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AGE-BASED DEMOGRAPHY AND REPRODUCTIVE ONTOGENY OF ANGELFISHES BELONGING TO THE FAMILY POMACANTHIDAE

Thesis submitted by
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at James Cook University

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General Abstract

There is an urgent need to understand population dynamics and life history characteristics of reef fishes that are highly exploited and limited in distribution to facilitate effective management and make predictions of potential impact. This is important within the pomacanthids family in which no demographic studies has been done. This study aims to explore the demographic features and reproductive ontogeny of a group of pomacanthids species, *Pomacanthus sexstriatus, Centropyge bicolor* and *C. loricul. P. sexstriatu* and *C. bicolor* were collected from the Great Barrier Reef and *C. loricul.* two species are in the first two species and from Kiritimati, Kiribati in the Central Pacific. An age-based approach combined with gonad histology was used in this thesis to examine the growth and longevity, reproductive ontogeny and the mechanism underlying larger male size in the study species.

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CHAPTER 1. GENERAL INTRODUCTION

An understanding of population dynamics and life history characteristics of harvested species is important to facilitate effective management and prediction of potential impact (Devlin and Nagahama 2002). The foundation of understanding population dynamics comes from age-based demographic parameters which provides estimates of age-specific growth, reproduction and mortality rates (Stearns 1992). This study aims to focus on the first two parameters: age-specific growth and reproductive biology of tropical coral reef fishes belonging to the family Pomacanthidae. This area of research has recently developed in tropical fisheries due to four major reasons: i) the need for management of fisheries, ii) an increase in the range of age-based studies in reef fishes including validations of increment periodicity in sectioned sagittal otoliths, iii) increasing evidence of decoupled size and age relationships and iv) long lifespan in coral reef fishes (Choat and Robertson 2002).

The form of growth of a species contributes to many important attributes of its life history such as variation in growth rate and adult size among sexual identity (Laman Trip 2004), and populations (Gust et al. 2002). Although reef fish display a wide variety of growth patterns two characteristic forms have been identified: continuous and asymptotic. In the first, size increases continuously throughout life resulting in determinate age from size (e.g. Some scarids: *Chlorurus gibbus* & *Scarus schlegeli* in Choat et al. 1996). The second form of growth is characterized by rapid initial growth within its early life history, after which growth asymptote or changes at a minimal rate towards adult size. In this type, numerous age classes accumulate within maximum size ranges (e.g. Acanthurids: *Ctenochaetus striatus* in Choat and Axe 1996; Lutjanids: *Lutjanus adetii* in Newman et al. 1996; Pomacanthids: *Centropyge bicolor* in Eagle 1997). One important characteristic that distinguishes the two is in the temporal pattern of growth. In continuous growth, growth occurs throughout its lifespan, while in species that acquire asymptotic size at age relationships, all or most of its growth is limited to the first 10 to 15% of its lifespan, so that any variation in growth therefore becomes limited to this timeframe (Laman Trip 2004).

In addition to the pattern of growth, differential growth rates among sexes are also a common feature of reef fish populations. Male-biased sexual size dimorphism, where males are relatively larger in size than females is a common phenomenon in coral reef fishes. This phenomena has been explained through the size advantage model by Ghiselin (1970) (Warner 1984). The model predicts protogynous reproductive ontogeny (where females change sex to males at larger sizes) to be selectively favored in species with polygynous social systems, such as in haremic species, so that there is a capacity for larger males (Warner 1984). Indeed, studies based on sex-specific size-at age in protogynous species have revealed a common trend of larger male sizes than females at any given age (Adams 2002; Munday et al. 2004; Walker and McCormick 2004). A modification of the size-advantage model can be observed in gonochoristic species in which growth rate in males is accelerated relative to females although such species do not obtain the benefits of protogynous sex-reversal (Newman et al. 1996; Laman Trip 2004).This suggests age-based mechanisms to be involved in male-biased size-at-age relationships.

Two mechanisms proposed to explain differential growth between sexes are i) “growth spurt” and ii) “juvenile growth” hypothesis. The time at which differential growth is established and the consequences of sexual function provides the basis to distinguish between these two hypotheses. In the “growth spurt” hypothesis, males become larger after sexual maturity or sex change, so that sexual function predicts relative growth between sexes (Choat et al. 1996; Walker and McCormick 2004). In the “juvenile growth” hypothesis, differential growth occurs very early in the juvenile stage, so that relative growth of juveniles determine sexual function (Francis and Barlow 1993). In most studies to date, larger male sizes in coral reef fishes are suggested to be explained by the “juvenile growth” hypothesis (Adams and Williams 2001; Laman Trip 2004; Munday et al. 2004). It is not clear what determines the mechanisms used, however there is an emerging pattern where longer lived species are usually explained through the juvenile growth hypothesis and perennial species through the growth spurt hypothesis. This remains an exploratory area of research.

Establishment of i) larger male size at age and ii) sexual function will differ in timeframe according to the form of growth and the type of reproductive ontogeny a species has. Studies to date have focused on protogynous fishes with continuous growth but less attention has been given to species with asymptotic growth, where these two activities are limited to the early parts of its life. What little study done on asymptotic species is limited to gonochoristic species such as acanthurids and lutjanids (Newman et al. 1996; Laman Trip 2004). It becomes more interesting in protogynous species where male recruitment has been discovered to be more complex. Males can recruit into the population via sexual differentiation occurring in the embryonic stage, by development as males from immature female gonads or by sex reversal after first functioning as a female (Adams 2002; Cole 2002; Fennessy and Sadovy 2002).

This study therefore aims to explore the demographic features and reproductive ontogeny of a group of protogynous coral reef species (Aldenhoven 1984; Moyer 1990) belonging to the family Pomacanthidae for which only one age-based study suggests asymptotic growth (in *Centropyge* sp by Eagle 1997). I chose to explore these objectives with respect to size and age relationships, sexual function and longevity. This study however does not serve to resolve the mechanism for variation in patterns of demographic features, however it demonstrates the problems and complexities faced in attempting to explore early growth in the study species, and therefore provide a foundation for future research in this area.

An important attribute to the study of angelfish is their high demand in the Global Marine Aquarium Trade (GMAT) due to their rarity at a local and global scale, and their striking color patterns (Allen et al. 1998; Sadovy and Vincent 2002). In contrast to preference for larger individuals in conventional fisheries which are the focus of most models, the aquarium trade is highly selective for small to intermediate fishes (2-15 cm) (Chung and Woo 1999; Sadovy and Vincent 2002), the consequence of which still needs to be discovered for angel fish. I chose three species for which phylogenetic information is available to enable evolutionary interpretation, a basal species: *Pomacanthus sexstriatus*, and two derived species: *Centropyge bicolor* and *C. loricula* (Bellwood et al. 2004). *P. sexstriatus* represent the larger angel fish (maximum size: 50cm), while the two *Centropyge* species represent the smaller species within the family (maximum size: 15cm) (Allen et al. 1998). Moreover, *C. loricula* is commonly exploited in great amounts for the GMAT from Kiritimati Island, Kiribati in the Central Pacific. While some research has focused on *Centropyge bicolor* (Aldenhoven 1984;

Aldenhoven 1986b; Aldenhoven 1986a; Eagle 1997), little has been done on *C. loricula* and *P. sexstriatus.*

This thesis first reviews the literature on the biogeography, life history and demography of pomacanthids and identifies areas of needed research (Chapter 2). Secondly, growth and longevity of the three study species is investigated (Chapter 3). This is followed by determination of the reproductive ontogeny through the histology of gonads (Chapter 4). Lastly, an attempt to explain the mechanism of relatively larger males with increasing age, demonstrates problems faced in two of the study species: *P. sexstriatus* and *C. bicolor* (Chapter 5). A general discussion and directions for future research concludes this thesis (Chapter 6).

CHAPTER 2. REVIEW OF THE BIOGEOGRAPHY, LIFE HISTORY AND DEMOGRAPHY OF ANGELFISHES (POMACANTHIDAE) WITH EMPHASIS ON ENDEMICS IN THE PACIFIC OCEAN

2.1. INTRODUCTION

Pomacanthids, commonly known as angelfishes, are an ecologically diverse group highly characteristic of coral reefs (Allen et al. 1998). Due to their accessibility to underwater observation and their global distribution patterns with representatives in all tropical seas, pomacanthids and their close relatives, the butterfly fishes (Chaetodontidae) are frequently used in biogeographical analyses of reef fish faunas (Steene and Allen 1978; Allen 1979; Springer 1982; Thresher 1991; Allen et al. 1998; Findley and Findley 2001; Bellwood and Wainright 2002). The biology of pomacanthids is, however, poorly understood. Information to date suggests high diversity but low abundance for most species (Allen et al. 1998), extended life spans (Eagle 1997) and complex patterns of reproductive biology (Bauer and Bauer 1981; Moyer 1990). Research suggests a reproductive strategy unique to this group generally involving daily pelagic pairwise spawning during sunset using a characteristic spawning ritual and posture, resulting in continuous and maximal larval dispersal (Bauer and Bauer 1981). The population dynamics and demography of angelfishes are still poorly understood. I approach the review firstly by exploring the taxonomic structure and ecological attributes of the pomacanthid fauna, and then explore the global species diversity and distribution patterns, with emphasis on the distribution ranges and patterns of endemics. It is not the scope of this review to explain historical mechanisms underlying these patterns, however these will be briefly discussed.

Terminology

a) Indo-west Pacific (IWP): includes the Indian Ocean and east to Easter Island in the Pacific Ocean.
b) East Pacific: including Clipperton, Galapagos, and coast of Mexico, to Costa Rica and Ecuador. The East Pacific is separated from Hawaii and the Line Islands in the Central Pacific by the East Pacific Barrier, an expanse of deep open ocean approximately 5000 km wide and after the closure of the Isthmus of

Panama, also isolated the East Pacific from the Atlantic ocean. The two regions have similar faunas and therefore called the East Pacific-Atlantic (EPA) region (Briggs 1996; Bellwood and Wainright 2002).

c) Endemic: species with distribution ranges less than 20,000 sq.km.
d) Panmictic: IWP species with distribution ranges greater than 160,000 sq.km.

2.2. THE STRUCTURE OF POMACANTHID FAUNAS

2.2.1. TAXONOMIC STRUCTURE

The family Pomacanthidae consists of 83 species within 8 genera (Allen et al. 1998). There is still a large discrepancy in conclusions over relationships within the family, demonstrating a need to re-examine its taxonomy (Allen et al. 1998; Chung and Woo 1998; Bellwood et al. 2004). Recent findings by Bellwood et al., (2004), exploring the phylogeny of 24 species representative of all pomacanthid genera and subgenera however, confirmed the monophyly of the family and genera, except the *Centropyge* genus. Moreover, subgeneric relationships were not fully supported (Figure 2.3). Interestingly, phylogeny of the study species demonstrated patterns where the genus *Pomacanthus* are basal, and the remaining genera derived.

Seventy species inhabit the IWP and the remaining in the EPA region (Table 2.1). *Apolemichthys, Chaetodontoplus*, *Genicanthus*, *Paracentropyge,* and *Pygoplites* genus inhabit the IWP only, while *Holacanthus* are found only in the EPA region. *Centropyge* and *Pomacanthus* species are present in both regions (Allen et al. 1998).

2.2.2. ECOLOGICAL ATTRIBUTES

Maximum size relationships

There are three size groups observed according to genera within the pomacanthids (Figure 2.1).

a. *Centropyge* and *Paracentroyge:* 50% of species within these genera have maximum sizes between 8-11cm. A few species of *Centropyge* species reach 14, 15 and 18 cm (displayed as outliers).

b. *Apolemichthys, Chaetodontoplus, Genicanthus* and *Pygoplites:* the maximum size of the majority of species range between 15-25cm. *Chaetodontoplus* and *Genicanthus* both have one species with 35cm.

c. *Holacanthus* and *Pomacanthus:* skewed towards larger maximum sizes; 50% of the genera fall between 25-42 and 38-46cm respectively. *Pomacanthus* are larger reaching 50cm with the lowest value of the range at 25cm (shown as an outlier).

Depth relationships

Pomacanthids are found from as shallow as 1 m to depths of 131m. There is considerable overlap between genera with respect to median depth ranges (Figure 2.2). Median depth ranges of the majority of *Genicanthus* and *Paracentropyge* species are highest, the majority found between 38-62m. The majority of *Centropyge, Chaetodontoplus*, *Holacanthus*, *Pomacanthus* and *Pygoplites* species have median depth ranges between 20-40m, while *Apolemichthys* species are skewed towards slightly deeper median depth ranges, mostly distributed between 20-54m. Species of *Centropyge, Apolemichthys* and *Paracentropyge* have the broadest median depth distribution, all skewed towards deeper median depth ranges. It is worth noting that median depths do not reveal wide or narrow depth ranges, however, they are a relative indicator of depth distribution.

Foraging patterns

The pomacanthids display three feeding types (Howe 1993; Allen et al. 1998; Aburto-Oropeza et al. 2000). The genera *Apolemichthys, Centropyge, Chaetodontoplus, Pomacanthus, Pygoplites* and *Holacanthus* are benthivores showing two of the three feeding modes. *Centropyge*s are herbivorous (feeding mode 1) and feed solely on algae, while the remaining genera are omnivorous (feeding mode 2) and consume sponges supplemented with algae and smaller amounts of zoantharians, tunicates, gorgonians, fish and invertebrate eggs, hydroids, and seagrasses (Hourigan et al. 1989; Sakai and Kohda 1995; Allen et al. 1998). Research on *Holacanthus passer* and *Pomacanthus zonipectus* at the Gulf of California suggest the importance of sponges and seaweeds in their diet (Perez-Espana and Abitia-Cardenas 1996). The third type of feeding mode is shown by *Genicanthus* species. These species are planktivorous aggregating well above the bottom, forming mid-water shoals that feed on zooplankton (especially pelagic tunicates), supplemented by benthic items including bryozoans, polychaetes, and algae (Allen et al. 1998). Possession of oesophageal papillae in the digestive tract is unique to this genus suggesting morphological specializations enabling them to exploit this food source (Howe 1993).

Exceptions to these feeding modes include coprophagy in *Holacanthus passer* and the Japanese *Centropyge interrupta,* which feed on faeces of plankton-feeding damselfishes and fairy basslets (Allen et al. 1998; Aburto-Oropeza et al. 2000). Furthermore, juveniles of *Pomacanthus paru* (french angelfish) and *P. arcuatus* (gray angelfish) are cleaner fishes and feed on parasites and mucus (DeLoach and Humann 1999; Sazima et al. 1999). Feeding ecology of the *Paracentropyge* is unknown.

2.3 BIOGEOGRAPHY OF POMACANTHIDS

2.3.1. SPECIES DIVERSITY: A GLOBAL VIEW

Pomacanthid species density contours (Figure 2.4) show general patterns congruent to marine groups such as fish, echinoids and corals (Thresher 1991; Bellwood and Wainright 2002) which distinguish between a species rich IWP, a depauperate EPA region and a center of diversity within the Indo-Australian archipelago (IAA) (Allen et al. 1998) also known as the East Indies region (Briggs 1996). The center of diversity within the Pomacanthid family encompasses part of the IAA (including the Great Barrier Reef, Philippines, Malay and PNG), east to Solomon Islands and Vanuatu, in parts of the Caroline Islands, in Palau and Yap and north to South Japan. This center of diversity contains more than 20 species and for the purpose of this study will be referred to as the “East Indies” following Briggs (1995).

Within the East Indies, there are two areas of highest species richness. These are located around the Philippines and Ryuku Islands, and at the eastern tip of PNG, both with 26 species, around which contours converge. There is a consistent decline in species diversity correlated with distance away from the East Indies in longitude, as reported in other studies (Springer 1982; Briggs 1996; Bellwood and Wainright 2002). There is a drop moving longitudinally east towards a depauperate East Pacific with one species at Clarion and two at the Galapagos, Easter Island and Clipperton Island. The Caribbean has a maximum of six species at the Lesser Antilles and Gulf of Mexico to Bermuda. Diversity also declines moving west to the Indian Ocean, however this is counteracted by high diversity at Sri Lanka and at East Africa with 12 and 15 species respectively. Latitudinal trends in species diversity are less distinct but are restricted to between 35^0N and 35^0S of the equator, due to lower temperatures above these latitudes (Briggs 1996). I examine species distribution and ranges with the aim to answer the following questions:

i. What species constitute the East Indies: wide distributors or endemics?
ii. What is the trend of species composition away from the East Indies?
iii. What is the geographic pattern of endemism?

2.3.2. GEOGRAPHIC DISTRIBUTION BY SPECIES

Pomacanthid species inhabiting the IWP region are distinct from those inhabiting the EPA region (Allen et al. 1998). Pomacanthid IWP species are either confined to the Western Pacific plate (excluding the East Pacific), the Indo-Australian plate (excluding the right side of the mid-Indian ridge), or to the West and Central Indian Ocean, or inhabit part of these plates with distribution ranges passing through the East Indies (see Figure 2.5 for plate illustration). The remaining are panmictic species, namely *Apolemichthys trimaculatus, Centropyge bispinosus, C. flavicauda, Pomacanthus imperator*, *P. semicirculatus* and *Pygoplites diacanthus* (Table 2.2). 41 out of 83 species from all IWP genera, have ranges passing through the East Indies. These include panmictic species and *Chaetodontoplus conspicillatus, C. duboulayi* and *C. meredithi,* limited to the Indo-Australian plate. There are 14 species confined to the Indian Ocean alone, found either in the west or northern Central Indian Ocean, in East and South Africa, to Madagascar, Reunion and Mauritius up to the Red Sea, or in the Central part of the Maldives, Sri Lanka and Chagos Island. The depauperate East Pacific and Atlantic Ocean are comprised of 4 and 9 species respectively.

2.3.3. GEOGRAPHIC DISTRIBUTION AND RANGE BY GENUS

Distribution ranges for the species suggest a high proportion (43/83) of endemic pomacanthids (< 20,000 sq.km) (Table 2.3, Figure 2.6). The 20,000-69,999 and 90,000-300,000 sq.km distribution categories hold 23 and 17 species respectively. An examination within the distribution categories at the genus level suggests 3 types of arrangement. The genus *Centropyge*s and the *Pomacanthus*
have distribution ranges spreading equally between the three categories, that is, species range from endemic to widespread within both the IWP and EPA region. *Pygoplite* is at the extreme with only one panmictic species. The majority of species within the genera *Apolemichthys, Chaetodontoplus, Genicanthus, Paracentropyge* and *Holacanthus* are limited in their distribution with fewer species having wide distribution ranges. In the next section, I will identify endemics and their geographic distribution and ranges.

2.3.4. GEOGRAPHIC PATTERNS OF ENDEMIC POMACANTHIDS

There are three classified types (a, b, c) of endemic pomacanthids in this review (Table 2.4). Endemics are either oceanic if geographic location is greater than 0.15 km; coastal if between 0.15-0.01 or continental if less than 0.01 km coastline/land area (Source of data: http://www.odci.gov/cia/publications/factbook).

Type a represent a high proportion (19/43) of the pomacanthid endemics with the most limited geographic distribution ranges (Table 2.5, Figure 2.7), most of which belong to the *Centropyge* genera. The majority are found in oceanic reef systems, particularly high in the Pacific Ocean including East Pacific, but also in Cocos Keeling and Christmas Island in the Indo-Australian plate and Ascension, Lesser Antilles, Curacao, Barbados and St. Lucia in the Atlantic Ocean. Continental and coastal endemics are located around the Indo-Australian plate, South Africa, and at Reunion and Mauritius Islands in the West Indian Ocean. Type a endemics, *Genicanthus takeuchi* and *Chaetodontoplus meredithi* pass through the East Indies.

As we move away from type a endemism, there is not only a wider distribution range, but there is a change from an oceanic to a more continental and/or coastal margin geographic distribution (Tables 2.6, 2.7, Figures 2.8 & 2.9). Endemics of type b and c constitute species from all genera except the *Pygoplites* genus, with *Centropyge* and *Chaetodontoplus* species constituting the majority with 5 species each (Allen et al. 1998). In addition, there is a higher degree of distribution within the East Indies. Although 12 endemics are distributed within the East Indies, a combined examination of all endemics suggests a high degree of distribution outside and peripheral to the East Indies. This pattern is seen in endemics from the families Chaetodontidae and Pomacentridae, implying the same mechanism operating in the 3 families, however this still remains obscure (Bellwood and Wainright 2002).

2.3.5. ENDEMICS OF THE PACIFIC OCEAN

Distribution on the Pacific plate reveal an interesting pattern. It holds 10 endemics of which are confined to anti-equatorial islands such as Hawaii and Cook Island to Easter Island in the South Pacific. Such islands are high islands believed to be recently formed (Springer 1982). Low island endemics consist of only *A. xanthopunctatus* confined to Kiribati and Caroline Island. Using historical evidence Springer (1982) stated the importance of the Pacific Island's western margin as a "boundary for the distribution and speciation of the pomacanthidae" and hence his view of the Pacific plate as a major biogeographic subunit of the IWP region. He supported his view by showing evidence of high endemism on the plate, and the fact that sister species of plate endemics do not occur on the Pacific Plate. The former is supported by our results, despite a more conservative approach in exclusion of species off the Philippine plates, which was included by Springer.

An examination of the whole of the Pacific Ocean (35°N 25°S 115°E 85°W) shows similar patterns of anti-equatorial distribution in 14 oceanic endemics out of a total of 28 endemic pomacanthids. Hotspots for coastal and/or continental Pacific endemics are situated within the East Indies in south China, Taiwan, Philippine plate to southern Japan in type b and c endemics and along the GBR in type a and c endemics.

2.3.6. MECHANISMS UNDERLYING BIOGEOGRAPHIC PATTERNS IN MARINE GROUPS

The mechanisms proposed to explain the congruent biogeographical patterns of species diversity in marine groups of reef fishes still remain unclear (Bellwood and Wainright 2002) but there have been, however, two approaches in explaining biogeography of marine organisms. Historical biogeography is based on the vicariance hypothesis that contribute to the geographical isolation of biotic populations, permitting those populations to speciate. These factors in turn affect the dispersal of organisms (Springer 1982; Becker 1999). Ecological biogeography is based on dispersal, life history, competition, and productivity. The dynamic nature of oceanographic processes and movement of larvae in water play a major role in connectivity between populations via dispersal of pelagic larvae between islands or marine systems (Becker 1999 and refs therein).

Reproductive parameters such as maximum size and larval duration have little effect on distribution ranges of IWP pomacanthids. In contrast, endemics have longer pelagic larval duration (Thresher and Brothers 1985; Allen et al. 1998) similarly reported in other reef fish families (Victor and Wellington 2000; Bellwood and Hughes 2001). This suggests that other mechanisms are of more importance. Recent research have found areas of shallow-water habitat to be an important component in explaining variation in species composition of fish and corals across the IWP (Becker 1999; Bellwood and Hughes 2001) providing direct evidence of the importance of shallow water substrate in maintaining high species diversity in the IAA, which others have tried to explain with models based on historical events. These models are:

1) “center of origin: patterns are shaped by dispersal and degree of isolation from IAA
2) IAA as a center of overlap or accumulation or
3) IAA as a center of survival, providing a refuge from higher extinction rates in peripheral locations” (Bellwood and Hughes 2001; Bellwood and Wainright 2002).

2.3.7. SPECIES DIVERSITY IN POMACANTHIDS IN RELATION TO SHALLOW WATER SUBSTRATE (SWS) IN THEPACIFIC OCEAN

The percentage of SWS within an area is representative of the connectivity between coral habitats, hence those with lower SWS represent isolated reef systems (Becker 1999). In such islands, larval retention and self-replenishment for population maintenance is expected (Harrison and Wallace (1990) in Becker 1999). Evidence that areas of SWS are a major factor in driving species richness in pomacanthids in the Pacific Ocean is supported by this study (Figure 2.10 and 2.11).

A more interesting observation was the relatively greater number (Bellwood and Hughes 2001; Bellwood and Wainright 2002) of species per 250sq.km within more isolated islands, which peaked at 14 species in Phoenix Island (Figure 2.12). Phoenix has low endemism (7.1%) within its pomacanthid fauna, however there is a transition towards higher endemism moving east from the East Indies towards more isolated islands (Figure 2.13). High endemism is characteristic in Cook Islands, Pitcairn, Clipperton, and Galapagos of 23%, 40%, 100%, 100% respectively, and in Hawaii in the North Pacific with 60% endemism. The increasing proportion of endemism moving east across the Pacific Ocean, indicates correlation of isolation and endemism in pomacanthids.

It is interesting to note however that this trend of high endemism is observed only in anti-equatorial isolated reef systems. Although Galapagos is equatorial, high endemism may be due to its isolation by the East Pacific Barrier. In contrast, equatorial isolated islands such as Line Islands in Kiribati and Marquesas have very low or no endemic species within the pomacanthid fauna. These patterns support Springer’s hypothesis (section 2.3.4).

2.4. PACIFIC ENDEMIC POMACANTHIDS IN THE GLOBAL MARINE AQUARIUM TRADE (GMAT).

71 pomacanthids, 27 of which are endemic, are exploited in the GMAT (Allen et al. 1998; Wood 2001; UNEP et al. 2002), constituting 8% (in quantity) of the total trade

(Source: http://www.marineaquariumcouncil.com; MAC 2002) (Table 2.8). There are 17 endemic pomacanthids harvested from the Pacific Ocean consisting of 6 type a, 3 type b and 8 type c endemics. Type c, *C. ferrugatus, P. zonipectus* and *H. passer* have the highest total export (between 15,000-20,000 specimens) between 1988-2001 (Figure 2.14) (UNEP et al. 2002). To a lesser extent, between 7,000-8,000 specimens of *C. duboulayi* and *C. potteri* and below 3225 specimens of *C. fisheri*, *A. xanthopunctatus*, *P. boylei* and *C. narcosis*, *C. shepardi, C. septentrionalis, C. caeruleopunctatus, G. semifasciatus*, *C. conpicillatus*, *C. personifer*, and *H. clarionensis* have been exploited for each species. Some endemics are currently exported from one source, eg *C. caeruleopunctatus* is exported only from the Philippines (UNEP et al. 2002).

Most records on catch per unit effort on a country to country, and reef to reef basis, at species level, suggests a risk of local depletion (Wood 2001). Evidence of angelfish endemic species depletion has been reported for *H. clarionensis* from Revillagigedo Archipelago off Mexico’s Pacific coast (Almenara-Rolden & Ketchum (1994) in Wood 2001). Similarly, juvenile *H. passer* collected in the Gulf of California has faced depletion (Arellano-Martinez et al. 1999). Anecdotal information on *C. duboylai* and *C. personifer*, both restricted to Australia, suggest depleted populations (QFMA (1999) in Wood 2001). There is a clear need for obtaining information on population dynamics especially of endemic species which are exploited in the marine ornamental trade.

