replicates at each station. (Solid lines, deep light-traps; dashed lines, shallow light-traps). Note the variable scale of the Y-axes.

*Photololigo* species can be described. Identification of *Photololigo* species using allozyme electrophoresis suggests that the two species are separated geographically across the Great Barrier Reef lagoon (Yeatman and Benzie, in press). This separation occurs in a region of the coastal lagoon where temperature-salinity data indicate heterogeneity. High numbers of juvenile *Photololigo* sp. A at stations close to the mainland suggests that spawning grounds for this species may be close to the coast, a feature typical for loliginid squid (Mangold, 1987). Furthermore, the presence of small and large individuals during summer and winter months indicates that spawning, hatching, and recruitment are not seasonal events. This characteristic may be more common for tropical species that tend to have shorter lifespans than temperate species (Jackson and Choat, 1992). Large numbers of small juveniles collected during the winter may be a function of slower growth during the winter (Jackson and Choat, 1992). Little is known about *Photololigo* sp. B adults; however, the presence of juveniles in this region suggests that an adult population does occur in the Townsville region and that spawning occurs throughout the summer. The identification of juvenile *Photololigo* was confirmed on a subsample of specimens captured during the summer. Conclusions drawn from this study are based upon the assumption that the offshore distribution pattern of the two species was consistent in all other months of sampling.

Juvenile squid are not easily sampled with towed nets (Vecchione, 1979; Vecchione and Gaston, 1985; Holme, 1974). They have highly developed sensory and locomotor systems (Boletzky, 1974) and it is likely that these animals are often undersampled because of net avoidance. Choat et al. (1993) have shown that plankton nets select for small larval fish, but larger

**Table 2**  
Catch per hour of light-trapping for each *Photololigo* species across the Great Barrier Reef Lagoon for eight months of summer sampling. *Photololigo* sp A at stations 19 km and 24 km and *Photololigo* sp B farther offshore.

Month	Species A		Species B				Total
	19km	24km	33km	43km	52km	61km	
<b>1990</b>							
OCT	2.38	1.81	0.25	0	0	0	0.62
NOV	0.75	8.25	5.42	—	0	0	2.88
DEC	1.88	8.00	0.50	0	0	0.75	2.63
<b>1991</b>							
JAN	0.42	1.33	9.42	3.58	1.00	1.13	2.91
OCT	1.67	2.17	4.25	0.25	0.67	0	1.50
NOV	1.83	4.83	2.17	0.42	0.50	0.25	1.67
DEC	10.67	24.42	1.92	0.17	0.50	0	6.28
<b>1992</b>							
JAN	0.83	1.75	1.67	0.17	0.08	0	0.75
TOTAL	2.59	6.31	3.30	0.72	0.36	0.17	2.33

**Table 3**  
Planned comparisons of juvenile *Photololigo* sp. A densities between depths, years, and sites.

Contrast	df	Contrast sums of squares	Mean squares	F-value	P>F
Depths	1	9.8165	9.8165	12.20	0.0006
Years	1	3.7565	3.7565	4.67	0.0320
Sites	1	8.6892	8.6892	10.80	0.0012
Residual	177	142.3838	0.8044		

**Table 4**  
Planned comparisons of juvenile *Photololigo* sp. B densities between depths and years.

Contrast	df	Contrast sums of squares	Mean squares	F-value	P>F
Depths	1	17.0607	17.0607	37.85	0.0001
Years	1	0.0438	0.0438	0.10	0.7554
Residual	335	148.7448	0.4507		

fish are captured from the same water column by using light attraction. Thorrold (1992), as well as this study, showed that light-traps are a useful technique for capturing juvenile squid. However, like most sampling techniques, the light-traps have biases. One problem is that light-traps sample an unknown volume of water. Nonetheless, they have

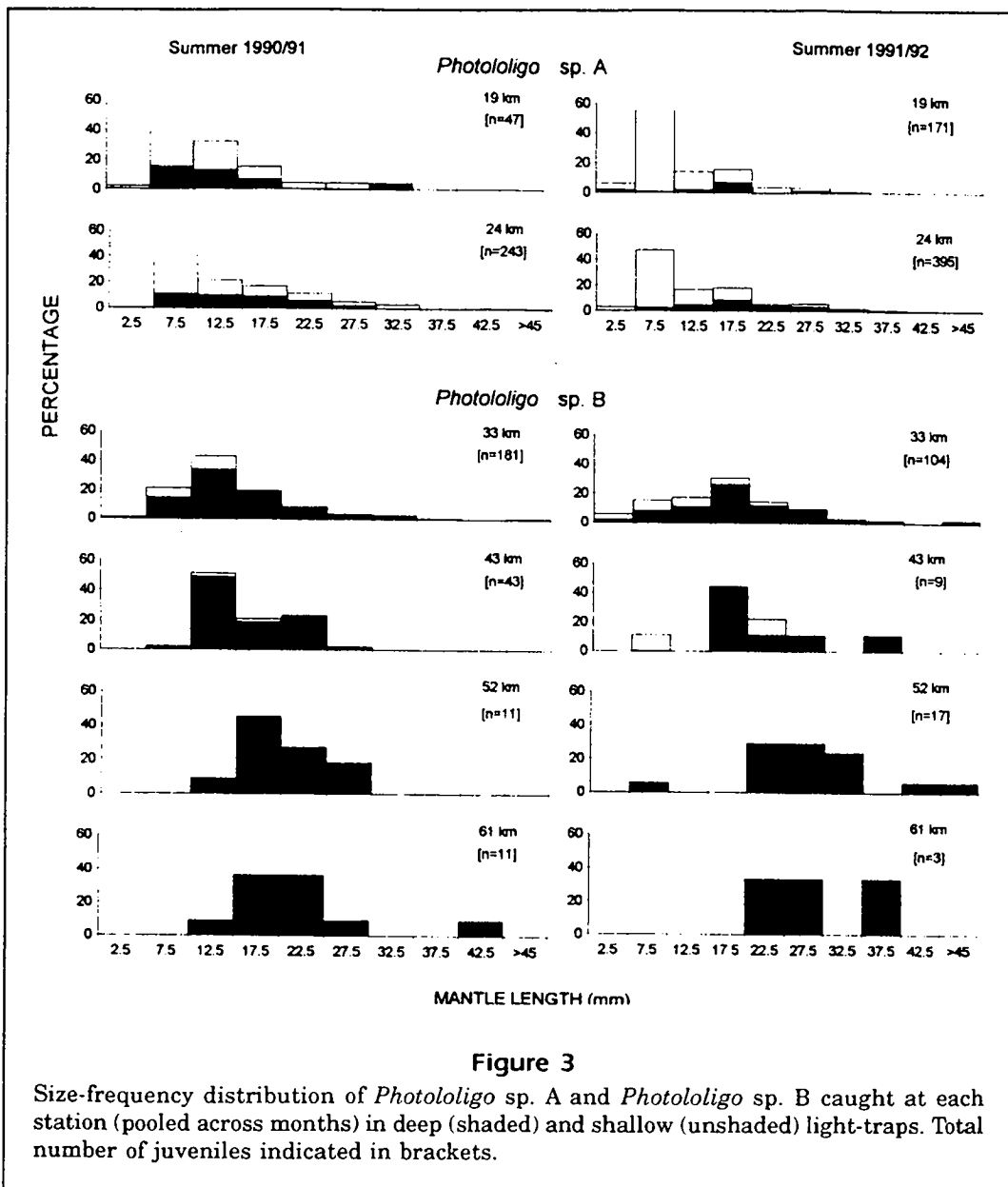


Figure 3

Size-frequency distribution of *Photololigo* sp. A and *Photololigo* sp. B caught at each station (pooled across months) in deep (shaded) and shallow (unshaded) light-traps. Total number of juveniles indicated in brackets.