2.5. ABUNDANCE AND DISTRIBUTION, LIFE HISTORY AND DEMOGRAPHY OF POMACANTHIDAE.

2.5.1. ABUNDANCE AND DISTRIBUTION

Qualitative data suggests rarity of Pomacanthid species in terms of abundance (Gaston (1994) in Eagle et al. 2001). Abundance within species, vary between countries and reefs, within reefs, with depth and microhabitat (Allen et al. 1998; Eagle et al. 2001; Wood 2001). Quantitative research by Eagle et al (2001) gives evidence of varying abundance and distribution patterns within *Centropyge* genus at Lizard Island, depending on the spatial scale examined. At the genus level, *Centropyge* species tend to be ecologically similar at the largest scale (whole reef) and at a microhabitat level, but different at an intermediate level (with depth). This highlights the importance of information on interspecific depth ranges. Assessment of density assessment especially on endemic angels has been a neglected part of research and is urgently needed at species and reef levels to further understand population dynamics.

2.5.2. LIFE HISTORY

Life history traits involve patterns in size and age, growth, mortality, and reproduction (Stearns 1992).

Larval development

Pomacanthidae species have planktotrophic pelagic larval stages in their development, ranging from 17 to 39 days, a period during which they are highly vulnerable to predators (Thresher and Brothers 1985; Allen et al. 1998). Larval duration varies according to species, endemics having relatively longer larval durations than widely distributed species (Thresher 1984; Thresher and Brothers 1985; Allen et al. 1998). In general, the larger the species, the shorter the duration of its pelagic larval stage in IWP pomacanthids (Thresher and Brothers 1985). Larvae of Caribbean pomacanthids have not been recorded in the oceanic plankton and are consequently thought to have a short planktotrophic larval or post-larval life, with rapid metamorphosis to the adult form (Munro et al. 1973).

Demography

Demographic parameters of reef fishes including estimates of growth, natural mortality and longevity (Meekan et al. 2001; Gust et al. 2002) are important in fisheries management as species with long life spans, delayed maturity, large body size and low rates of natural mortality and recruitment are relatively more susceptible to exploitation (Adams (1980) and Jennings et al (1998) in Russ and Alcala 1998). This field has recently become a central element of fisheries research in tropical reef fish, however, it is lacking within the pomacanthid family.

Otolith based studies on growth patterns are limited to one study by Eagle (1997) on the *Centropyge* genus. A common asymptotic growth pattern was observed. Growth rate was rapid in the first five years, followed by slow growth for the next five years, after which maximum length was reached. Minimum age of sex change in the *Centropyge* genus was consistent at five years of age indicating developmental control, but more interestingly, it is after this age that growth began to slow down, despite the interspecific differences in maximum longevity. Furthermore, females changed sex at later ages in species with longer lifespans, highlighting the importance of longevity on reproductive success (Eagle 1997). Age of first reproduction is probably reached after one to two years of age, however this could be confirmed by histological observation through the presence of vitellogenic and hydrating eggs (Hourigan and Kelley 1985).

Otolith based research has found longevity within the pomacanthids to be of a wide range. Maximum longevity have been recorded to range between nine years in the smaller *Centropyge* species (Eagle 1997) and 60 years in the larger bodied *Pomacanthus* species (Ackerman personal observation). In a scale analysis based study by Chung and Woo (1999), *P. imperator* was reported to reach 16 years but predicted to have a lifespan of up to 60 years. This study faces inaccurate results due to low precision, difficulties in annuli reading and increased inaccuracy when growth becomes asymptotic (Chung and Woo 1999). This contrasts with longevity estimates between 1 to 13 years based on natural mortality rate (Aldenhoven 1986b) and survival in aquaria (Bauer and Bauer 1981; Aldenhoven 1986b). The former prediction assumed consistent growth rates within a species lifetime which has been proven otherwise by age-based studies (Eagle 1997), while the latter does not necessarily predicate wild conditions (Bauer and Bauer 1981) and hence yield biased results.

No age-based research on natural mortality has been done, however, studies on *C. bicolor* by Aldenhoven (1986) suggest natural mortality rates to vary tenfold between sites (small scale). In addition there was a trend of decreased mortality with size, however, this was not statistically significant due to insufficient data.

Reproductive behaviour and biology

Pomacanthids share a general reproductive strategy distinct from other reef pelagic spawners (Moyer and Nakazono 1978; Bauer and Bauer 1981). They have haremic social organizations consisting of a single male and several females (2-7) within a constant territory (Thresher 1982; Moyer et al. 1983; Thresher 1984; Moyer 1990). However monogamous pairing is also common in larger bodied *Pomacanthus* species due to large territories required for foraging habits (Neudecker 1985). Evidence also suggests a relationship between haremic sizes, species, and habitat structure, which is suggested to limit population size and sex ratio (Lobel 1978; Moyer and Nakazono 1978; Thresher 1984; Moyer 1990). Each harem is arranged in size dominant hierarchies in which the male is the largest dominant fish. Males maintain dominance over females within home ranges, keeping them away from adjacent males by rushing and circling in order to attain the greatest reproductive fitness (Moyer and Nakazono 1978; Hourigan and Kelley 1985).

Most pomacanthids show permanent or temporary sexual dichromatism and/or size dimorphism (Moyer 1990). Sexual dimorphism and haremic social organization is characteristic of most angel fish (Moyer 1990). As these feastures are thought to be related to protogynous hermaphroditism (Robertson and Hoffman (1977) in Hourigan and Kelley 1985), protogyny has been assumed to be universal in the family (Neudecker and Lobel 1982), however this would be disclosed with detailed histological (Moyer and Nakazono 1978; Hourigan and Kelley 1985), behavioural (Moyer and Nakazono 1978) and age-based research (Eagle 1997).

Studies on fecundity (reproductive effort) report no relationship between female body size and the spawning frequency within the breeding season of the Japanese endemic, *C. ferrugatus*, possibly due to protogyny (Sakai 1996). In contrast to gonochoristic reef fishes, which invest energy into egg production, the *Centropyge* invest more on becoming a larger female for a higher chance of becoming a dominant male (Sakai 1996).

2.6. CONCLUSION

In conclusion, species density patterns distinguish between high diversity (20-26 species) within the East Indies (West Pacific), and a depauperate East Pacific-Atlantic region. The area of shallow water substrate (SWS) within the Pacific Ocean was observed to be an important factor in driving species diversity. Phoenix Island was the most diverse in terms of species per unit area of SWS within the family Pomacanthidae. It marks a transition area moving east across the Pacific Ocean to higher endemism level found in anti-equatorial isolated oceanic reef systems. Evidence of local depletion in some endemics highlights the need for research on demography, a neglected area of research. This will contribute in understanding population dynamics and the impact of harvesting on potentially long-lived and fast growing pomacanthids.

2.7. PREDICTIONS FOR PATTERNS IN THE PACIFIC OCEAN

Pomacanthid endemics become geographically distributed in isolated oceanic reef systems moving east across the Pacific Ocean. Biological features such as reproductive and demographical parameters may differ between endemics inhabiting oceanic isolated reef systems and those inhabiting continental masses. Studies on demographical parameters suggest higher longevity, lower natural mortality and larger body sizes as characteristic features of endemic damselfish of the isolated islands of Galapagos and Baja California compared to continental reef systems of Panama, in the East Pacific (Meekan et al. 2001). Oceanographical phenomena such as El Nino, can affect the reproduction and the survival of benthic and pelagic larvae via reduced reproductive success and thus reduced recruitment (Mora and Ospina 2001). A longer reproductive lifespan would contribute in maintaining populations in isolated reef systems. It is therefore my prediction that pomacanthid endemics of oceanic isolated islands have relatively longer lifespans and lower natural mortality than those of continental reef systems.

All endemic pomacanthids studied to date are seasonal spawners. It is possible that endemics of isolated reef systems, especially type a endemics will synchronize spawning seasonally with ocean current patterns that will favour return of larvae to home reefs. Hence, endemics will be abundant within their range as in the family Chaetodontidae (Allen et al. 1998).

2.8. RESEARCH NEEDED ON ENDEMIC POMACANTHIDS IN THE PACIFIC

i. There is an urgent need for otolith-based demographical research to understand population dynamics of pomacanthids and the impacts of harvesting. Urgent attention is needed in endemics with relatively long lifespans such as in the *Pomacanthus* genus which are collected at juvenile stages so that recruitment to the spawning stock is endangered. In addition, *Centropyge* species, which constitutes the majority of endemics, are widespread in distribution, and exploited at all sizes suggests these are important genera to study.

ii. Research on endemics of isolated reef systems which are more vulnerable to overexploitation.

iii. Density assessments and research on patterns of recruitment to compliment demographic studies for better understanding of population dynamics. Assessment is needed at species and reef level.

Table 2.1. Classification and regional distribution within the Pomacanthidae family (Source: Allen et al. 1998).

Genus	**Subgenera**	**Species**	**Geographic region**	**# of species**
Apolemichthys		*Apolemichthys arcuatus, A. griffi, A. guezei, A. kingi, A. trimaculatus, A. xanthopunctatus, A. xanthothis,A. xanthurus*	IWP	8
Centropyge	Centropyge	*Centropyge aurantia, C. bicolor, C. colini, C. eibli, C. flavissima, C. heraldi, C. multispinus, C. narcosis, C. nox,* *C. tibicen, C. vroliki.*	IWP	11
	Xiphypops	*C. acanthops, , C. bispinosa, C. debelius, C. ferrugata, C. fisheri, C. flavicauda, C. flavipectoralis, C. hotumata, C. interrupta, C. joculator, C. loricula, C. multicolor, C. naharckyi, C. nigriocella, C. potteri, , C. shepardi.*	IWP	16
	Xiphypops	*C. argi, C. aurantonota, C. resplenden*	East Pacific-Atlantic	3
Chaetodontoplus		*Chaetodontoplus ballinae, C. caeruleopunctatus,* *C. chrysocephalus, C. conspicillatus, C. duboulayi,* *C. melanosoma, C. meridithi, C. mesoleucus, C. niger,* *C. personifer, C. septentrionalis.*	IWP	11
Genicanthus		*Genicanthus bellus, G. caudovittatus, G. lamarck,* *G. melanospilos, G. personatus, G. semicinctus, G. semifasciatus, G. spinus, G. watanabei, G. takeuchi.*	IWP	10
Holacanthus	Angelichthys	*Holacanthus africanus, H. bermudensis, H. ciliaris.*	Atlantic	3
	Holacanthus	*H. tricolor.*	Atlantic	1
	Plitops	*H. clarionensis, H. limbaughi, H. passer.*	East Pacific	3
Paracentropyge		*Paracentropyge boylei, P. multifasciata, P. venusta.*	IWP	3
Pomacanthus	Euxiphipops	*Pomacanthus navarchus, P. sextriatus,* *P. xanthometopon.*	IWP	3
	Pomacanthodes	*P. annularis, P. asfur, P. chrysurus, P. imperator,* *P. maculosus, P. semicirculatus, P. striatus.*	IWP	7
	Pomacanthodes	*P. zonipectus.*	East Pacific	1
	Pomacanthus	*P. arcuatus, P. paru.*	Atlantic	2
Pygoplites		*Pygoplites diacanthus.*	IWP	1

Table 2.2. Pomacanthid species according to distribution types, showing maximum sizes in brackets (Source: Allen et al. 1998).

Ocean Basin	Species list
IWP: Panmictic distribution	*Apolemichthys trimaculatus (25)* *Centropyge bispinosa (10) C. flavicauda (6),* *Pomacanthus imperator(38) , P. semicirculatus (35),* *Pygoplites diacanthus (25).*
Indian Ocean East and Central parts	*Apolemichthys guezei (15), A.xanthotis (15), A.xanthurus (15), A. kingi (20),* *Centropyge debelius (9), C. flavipectoralis (10), C. multispinus (9), C. acanthop (8)* *Genicanthus caudovittatus (20),* *Pomacanthus asfur (40), P. maculosus (50), P. chrysurus (33), P. rhomboides (46).*
Limited to Western Pacific Plate only (excluding East Pacific)	*Apolemichthys xanthopunctatus (25), A. arcuatus (18),* *Centropyge potteri (10), C. narcosis (8), C. naharckyi (9), C. fisheri (6), C. hotumata (8),* *C. multicolor (9), C. nigriocella (6),* *Genicanthus spinus (35), G. personatus (21),* *Paracentropyge boylei (7)*
Limited to Indo-Australian Plate only (excluding west of Mid-Indian ridge)	*Centropyge joculator (9),* **Chaetodontoplus conspillatus (25), *C. duboulayi (25), C. personifer (35), *C. meredithi (25),C. ballinae (20),* *Genicanthus semicinctus (18),*
Species with ranges passing through the East Indies; Distribution also partly in Indo-Australian, and/or Philippine, Malay and Western Pacific plate	*A. griffisi (25),* *Centropyge ferrugata (10),C. aurantia (10), C. eibli (11), C. vroliki (12), C. flavissima (14), C. heraldi (10), C. bicolor (15), C. interrupta (15), C. nox (9), C. tibicen (18), C. shepardi (12), C. loricula (10),* *C. colini (9),* *Chaetodontoplus septentrionalis (20), C. chrysocephalus (22), C. caeruleopunctatus (14),* *C. niger (35), C. mesoleucus (18), C. melanosoma (20),* *Genicanthus bellus (18),G. melanospilos (18), G. lamarck (23),G. watanabei (15), G. semifasciatus (21), G. takeuchi (25),* *Paracentropyge venusta (12), Paracentropyge multifasciata (10),* *Pomacanthus navarchus (25) , P. sextriatus (46), P. annularis (45), P. xanthometopon (38),*
East Pacific	*Holacanthus limbaughi (24), H. passer (25), H. clarionensis (20),* *Pomacanthus zonipectus (48).*
Atlantic Ocean	*Centropyge argi (6.5) , C. aurantonota (6), C. resplendens (6),* *Holacanthus africanus (45), H. bermudensis (38), H. ciliaris (45), H. tricolor (30),* *Pomacanthus arcuatus (50), P. paru (38).*

*Species with distribution ranges passing through the GBR within the East Indies.

Table 2.3. Number of pomacanthid species within distribution range categories according to genus (km.sq). Acquired using Sigma scan 4. Distribution range values: by 1000's.

Genus	<5	5 - 9.9	10 - 19.9	20 - 29.9	30 – 39.9	40 – 49.9	50 – 59.9	60 – 69.9	70 – 79.9	90 – 99.9	100 - 199.9	200 – 299.9	Total
Apolemichthys	2	3	1	0	1	0	0	0	0	0	1	0	8
Centropyge	6	3	4	1	3	1	1	3	0	1	6	1	30
Chaetodontoplus	4	2	2	1	1	0	0	1	0	0	0	0	11
Genicanthus	2	3	1	0	1	0	0	2	0	0	1	0	10
Paracentropyge	1	1	0	0	0	0	0	0	0	0	1	0	3
Pomacanthus	0	1	2	2	1	0	1	1	0	2	1	2	13
Pygoplite	0	0	0	0	0	0	0	0	0	0	0	1	1
Holacanthus	2	2	1	0	2	0	0	0	0	0	0	0	7
Total	17	15	11	4	9	1	2	7	0	3	10	4	83

Table 2.4. Definition of Endemism for the purpose of this review.

Endemic Type	***Definition***
Type a	Distribution range < 5000 km.sq and/or restricted to one or two isolated islands such as Hawaii and Johnston Island; 19/83 species.
Type b	Distribution range between 5,000-10,000 km.sq and/or species which occur in 2 separate islands over this range; eg *Chaetodontoplus niger* which have been found in Macclesfield Bank, and also in Soutern Honshu, Japan; 13/83 species.
Type c	Distribution range: 10,000-20,000 km.sq; 11/83 species.

Table 2.5. Type a endemism with respective geographic distribution and range.

Region	**Geographic distribution**	**Gepgraphic location: Oceanic, coastal or continental**	**Species**	**Distribution range (km.sq)**
W. Indian	Reunion, probably also in Mauritius and Madagascar	Coastal	*Apolemichthys guezei*	964.604
	B/w Kosi Bay & Aliwal Shoal (off S.Africa)	Continental	*Apolemichthys kingi*	2421.772
	Mauritius & Reunion	Coastal	*Centropyge debelius*	923.557
Pacific plate	Hawaii and Johnston	**Oceanic**	***Apolemichthys arcuatus***	9974.415
	Mainly Johnston but also Hawaii	Oceanic	*Centropyge nahackyi*	4535.691
	Hawaii; rare at Johnston	Oceanic	*Centropyge fisheri*	5643.959
	Hawaii Island; common in northwestern islands	Oceanic	*Genicanthus personatus*	5418.201
	Hawaii; rare at Johnston	Oceanic	*Centropyge potteri*	6136.523
	Cook Island	Oceanic	*Centropyge narcosis*	1067.221
	Rarotonga	Oceanic	*Paracentropyge boylei*	1190.362
East Indies	Bonin and Marcus Is in NW Pacific	Oceanic	*Genicanthus takeuchi*	1765.02
Indo-Aust plate	Cocos Keeling & Christmas Is	Oceanic	*Centropyge joculator*	2544.913
Indo-Aust plate	West.Australia	Continental	*Chaetodontoplus personifer*	3878.939
Indo-Aust plate	East.Australia from Qld (rare on GBR) to NSW (Sydney); Lord Howe Island	Continental	*Chaetodontoplus meredithi*	3837.892
Indo-Aust plate	NSW and Lord Howe	Continental; oceanic	*Chaetodontoplus ballinae*	759.3691
East Pacific	Clipperton. Island	Oceanic	*Holacanthus limbaughi*	1190.362
East Pacific	East Pac: Clarion Is (common), other islands of Revillagigedos Gp, Clipperton Is (not common); tip of Baja California	Oceanic	*Holacanthus clarionensis*	4125.221
Atlantic	S.portion of Caribbean Sea (including Lesser Antiles, Curacao, Barbados, St.Lucia) to Brazil	Oceanic	Centropyge aurantonota	3324.805
Atlantic	East Atlantic: Ascension Island	Oceanic	*Centropyge resplendens*	1990.778

Table 2.6. Type b endemic species with respective geographic distribution and range.

Region	Geographic distribution	Geographic location: oceanic, coastal or continental	Species	Distribution range
W. Indian	Red Sea; Gulf of Aden; Oman and Arabian Gulf	**Continental**	***Apolemichthys xanthotis***	9132.952451
W. Indian	Mauritius/Reunion; Maldives; Sri Lanka, east coast of India	Oceanic; coastal; continental	*Apolemichthys xanthurus*	6670.133812
W. Indian	Sri Lanka & Maldives, east coast of India	Oceanic; coastal; continental	*Centropyge flavipectoralis*	5643.95938
W. Indian	W. Indian Ocean: Red Sea & Gulf of Aden, south to Zanibar	Continental	*Pomacanthus asfur*	9584.469201
Pacific plate	Austral; Pitcairn (including Ducie); Rarontonga	Oceanic	*Genicanthus spinus*	7655.261268
East Indies	Philippine archipelago	Coastal	*Chaetodontoplus caeruleopunctatus*	6690.657301
East Indies	Macclesfield Bank (b/w Vietnam & Philippines); and south Honshu, Japan	Coastal	*Chaetodontoplus niger*	2791.194457
East Indies	S.China & Taiwan, north to S.Japan	Coastal; continental	*Chaetodontoplus septentrionalis*	9317.663848
East Indies	N. Philippines; Taiwan; Ryuku and Izu Islands, S.Japan	Coastal	*Genicanthus semifasciatus*	9481.851758
East Indies	N.Philippines to Taiwan; also at Ryukus and Izu Islands	Coastal	*Paracentropyge venusta*	6793.274744
Indo-Aust plate	Lord Howe; Kermadec Island	Oceanic	*Genicanthus semicinctus*	1046.697921
Atlantic	E.coast of N.America (N.Carolina to S.Florida), Bahamas, Bermuda, Gulf of Mexico	Continental; oceanic	*Holacanthus bermudensis*	5602.912402
Atlantic	East Caribbean: W. African coast b/w Senegal and Congo; common off Ghana	Continental	*Holacanthus africanus*	8127.301507

Table 2.7. Type C Endemic species with respective geographic distributions and range.

Region	**Geographical distribution**	**Geographic location: oceanic, coastal or continental**	**Species**	**Distribution range**
W. Indian	East Africa to Red Sea; Gulf of Aqaba (common); Mauritius	Continental; coastal	*Genicanthus caudovittatus*	13217.12669
W. Indian	Arabian Peninsula; Red Sea; Gulf of Oman; Arabian Gulf; Kenya	Continental	*Pomacanthus maculosus*	18040.14653
Pacific plate	Kiribati (all); Caroline	Oceanic	*Apolemichthys xanthopunctatus*	19066.32096
Pacific plate	Austral, Rapa, Pitcairn, Easter Island	Oceanic	*Centropyge hotumatua*	14120.16019
East Indies	Japan's southern coast; to NE Hawaiian islands (Midway & Kure)	Oceanic; coastal	*Centropyge interrupta*	11041.6369
East Indies	From S. Japan to Philippines; common at Okinawa	Coastal	*Centropyge ferrugata*	18430.09281
East Indies	Mariana and Bonin Islands, extending N to Izu Islands	Oceanic; coastal	*Centropyge shepardi*	11021.11341
Indo-Aust plate	Northern Australia; from NW Aust to QLD; S.parts of Indonesia, Aru Islands and S. PNG	Continental	*Chaetodontoplus duboulayi*	10384.88526
Indo-Aust plate	S. GBR to central NSW; New Caledonia, Lord Howe, Norfolk Island	Continental; coastal	*Chaetodontoplus conspicillatus*	11924.14691
East Pacific	Baja California, Sea of Cortez to coastal Peru; offshore islands of Revillagigedos and Galapagos	Continental; oceanic	*Pomacanthus zonipectus*	13176.07972
East Pacific	E.Pacific: Gulf of California to Ecuador and Galapagos Is	Continental; oceanic	*Holacanthus passer*	13483.93205

Table 2.8. Harvested endemic species belonging to the Pomacanthidae family (Allen et al. 1998; UNEP et al. 2002a). ** in Allen et al, 1998.

Species	**Endemic type**	**Geographic distribution**	**Country of export**	**Region**
Centropyge potteri	A	Hawaii (common), rare at Johnston.	Hawaii	West Pacific
C. fisheri	A	Hawaiian Islands and Johnston (rare).	Hawaii	West Pacific
Apolemichthys xanthopunctatus	C	Kiribati, Kapingamarangi, east Caroline Is.	Hawaii	Central Pacific
Paracentropyge boylei known as Centropyge boylei in GMAD	A	Cook Islands	Cook Islands	Central Pacific
Centropyge narcosis	A	Cook Islands	Cook Islands	Central Pacific
C. ferrugata	C	From S. Japan to the Philippines. Common at Okinawa.	Philippine, Indian Ocean, Unknown	East Indies
C. shepardi	C	Mariana and Bonin Islands, extending north to Izu Islands, Japan.	Guam	East Indies
C. interrupta	C	Japan’s southern coast; to NE Hawaiian Is (Midway & Kure)	Maldives	East Indies
Chaetodontoplus septentrionalis	B	Southern China and Taiwan, north to S. Japan.	Unknown	East Indies
C. caeruleopunctatus	B	Philippine archipelago	Philippines	East Indies
Genicanthus semifasciatus	B	N. Philippines, Taiwan, Ryuku and Izu and S. Japan.	Indian Ocean, Unknown	East Indies
Chaetodontoplus conpicillatus	C	S. Coral sea, S. GBR, central NSW, New Caledonia, Lord Howe, and Norfolk.	Australia	Indo-Australian plate
C.duboulayi	C	Norhern parts of Australia, including GBR.	Australia	Indo-Australian plate
C. personifer	A	Western Australia.	Australia	Indo-Australian plate
Pomacanthus zonipectus	C	Baja California, Sea of Cortez, to coastal Peru. Galapagos and Revillagigedos.	Mexico, Central America, Sea of Cortez, Unknown.	East Pacific
Holacanthus clarionensis	A	Clarion (common), other islands of Revillagigedos Gp, Clipperton Is (lesser extent), S. tip of Baja California.	Mexico, Central America	East Pacific
Holacanthus passer	C	Baja California, Sea of Cortez, to coastal Peru. Galapagos and Revillagigedos.	Mexico, C. America, Unknown	East Pacific
Apolemichthys xanthotis	B	Red Sea, Gulf of Aden, Oman, and Arabian Gulf.	Yemen	Indian Ocean
A. xanthurus	B	Mauritius, Maldives, Sri Lanka and east coast of India.	Red Sea	Indian Ocean
Centropyge flavipectoralis	B	Maldives, Sri Lanka, east coast of India.	Maldives, Sri Lanka	Indian Ocean
C. joculator	A	Cocos Keeling & Christmas Is.	W. Australia	Indian Ocean
Genicanthus caudovittatus	C	East African coast, from Mozambique northwards to the Red Sea, Mauritius.	Philippines, Saudi Arabia, Red Sea	Indian Ocean
Pomacanthus asfur	B	Red Sea, Gulf of Aden, south to Zanibar.	Sri Lanka, Saudi Arabia, Bahrain, Yemen, Red Sea	Indian Ocean
P. maculosus	C	Red Sea, Gulf of Oman (common near Muscat), Arabian Gulf.	Sri Lanka, Bahrain, Saudi Arabia, Yemen, Africa, Red Sea	Indian Ocean
Centropyge aurantonota		Lesser Antiles, Curacao, Barbados, St. Lucia, to Brazil.	S. America, Brazil, Unknown.	Atlantic Ocean
Holacanthus berrnudensis	B	E.coast of N.America (N.Carolina to S.Florida), Bahamas, Bermuda, Gulf of Mexico	USA	Atlantic Ocean
H. africanus	B	West African coast between Senegal and the Congo. Common off coast of Ghana.	W. Africa	Atlantic Ocean

Table 2.9. Spawning seasonality and lunar periodicity in pomacanthids.