Table 5

Results of the multiway frequency analysis to examine changes in the size distribution of *Photololigo* sp. A between time of night and depth.

Source	df	$\chi^2$	P
Depth	1	92.8	0.00
Time	1	25.57	0.00
Depth x Time	1	0.19	0.66

been validated as useful devices for monitoring relative abundance patterns in larval supply of pelagic juvenile fish at fixed locations (Milicich et al., 1992).

Great care needs to be exercised when interpreting catch rates from different locations because changes in water transparency can bias light-trap efficiency. Similarly, it is not possible to quantitatively compare catches from drifting and anchored light-traps (Thorrold, 1992). This is because the former act as lagrangian drifters and sample photopositive organisms from within a constant light pool. In contrast, the moored light-traps experience a variable water flow that may greatly increase the volume of water swept in an hour of sampling. Despite more intensive sampling on the reefs, catches of *Photololigo* were low and we conclude that spawning does not occur near the reefs and that juvenile *Photololigo* individuals are concentrated in the lagoon. In the

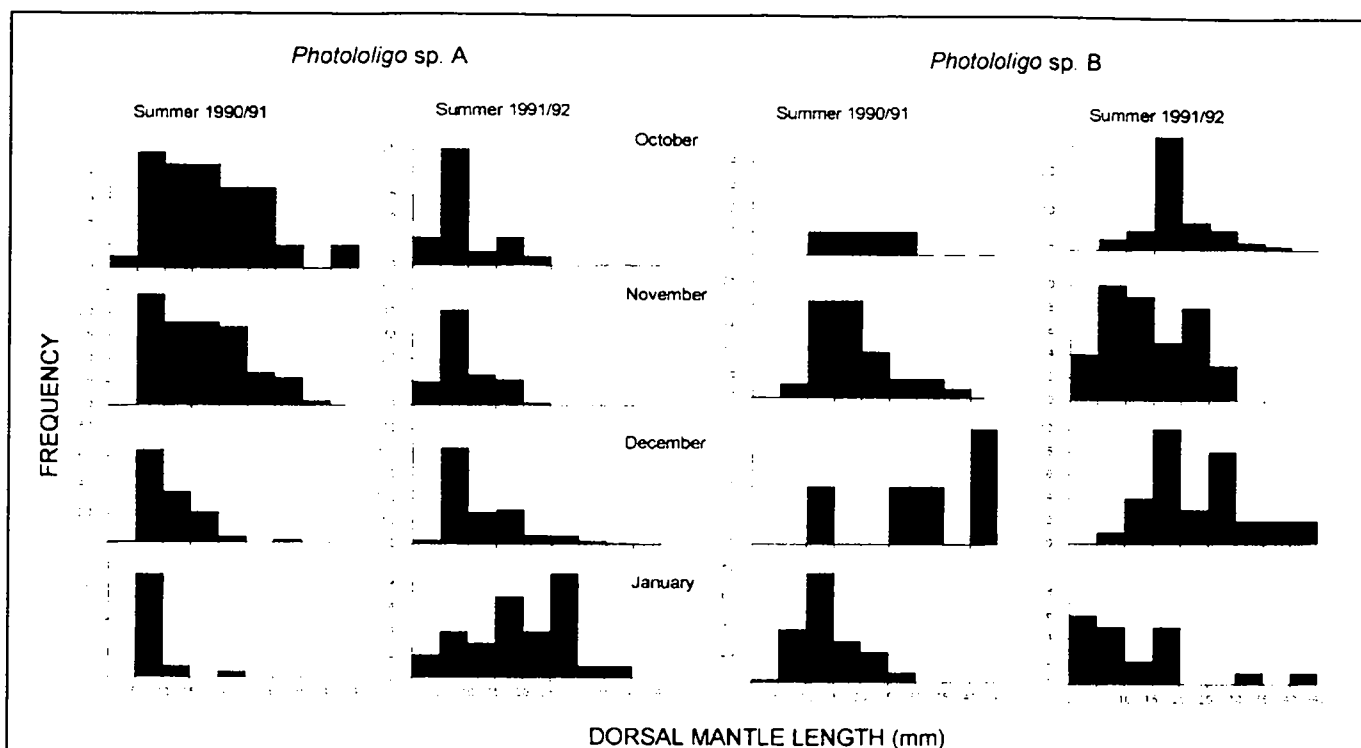


Figure 4

The size-frequency distribution of juvenile *Photololigo* sp. A and *Photololigo* sp. B during eight months of summer sampling. Size classes are mid-points of each class. Data are pooled across depths and stations.

present study, a gradient of turbidity across the shelf makes it possible that inshore catches would underestimate abundance if corrected for diminishing light-pools. However, if the error was significant, it would only exaggerate, not diminish, our observation that juvenile squid were more abundant within the coastal lagoon.

High catches of juvenile squid in the coastal lagoon were at locations where discontinuities were often observed in surface temperature and salinity. Hydrodynamic modelling of this region suggests that the coastal lagoon is often subject to velocity shear (King and Wolanski, 1992). Water in the lagoon typically flows southward under the influence of the poleward East Australian Current, which pushes water onto the outer shelf and through the reef matrix, especially through channels like the Magnetic Passage. Under typical south-easterly wind conditions the shallow body of water trapped against the coast moves in the opposite direction, northwards. The result is a velocity shear between the two water masses and a

Table 6

Analysis of variance examining differences between densities of *Photololigo* sp. A at the 19 km station between summer months of 1990/91 and 1991/92 and winter months of 1991.