Species	**Lunar periodicity**	**Spawning season**	**Region of study**	**Latitude**	**End or wide**	**Reference**
C. bicolor	-	Not complete	Australia	Tropical	Widespread	Bauer & Bauer, 1981
C. interruptus	-	May-October	S. Japan	Subtropical	Endemic type c	Moyer & Nakazono, 1978
C. potteri	Full moon phases	Dec-May	Hawaii	Tropical	Endemic type a	Lobel, 1978
C. flavissimus	Full moon phases	Not complete	Enewetak, Marshall	Tropical - Equatorial	Widespread	Lobel, 1978
C. flavissimus	Semi-lunar non-spawning period	Not complete	Enewetak, Marshall	Tropical - Equatorial	Widespread	Thresher, 1982
C. flavissimus	-	Not complete	Tahiti	Tropical	Widespread	Bauer & Bauer, 1981
C. ferrugata	-	early May-mid November	Okinawa, S. Japan	Subtropical	Endemic type c	Sakai, 1996
C. loriculus	Semi-lunar non-spawning period	Not complete	Enewetak, Marshall	Tropical - Equatorial	Widespread	Thresher, 1982
C. bispinosa	Semi-lunar non-spawning period	Not complete	Enewetak, Marshall	Tropical - Equatorial	Widespread	Thresher, 1982
C. tibicen	-	??				Thresher, 1982
C. argi	-	Not complete	Curacao, Cayman,	Tropical	Caribbean	Moyer et al, 1983; Bauer & Bauer, 1981
P. imperator	Semi-lunar non-spawning period	Not complete	Enewetak, Marshall	Tropical - Equatorial	Widespread	Thresher, 1982
P. arcuatus	-	Feb-June, peak in March	Oceanic banks & Jamaica	Tropical	Carribean	Munro et al, 1973
P. paru	-	May, incomplete	Oceanic banks & Jamaica	Tropical	Carribean	Munro et al, 1973
P. diacanthus	Semi-lunar non-spawning period	Not complete	Enewetak, Marshall	Tropical - Equatorial	Widespread	Thresher, 1982
H. tricolor	-	Feb & May, April	Oceanic banks & Jamaica respectively	Tropical	Carribean	Munro et al, 1973
H. tricolor	8 days before full moon	Not complete	St Croix, Caribbean	Tropical	Carribean	Neudecker & Lobel, 1982
H. tricolor	-	Spawned most of year	Puerto Rico, Caribbean	Tropical	Carribean	Moyer et al, 1983
H. ciliaris	-	Jan-Aug, peak in April; no sample in March & Nov	Oceanic banks & Jamaica	Tropical	Carribean	Munro et al, 1973
H. passer	No lunar: based on 3 wk observation in Moyer	Females: June-Oct; males: April-Nov	Gulf of California	Tropical/subtropical	Endemic type c	Arellano-Martinez et al, 1999; Moyer et al, 1983

Figure 2.1. Maximum size range of Pomacanthidae according to genera showing lower, upper and middle quartiles and outliers (o and *).

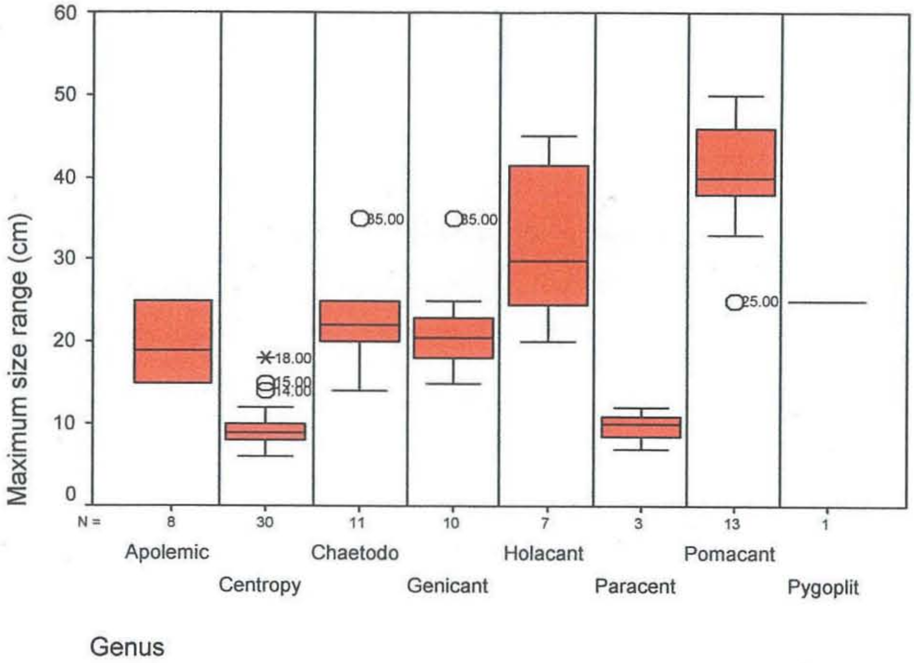


Figure 2.2. Box plot showing median depth ranges of Pomacanthids according to genus, showing lower, upper and middle quartiles and outliers (o and *).

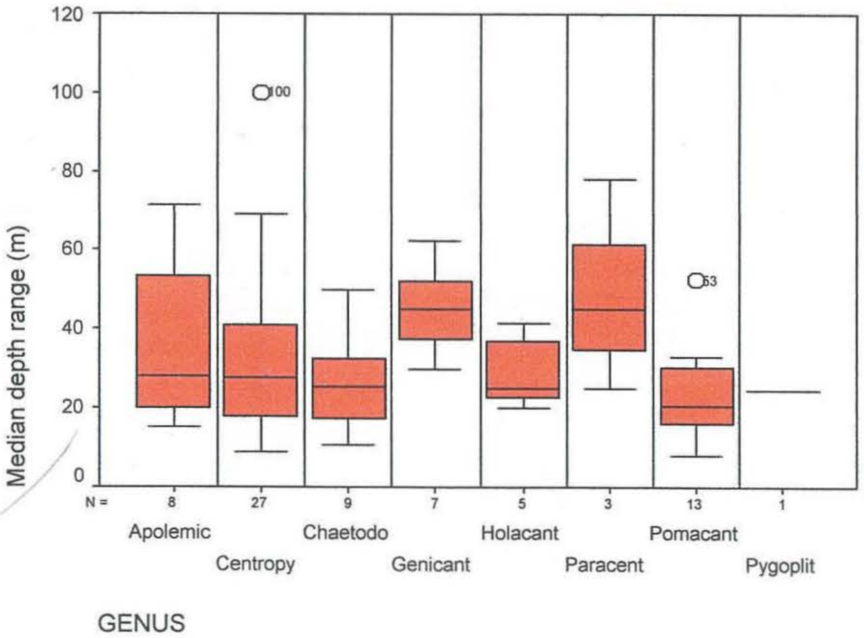


Figure 2.3. Phylogenetic tree of 24 chosen species of Family Pomacathidae relative to the Family Chaetodontidae. Figure modified from Bellwood et al (2004).

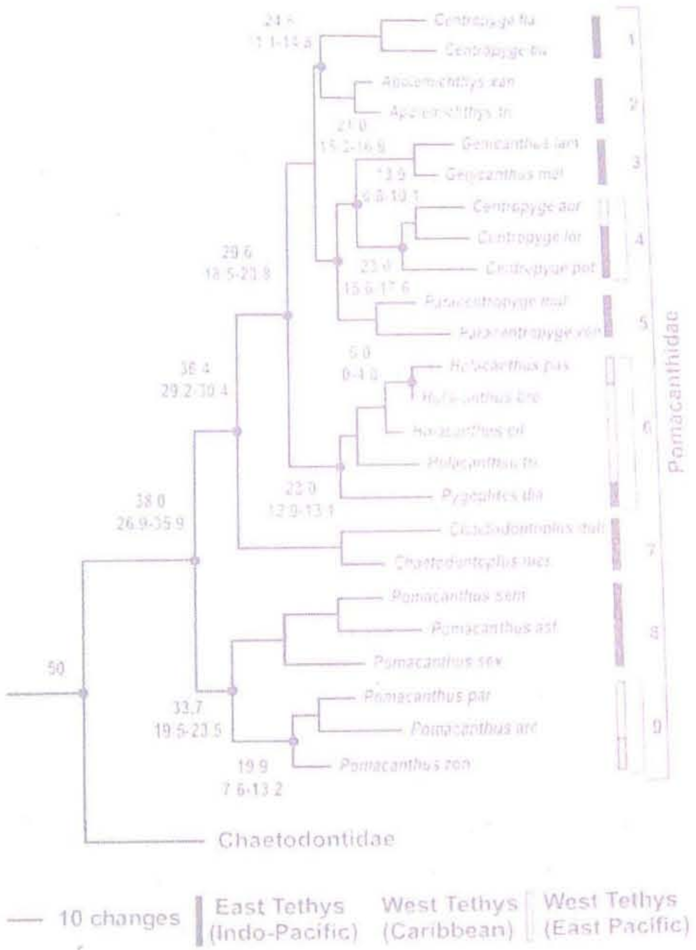


Figure 2.4. Species density contour map of the family Pomacanthidae. Colored lines represent level of species diversity (see keys). Values represent number of species.

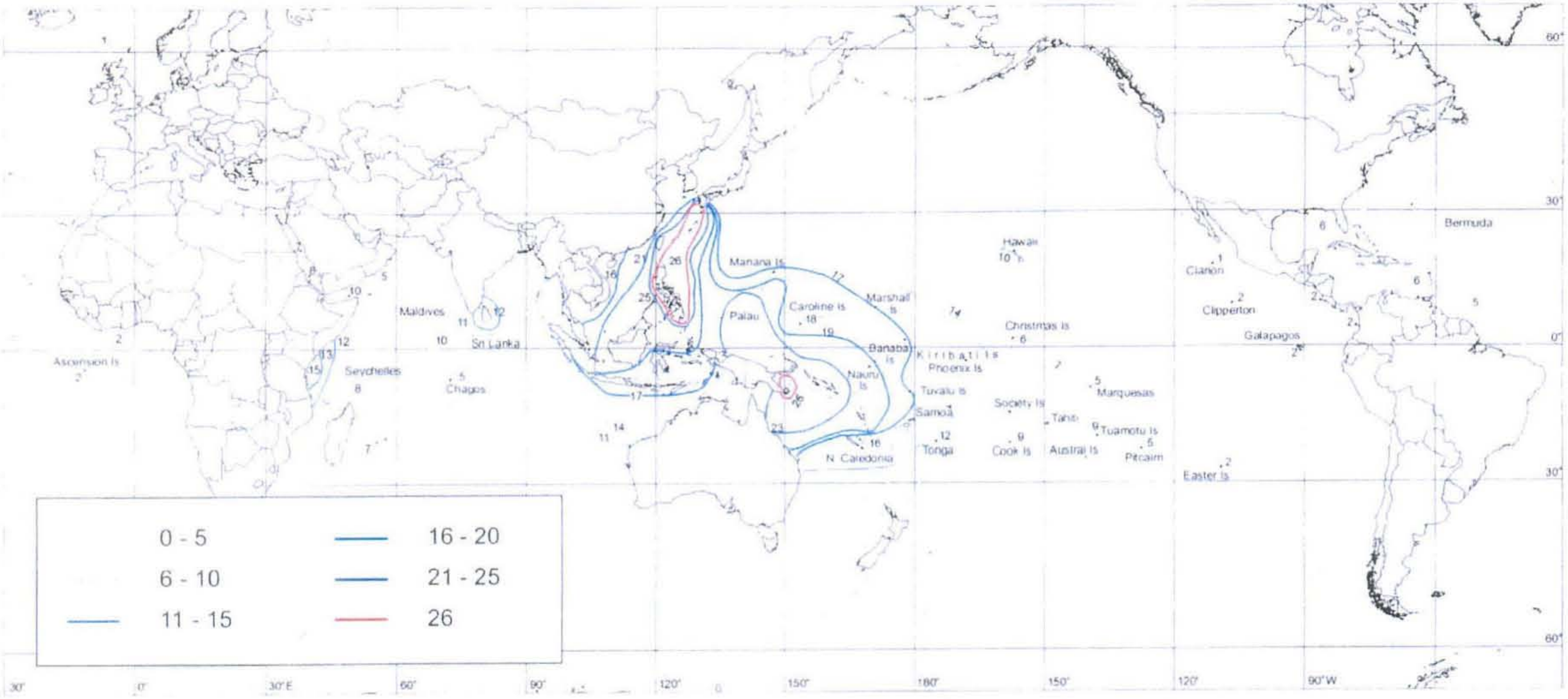


Figure 2.5. Geotectonic features of the world (dashed lines denote margins of major lithorspheric plates; arrows indicate direction of plate movements and subduction along deep-sea trenches; heavy dark lines denote deep-sea trenches; dotted lines indicate prominent inactive ridges in Indian Ocean (Springer 1982).

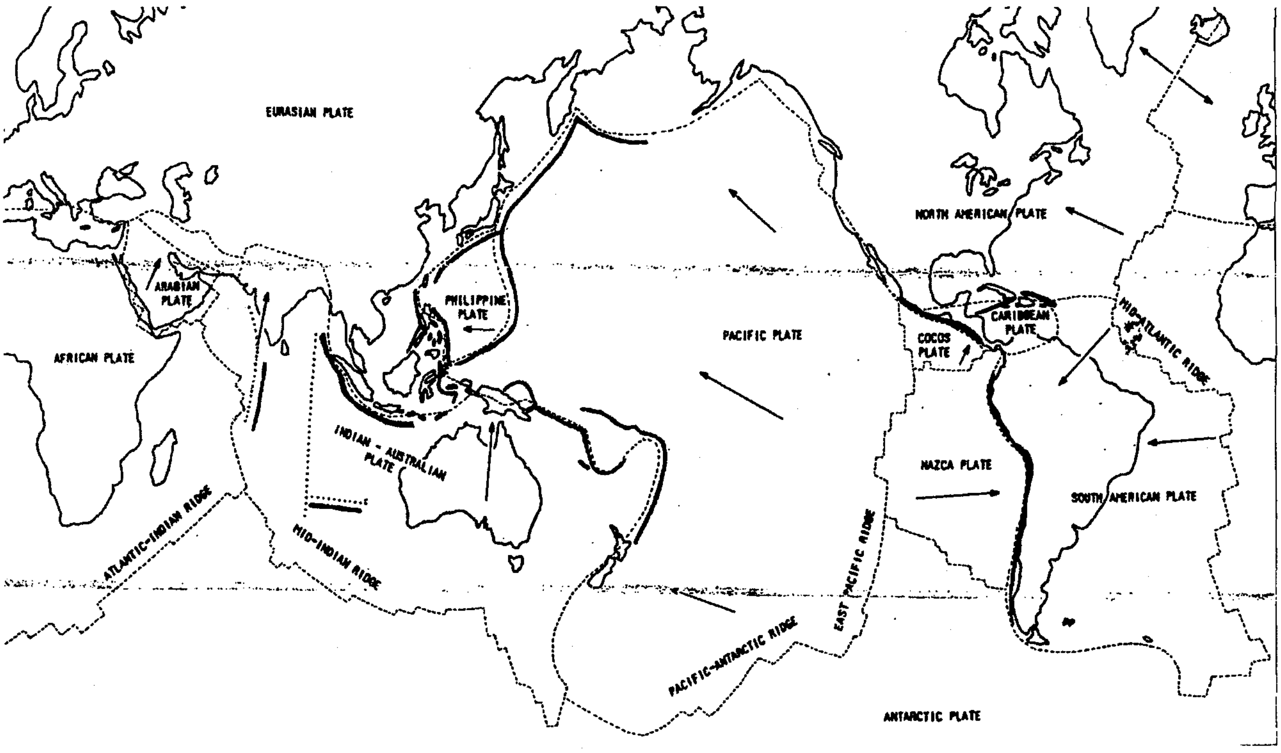


Figure 2.6. Frequency distribution of pomacanthid species according to distribution range categories.

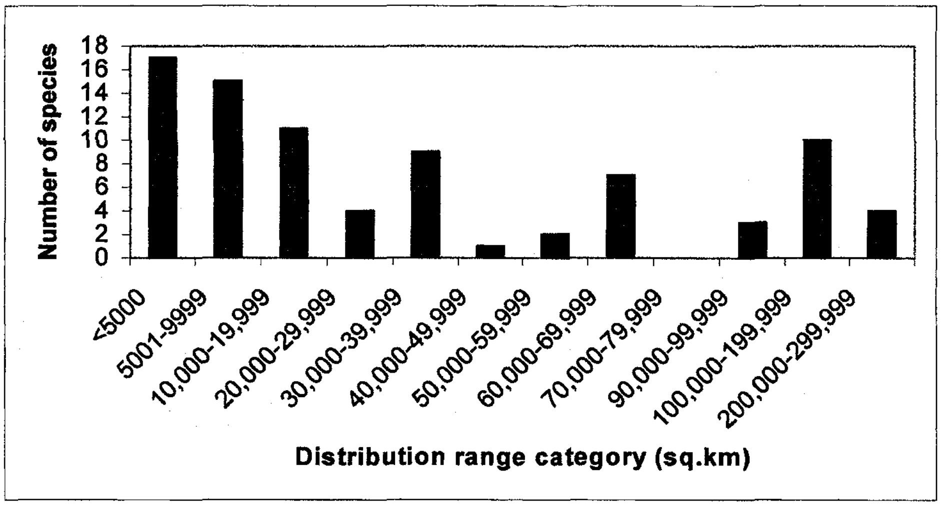


Figure 2.7. Distribution of type a endemic pomacanthids species. Lines indicate distribution ranges of chosen species. Numbers represent pomacanthids (see key).

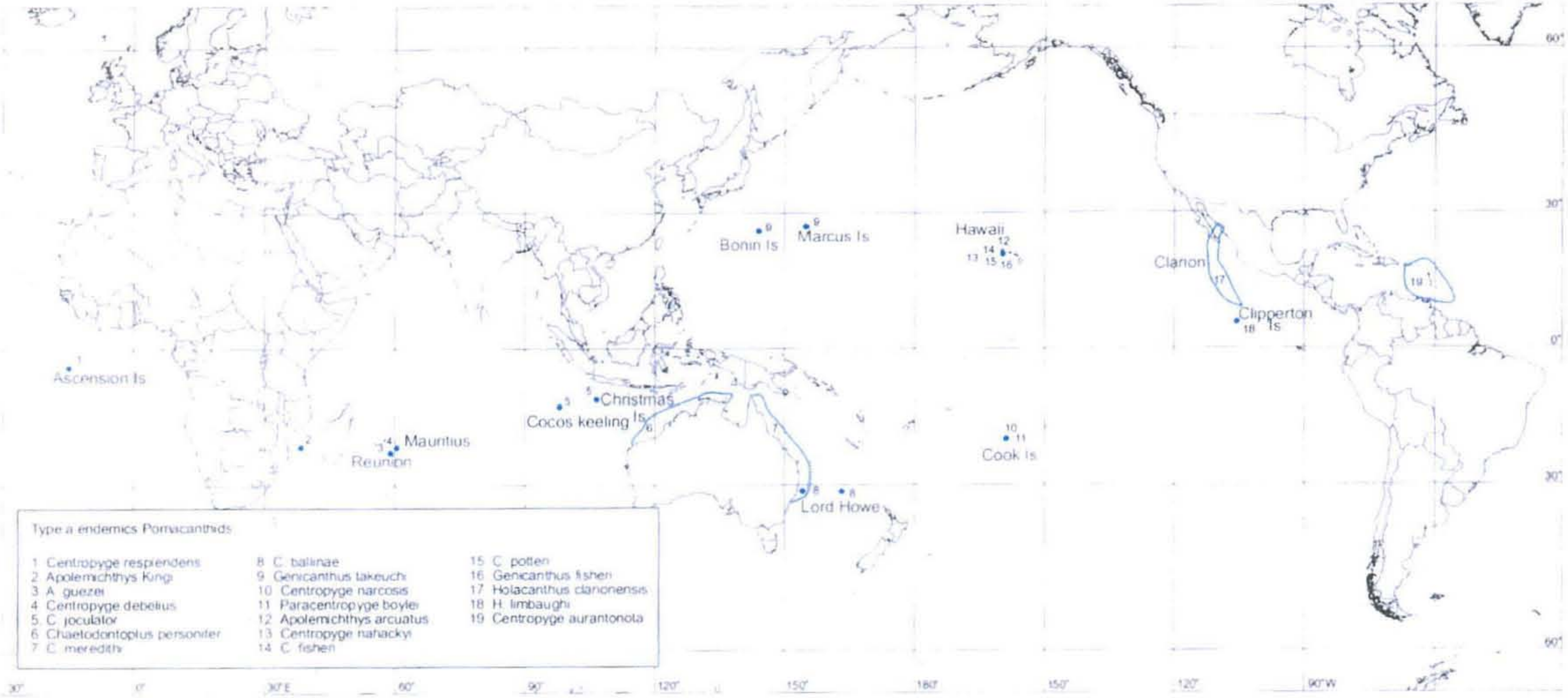


Figure 2.8. Distribution of type b endemic pomacanthid species. Lines indicate distribution range of chosen species. Numbers represent pomacanthid species (see key).

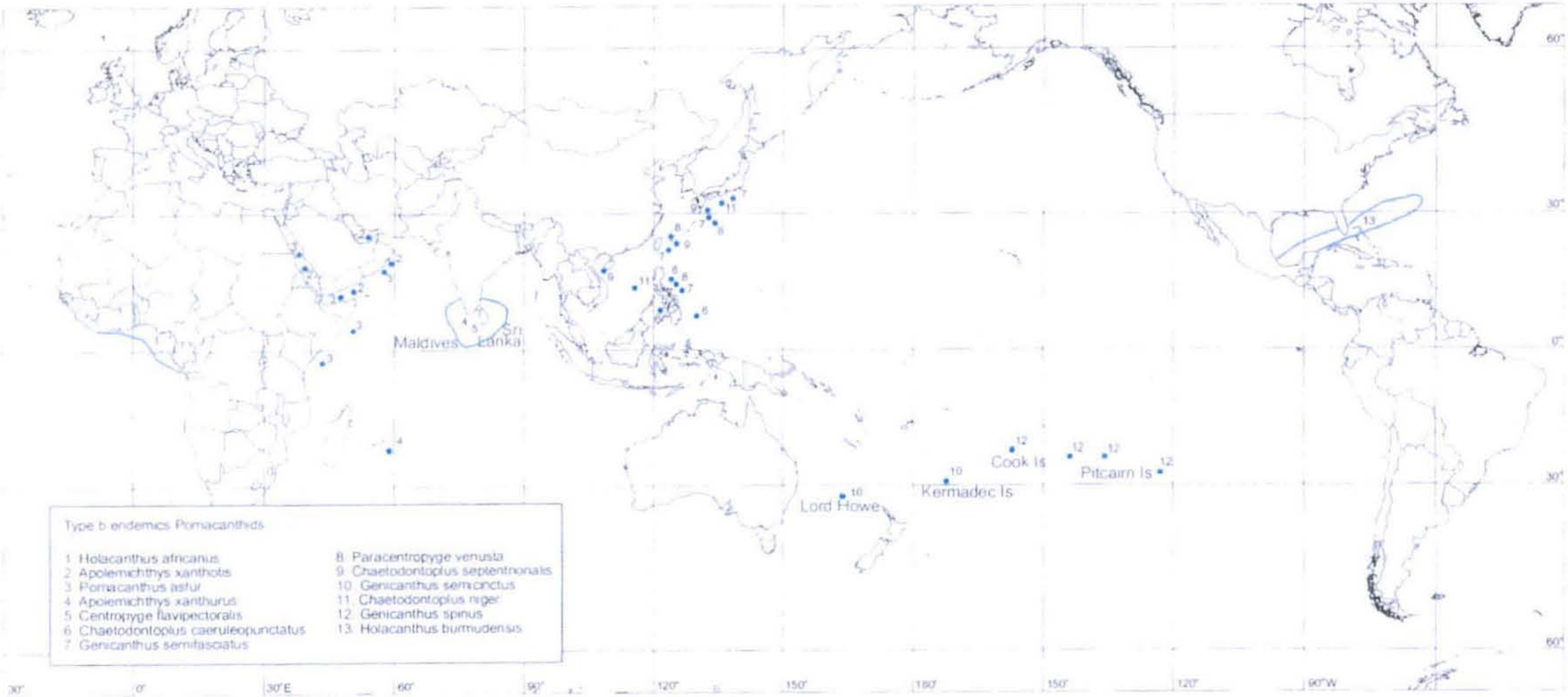


Figure 2.9. Distribution of type c endemic pomacanthid species. Lines indicate distribution ranges of chosen species. Numbers represent species (see key).

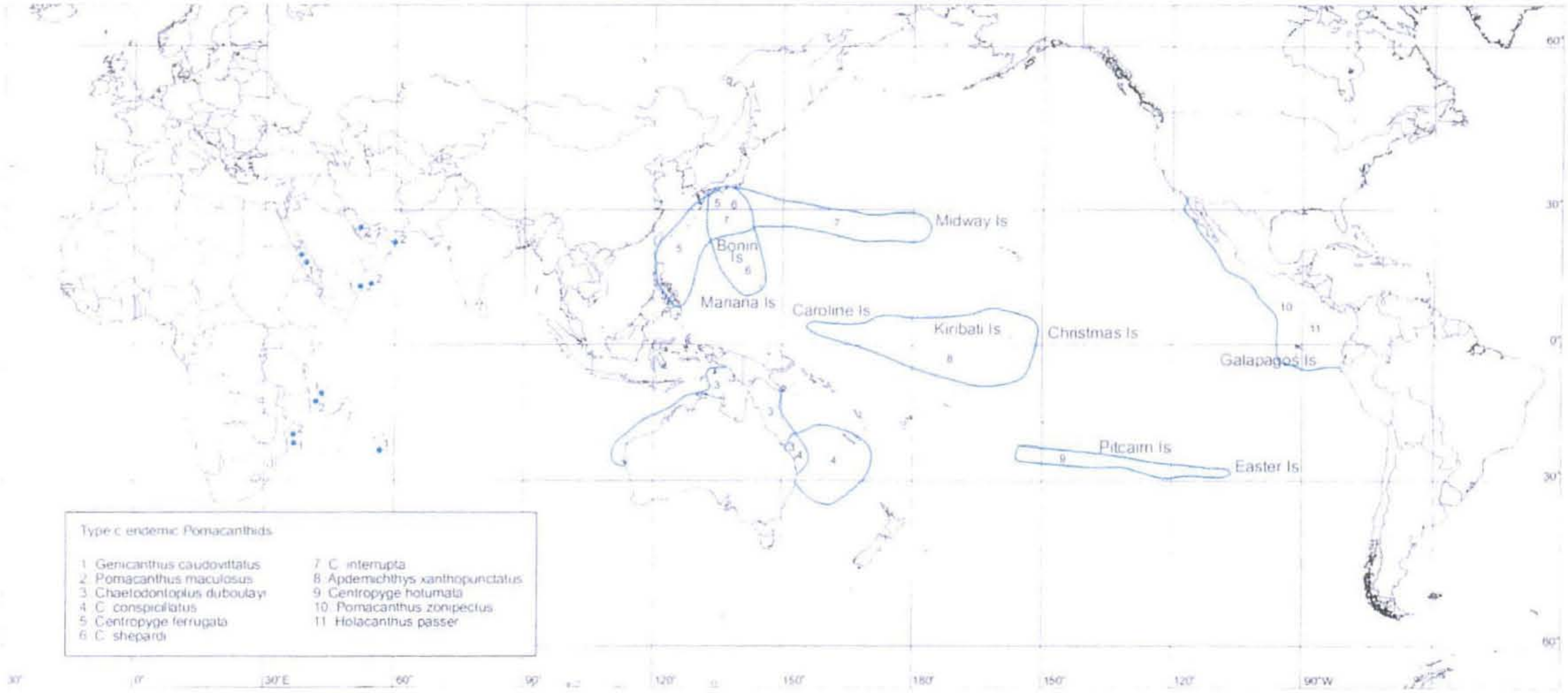


Figure 2.10. Histogram of total area of shallow water substrate (SWS) moving longitudinally away from the center of diversity within the Pacific Ocean (Source of area SWS: Becker 1999).

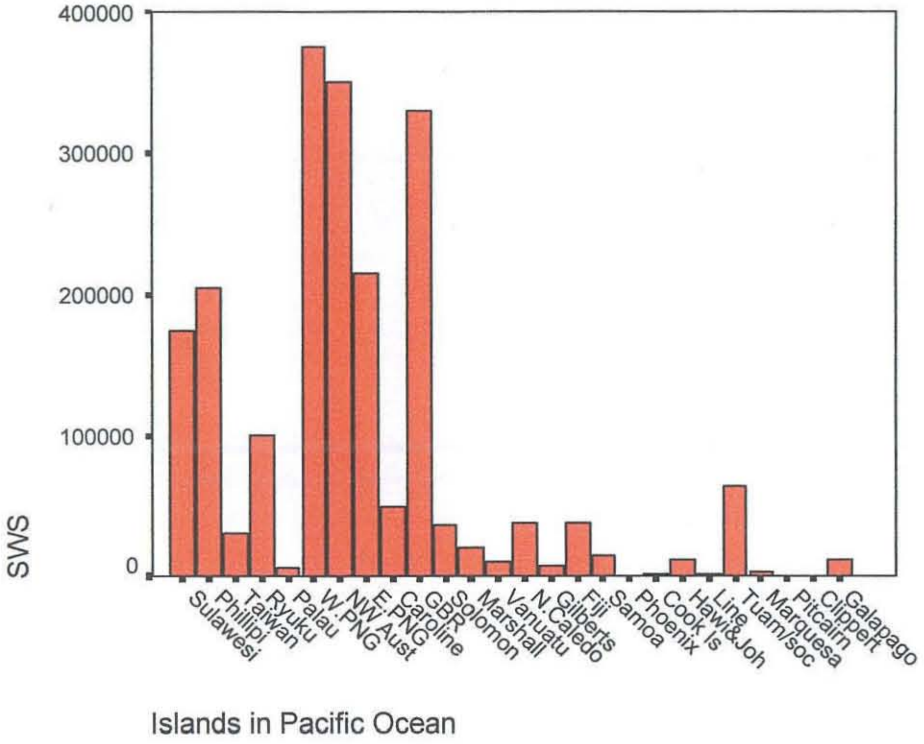


Figure 2.11 Frequency distribution of pomacanthid species moving longitudinally away from the center of diversity in the Pacific Ocean.

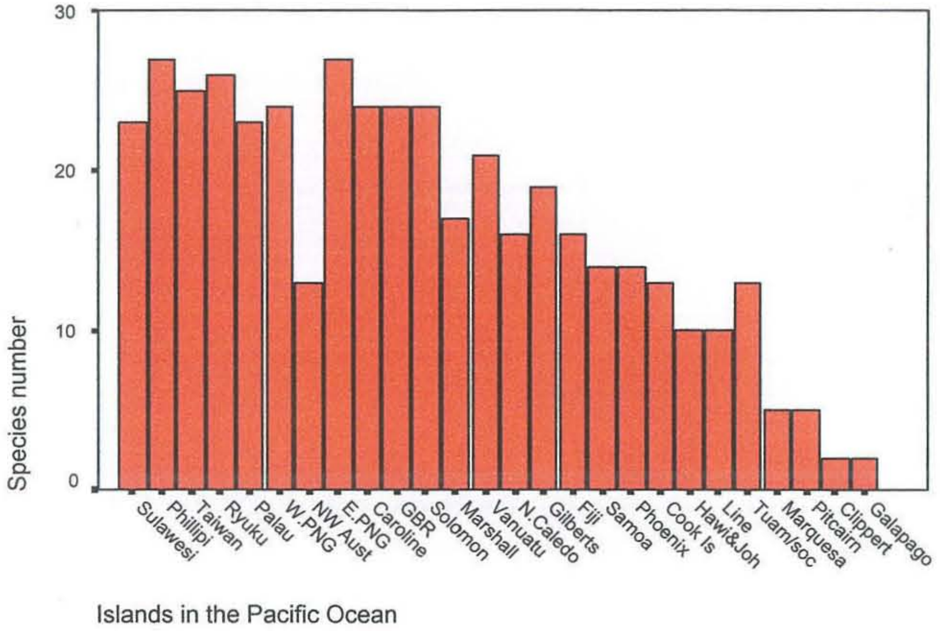


Figure 2.12. Number of pomacanthid species per unit area of shallow water substrate (SWS) moving longitudinally away from the East Indies in the Pacific Ocean.

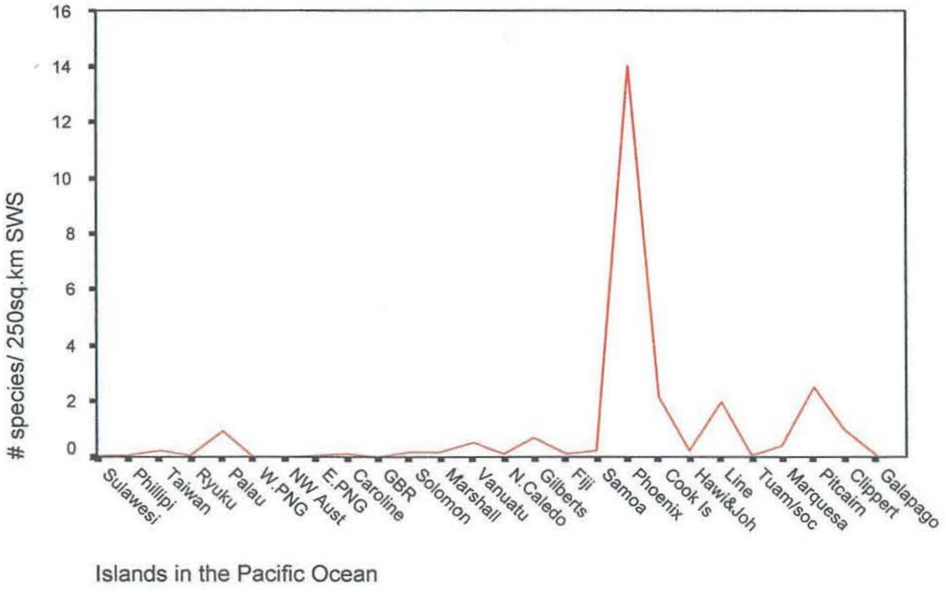


Figure 2.13. Relative % of endemism (combined) in Pomacanthid fauna within each island moving longitudinally away from the East Indies in the Pacific Ocean.

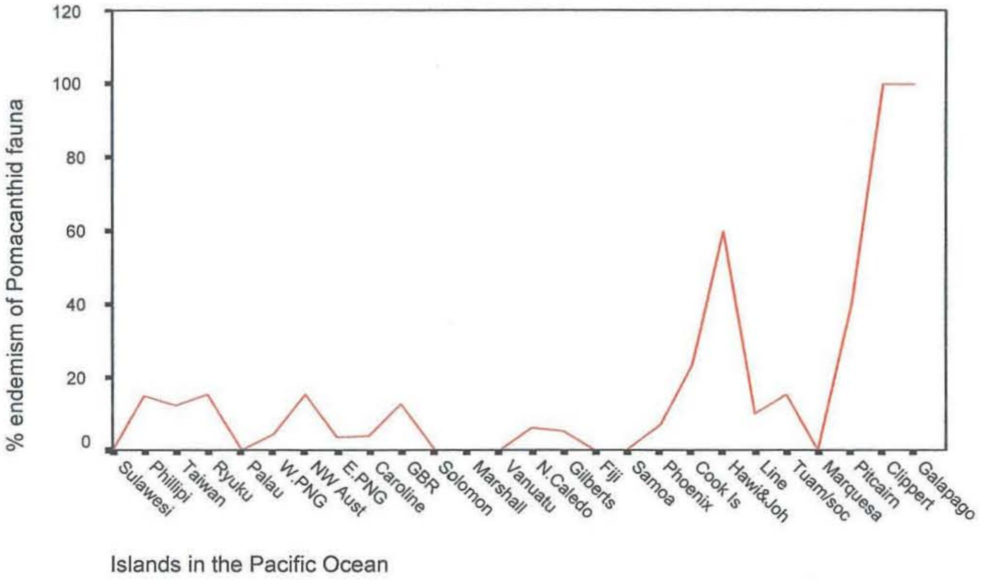
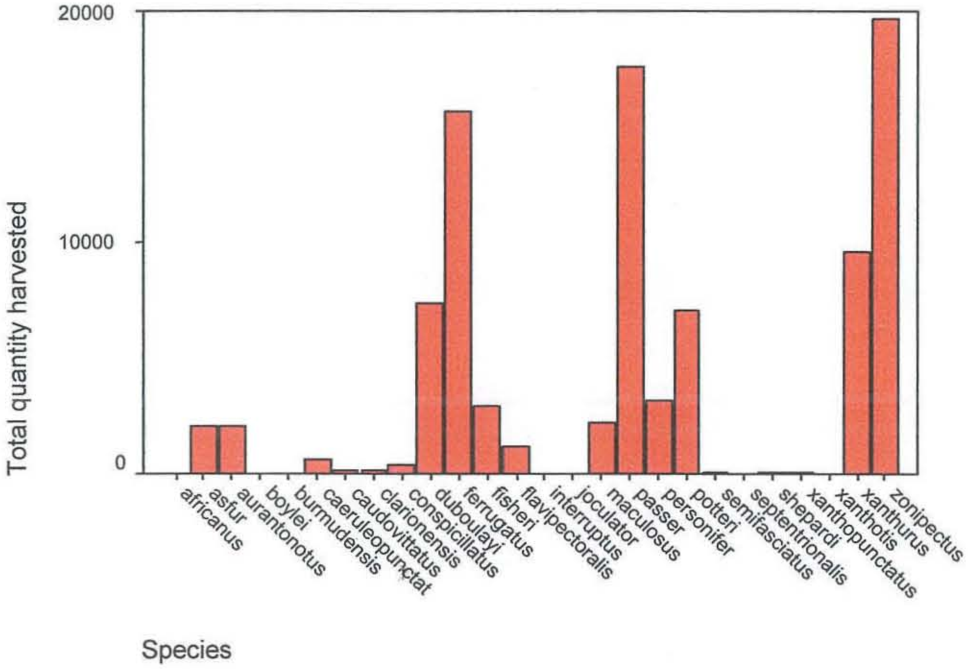


Figure 2.14. Total harvest of Pomacanthid endemics in the GMAT between 1988 to 2001 (Source of data: UNEP et al. 2002b: http:www.unep-wcmc.org/marine/GMAD/).



CHAPTER 3. GROWTH AND LONGEVITY IN THREE ANGELFISH SPECIES

3.1. INTRODUCTION

Estimation of growth patterns and maximum longevity are an important initial step in the investigation of recruitment rates and resilience to mortality (Ferreira and Russ 1992; Choat et al. 2003). This is especially crucial in fishes characterized by demographic features that make them more susceptible to exploitation. These include long-lives, which are usually associated with delayed maturity, low rates of natural mortality and recruitment (Jennings et al. 1996 in Russ and Alcala 1998) (refs in Newman et al. 1996). Collection of such information is however biased towards common coral reef fishes such as lutjanids (Newman et al. 1996; Kritzer 2002), serranids (Ferreira and Russ 1992; Adams and Williams 2001), acanthurids (Choat and Axe 1996), damsel fish (Meekan et al. 2001) and scarids (Choat et al. 1996; Choat and Robertson 2002; Gust et al. 2002). Susceptibility of fishes that are also characterized by rarity both globally and locally such as in some angel fish is unclear but may be more pronounced. Despite their high exploitation in the Global Marine Aquarium Trade (GMAT), the literature still lacks information even on growth and longevity in this family.

An extensive number of literature is available on pomacanthids, however these have focused on reproductive and social behaviour (Bauer and Bauer 1981; Neudecker and Lobel 1982; Thresher 1982; Moyer 1984; Moyer 1990), feeding (Hourigan et al. 1989; Sakai and Kohda 1995; Perez-Espana and Abitia-Cardenas 1996; Martinez-Diaz and Perez-Espana 1999) and phylogenetic relationships (Chung and Woo 1998; Bellwood et al. 2004). Maximum longevity is estimated to range from nine to 23 years in the smaller genus *Centropyge* (Eagle 1997) and may reach up to 60 years in the larger genus *Pomacanthus* (John Ackerman, pers. comm.). Other attempts to estimate longevity have been through scale analysis (Chung and Woo 1999) and calculated from mortality rates using a size-based approach (Aldenhoven 1986b), the accuracy of which are still questionable (Choat and Robertson 2002). There has been no further attempt to derive demographic features of this family using an age-based approach.

The present study explores the growth patterns of three angelfish species: two of which are common on the Great Barrier Reef: *Pomacanthus sexstriatus* and *Centropyge bicolor,* and one harvested in great amounts for the GMAT from Kiritimati Island, Kiribati in the Central Pacific: *C. loricula.* I examined size-at-age relationships and longevities with respect to sexual identity. Specifically I aim to answer the following questions:
i). What is the maximum lifespan?
ii). What is the form of the growth curve?
iii). Is there sex-specific patterns of growth and longevity?

3.2. MATERIALS AND METHODS

3.2.1. STUDY SPECIES

C. bicolor and *P. sexstriatus* are widespread species, distributed within the Indo-Pacific region, while *C. loricula* is limited to the West and Central Pacific (Allen et al. 1998). The *Centropyge* species are similar in having polygamous social systems consisting of one dominant large male and several smaller females arranged in size-dominant hierarchies, each male spawning with all or most females (Aldenhoven 1984; Moyer 1990). Very little is known of the social system of *P. sexstriatus*, however they have often been seen in pairs, one always larger than the other (Howard Choat personal observation, Thresher 1984). They have two color morphologies, when juvenile and adult and begin color transformation into the adult morphology at 8-15 cm, at which stage they are no longer cryptic in shallower protected reefs (Allen et al. 1998). They have relatively larger territories required for foraging habits (Neudecker 1985).

3.2.2. SAMPLE COLLECTION

A total of 69, 87 and 153 specimens of *P. sexstriatus*, *C. bicolor* and *C. loricula* respectively were collected. The majority of the samples of *P. sexstriatus* and all individuals of *C. bicolor* were collected by spearing between the 14th and the 21st of March 2002 from Lizard Island (145.27°E, 14.4°S,), and adjacent reefs in the Great Barrier Reef, Australia. Additional samples of *P. sexstriatus* were collected from reefs off Townsville (146.48°E, 19.13°S: 30th May to 1st June 2003), One Tree (152.3°E, 23.42°S: 14-16th April 2003), and again in Lizard Island (11-18th October 2003). Previous samples dating back to as early as 1996, 1997, and 1999 were also included (n = 3). *C. loricula* mortality individuals were bought off aquarium companies in Kiritimati Island (157.1°W, 1.42°N) in Kiribati between 11th July and 11th August 2003. Selective sampling in *P. sexstriatus* was limited to adults (distinguished by different morphological colour), due to the cryptic nature of juveniles making them difficult to collect. Sampling of *C. bicolor* and *C. loricula* provided representative estimates of most size classes. Size ranges acquired for each species were 130-400mm FL, 60-120 mm FL and 51-81 mm TL in *P. sexstriatus, C. bicolor* and *C. loricula* respectively.

Fish samples of *P. sexstriatus* and *C. bicolor* were stored on ice and processed in the laboratory within 6 hours of capture. Some samples of *P. sexstriatus* and all individuals of *C. loricula,* however were stored frozen for periods from 2 days to two months. Total and fork lengths were measured to the nearest millimeter. Both sagittal otoliths were removed from the otic capsules through sectioning of the skull, cleaned in distilled water and ETOH and stored dry. Gonads were removed and weighed to the nearest 10^{-2} g. Sex was determined initially upon macroscopic examination of gonads (before histology) and the tissue preserved in Formal Acetic Acid Calcium (FAAC).

3.2.3. OTOLITH PROCESSING

One of each pair of sagittae was chosen haphazardly and weighed to the nearest 10^{-4} g. Otoliths were transversely sectioned by grinding. The sagitta was mounted transversely in thermoplastic cement Crystal bond ™ on a slide edge and ground down to the nucleus with 800 grade for *P. sexstriatus* and 1200 grade abrasive paper for *C. loricula* and *C. bicolor*. The sagitta was then positioned on a second slide and the opposite side was ground down to the nucleus. Grinding was carried out on a modified lapidary grinding apparatus. To improve optical quality the polished sagitta section was covered with Crystal bond ™ prior to age estimation. Sectioned otoliths less than one years of age, were further polished with 3.0 and 9.0 μm lapping film until sub-annual rings were visible under high-power microscope. Slides were remounted with Crystal bond $^{\text{TM}}$ thermoplastic cement.

3.2.4. AGEING FROM ANNUAL OTOLITH INCREMENTS

No validation on annual sagittal increments within the Pomacanthidae family has been done, however with confirmation of the annual nature of sagittal increments from a variety of coral reef fishes ((Acanthuridae) Choat and Axe 1996; (Scaridae) Choat et al. 1996; (Lutjanidae) Newman et al. 1996; (Serranidae) Adams 2002), it was assumed that sagittal increments of alternating opaque and translucent bands were annual rings. Counting of annual otolith increments was done under a stereomicroscope at 3x magnification for *C. bicolor* and *C. loricula* and 2x magnification for *P. sexstriatus* under transmitted light. Age was estimated after agreement of two independent counts, counted on different days. A third count was done on disagreement of the initial two counts, upon which a match was accepted. If there was still a difference, counting was repeated again.

3.2.5. AGEING FROM DAILY OTOLITH INCREMENTS

Daily otolith increments were counted under high power microscope for individuals younger than one year old. Each sub annual ring was assumed to represent daily rings. Three independent counts were accepted if they were within 10% of each other. They were averaged and divided by 365 days to give an age in years. This was done for the two *Centropyge* species only.

3.2.6. GONAD HISTOLOGY

A total of 64, 84 and 144 gonads of *P. sexstriatus, C. bicolor* and *C. loricula* respectively were used for histology. Transverse sections were dehydrated overnight and embedded in paraffin wax the next morning. Embedded gonads were sectioned at 5µm on a micrometer and mounted on glass slides, then stained with Mayer’s alum haemotoxylin and Young’s eosin-erythrosin stains. Each section was examined under a high-powered microscope to determine the sexual identity of the gonad of each individual.

3.2.7. ANALYSIS OF DATA

3.2.7.1. GROWTH TRAJECTORIES

The von Bertalanffy Growth Function (VBGF) gave the best model fitted to size at age data for all species. The von Beratalanffy growth function had an advantage of being easily constrained to take into account younger fish usually absent from datasets (Meekan et al. 2001) providing “biologically tenable” growth parameter estimates (Grandcourt 2002).

The VBGF equation is:

$L_t = L_\infty * (1 - e^{-k(t-to)})$;

where L_t = length at time, t; L_∞ = mean asymptotic length; k = rate at which the curve approaches asymptote (or curvature coefficient); t_o = theoretical age at which length is zero. To examine growth patterns in each species, pooled size at age data were fitted to the VBGF. Similarly, to examine sex specific growth, male and female size at age data were fitted into the VBGF growth trajectories for all species. Due to sensitivity of VBGF growth parameters to absence of young juveniles (Kritzer 2002), intercepts of all growth trajectory curves were constrained to confamiliar size at settlement L_0 (length at age 0)of 24.6 mm (Leis and Rennis 1983) in all study species. In the *P. sexstriatus* sample, all undifferentiated and immature bisexual individuals were incorporated in both sex specific data as they were distributed at ages younger than male and female differentiation. This allowed a better fit on the VBGF curve.

3.2.7.2. SEX-SPECIFIC MEAN SIZE AT AGE

To examine if mean size at age was different between male and female, a one-way analysis of variance ANOVA was performed on the mean body length of both sexes where VBGF mean asymptotic body lengths had been reached. Age ranges at which mean body length comparison was performed was therefore limited to ages where both sexes existed. Accordingly, ANOVA was performed on the mean body length between age ranges 10-41, two-10 and two-five years old in *P. sexstriatus, C. bicolor* and *C. loricula* respectively.

3.2.7.3. SEX-SPECIFIC MAXIMUM LONGEVITY AND BODY LENGTH OF OLDEST INDIVIDUALS

Mean maximum longevity (mean T_{max}) was calculated as the average age of 10% of the oldest individuals (as done in Choat and Robertson 2002; Laman Trip 2004). To examine sex-specific differences in mean T_{max}, confidence intervals around this parameter was calculated using a bootstrapping method (Haddon 2001) for both male and female populations. This involved resampling 1000 times with replication maintaining original sample size (n) of both male and female population, where n represents the actual sample size of the oldest 10% of a population. This analysis for male and female populations resulted in 1000 values of mean T_{max} (modified from method used by Laman Trip 2004). Subsequently estimates of mean T_{max} were bias corrected by subtracting the mean of the bootstrap estimates from the original mean T_{max} (Haddon 2001). Confidence intervals were calculated as: CI = 1.96*SE of bootstrapped estimates (Haddon 2001). To examine differences in sex specific mean T_{max}, mean parameter values were plotted with confidence intervals and compared visually.

A comparison of sex-specific body length within the mean T_{max} was performed using the same method as done to mean T_{max}. This involved resampling 1000 times body lengths within both male and female populations while maintaining original sample size (n), n being the actual sample size of the oldest 10% of each population. This resulted in 1000 values of mean body lengths within the mean T_{max}. Mean body lengths of T_{max} were bias corrected for by subtracting the mean of bootstrapped estimates from the original mean of body length of mean T_{max}. Confidence intervals was then calculated as: CI = 1.96*SE of bootstrapped estimates (Haddon 2001). To examine any difference in the body length of the oldest male and female individuals, the mean parameter values were plotted for both sexes with confidence intervals and compared visually.

3.3. RESULTS

Size at age plots illustrated firstly a large range of maximum lifespan between the three study species (Figure 3.1). The large angel fish, *P. sexstriatus* are longer lived, reaching maximum lifespan of 58 years, while the smaller species, *C. bicolor* and *C. loricula* are relatively shorter lived, at 17 and 8 years respectively. Secondly, the three study species displayed asymptotic growth curves, in which most growth occurred early in its life history, so that after reaching asymptotic growth, size is no longer a good predictor of age. Mean asymptotic body lengths of 291.73, 99.54 and 72.45 mm were estimated for *P. sexstriatus*, *C. bicolor* and *C. loricula* respectively (Table 2.1). The time period at which asymptotic lengths are reached is positively related to the maximum lifespan a species has, so that a mean asymptotic length is reached after 20 years in the longer-lived *P. sexstriatus* (Figure 3.1) but after two years in the shorter-lived *Centropyge* species. Lastly, there was very high variation in the size at age plots around the fitted VBGF curves. This was very obvious in *P. sexstriatus*.

Gonad histology revealed an unusual ontogeny of sexual differentiation in *P. sexstriatus*, in which sexual identity (male or female) is established as late as 10 years of age. Sexual differentiation early in life initiates as undifferentiated and immature bisexuals, where immature bisexuals persist for an extended period of up to 12 years. One immature bisexual was also found in *C. loricula*, however there was no evidence that this initiated sexual differentiation, as female differentiated at an earlier age. Females initiate sexual differentiation in both *C. bicolor* and *C. loricula*, after which males are recruited at a later age of 2 years and 1 year old respectively. Partitioning of size at age plots by sex displayed a greater fit to the VBGF curves in the three species (Figure 3.2). Size at age plots by sex displayed evidence of sex-specific differences in mean body length, where males reached greater mean asymptotic body lengths in the three study species. Mean asymptotic length was reached between two and three years in both males and females of *C. bicolor* (Figure 3.2 b) and *C. loricula* (Figure 3.2 c). However, growth differed between sexes in *P. sexstriatus* (Figure 3.2 a). Mean asymptotic body length was reached earlier in females at 9 years of age, while male growth was still increasing at a minimal rate, and started to asymptote after 20 years of age. Mean body length of males was significantly higher than those of females in

P. sexstriatus (age ranges 20-41 years, F = 26.76, P = 0.000), *C. bicolor* (age ranges 2-10 years: F = 24.4, P = 0.00) and *C. loricula* (age ranges 2-5 years: F = 150.96, P = 0.000).

Differential mean maximum longevity, T_{max}, between sexes was observed in the three study species, where males were always older than females (Figure 3.3a-c). The mean body length of individuals within the T_{max}, was also directed towards larger males compared to females in two of the study species, *P. sexstriatus* (Figure 3.3 I) and *C. loricula* (Figure 3.3 III). Mean body length of the oldest individuals in *C. bicolor* was however similar between males and females (Figure 3.3 II).

Figure 3.1. Size at age plots with fitted von Bertalanffy growth function (VBGF) curves for study species: *P. sexstriatus*, *C. bicolor* and *C. loricula*. Length intercept of the growth function constrained: length at age 0, L_0 = 24.6 mm for all species.