Source	df	Contrast sums of squares	Mean squares	F-value	P>F
Month	11	1118.200	101.654	9.55	0.0001
Residual	214	2277.910	10.644		

zone of low residual displacement. Modelling studies suggest that the cross-shelf location of this feature, referred to a separation front (King and Wolanski, 1992), will shift seawards as the wind strength increases and vice versa. This mobility of the frontal region is consistent with the daily and monthly variability of salinity and temperature at the surface indicated by our physical monitoring during the second summer.

This low-shear zone is identified as a significant place for aggregation of planktonic organisms. Cross-shelf studies have shown highest abundances of larval reef fishes in a similar location near the

caps center of the Great Barrier Reef lagoon (Thorrold, in press). These catches included individuals taken from reefs farther offshore, as well as piscivorous larvae of various scombrids from inshore (Thorrold, 1993). It is not clear whether aggregation of these stages is passive, due to hydrodynamics, or the result of attraction to the coastal boundary area by enhanced secondary productivity in this frontal zone

(Thorrold and McKinnon, 1992). This discontinuity may be a mechanism separating the two *Photololigo* species geographically. The separation of juvenile cephalopod species in the Gulf Stream east of New England is thought to be closely related to meso-scale hydrological features (Vecchione and Roper, 1986). The importance of hydrological features in aggregating juvenile squid has been identified in a

number of species (Rodhouse and Clarke, 1985; Brunetti and Ivanovic, 1992; Rodhouse et al., 1992). This suggests that these areas are ecologically important for juvenile squid.

The second way in which shelf-scale hydrodynamics affects the stability of the water column is the intrusion of upwelled waters from the shelf-break driven onto the shelf by variations in the speed and position of the East Australian Current. These cold intrusions can be tracked into the Great Barrier Reef lagoon (King and Wolanski, 1992) and the strong thermal stratification observed in January 1992 was consistent with an intrusion at this time. A cold bottom layer at 33 km was evident on one night in November, but the inner stations were not stratified. The presence of juvenile *Photololigo* at most stations in all months, despite a range of physical conditions, suggests juvenile *Photololigo* can tolerate substantial environmental variation. This tolerance is consistent with a nonseasonal reproductive strategy, which is

essential for a species that lives for only four months.