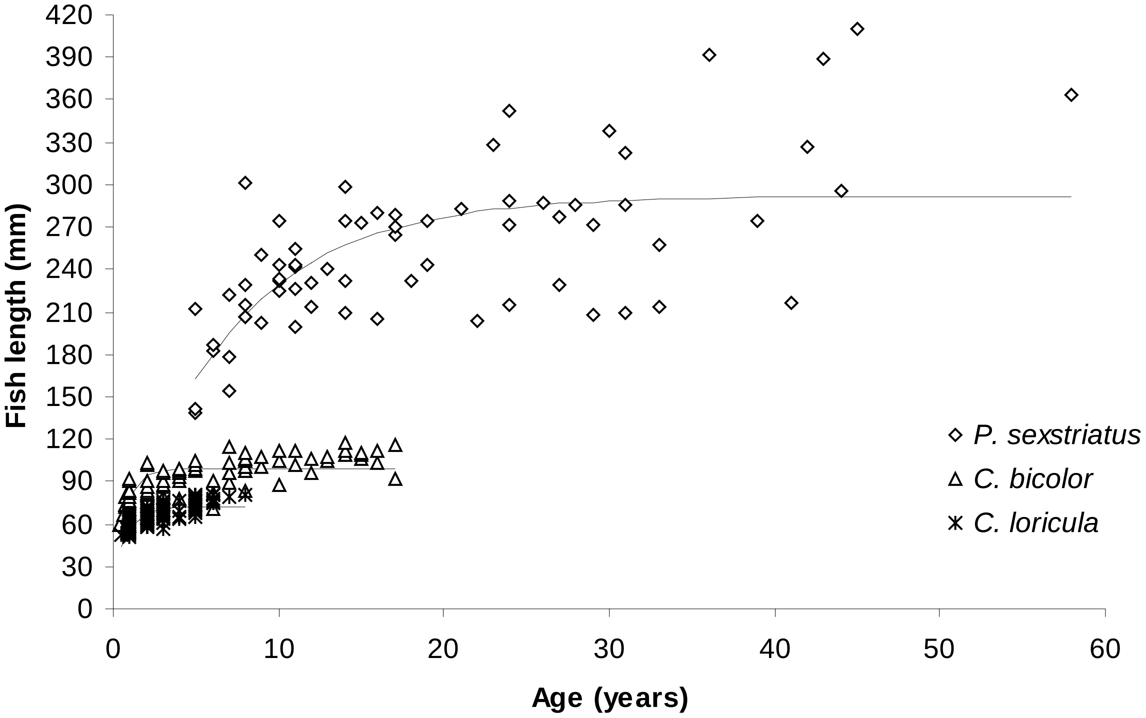
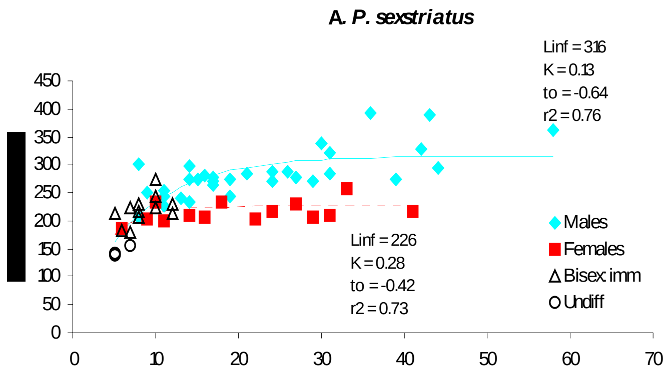
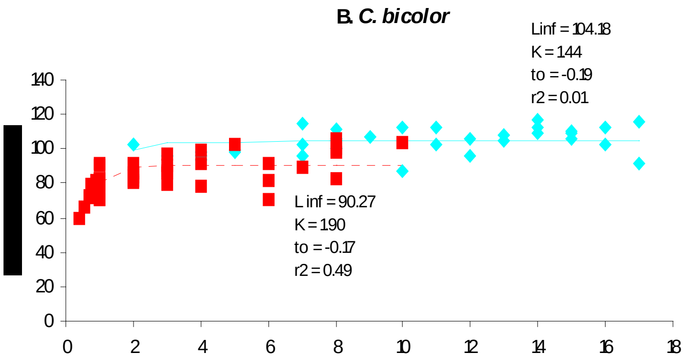


Table 3.1. Von Bertalanffy parameter estimates of size at age data for study species. L_{inf} = mean asymptotic body length, k = coefficient of growth, t_o = age of fish at length 0, r^2 = coefficient of determination, n = sample size. Lengths are FL.

VBGF parameter estimates	***P. sexstriatus***	***C. bicolor***	***C. loricula***
L_{inf}	291.73	99.54	72.45
K	0.15	1.32	1.10
t_o	-0.61	-0.21	-0.38
r^2	0.41	0.54	0.56
N	69	87	153
Body length range (mm)	130-400 FL	60-120 FL	51-81 TL

Figure 3.2. Sex-specific size at age plots with fitted von Bertalanffy growth function (VBGF) curves for *P. sexstriatus* (A), *C. bicolor* (B) and *C. loricula* (C). Length intercept of the growth function constrained: length at age 0, L_0 = 24.6 mm for both sexes. VBGF parameters shown for each sex.





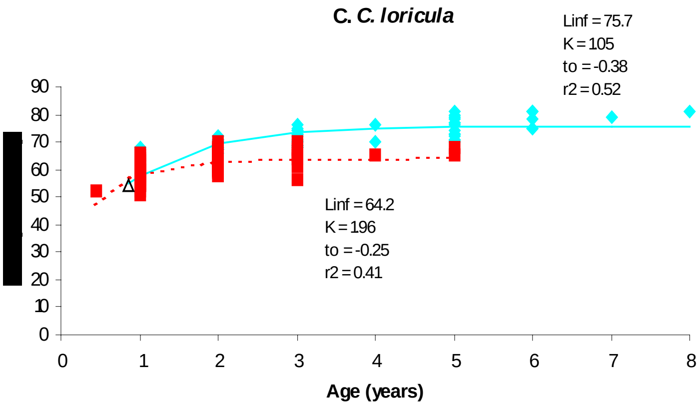
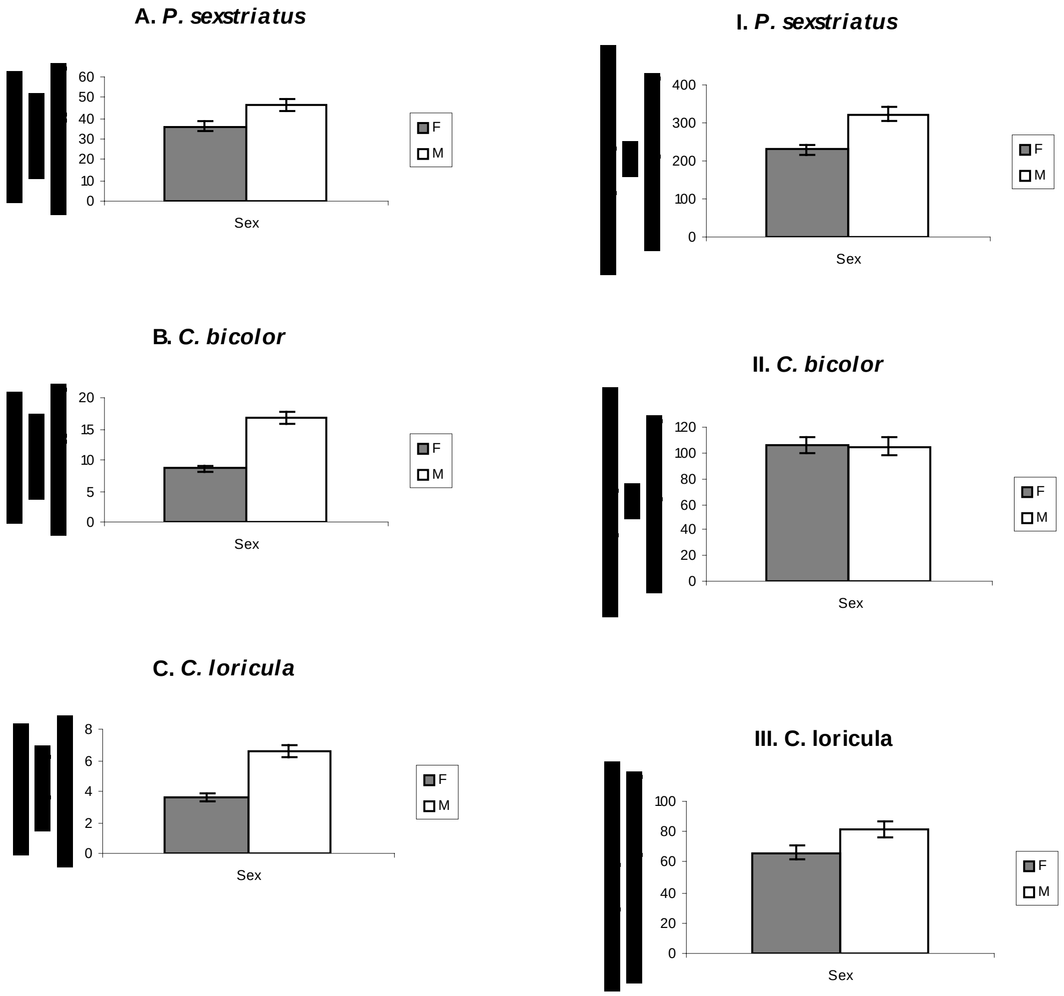


Figure 3.3. Bootstrapped estimates of sex-specific mean maximum longevity and body length of oldest individuals in P. sexstriatus _(A & I),_ C. bicolor _(B & II) and_ C. loricula _(C & III). Confidence intervals calculated as 1.96*SE (Haddon, 2001)._



3.4. DISCUSSION

Examination of size-at-age relationships and longevities with respect to sexual identity revealed a high level of demographic complexity within the family Pomacanthidae. The study species showed patterns that were similar to the related acanthurid fishes, primarily a very characteristic growth curve with initial rapid growth reaching an asymptotic size early in life, and a decoupling of age and size. This growth pattern was consistent despite a diverse range of maximum longevity estimates among the three study species. Moreover there was clear evidence of differential growth and longevity between sexes in all three species resulting in larger sized males at any given age, and a consistent pattern of relatively higher maximum longevity in males. A more interesting finding involved analysis of gonad ontogeny in *P.sexstriatus*, which suggested an unusual extended period of sexual differentiation with juvenile fishes exhibiting a prolonged bisexual stage.

Higher maximum longevity in larger Pomacanthid species

Results of this study revealed a pattern of relatively greater lifespan in the larger pomacanthid species conforming to predictions made by Chung and Woo (1999). Differences in lifespan were three-fold between *P. sexstriatus* and *C. bicolor*, and six-fold relative to *C. loricula*. In an evolutionary sense, it can be predicted that the larger basal species belonging to the *Pomacanthus* genus may be long-lived, while the smaller derived species belonging to the *Centropyge* genus may be relatively shorter lived (Bellwood et al. 2004), which may suggest greater fluctuations in recruitment levels in the larger angel fish speciess. Examination of lifespan and recruitment levels of a range of *Pomacanthus* and *Centropyge* species will disclose this hypothesis of phylogenetic structure within the family. Longer life span is usually associated with delayed sexual maturity, low natural mortality, growth rates and recruitment levels (Russ and Alcala 1998). While these life history features are characteristic of fishes more susceptible to exploitation, knowledge of the intensity of fishing is also needed to make predictions on the decline and recovery of such species (Russ and Alcala 1998).

Distinctive asymptotic growth patterns

Analysis of growth patterns revealed distinctive asymptotic growth curves in the three study species confirming previous findings by Eagle (1997). There is increasing evidence that this type of growth is characteristic of a number of reef fishes including lutjanids (Newman et al. 1996), chaetodontids (Berumen in press), acanthurids (Choat and Axe 1996; Laman Trip 2004) and a number of scarid species (Choat et al. 1996; Munday et al. 2004). Asymptotic growth involves fast initial growth where asymptotic size is reached within the first 15-30% of a species’ life, resulting in decoupling of size and age, and accumulation of a large range of older individuals within the asymptotic size range, implying the importance of rapid early growth. It is necessary for future research to include younger individuals such as juveniles younger than five years of age in *P. sexstriatus* for a better understanding of the initial ascending trajectory in angelfish. Asymptotic growth may be interpreted with respect to life history trade-offs between growth and reproduction, where energy expenditure shift towards initial gonadal production that results in slow or negligible growth (Chung and Woo 1999; Choat and Robertson 2002). As there is evidence that the period at which asymptotic size is reached is correlated with maximum lifespan of a species, sexual maturity may be attained later in life in longer-lived species. This could be investigated upon examination of age of first sexual maturity with respect to size and age data. Alternatively, fast initial growth may be a mechanism to escape predators, so that adult size is reached early in life. This is especially evident in the juvenile *P. sexstriatus* (distinguished by different morphological colour) which are cryptic and usually hidden in refuges (Allen et al. 1998) possibly to escape predation.

Sex-specific differential growth and longevity

Evidence from this study suggests sex-specific differential growth as a mechanism to explain larger males relative to females at any given age range in all populations of study species. Moreover, asymptotic growth was maintained within the male and female population in all three study species, however asymptotic size was reached at different ages in *P. sexstriatus* only. This may suggest firstly that production of female reproductive gametes expend proportionally more energy than male's resulting in greater net fitness returns towards somatic growth in males (Warner 1984; Newman et al. 1996). This is a common phenomena in polygynous reef species where males monopolise a few females (Warner 1984). Secondly, the selective pressures causing the decline in growth rate is sex-specific in *P. sexstriatus* only. In contrast, *C. bicolor* and *C. loricula* conformed to patterns found in the acanthurid *Ctenochaetus striatus*, where the age at which growth trajectories asymptote is the same in both sexes (Laman Trip 2004). Whether this is a cause of shift in energy expenditure from growth to gonad production would be an interesting topic to investigate. This would predict similar ages of maturity between sexes in the *Centropyge* species, but differing in *P. sexstriatus*. Lastly, as protogyny has been assumed to be a universal feature of the family, differential growth may either be established early in its life where faster growing females become males (Juvenile growth hypothesis: Laman Trip 2004) or through a growth spurt after sex change from a female to a male (Growth spurt hypothesis: Walker and McCormick 2004).

A consistent pattern of longer lifespan in males was always observed, suggesting the potential for all females to change sex to males after a given maximum age. Moreover, the size of the oldest female and male population were similar in *C. bicolor,* which coincides with the presence of a few large females within the male growth schedule. This suggests the potential for these individuals to change sex to males upon removal of the dominant male of a harem (Sakai et al. 2003b). This implies that mechanisms for differential growth may differ between *C. bicolor*, and the remaining two species. I would predict therefore that *C. bicolor* would establish differential growth through the 'juvenile growth hypothesis' evident by the presence of a few large females within the male growth schedule, but through the 'growth spurt hypothesis' in *P. sexstriatus* and *C. loricula,* where growth increases only after becoming a male.

Complex gonad ontogeny in P. sexstriatus

There is evidence of complex gonad ontogeny in *P. sexstriatus,* which differs from the common protogynous gonad ontogeny of scarids. Male recruitment in scarids involves differentiation from immature males and/or immature females which change sex to become males (Choat and Robertson 1975). Instead gonad ontogeny involved immature bisexuals existing as juveniles that had prolonged differentiation periods of up to 12 years. While gonad ontogeny may differ from scarids, male recruitment (and hence reproductive ontogeny) may be similar (Devlin and Nagahama 2002), but needs to be investigated with respect to age-based sexual maturation and growth.

CHAPTER 4. REPRODUCTIVE ONTOGENY OF THREE POMACANTHID SPECIES.

4.1. INTRODUCTION

Reproductive ontogeny is defined as the reproductive development throughout a species life history (Warner 1984), also known as sexual pattern or sexual ontogeny (e.g. in Adams 2002). Teleosts have a diverse range of reproductive ontogenies ranging from gonochorism to functional hermaphroditism. Gonochorism, where males and females function separately throughout life, is most widely known, while functional hermaphroditism, where a proportion of a population may function as both sexes in their lifetime is being reported more and more in reef fishes (Warner 1984) (see Figure 5.1). Functional hermaphroditism may be expressed in two ways depending on the timing of male and female function following maturity, either sequential or simultaneous (Warner 1984). Sequential hermaphroditism is characterized by individuals that function as both sexes at different times in their life history. The sequence can be either protogynous (initial functional sex as female followed by a change of sexual function to male) or protandrous (initial functional sex as male followed by female) (Sadovy and Shapiro 1987). The defining feature of sequential hermaphroditism is that simultaneous activity does not occur in the same individual (Warner 1984; Sadovy and Shapiro 1987). Simultaneous hermaphroditism occurs in some serranid species where a mature gonad of an individual may contain sexually mature elements of both sexes (Fischer 1980; Petersen 1987; Sadovy and Shapiro 1987). Such species characteristically pair spawn with individuals alternating in function first as one sex then as the other (Fischer 1980). However this is a condition only seen in a minority of reef fishes (Petersen 1987). Most studies on the reproductive biology of reef fishes have implicated either gonochorism or sequential hermaphroditism.

Protogynous male recruitment occurs through two developmental pathways, either from sexually immature juveniles (resulting in primary males) or from sexually mature females (resulting in secondary males) (Sadovy and Shapiro 1987). Protogynous species with male recruitment occurring through both of these developmental pathways are *diandric,* while those from functional females only are *monandric* (Sadovy and Shapiro 1987). Recent studies have indicated additional complexities in the sexual ontogeny of reef fishes. Some species (primarily gobies (Munday et al. 1998) and a pomacanthid: *Centropyge ferrugata* (Sakai et al. 2003a) that display sequential hermaphroditism may have bi-directional sex change in which the direction of sex change is not fixed but can occur in both directions.

Gonadal development within each form of reproductive ontogeny can be highly variable among species. Sexual differentiation, which is the process of gonadal development (after sex has been determined through genetic and environmental processes and variables (Devlin and Nagahama 2002) therefore becomes a very important component in the analysis of reproductive ontogeny. The mode of gonadal development shall interchangeably be referred to as sexual differentiation pathways in this study. In gonochoristic species, at least three sexual differentiation pathways have been described which illustrates that the final function of sex may not reflect the initial gonadal development (Devlin and Nagahama 2002). In the first type, undifferentiated gonads directly become testes or ovaries, which then develop into functional testes or ovaries (Yamamoto 1969). In the second type of gonochoristic sexual differentiation, undifferentiated gonads all become ovarian. Some of these gonad stages may become intersexuals (having immature germ cells of both sexes and may also be called immature bisexuals), eventually becoming testes. The rest remain as ovaries and later become functional (Yamamoto 1969). In the third type, undifferentiated gonads all become intersexual gonads, some of which become testes and others ovaries. This type of sexual differentiation has recently been found in the gonochoristic grammatid, *Gramma loreto* (Asoh and Shapiro 1997) and serranid *Epinephelus striatus* (Sadovy and Colin 1995). While gonad ontogeny is known to be variable within gonochoristic species, it becomes even more complex within functional hermaphrodites, especially in sequential hermaphrodites (Adams 2002; Chan and Sadovy 2002; Cole 2002). Given, the complexity in gonad development from as early as the juvenile stage, distinguishing between sex differentiation in immature and mature stages, that is, development before and after sexual maturity, is important in diagnosing the sexual ontogeny of reef fishes.

An important reason for distinguishing between a species' immature and mature gonad stage is related to the discrepancy in the definition of hermaphroditism. According to Atz (1964) (cited in Yamamoto 1969), "an individual is hermaphroditic if it bears recognizable male and female tissues". This term is known as anatomical hermaphroditism (Adams 2002). This term not only includes juvenile hermaphrodites such as intersexuals in gonochoristic species, but also includes mature individuals that undergo sex transition (functional hermaphrodites). To diagnose species that are functional hermaphrodites from gonochoristic species with juvenile hermaphroditism, reproductive ontogeny must be defined based on functional criteria of each gonadal stage (Sadovy and Shapiro 1987). That is, reproductive ontogeny within a species must be interpreted based on the reproductively mature and therefore functional gonad stages only rather than on sexual differentiation pathways or structures observed at a particular life history stage (Devlin and Nagahama 2002).

Sexual maturation, or the functional expression of sex, can be recognized through mature stages of germinal cells and structural features, for example, the presence of vitellogenic oocytes in ripe females, or evidence of prior spawning in resting mature females through degenerating vitellogenic oocytes, a thick gonad wall, post ovulatory follicle or intralamellar muscle bundles (Samoilys and Roelofs 2000; Adams 2002; Chan and Sadovy 2002). Species may vary in gonadal features by which sexual maturation may be recognized, hence species need to be observed separately, and in some cases, new criteria may need to be developed to describe each gonad stage. Therefore to analyze the mode of sexual differentiation, an age-based examination of all stages of gonad development from juvenile through to adult stage is critical (Devlin and Nagahama 2002).

Confirmation of functional hermaphroditism comes from obtaining transitionals examined histologically after experimental manipulation of social systems (Sadovy and Shapiro 1987). Examples of such experiments were done with two angelfish, *Centropyge bicolor* and *C. interruptus*, which both live in male-dominated harems with three or more females. Sex transition of the largest mature female into a functional male after experimental male removal indicated functional hermaphroditism (Moyer and Nakazono 1978; Aldenhoven 1984). Alternatively, the presence of mature sex transitional gonads from random samples of a species may confirm functional hermaphroditism (Sadovy and Shapiro 1987). A gonad undergoing sex transition is recognized by degeneration of mature germ cells of the former functional sex, and proliferation of germ cells of the resting mature gonad of the former (Aldenhoven 1984; Adams 2002).

The very few histological studies on Pomacanthidae have shown that the most common form of reproductive ontogeny is protogyny. This has been recorded in three pomacanthid genera, *Centropyge* (Moyer and Nakazono 1978; Bruce 1980; Aldenhoven 1984; Lutnesky 1988; Lutnesky 1996; Eagle 1997), *Holacanthus* (Hourigan and Kelley 1985), and *Genicanthus* (Bruce 1980; refs in Thresher 1984). Past authors have assumed protogynous hermaphroditism as a universal feature in the family based on close similarities in reproductive strategies and once thought close phylogenetic relationships (e.g., Neudecker and Lobel 1982). A recent study by Bellwood et al. (2004), however found very distinct relationships even among pomacanthid species, suggesting that protogyny may not be as common in the family as previously thought.

For this study I chose three species from different phylogenetic levels, one basal species, *Pomacanthus sexstriatus,* and two derived species, *Centropyge bicolor* and *C. loricula* (see Figure 3 in Bellwood et al. 2004). Histological studies have been done on *C. bicolor* (Aldenhoven 1984), where *monandry* has been suggested, however nothing is yet known on the reproductive ontogeny of *P. sexstriatus* and *C. loricula*. While detailed analysis of sexual differentiation has been made for *C. bicolor,* all studies are size-based. This methodology has recently been criticized as it does not necessarily reveal the age of an individual due to asymptotic growth in pomacanthids (Eagle 1997). Furthermore, differential growth between males and females (Chapter 3) contributes to the problem of predicting age from size. Thus using age-based histological analysis may provide new information on *C. bicolor*.

Therefore in this chapter, we study the mode of sexual differentiation with respect to age-based growth of three pomacanthid species, *P. sexstriatus, C. bicolor* and *C. loricula* with the aim to

i. determine the age at first sexual maturity and sexual transition if present
ii. examine the pattern of sexual differentiation in immature individuals
iii. provide an ontogenetic analysis of the different stages of gonad maturation

iv. describe morphological criteria of the testis associated with different forms of male recruitment and

v. identify important features for diagnosis of protogynous hermaphroditism (refer to Figure 4.2 for diagrammatic figure of aims).

4.2. MATERIALS AND METHODS

4.2.1. STUDY SPECIES

A description of the study species *Pomacanthus sexstriatus, Centropyge bicolor* and *C. loricula* are described in Chapter 3, section 3.2.1.

4.2.2. SAMPLE COLLECTION

A total of 69, 87 and 153 specimens of *P. sexstriatus, C. bicolor* and *C. loricula* respectively were collected initially for the purpose of constructing growth and longevity estimates (Chapter 3, section 3.2.2). Measurements such as total and fork length, and ageing was done as described in Chapter 3, section 3.2.2-3.2.5. Fish samples of *P. sexstriatus* and *C. bicolor* were stored in ice and processed in the laboratory within 6 hours of capture, with the exception of some samples of *P. sexstriatus* and all individuals of *C. loricula,* which were stored in the refrigerator for as long as two days to two months before processing. Gonads were removed and weighed to the nearest 10^{-2} g. Sex was determined initially on macroscopic examination of gonads and the tissue preserved in FAAC.

4.2.3. GONAD HISTOLOGY

A total of 64, 84 and 144 gonads of *P. sexstriatus, C. bicolor* and *C. loricula* respectively were used for histology. As previous research has suggested no consistent pattern in sexual development along the length of the gonad of *C. bicolor* (Aldenhoven 1984) and in several serranids (Samoilys and Roelofs 2000; Fennessy and Sadovy 2002), sections were assumed to be homogeneous throughout the gonads of each species studied. Furthermore, Aldenhoven (1984) confirmed consistent gametogenesis between the large and small gonad lobes, hence, to standardize the method, most transverse sections were taken from the medial part of the larger gonad lobe. Transverse sections were dehydrated overnight and embedded in paraffin wax the next morning. Embedded gonads were sectioned at 5µm on a micrometer and mounted on glass slides, then stained with Mayer's alum haemotoxylin and Young's eosin-erythrosin stains. Each section was examined under a high-powered microscope to determine the developmental stage of the gonad of each individual.

4.2.4. DEVELOPMENTAL STAGING

4.2.4.1. DEVELOPMENTAL STAGES OF OOCYTES

There are 5 stages in oocyte development after a primary stage called oogonia. Germ cell mass increase from stage I-V through meiosis (adapted from Aldenhoven 1984; Hourigan and Kelley 1985; Takashima and Hibiya 1995; Cole 2002).

Primary stage: Oogonia – single cells on periphery of lamellae. The nucleus is large and surrounded by a thin band of lightly staining cytoplasm.

Stage I: Chromatin nucleolar stage - Contains a single centrally located nucleolus. Cytoplasm is thinner than diameter of nucleus (stains purple).

Stage II: Perinucleolar stage - Numerous nucleoli located close to the nucleus. Cytoplasm layer is thicker than diameter of nucleus (stains purple).

Stage III: Cortical alveolar stage - First appearance of cortical granules (small, spherical, pale staining) near periphery of cell membrane; numerous oil droplets surround nucleus; vitelline granules (spherical bodies) in rest of cytosplasm; nuclear cytoplasm is fibrillar rather than granular; oocyte is surrounded by a layer which we will call the zona radiata in this study (stains light pink-purple).

Stages I - III are called the previtellogenic stages, and will now be referred to as such in the rest of this study.

Stage IV: Vitellogenic stage - Large granular oocyte; vitelline granules fills large proportion of cytoplasm; zona radiata obvious (stains pink).

Stage V: Final maturation and/or hydrated stage - Oocytes with obvious coalescence of oil droplets; and oocytes in which vitelline vesicles form sheet like appearance (stains pink).

4.2.4.2. DEVELOPMENTAL STAGES OF SPERMATOCYTES

In contrast to oocyte developmental stages, which grow in germ cell mass as they proceed from stages I – V, there is a decrease in germ cell mass during the spermatogenic process. There are five stages of spermatocytes: spermatogonia, primary spermatocytes and secondary spermatocytes, spermatids (round cells containing round nuclei), and spermatozoa (smallest spermatogenic germ cells with tails) (Aldenhoven 1984; Takashima and Hibiya 1995). Stages I - IV are in crypt formation and become disrupted as they reach the spermatozoa stage. Each stage is homogeneously enclosed in spermatocysts so that the same staged spermatocytes are in each spermatocyst.

4.2.4.3. DEFINITIONS AND CRITERIA USED IN DETERMINING GONADAL DEVELOPMENTAL STAGES.

For the assessment of functional reproductive ontogeny for each species, general terms used in this study are defined, with reference to works already done on the pomacanthids (e.g., Moyer and Nakazono 1978; Aldenhoven 1984; Hourigan and Kelley 1985), and others such as in serranids (e.g., Ferreira 1993; Samoilys and Roelofs 2000; Adams 2002; Fennessy and Sadovy 2002), and damselfish (Cole 2002). In addition, authors that have made important contributions on the analysis of gonad ontogeny (Sadovy and Shapiro 1987; Takashima and Hibiya 1995) were also referred to.

DEFINITIONS AND CRITERIAS

Functionality

A *functional* individual is defined in this study as any *mature* individual, whether active or inactive at the time of capture. A mature individual is identified by either the latest stage of oocytes (vitellogenic) or spermatocytes (spermatozoa) present in a gonad (Samoilys and Roelofs 2000) or by features representative of prior spawning in seasonal spawners (Adams 2002). For example, evidence of previous spawning as a female may be indicated by a thicker gonad wall, larger gonad area, intra-lamellar muscle bundles and/or post ovulatory follicles in mature resting or mature spent female serranids (Samoilys and Roelofs 2000; Adams 2002; Fennessy and Sadovy 2002). A functional male is indicated by the presence of spermatozoa and/or a sperm duct through which sperm can exit in a testis (Sadovy and Shapiro 1987; Cole 2002). Sperm ducts that run within the gonadal wall are termed ‘sperm sinus’ and those that run along the medial hilar regions of the gonadal lobes are ‘medial sperm ducts’ (Asoh 2004).

Two developmental stages were distinguished to address problems of assessing functional sexual differentiation pathways. These were transitional and immature bisexual gonad stages, in which the former indicates functional sex change, and the latter indicates a gonad of immature germ cells of both sexes with unknown direction and does not imply sexual function (as done by Chan and Sadovy 2002). The term bisexual rather than intersexual was used in this study.

Transitional sex change

A transitional gonad is defined as one in which there is degenerating vitellogenic oocytes and proliferating male germ cells (Sadovy and Shapiro 1987). Additionally an inactive mature female gonad with proliferating spermatocytes is also a transitional (Adams 2002). In a study on *C. bicolor* by Aldenhoven(1984), transitional individuals that were five, 10 and 20 days old, were obtained after experimentally removing dominant males from single-male harems in the field. Results from Aldenhoven’s (1984) study observed three distinct stages of the transitional phase that progressed over the time period. The first stage contained previtellogenic oocytes, degenerating vitellogenic oocytes but no proliferating sperm crypts. The second stage followed the definition by Sadovy and Shapiro (1987). The last stage followed that defined by Adam’s (2002). Day 20 transitionals had become fully functional males. These three stages were therefore used to define transitionals in this study.

Gonadal Developmental Stages

P. sexstriatus individuals were classified as either undifferentiated, immature bisexual, immature female, mature female (ripe or running ripe), immature male or mature male (Table 4.1, Figure 4.3, 4.4); *C. bicolor* as either immature female, mature female (ripe or running ripe), transitional or mature male (Table 4.2, Figure 4.5, 4.6);

and *C. loricula* as either an immature bisexual, mature female (resting/spent, ripe, or running ripe), or a mature male (inactive undifferentiated, inactive mature, or active mature) (Table 4.3, Figure 4.7, 4.8).

4.2.5. ANALYSIS OF DATA

4.2.5.1. SIZE AND AGE FREQUENCY DISTRIBUTION

Identified reproductive categories were incorporated into size and age frequency distributions to examine the patterns of gonad development with size and age in each species.

4.2.5.2. SIZE AND AGE OF 1st SEXUAL MATURITY AND SEXUAL TRANSITION

Size and age of sexual maturity was obtained based on the minimum size and age of first maturity. Similarly size and age of sexual transition was considered as the size and age of the first sexual transitional individual found.

Table 4.1. Gonad developmental stages in *Pomacanthus sexstriatus* based on histological examination and with reference to Aldenhoven (1984), Hourigan and Kelley (1985), Adams (2002), Cole (2002), and Munday (pers comm.).

Reproductive category/ stage	**Sexual maturity or gonad developmental stages**	**Histological description**
Undifferentiated	Undifferentiated	Gonad filled of tissue other than spermatogenic or oogenic tissue
Bisexual immature	Immature bisexual	Gonad containing stage I and II oocytes intermixed with testicular tissue of early spermatocytes. Germ cells show no sign of degeneration or proliferation (c.f transitionals). Immature status supported by thin film as gonad wall.
Female immature	Immature	Gonad containing previtellogenic stage I-III oocytes (stains purple). Small gonad area. No evidence of prior spawning as a female by the presence of intralamellar muscle bundles, atretic oocytes or post ovulatory follicles.
Female: mature	Ripe	Large gonad with lamellae containing up to vitellogenic oocytes. May contain intralamellar muscle bundles, atretic vitellogenic oocytes and post ovulatory follicles.
Female: mature	Final maturation/ Running ripe	Large gonad with lamellae containing up to coalesced or hydrated oocytes. May contain intralamellar muscle bundles, atretic vitellogenic oocytes and post ovulatory follicles.
Male: immature	Inactive immature	Proliferating spermatogenic tissue (up to spermatid stage) in a presumably previous immature bisexual gonad with previtellogenic oocytes. Has taken the shape of a testes, but sperm duct is not fully formed yet.
Male: mature	Active mature	Well-developed spermatogenesis, up to spermatozoa stage filling the medial sperm duct. Stage I oocytes may be present.

Table 4.2. Gonad development stages in *Centropyge bicolor* based on histological examination and with reference to Aldenhoven (1984), Hourigan and Kelley (1985), and Adams (2002).

Reproductive category/ stage	**Gonad developmental stage (or sexual maturity)**	**Histological description**
Female immature	Immature	Small compact ovary containing previtellogenic oocytes only. Thin gonad wall. No evidence of prior spawning by the presence of lamellar core tissue or brown bodies in the lamellae. No sperm crypts present.
Female mature	Ripe	Large ovary in active vitellogenesis, up to vitellogenic oocytes. May contain degenerating eggs. No sperm crypts present.
Female mature	Running ripe/ final maturation	Large ovary in active vitellogenesis, but oogensis has reached the hydrated (stage V) oocytes May contain degenerating eggs. No sperm crypts present.
Transitional	Sex changed mature female	Three sequential transitional stages according to Aldenhoven (1984): 1. Gonads with degenerating vitellogenic oocytes; previtellogenic oocytes present; usually no sperm crypts present yet (day 5 transitionals), 2. Gonad with degenerating vitellogenic oocytes and proliferating spermatocytes 3. Gonad with previtellogenic oocytes and proliferating sperm crypts throughout lamellae; lamellar core tissue and brown bodies are abundant (day 10 transitionals) (This stage is equivalent to proliferation of testicular tissue in a mature resting female (see table 4.3 for definition of resting female) Transitional gonads have thick gonad walls; not reached spermatozoa stage, and dorsal sperm sinus is not yet fully developed.
Male mature		Well developed spermatogenesis, up to spermatozoa stage and developed peripheral sperm sinus. Recently sex changed males may still have previtellogenic oocytes, and a lamellar organization but still have features of a mature male. All males retain the former ex-ovarian lumen.

Table 4.3. Gonad development stages in *Centropyge loricula* based on histological examination and with reference to Aldenhoven (1984), Hourigan and Kelley (1985), Samoilys and Roelofs (2000), Adams (2000).

Reproductive category/stage	**Sexual maturity or gonad developmental stages**	**Histological description**
Bisexual: immature	Bisexual: immature	Previtellogenic oocytes intermixed with testicular tissue of early development in an ovarian gonad. Germ cells show no sign of degeneration or proliferation (c.f transitionals).
Female: mature	Mature resting	Ovary filled with previtellogenic oocytes in non-compact lamellae. Evidence of prior spawning: thick gonad wall and atretic oocytes. Sperm crypts may be present.
Female: mature	Mature ripe	Large ovary with thick gonad wall containing up to vitellogenic stage oocytes, atretic oocytes may also be present. Sperm crypts may be present.
Female: mature	Mature running ripe	Large ovary with thick gonad wall; hydrated oocytes present, usually with degenerating vitellogenic oocytes; post-ovulatory follicles, muscle bundles may be present. Sperm crypts may be present peripherally in gonad wall or within lamellae.
Female: mature	Mature spent	Large ovary with thick gonad wall filled with previtellogenic and atretic vitellogenic oocytes. Lamellae is disorganized and disrupted. Brown bodies are common. Sperm crypts may be present peripherally in gonad wall or within lamellae. Sperm crypts may be present.
Male: mature	Inactive undifferentiated: mature	Testis in partial lamellar form in which tissue is undifferentiated, so that there is no sperm crypts present. Lots of brown bodies; sperm sinus not so distinct.
Male: mature	Inactive mature	Testis in partial lamellar form in which spermatogenesis is up to the secondary spermatocyte stage; sperm sinus not so distinct.
Male mature	Active mature	Testis in partial lamellar form in which spermatogenesis is up to the spermatozoa stage filling the sperm sinus.

4.3. RESULTS

4.3.1. SEX-SPECIFIC SIZE AND AGE FREQUENCY DISTRIBUTION

Size frequency distribution

There were three similar attributes among the three species displayed in their size frequency distribution (Figure 4.9). Firstly they all displayed bimodal frequency distribution between females and males (both immature and mature), where males were distributed in larger size classes. Secondly, there was a slight overlap between female and male size ranges and thirdly, maturity in females was accomplished at a smaller size compared to males.

Size range of *P. sexstriatus* ranged from 130-400 mm SL (Figure 4.9a). There was a gradual shift in reproductive category with size, from individuals with undifferentiated gonads to immature bisexuals, followed by immature females, immature male, mature females to a mature male. The size range of immature bisexuals (171 > FL < 280 mm) fully overlapped those of immature females (181 > FL < 210 mm), an immature male (201 > FL < 210 mm) and mature females (201 > FL < 260 mm), and slightly with mature males (221 > FL < 280 mm). The overlap between female-male was between 221-260 mm FL.

Size ranges in *C. bicolor* and *C. loricula* were relatively smaller than *P. sexstriatus* at 60-120 mm FL and 51-81 mm TL respectively. These two species were similar in having females as the smallest individuals that were immature in *C. bicolor*. In *C. bicolor*, gonad categories shifted with size from an immature female to mature females to mature males. Two transitionals were observed within the female-male overlap (86 > FL < 110 mm) at size category 96-100 mm FL (Figure 4.9b). In *C. loricula*, however, gonad development initiated as mature females (51 > TL < 70 mm) and shifted to mature males (66 > TL < 81 mm) with size, overlapping between 66-70 mm TL (Figure 4.9c). The only immature individual in *C. loricula* was an immature bisexual of 54 mm TL.

Age frequency distribution

The three similar attributes seen in size distribution in all the three species were retained only in *C. bicolor* and *C. loricula* upon examining age frequency distribution (Figure 4.10). These were a sex-specific bimodal distribution, with males distributed in the older age classes, a slight overlap between male and female age classes, and maturity accomplished at an earlier age in females than males. One similar attribute in age distribution in all three species was males attaining longer lifespan than females.

P. sexstriatus ranged in age from five to 58 years (Figure 4.10a). There was less distinction in the shift in gonad development with age due to high overlap between most reproductive categories. Individuals shifted from undifferentiated gonads (5 >age < 7 years) to immature bisexuals (6 > age < 12 years) and immature females (6 > age < 11 years), followed by an immature male (8 years), mature males (8 > age < 58 years) which fully overlapped the age range of mature females (10 > age < 41 years). Interesting observations made in the age frequency distribution in *P. sexstriatus* were firstly, that immature bisexuals, which fully overlapped females (immature and mature) in size, are instead fully overlapped in age range by the latter (6 > age < 41 years). *P. sexstriatus* females are therefore diverse in age groups, reaching very high longevity despite their small size range. Secondly, immature individuals were still observed after the first age of maturity in both sexes, up to 12 yrs in immature bisexuals and 11 yrs in immature females. Age frequency distribution in this species displayed a very different story to that of size.

Compared to *P. sexstriatus, C. bicolor* and *C. loricula* had relatively lower longevities at 17 and eight years respectively. Shifts in gonad development with age was similar with size in both species. This involved a shift from immature females to mature females to mature males in which transitionals were found at three and five years within female-male overlaps (2 > age < 10 years) in *C. bicolor* (Figure 4.10b). A shift in gonad categories in *C. loricula* was maintained with age, from mature females to mature males, in which an immature bisexual was found as the second youngest individual (Figure 4.10c).

As patterns observed in frequency distribution with size and age were only retained in *C. bicolor* and *C. loricula*, the mode of sexual differentiation should be best interpreted based on age for more accurate results.

4.3.2. SIZE AND AGE OF FIRST SEXUAL MATURITY AND SEX CHANGE

Size at first maturity was accomplished at smaller sizes in females when compared to males in all three species (*P. sexstriatus:* 201-210 mm FL; *C. bicolor:* 66-70 mm FL; and *C. loricula*: 51 mm TL)(Table 4.4). The first mature males were found at larger sizes between 220-230 mm FL, 86-90 mm FL and at 66 mm TL in *P. sexstriatus, C. bicolor* and *C. loricula* respectively. This pattern was reflected in the age at first maturity in females and males in both *C. bicolor* (female: 0.56yrs; male: 2yrs) and *C. loricula* (female: 0.44yrs; male: 1yr). As the youngest individual was mature, the age of 1st maturity may possibly be earlier in *C. loricula*. Age of first maturity however differed in *P. sexstriatus* in having males mature earlier (at 8 yrs) than females (at 10 yrs). Sex change was revealed by the presence of two sexual transitional individuals found in *C. bicolor* only, between sizes 96-100 mm FL and ages three to five years.

Table 4.4. Sex specific size and age of 1st sexual maturity in *P. sexstriatus, C. bicolor* and *C. loricula*.

Species	**Gender**	**Size of 1st sexual maturity**	**Age of 1st sexual maturity**
P. sexstriatus	Female	201-210 mm FL	10 yrs
	Male	220-230 mm FL	8 yrs
C. bicolor	Female	66-70 mm FL	0.56 yrs
	Male	86-90 mm FL	2 yrs
C. loricula	Female	51 mm TL	0.44 yrs
	Male	66 mm TL	1 yr

4.3.3 PATTERN OF SEXUAL DIFFERENTIATION IN IMMATURE INDIVIDUALS

P. sexstriatus had a unique pattern of sexual differentiation of immature individuals that was widely distributed in age range (Figure 4.9a, 4.10a). Nineteen individuals (i.e., about 30% of sample) were classified as immature, of which there were four types: undifferentiated (n = 3), immature bisexual (n = 12), immature male (n = 1) and immature female (n = 3) individuals. Interestingly, immature individuals in *P. sexstriatus* may remain immature up to seven years in undifferentiated individuals, 11 in immature females and 12 years in immature bisexuals. Based on frequency distribution with age, sexual differentiation commences with undifferentiated gonads, and may differentiate to either immature bisexual or immature females. As there is full overlap of immature females by bisexuals, it is also possible that immature bisexuals may differentiate to become immature females or vice versa. As immature males were found later in life history, it is likely that they differentiate from either immature females via a bisexual stage or directly from immature bisexuals.

Immature individuals in both *C. bicolor* and *C. loricula* comprised of one individual each, an immature female and an immature bisexual respectively. The immature female was the smallest (60-65 mm FL) and youngest (0.38 yrs) individual in *C. bicolor* (Figure 4.9b, 4.10b). The immature bisexual in *C. loricula* however was larger (at 54 mm FL) than the size of 1st maturity in females (51mm FL) but was the second youngest at 0.87 yrs (compared to the age of 1st maturity in females at 0.45 yrs) (Figure 4.9c, 4.10c).

4.3.4. AGE-BASED ONTOGENETIC ANALYSIS OF THE DIFFERENT STAGES OF GONAD DEVELOPMENT

4.3.4.1. *P. sexstriatus*

All gonads in *P. sexstriatus* commence ontogeny as undifferentiated gonads and differentiate to either immature bisexual or immature female gonads (Figure 4.11). As there is full overlap of immature females by bisexuals, it is also possible that immature bisexuals may differentiate to immature females or vice versa. There are three possible pathways to male recruitment starting at eight years of age. The first arises directly from immature bisexual gonads. Evidence of this route was seen morphologically in the only immature male which contained proliferating spermatogenic tissue (up to spermatid stage) in a presumably previous immature bisexual gonad with lots of previtellogenic oocytes (Figure 4.3e). This stage had taken the shape of testes, but a sperm duct had not fully formed yet. Immature males then mature and become functional at eight years old. Male recruitment may also arise through direct differentiation from immature females. This involves differentiation into a bisexual stage, which then become a testis. A reason to suggest this may not happen is because no immature bisexual gonads were ovariform and lamellar in organization. The third possible pathway of male recruitment suggest functional sex transition. The fact that males were relatively longer-lived may suggest male recruitment through sex change of all functional females. While this is certainly possible, there was no transitional to support this. Furthermore, testes morphology does not suggest secondary formation from females through features such as an ex ovarian lumen or lamellar organization (Sadovy and Shapiro 1987). Sexual differentiation through direct differentiation of immature bisexuals to males only suggests a gonochoristic reproductive ontogeny in *P. sexstriatus.*

Female recruitment may be through either differentiation of immature bisexual gonads to a female gonad, or through maturation of immature females, which differentiate from undifferentiated gonads.

4.3.4.2. *C. bicolor*

Sexual differentiation in *C. bicolor* was typical of that of protogynous species, where gonads commenced as immature females and mature at the age of 0.56 years, after which they change sex to become functional males at 2 yrs of age (Figure 4.12). The absence of small or young immature males were not found suggest *monandry* in *C. bicolor*, so that male recruitment is through sex transition of females only. This was supported by females not living the full lifespan of *C. bicolor*.

4.3.4.3. *C. loricula*

Sexual differentiation in *C. loricula* was not as distinct as observed in *C. bicolor*. Individuals younger than the age at first maturity in females (0.44 years) were not obtained in the sample, therefore immature bisexual individuals may not necessary commence ontogeny in *C. loricula* (Figure 4.13). There is however potential for protogyny. This is evident by the absence of young mature males, later male recruitment (1 year old), and males reaching relatively longer lifespans similar to *C. bicolor.* This is supported by the testes morphology in which there is evidence of secondary formation through the presence of an ex-ovarian lumen, lamellar organization and peripheral sperm duct (Sadovy and Shapiro 1987). The first two gonadal features were however present as early as in the immature bisexual stage which may equally suggest direct differentiation of males from immature bisexuals. It is therefore unclear from this study which pathway is taken. Nevertheless, if sexual differentiation occurs through both pathways of male recruitment, *C. loricula* would be a functional diandric protogynous hermaphrodite. This needs to be confirmed by identifying a transitional. The difficulty faced in finding transitionals in this species was the presence of sperm crypts even throughout the functional female gonads. The status of individuals which had sperm crypts in resting mature female gonads as transitionals was therefore questioned. Alternatively, if sexual differentiation involved the latter pathway of male recruitment only, it would suggest gonochorism in *C. loricula*.

Female recruitment may occur through direct differentiation from immature bisexual gonads (which may explain the sperm crypts observed in all female stages) or through direct differentiation from gonads of individuals younger than the age of first maturity, which were not obtained in this study (Figure 4.13).

4.3.5. MORPHOLOGICAL CRITERIA OF TESTIS ASSOCIATED WITH DIFFERENT FORMS OF MALE RECRUITMENT

4.3.5.1. *P. sexstriatus*

Male recruitment directly from immature bisexuals (Figure 4.3 a-d) resulted in testes that lacked some common features of secondary formation from females in the form of an ex-ovarian lumen or lamellar organization as seen in some secondary males of scarids and wrasses (Choat and Robertson 1975; Sadovy and Shapiro 1987). Instead all functional testes were always filled with spermatogenic tissue and occasionally with early stages of oogenesis (oogonia) in the small recent males (Figure 4.3e). Oogonia in these smaller testes were always located peripherally on the lateral side of the gonad. Spermatogenesis was continuous in the majority of the testes with sperm ducts located in the medial region of all testes. Cracks were found alongside the medial blood vessel in immature bisexuals. As the position was identical to sperm ducts seen in an immature male, it was identified as potential precursory sperm ducts.

4.3.5.2. *C. bicolor*

As male recruitment was through sexual transition of all functional females (which directly differentiated from immature females), all testes showed evidence of secondary male formation from a mature female by the presence of an ex ovarian lumen that is located laterally to the gonad wall, and a peripheral sperm sinus located within the former lateral ovarian gonad wall (Figure 4.6d) (Sadovy and Shapiro 1987). The laterally located ex-ovarian lumen results from a lateral ovarian lumen in functional females (Figure 4.5d). The sperm sinus was not always evident in every section. One transitional individual (Figure 4.6a) retained a lamellar organization and stage I-II oocytes, which eventually disappeared so that the lamellae coalesced to form a continuous sheath of testicular tissue in functional males. Spermatogenesis was continuous at all stages and therefore functional in all male testes obtained.

4.3.5.3. *C. loricula*

Most *C. loricula* testes resembled those of secondary testes, retaining partial lamellar organization, a central ex ovarian lumen, and a peripheral sperm sinus within the whole length of the gonad wall (Figure 4.8c,d). In some testes, the lamellae have opened up, so that they were organized in a straight plane along the whole length of the gonad wall (Figure 4.8d). Sperm sinus was not always distinct within most testes. Although testes types support secondary derivation from functional female ovaries, sexual differentiation which involves juveniles (immature bisexuals) that already developed a central ex ovarian lumen and lamellar organization may equally suggest direct differentiation from such individuals.

4.3.6. IMPORTANT FEATURES IN DIAGNOSING PROTOGYNY IN *C. bicolor*

In *C. bicolor*, protogyny was confirmed by the presence of two transitional individuals. Transitional gonads were both in the 3rd stage of transition according to studies by Aldenhoven (1984), showing no evidence of degenerating vitellogenic oocytes, but only of proliferation of sperm tissue. Evidence of former female function became important in distinguishing a transitional gonad in *C. bicolor*. This involved the presence of a large amount of brown bodies, and a thick gonad wall. No spermatozoa were present, and dorsal sperm sinus was not yet evident.

4.4. DISCUSSION

4.4.1. THE NATURE OF REPRODUCTIVE ONTOGENIES AND THE SIGNIFICANCE OF PROTOGYNY IN THE FAMILY POMACANTHIDAE

The present study suggests protogyny may not be as common in the Family Pomacanthidae as previously assumed. *C. bicolor* was confirmed to be monandric, while *P. sexstriatus* suggested a gonochoristic reproductive ontogeny, the first time this has been reported within the family. Sexual ontogeny in *C. loricula* remain unclear, but may either be gonochoristic or diandric. Combined with findings of reproductive ontogeny from other histological studies, an emerging pattern among species of the Family Pomacanthidae from different phylogenetic hierarchies appears (Figure 4.14). Generally, protogyny has only been observed in representatives from genera *Centropyge, Genicanthus,* and *Holacanthus* (Moyer and Nakazono 1978; Bruce 1980; Aldenhoven 1984; Hourigan and Kelley 1985; Lutnesky 1988; Moyer 1990; Lutnesky 1996) some of which are indicated in Figure 4.14. This pattern suggests basal species to be gonochoristic and derived species to be protogynous hermaphrodites. It can therefore be predicted that protogyny is a derived condition from a gonochoristic reproductive ontogeny.

This trend reflects intra-specific hierarchy in size and social structure. The basal group (genus *Pomacanthus*) represents the largest species, which are usually pair forming, while the derived species are smaller and polygamous. *Pomacanthus* species can reach maximum lengths of 50 cm and are usually pair-forming. Although other reports have suggested polygamy, for example, in the western Atlantic *Pomacanthus arcuatus* (promiscuous), and *Pomacanthus paru* (polygamous or monogamous depending on its location) (refs in Moyer 1990), most observations suggest monogamy (Choat personal observation). Derived species however are represented by relatively smaller sized individuals (*Centropyge:* maximum size of 18 cm, *Apolemichthys*: 25 cm, *Genicanthus*: 35 cm, *Paracentropyge*: 12 cm, *Holacanthus:* 45 cm, *Pygoplites*: 25 cm, *Chaetodontoplus*: 35 cm) (Allen et al. 1998). They are group formers either forming male-dominated haremic systems (in most *Centropyge* sp, *Holacanthus tricolor, Pygoplites diacanthus*, *Chaetodontoplus mesoleucus*) (Moyer 1990) or lek-like structures (in *Genicanthus* sp) (Moyer 1984; Moyer 1990). This pattern and prediction will however need verification through age-based histological studies on more of the basal group, such as the *Pomacanthus sp,* and derived species from which no studies have yet been done, including genera *Apolemichtys*, *Chaetodontoplus, Paracentropyge*, and *Pygoplites*. Furthermore, integrated research which also looks at the social structures of *Pomacanthus sp* is needed.

4.4.2. THE MODE OF SEXUAL DIFFERENTIATION

The main findings of this study suggested high complexity in the mode of sexual differentiation among species of the Family Pomacanthidae. One study species, *P. sexstriatus* suggested a gonochoristic ontogeny with an extended period of sexual differentiation in immature individuals exhibiting a bisexual and female stage. This is followed by differentiation as either a functional male or female in the former, and towards functional females only in the latter stage. In contrast, *C. bicolor* demonstrated a *monandric* protogynous pattern of sexual ontogeny with males being recruited from all functional females through sexual transition. Still in another species, *C. loricula,* the mode of sexual differentiation is unclear, and may either be diandric or gonochoristic.

Interestingly sexual differentiation in *C. loricula* involved a bisexual immature stage similar to *P. sexstriatus*.