During the night there was little evidence of a pronounced vertical migration such as the mass aggregations of juvenile *Loligo* spp. on the benthos (Vecchione and Gaston, 1985) or the general movement to the surface by juvenile *L. pealei* (Vecchione, 1981). The absence of vertical movement during the night suggests that the observed ontogenetic shift of *Photololigo* sp. B farther offshore and deeper is real and not a product of location confounded with time of night when sampling occurred. However, as was noticed in the catch-per-unit-of-effort values, both species are caught in relatively low numbers; hence, conclusions based on small differences that are not significantly different are limited. There was a problem with low numbers in all spatial and temporal trends described. However, this was a preliminary study with just two hours of sampling at each station per night. More intensive sampling in bound-

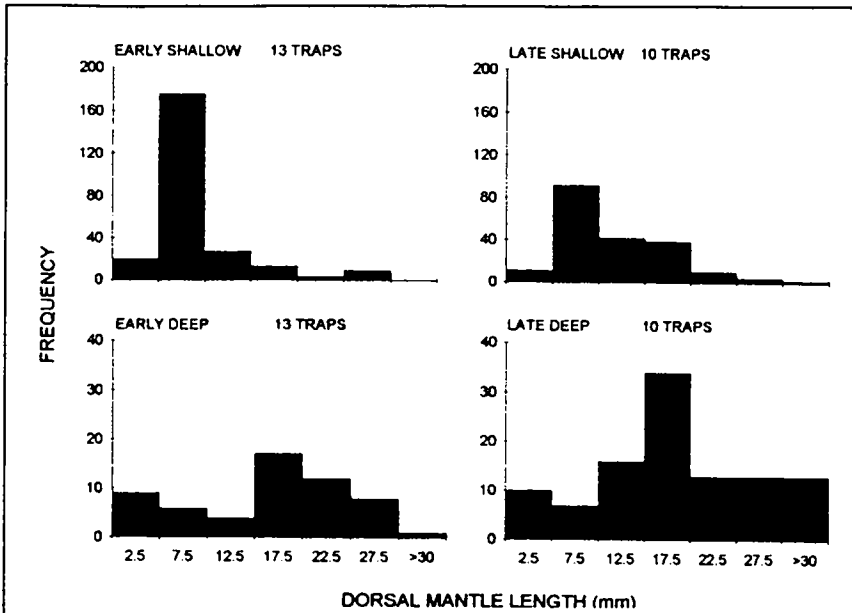


Figure 5

Size-frequency distributions of juvenile *Photololigo* sp. A from the two inshore stations at two sampling depths (pooled across the summer months 1991/92), captured early (before 2400 hrs) and late (after 2400 hrs) in the night.

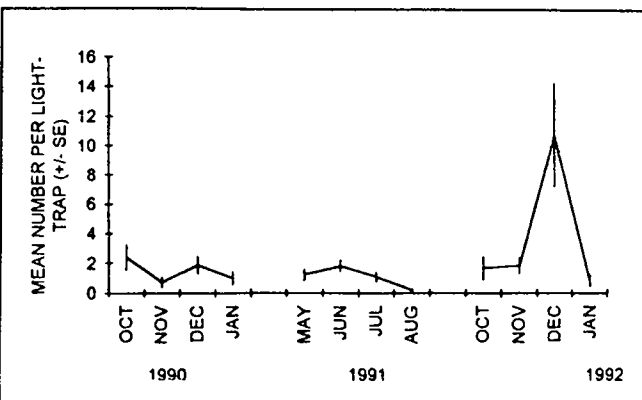
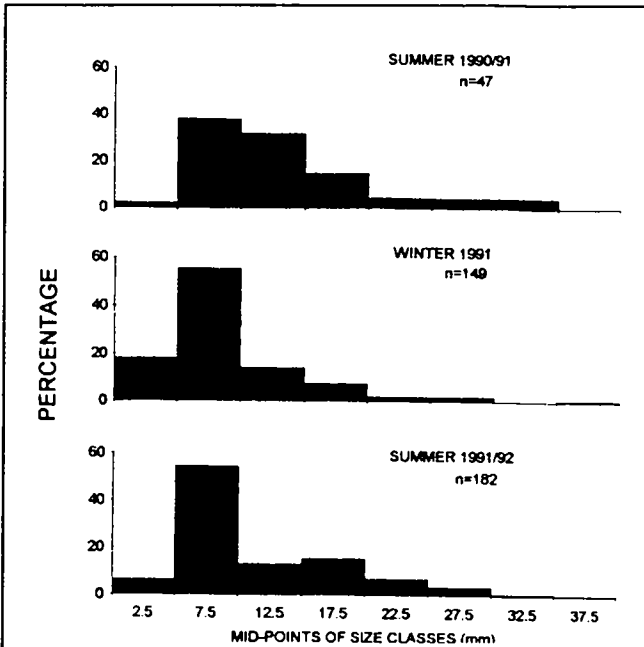