P. sexstriatus

The gonochoristic mode of sexual differentiation differs from those already described by Yamamoto (1969), Asoh and Shapiro (1997) and Sadovy and Collin (1995). All gonads in *P. sexstriatus* commence ontogeny as undifferentiated gonads as late as 5 years of age. They differentiate to either immature bisexual or immature female gonads at eight years of age. This is similar to the early mode of differentiation in the gonochoristic serranid *Epinephelus striatus* (Sadovy and Colin 1995). Immature bisexual gonads contain immature germ cells of both sex with no sign of degeneration or proliferation (Chan and Sadovy 2002) in a non-ovarian gonad. Sexual differentiation pathways involving a juvenile bisexual stage is consistent with sexual ontogeny described in many gonochoristic species. For example, juveniles of the serranid *Epihephelus akaara*, grammatid *Gramma loreto* and sea breams differentiate first as immature bisexuals and mature directly either to a male or a female (termed as late gonochorism in Buxton and Garratt 1990; Asoh and Shapiro 1997; Tanaka et al. 1990 in Chan and Sadovy 2002). An explanation of an extended period of the immature bisexual stage (up to 12 years) and the immature females is not yet clear. It does suggest however the flexibility in gonad differentiation towards either a male or female, which may reflect a social system in which finding a partner, may be difficult.

Distinct features of these gonads were potential precursory sperm ducts appearing as cracks along a medial blood vessel. Formation of precursory sperm sinus early in juveniles is typical of gonochoristic species (e.g., in the grammatid, *Gramma loreto* in Asoh and Shapiro 1997). These sperm sinuses are retained in testes but lost in differentiated ovaries (Asoh and Shapiro 1997). Precursory sperm sinuses may also develop prior to sexual maturation in some protogynous species. For example, in the diandric protogynous serranids, *Plectropomus leopardus* and *P. maculatus,* precursory sperm sinuses occur in immature bisexual gonads, but are distinguished from gonochoristic species in retaining sperm sinuses in mature females prior to sex change (Adams 2002). Early formation of a sperm sinus allows flexibility in sexual differentiation towards primary or secondary males (Adams 2002). This mechanism of attaining potential precursory sperm sinuses early in sexual differentiation well before gonadal maturation may equally allow flexibility in sexual maturation towards a functional male in *P. sexstriatus*. Subsequently they may be lost in some juvenile bisexuals differentiating into females. Future studies need to include samples of juveniles younger than 5 years to confirm initiation of sexual differentiation in *P. sexstriatus*.

C. bicolor **and** ***C. loricula***

Sexual development in *C. bicolor* was very distinct with a monandric protogynous hermaphroditism in which gonads commenced differentiation as immature females. These females mature before males, as early as 0.561 (205 days) years and all recruit into the male population through sex change at a minimum age of three years. Diagnosis of monandric protogynous hermaphroditism in *C. bicolor* was supported by a bimodal size and age distribution so that there were no young mature males (presumably primary males), and the presence of two transitional gonads. This is consistent with reported monandric protogyny in other *Centropyge* sp (e.g., *C. interruptus*: Moyer and Nakazono 1978; *C. multispinus*: Bruce 1980; *C. bicolor*: Aldenhoven 1984; *C. potteri*: Lutnesky 1988; Lutnesky 1996) in which all males were secondarily formed through sex change from functional females. Protogyny is a common phenomena in polygamous species in which large males monopolize mating through female defense and exclusion of smaller males (Warner 1984). Moreover it is favoured over gonochorism in species which acquire such social systems (Warner 1984). *Centropyge* sp have strict haremic social systems based on size hierarchies, so that the largest male dominates two or more females (Aldenhoven 1984; Moyer 1990), which change sex to male upon removal of the dominant male (Moyer and Nakazono 1978; Thresher 1982; Moyer 1984; Sakai and Kohda 1997; Sakai et al. 2003b).

There has been no study to confirm polygamy in *C. loricula,* however, results of this study suggest two possible pathways of male recruitment. The first pathway is through maturation of juvenile bisexuals into females, which presumably all recruit into the male population through sexual transition. While there was no transitional individual to confirm this, demographic features similar to *C. bicolor* suggested protogyny. This included bimodal age frequency distribution, where males were distributed in older age-classes, and females did not grow to full lifespan of males. In addition all testes were secondary in nature, acquiring peripheral sperm sinus, an ex-ovarian lumen and partial lamellar organization (Choat and Robertson 1975; Sadovy and Shapiro 1987). These features however do not hold conclusive evidence of a functional female changing sex to male. As immature bisexuals had already retained these anatomical features, males may have equally differentiated directly from immature bisexuals. A mode of sexual differentiation, which involves both pathways of male recruitment, will suggest *diandry*, however if it involves only the latter, reproductive ontogeny in *C. loricula* would be gonochoristic. Future research needs to include immature individuals younger than the age of sexual maturity to confirm initiation of sexual differentiation.

4.4.3. MORPHOLOGICAL CRITERIA OF THE TESTIS ASSOCIATED WITH DIFFERENT FORMS OF MALE RECRUITMENT

Testes, which correspond to a ‘secondary testes’ of protogynous species, may Develop through two ways. The first is through sexual transition of functional females (Choat and Robertson 1975; Sadovy and Shapiro 1987). Alternatively, they may develop directly from juvenile gonads that are ovarian and had already attained a lumen (Shapiro and Rasotto 1993; Sadovy and Colin 1995; Asoh 2004) and in some, potential sperm sinuses (Adams 2002). Consequently, sperm ducts run within the testis wall in the form of sperm sinuses as these gonads differentiate or change sex to function as males (Asoh 2004). In either pathway taken, males retain an ex-ovarian lumen, a lamellar organization and develop peripheral sperm sinuses (Sadovy and Shapiro 1987).

Results of this study revealed *C. bicolor* and *C. loricula* testes that corresponded to secondary testes. In *C. bicolor*, male recruitment through sex change of functional females only, resulted in testes that retained a lateral ex-ovarian lumen, lamellar organization and peripheral sperm sinus within the lateral side of the former gonad wall. Lamellar organization is eventually lost in all later functional testes. Studies on *C.*

bicolor by Aldenhoven (1984) proposed this to be due to fusion of lamellae in which spermatogenic tissue arose and proliferated from within the lamellae. This happens during and after sexual transition, forming a continuous mass of spermatogenic tissue in all functional testes.

Similarly, all testes in *C. loricula* hold partial lamellar organization, central ex ovarian lumen, and peripheral sperm sinuses within the whole length of the gonad wall. Males may develop from juvenile bisexuals that are ovarian similar to those in the gonochoristic serranid, *Epinephelus striatus* (Sadovy and Colin 1995), and grammatid *Gramma loreto* (Asoh and Shapiro 1997). Males may also be recruited through sex change of females. This scenario is similar to that seen in the scarid *Sparisoma cretense*, which initiates gonad ontogeny through an ovarian gonad with a lumen (de Girolamo et al. 1999). No transitional was found, however, all testes were ‘secondary’ in nature (de Girolamo et al. 1999). Furthermore, demographic results supported small and presumably younger males than the age of first maturity in females, which suggested direct differentiation from immature females (de Girolamo et al. 1999).Further, de Girolamo et al. (1999) concluded a gonochoristic reproductive ontogeny with potential for diandry. They recognized the high degree of variability in sexual ontogeny between populations and suggested further sampling of other populations. Juvenile sexual differentiation is needed to verify which mode of sexual differentiation is taken, and important features in identifying a transitional is also critical before reaching conclusive suggestions about the reproductive ontogeny of *C. loricula*.

Male recruitment in *P. sexstriatus* involved direct differentiation from immature bisexual gonads that were not ovarian. This resulted in testes morphology that lacked common features of ‘secondary testes’. Early immature testes however contained early stages of oogenesis (oogonia) from the former immature bisexual gonads. These eventually disappeared later in functional testes, which were always filled with spermatogenic tissue, and developed medial sperm ducts. Medial sperm ducts in primary males of some diandric labroid species develop from gonads that were oocytic but without a lumen or spermatogenic tissue (Shapiro and Rasotto 1993). For example, sperm ducts of *Thalassoma bifasciatum* consequently run in the medial hilar regions of gonadal lobes throughout their length (Shapiro and Rasotto 1993). Similarly, the absence of a lumen in the immature bisexual of *P. sexstriatus* may have given rise to a medial sperm duct. Similar to *C. loricula*, future research need to include in samples of early juveniles to confirm the proposed hypothesis of gonad ontogeny.

4.4.4. IMPORTANT FEATURES IN DIAGNOSING PROTOGYNY IN *C. bicolor*

Important criteria for diagnosis of protogyny in some of the most commonly studied fishes, the labroids are the formation of ducts during the transitional phase so that they are entirely new structures (Choat and Robertson 1975; Shapiro and Rasotto 1993). This occurs concomitant with germinal changes (degeneration of female tissues and proliferation of male tissue) associated with sex change (Sadovy and Shapiro 1987). Moreover, the presence of a peripheral sperm sinus in the gonad wall of testes is important in the diagnosis of protogyny (Sadovy and Shapiro 1987).

Evidence from this study suggests however that these criteria may differ at the species level. *C. bicolor* transitional individuals corresponded to the 10-day transitional gonads found in Aldenhoven (1984) (see Table 4.2), in which there were no degenerating vitellogenic oocytes, but only proliferating sperm tissue. While this study was not able to distinguish whether sperm ducts were new structures following sex change, evidence to suggest they are comes from a study on the same species by Aldenhoven (1984). Twenty days after male removal from a harem, sperm ducts of gonads of former dominant females had formed with abundant spermatozoa in them (Aldenhoven 1984). Sexual transition in *C. bicolor* can therefore be presumed to involve secondary formation of the sperm sinus, however the process of degeneration of female germ cells and proliferation of male germ cells may occur sequentially over a period of about 20 days (Aldenhoven 1984).

Evidence of former female function therefore became important in distinguishing a transitional gonad in *C. bicolor*. Transitional direction from mature females included a thick gonad wall, lamellar organization, and an ovarian lumen remnant. The importance of former female function as criteria in diagnosis of protogyny is observed in the diandric serranids, *Plectropomus leopardus* and *P. maculatus* (Adams 2002). The sperm sinus is formed as early as in immature females (termed immature bisexuals), so that the sperm sinus is not a new structures in secondary testes. Furthermore, a peripheral sperm sinus is developed in both primary and secondary testes when fully developed so that it is difficult to distinguish them. Structural features that become important in diagnosis of protogyny in these species are those indicating previous spawning such as a thick gonad wall, intra-lamellar muscle bundles, post-ovulatory follicles and the presence of atretic vitellogenic oocytes (Adams 2002). It would be interesting to further investigate and define important criterias in transitionals of species like *C. loricula* in which, sperm tissue is maintained within gonads from the juvenile stage all throughout its mature female stage. Other features such as formation of sperm ducts may become more important.

4.4.5. FUTURE DIRECTIONS AND RESEARCH

With regards to the aims of this study, some questions still remain on

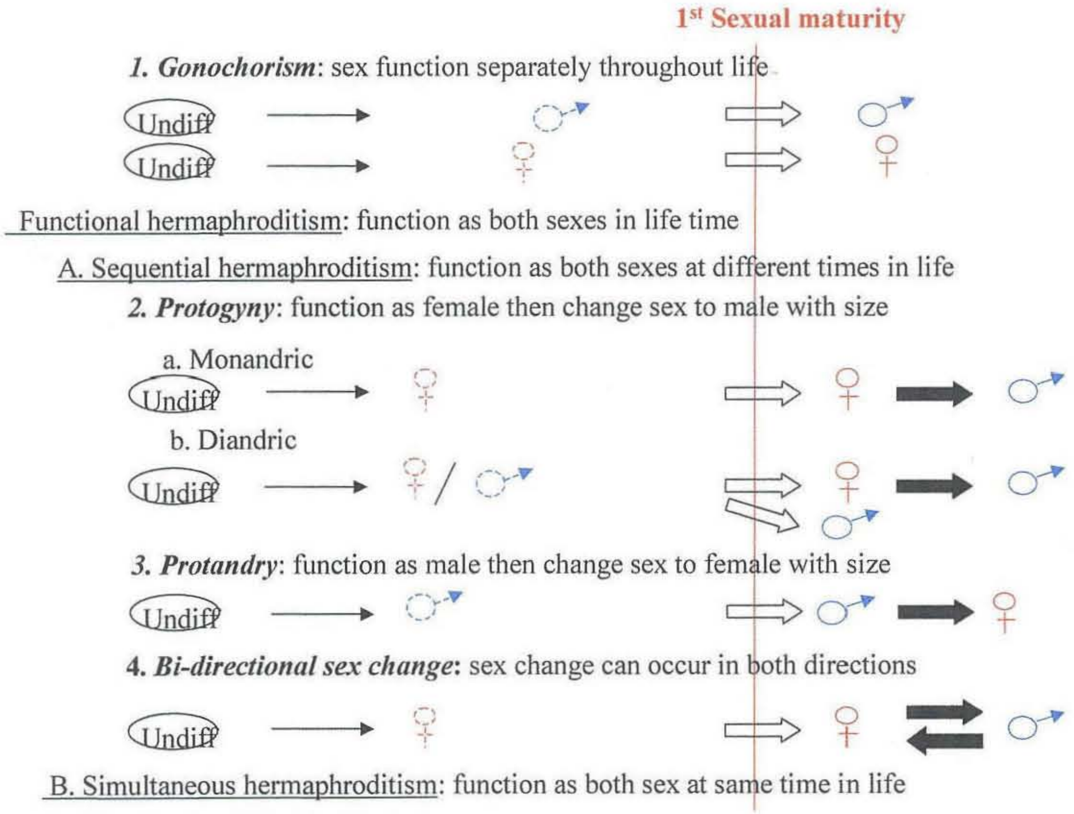
i. the mode of sexual differentiation in the very early life history of *P. sexstriatus* and *C. loricula,*
ii. whether *C. loricula* is in fact protogynous, and hence criteria for classification of a transitional.

Specifically, to follow on from this study and to disclose its gaps and shortcomings, the following directions are recommended:

- Include in samples, juveniles younger than 5 years of age and smaller than 170mm FL in *P. sexstriatus* and younger than 0.44 years (161 days)and smaller than 50mm in *C. loricula*, to explore initial gonad ontogeny.
- Re-examine gonad ontogeny of *C. loricula* in detail from a sample that covers a wide range of size and ages, and preserving gonads in FAAC on the day of capture. Find important criteria to distinguish a transitional.
- Detailed examination of the mode of sexual differentiation in other *Pomacanthus* species to verify if gonochorism with an extended immature bisexual stage is indeed a generic feature. This should be integrated with studies of social systems to relate it to its corresponding reproductive ontogeny.
- Examine phylogeny of a wider range of species within the Family Pomacanthidae to get a greater picture of the trends observed in this study, that derived species are protogynous and are derived from a basic gonochoristic ontogeny in the larger basal species.

- On a more broader perspective, samples from other geographic locations to examine if the mode of sexual differentiation in species of the Family Pomacanthidae is similar in different regions, e.g., in central areas along the and edge of distributions as done in Meekan et al. (2001) on demographic features.

Figure 4.1. Forms of reproductive ontogeny in reef fishes. Arrows indicate sexual differentiation, at which they first become mature (empty block arrows) or functionally sex change (filled block arrows). Immature individuals have dotted symbols; mature individuals with fully lined symbols; undiff = undifferentiated.



1. Gonochorism: sex function separately throughout life

Functional hermaphroditism: function as both sexes in life time

A. Sequential hermaphroditism: function as both sexes at different times in life

2. Protogyny: function as female then change sex to male with size

a. Monandric

b. Diandric

3. Protandry: function as male then change sex to female with size

4. Bi-directional sex change: sex change can occur in both directions

B. Simultaneous hermaphroditism: function as both sex at same time in life

Figure 4.2. Aims of Chapter 4. Displayed on a sample of a sex-specific size at age plot. Not to scale.

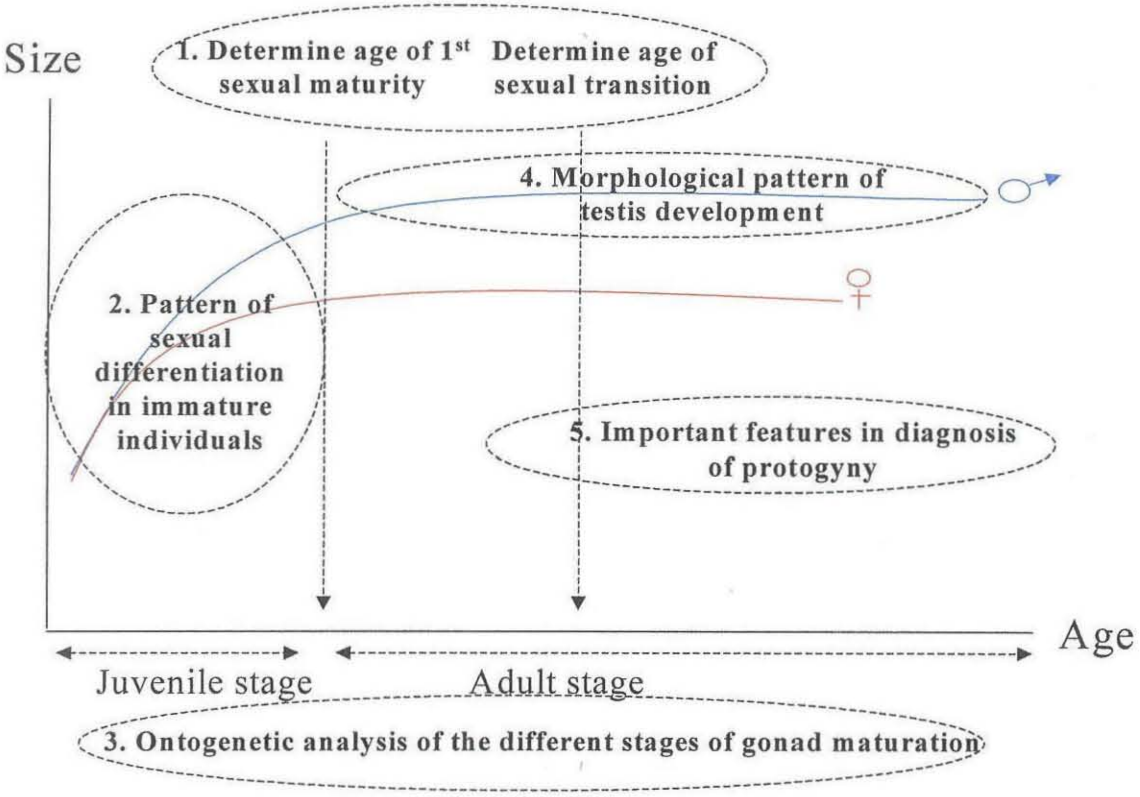


Figure 4.3. ***P. sexstriatus***. Histological transverse section of immature individuals. **A-D** Immature bisexuals contain stage I previtellogenic oocytes (St I) and spermatocytes (SC) showing no degeneration or proliferation of both sexual germ cells. Potential precursory medial sperm ducts (PPMSD) appear as cracks alongside the medial blood vessel (MBV) in some individuals. **E** Immature male: testes containing proliferating sperm tissue (ST) and stage I oocytes. **F** Immature females: ovary with thin gonad wall (c.f mature females); lamellae filled with previtellogenic oocytes, mostly stage I-II.

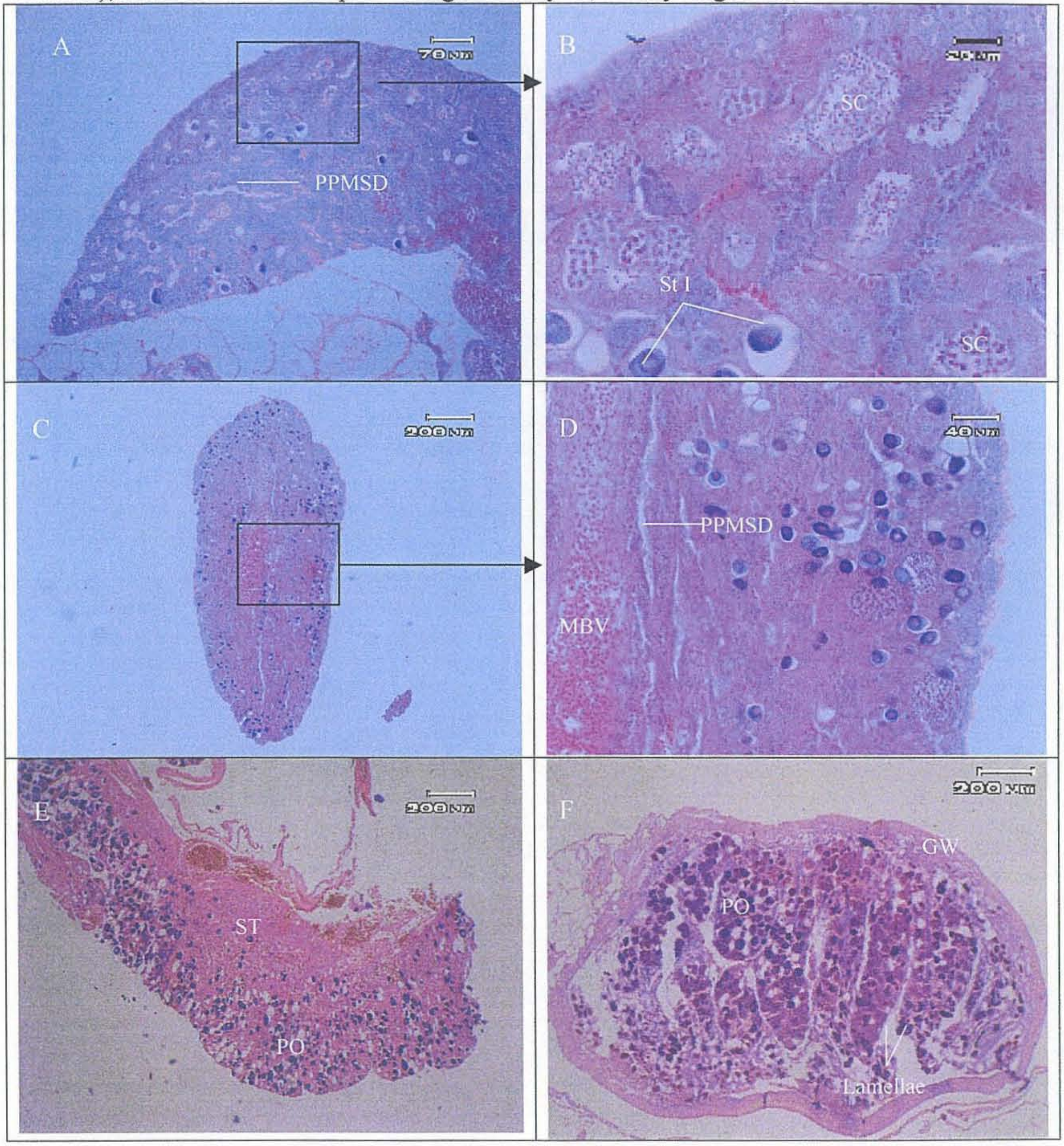


Figure 4.4. ***P. sexstriatus.*** Mature individuals. **A** Early staged-mature male: some spermatozoa in sperm ducts (SD). **B-C** Later staged-mature males showing very distinct sperm ducts filled with spermatozoa (SZ). Stage I oocytes may be present in some mature male testes. **D** Mature female gonads are large and have thick gonad wall (GW) and contain at least vitellogenic oocytes (St IV). **E** Running ripe mature female containing coalesced oocytes (St V). **F** Sign of previous spawning in mature females: atretic stage IV oocytes and muscular bundles (MB).

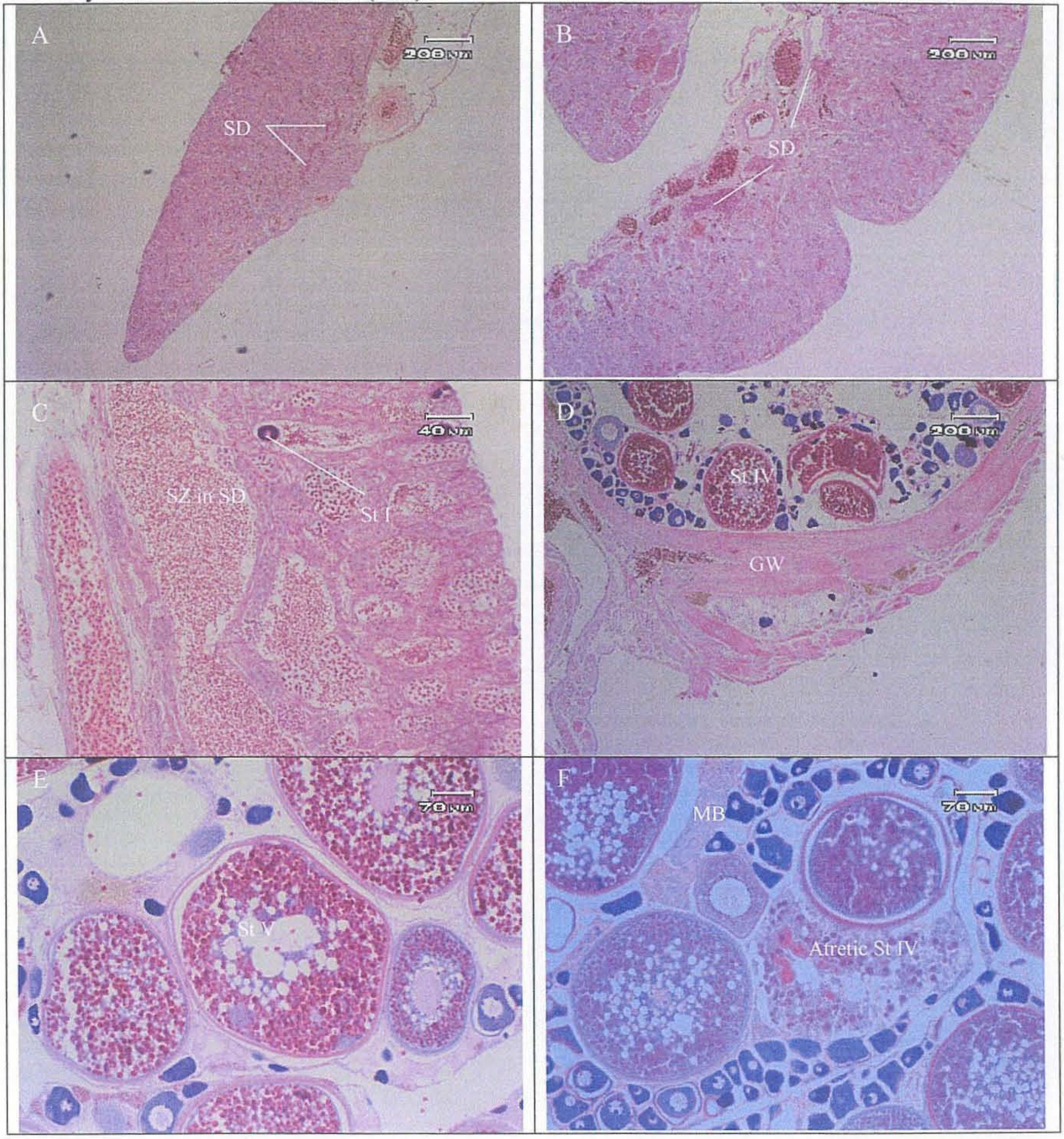


Figure 4.5. *C. bicolor*. Histological section of female gonads. **A** Immature female have small compact gonads containing stages I-III previtellogenic oocytes (St I-III). **B-D** Mature female gonads with at least vitellogenic oocytes (St IV), which sequentially become coalesced (**C**) and then hydrated (St V) (**D**) before spawning.