Figure 6

Catches of juvenile *Photololigo* sp. A at the 19-km station over twelve months: during summer 1990/91, winter 1991, and summer 1991/92. (Data pooled across depth and nights.)



**Figure 7**

Size-frequency distributions of juvenile *Photololigo* sp. A at the 19 km station during the summer and winter months. (Numbers are pooled across months, depth, and nights.)

ary waters, both vertical and horizontal, is needed to understand how juvenile squid react to the physical environment. This study has shown that light-traps are useful devices for catching juvenile squid, providing a basis for a more intensive study of the early life-history of squid.

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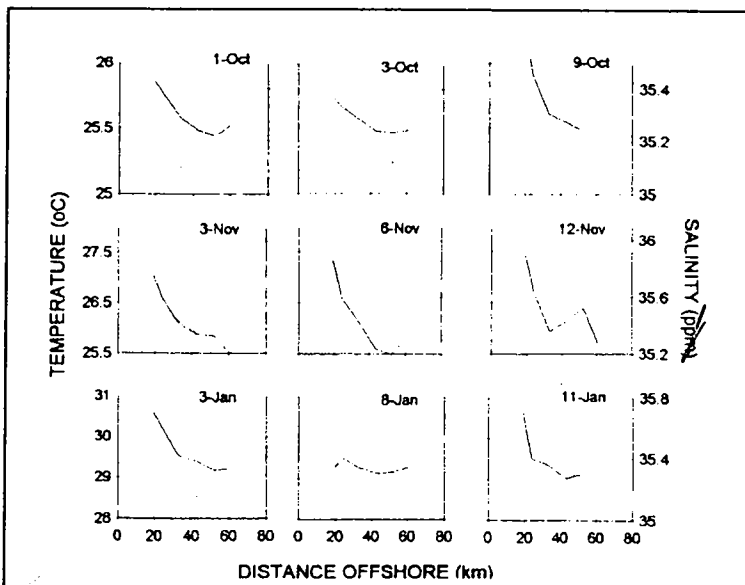
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**Figure 8**

Surface temperature (dashed) and salinity (solid) profiles across the Great Barrier Reef Lagoon for each night of sampling in October and November 1991 and in January 1992. The Conductivity Temperature Device failed during the December cruise.

9

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Table 7

Depth of the thermocline (m) at each station on each night of sampling during the four months of the 1991/92 summer.

Sampling period and situation	Depth of thermocline		
	Day 1	Day 2	Day 3
<b>October 1991</b>			
19 km	14	11	Absent
24 km	13	14	Absent
33 km	10	13	Absent
43 km	10	Absent	Absent
52 km	25	Absent	Absent
61 km	31	Absent	—
<b>November 1991</b>			
19 km	Absent	Absent	Absent
24 km	Absent	Absent	Absent
33 km	Absent	Absent	20
43 km	Absent	Absent	22
52 km	Absent	Absent	25
61 km	Absent	Absent	29
<b>January 1992</b>			
19 km	7	7	9
24 km	9	9	9
33 km	11	13	12
43 km	15	15	18
52 km	28	24	27
61 km	31	47	—

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