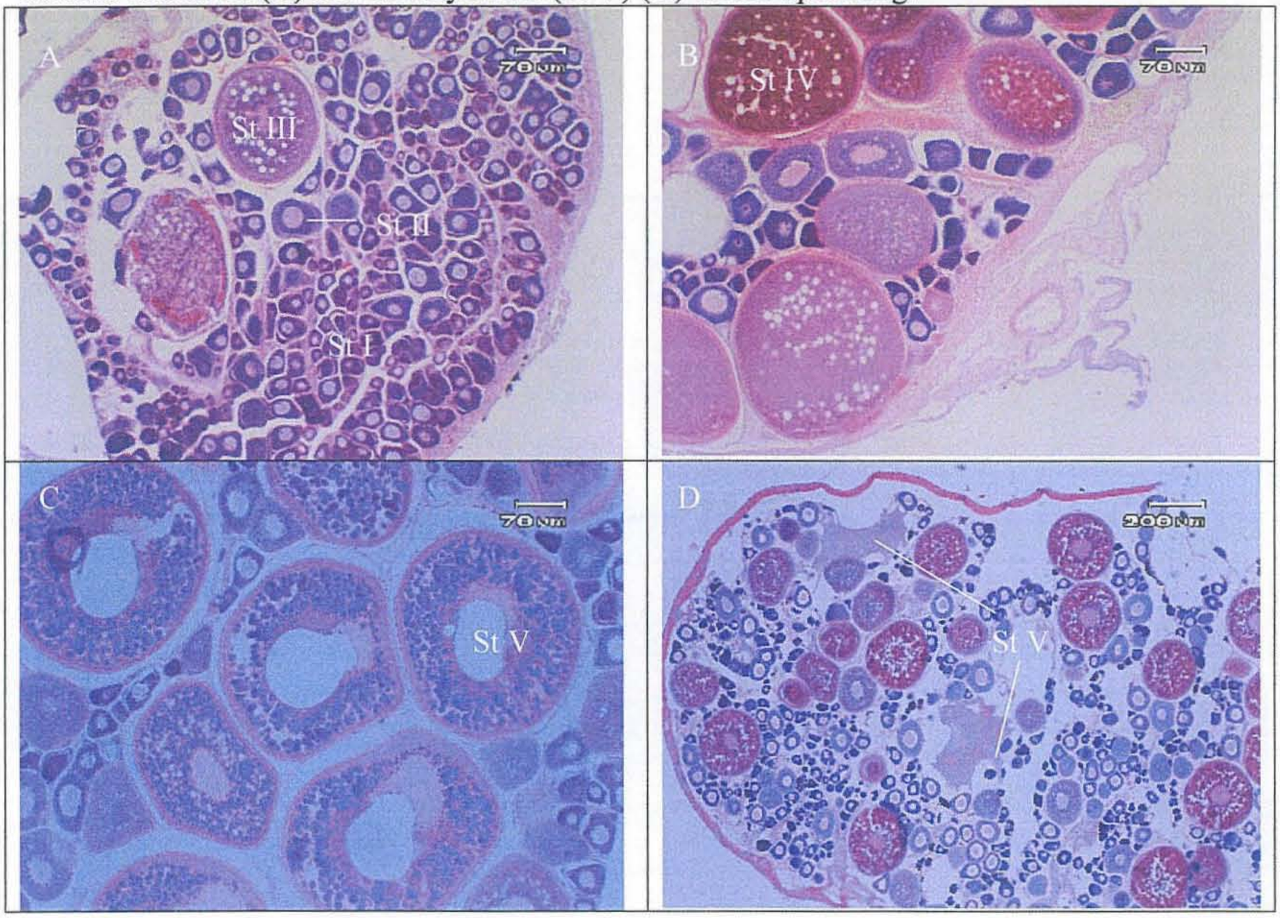


Figure 4.6. ***C. bicolor***. **A-C** Stage 3 transitional gonads contain previtellogenic oocytes and proliferating sperm tissue in a previously functional female gonad (Aldenhoven 2004, Adams 2002). Previous female function is evident through the thick ex-ovarian wall (EOW) and brown bodies (BB). **D** Mature male testes develop peripheral sperm sinus (SS) within the ex-ovarian wall (EOW) that is filled with spermatozoa. Ex-ovarian lumen (EOL) is retained in all mature males.

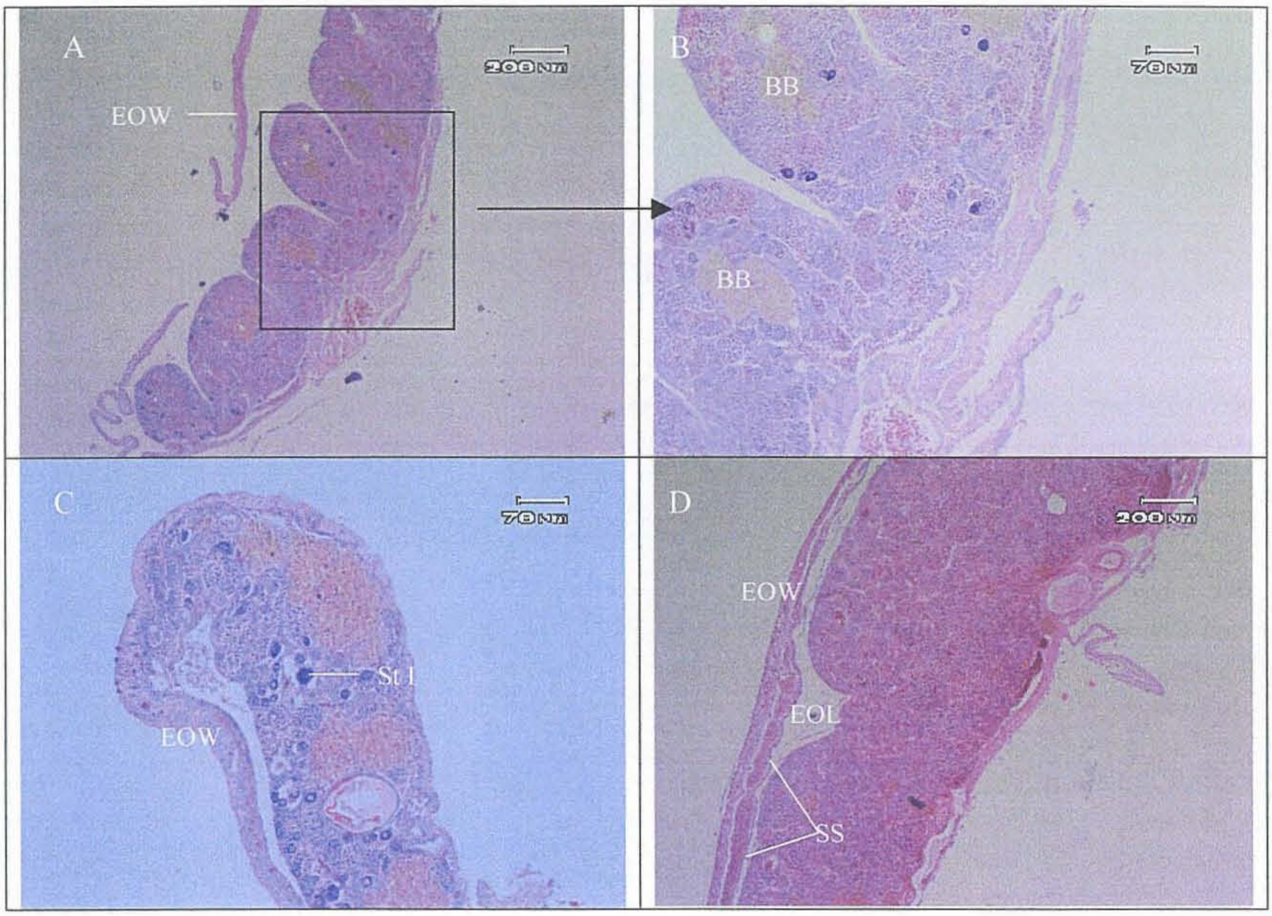


Figure 4.7. *C. loricula.* **A-B** Immature bisexual gonad containing previtellogenic oocytes intermixed with early spermatogenic tissue in an ovarian gonad with a central ovarian lumen (OL). **C-D** Sperm crypts usually present in all stages of mature females. **E** Mature spent female: large ovary containing atretic vitellogenic oocytes. Lamellae is disorganized and disrupted. **F** Mature resting female: ovary comprises of previtellogenic stage I oocytes in a non-compact lamellae that is disrupted and disorganized.

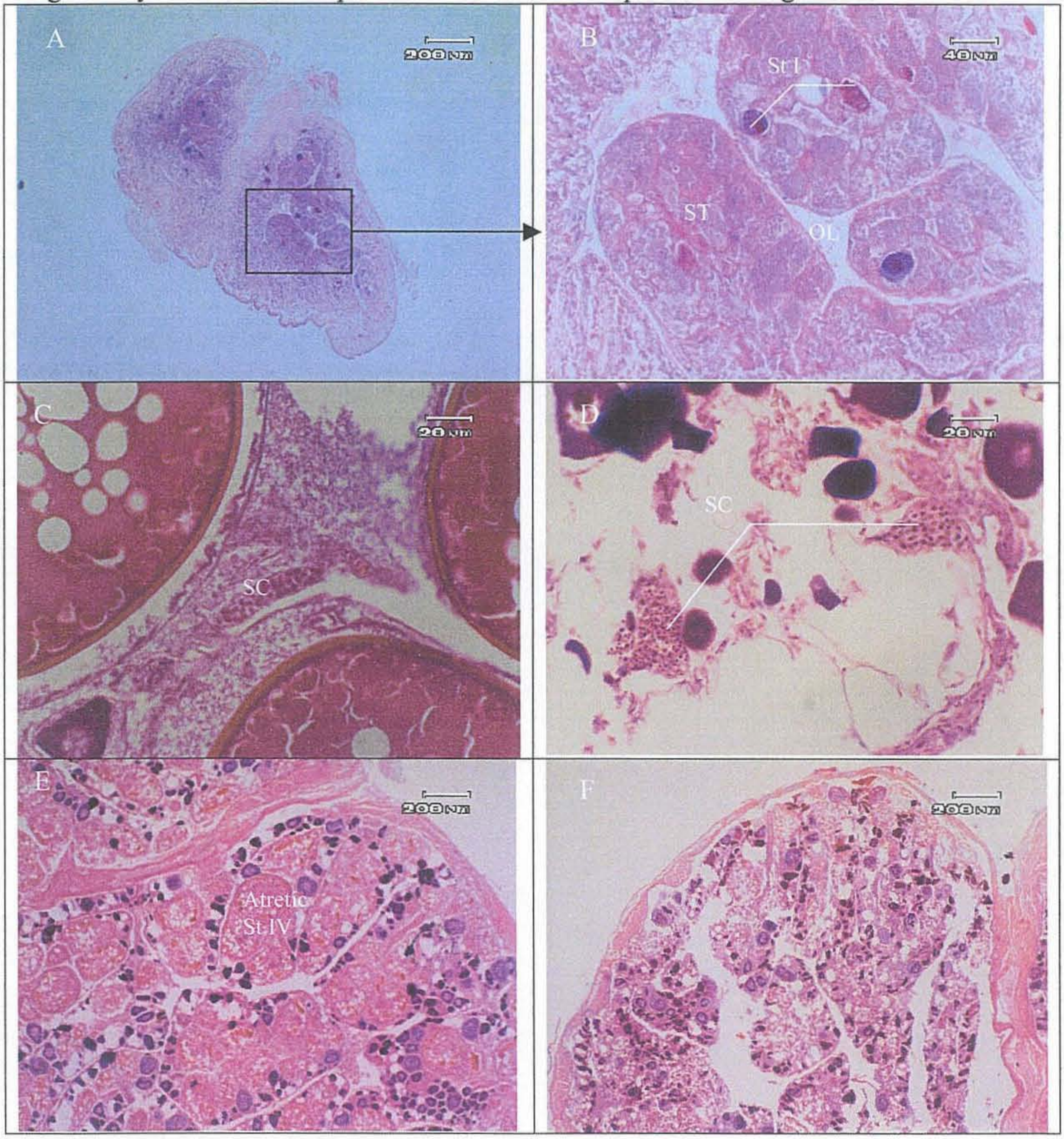


Figure 4.8. ***C. loricula***. **A** Active mature male: testis in partial lamellar form showing an ex-ovarian lumen (EOL). **B** Active mature male: testis is fully extended showing very distinct sperm sinus filled with spermatozoa. **C** Inactive mature male: spermatogenesis is up to the secondary spermatocyte stage (SS). **D** Inactive undifferentiated mature male: tissue in testis is undifferentiated (UT); no spermatogenic activity.

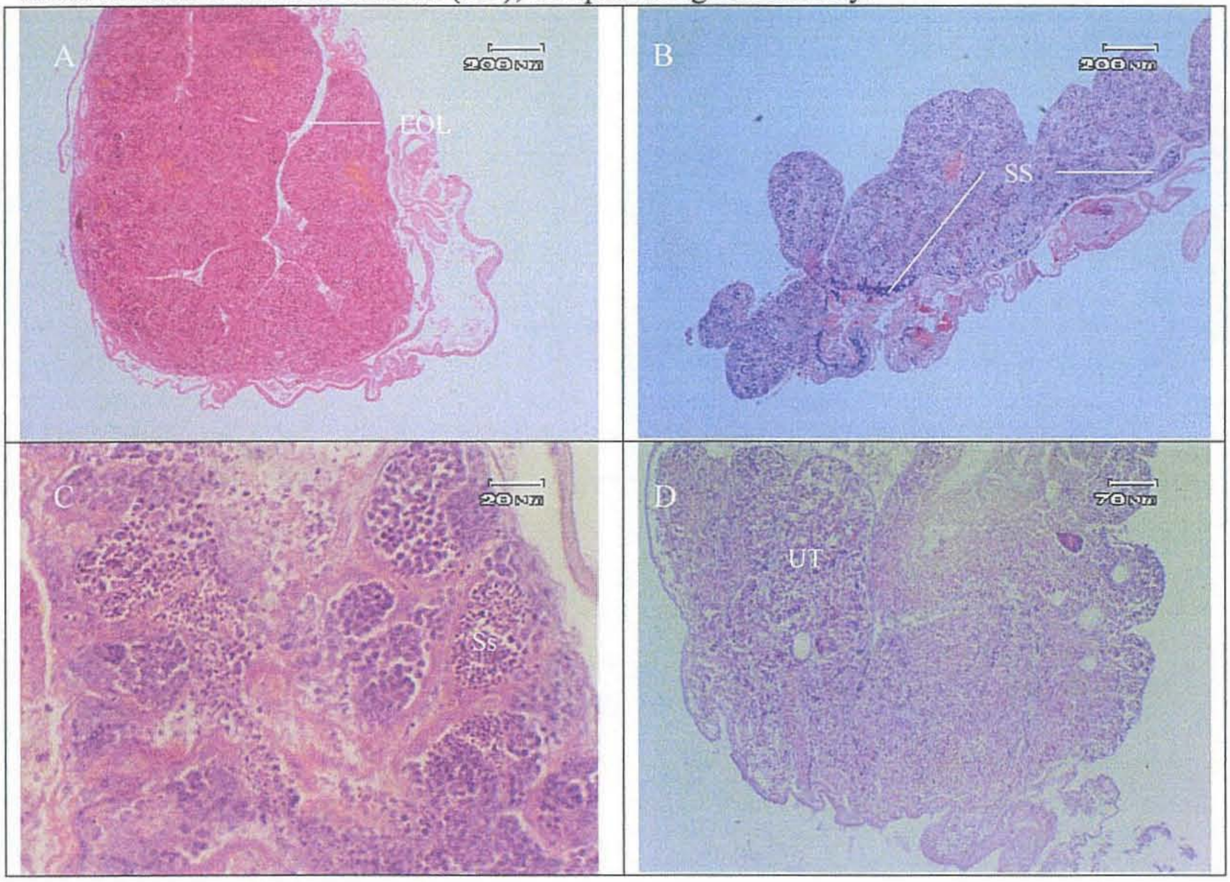
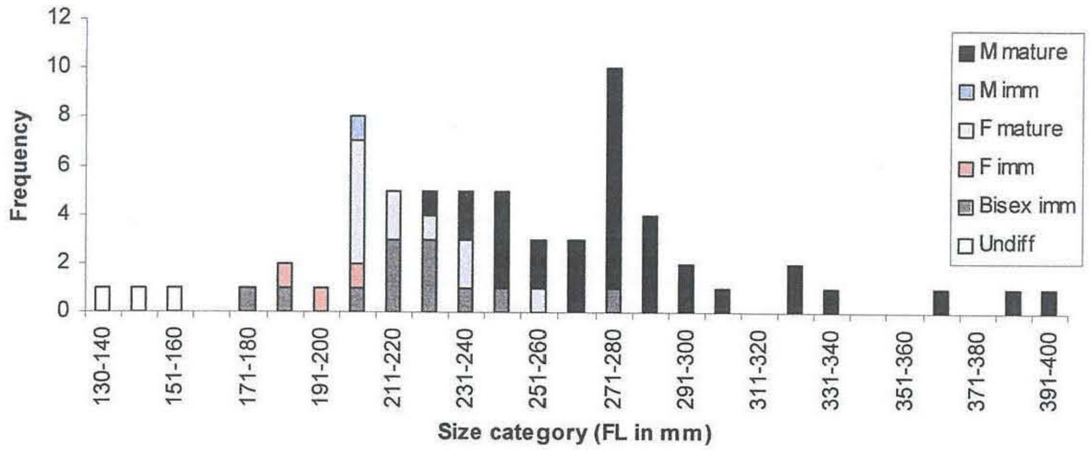
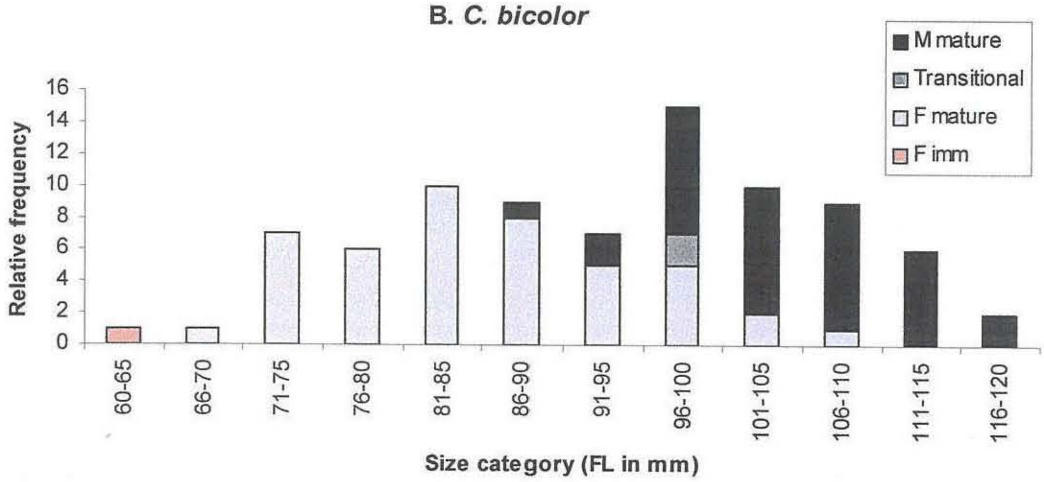


Figure 4.9. Size frequency distribution by reproductive category for *P. sexstriatus* (A), *C. bicolor* (B) and *C. loricula* (C).

A. *P. sexstriatus*



B. *C. bicolor*



C. *C. loricula*

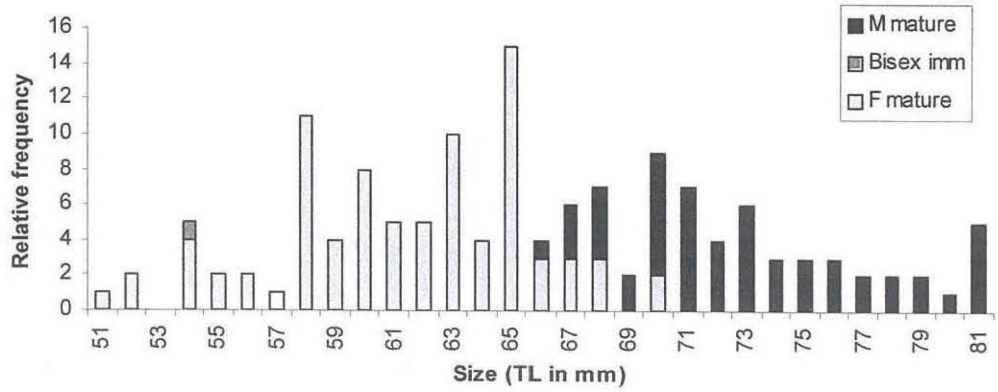
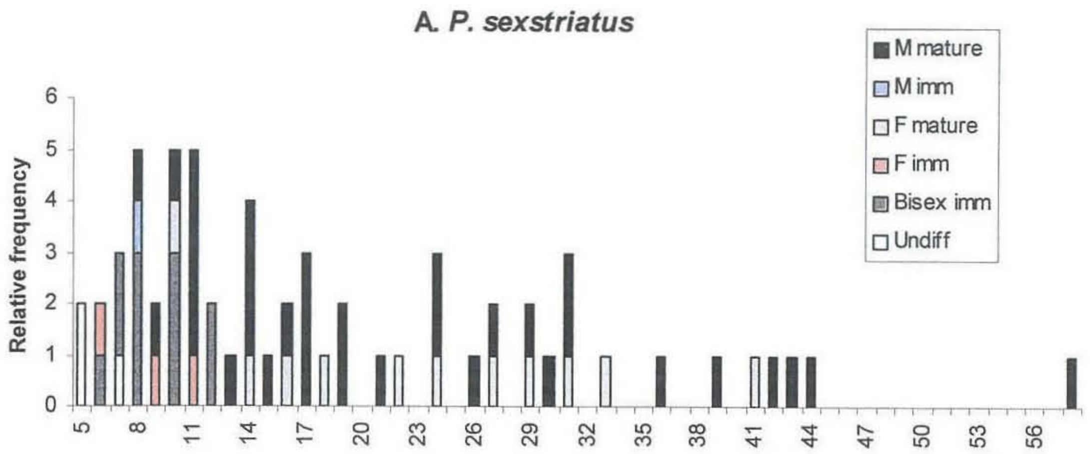
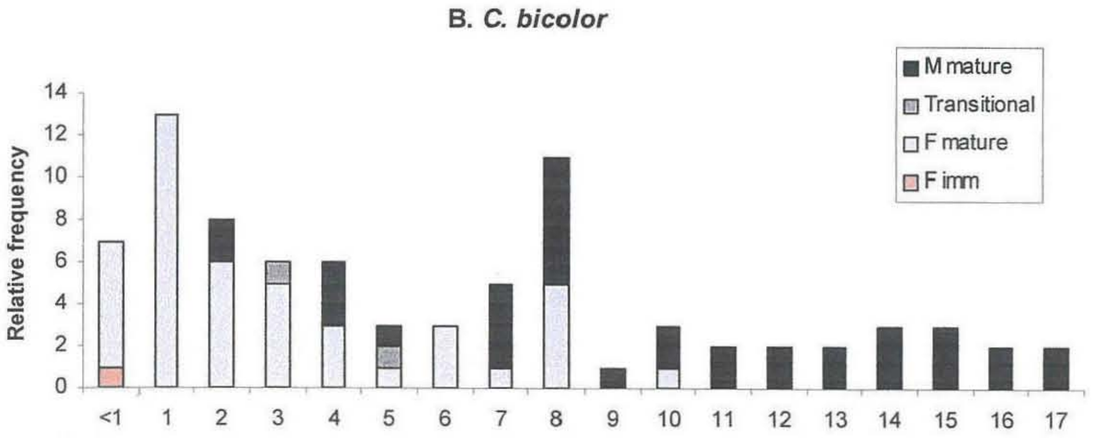


Figure 4. 10. Age frequency distribution by reproductive categories for *P. sexstriatus* (A), *C. bicolor* (B) and *C. loricula* (C).





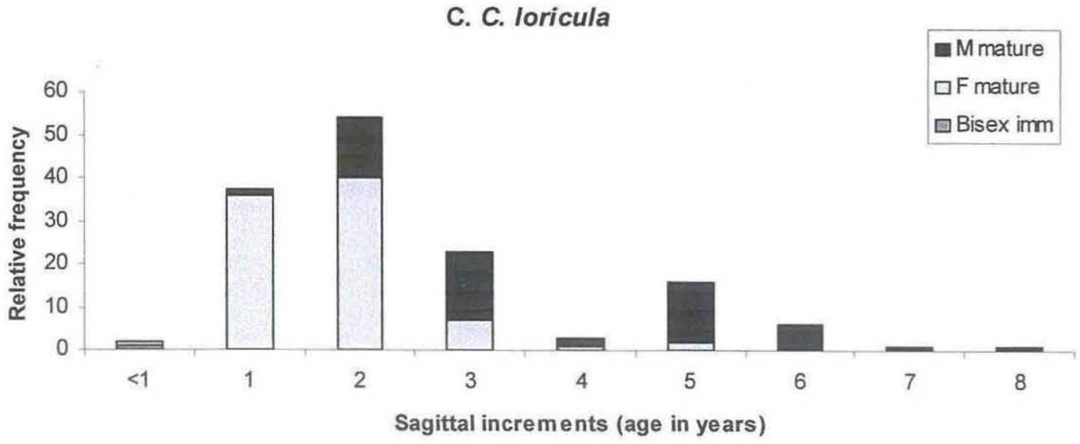


Figure 4.11. Possible sexual differentiation pathways for *P. sexstriatus*. Reproductive categories organized according to where it best fits on sex-specific size at age plots. The extent of ovals represent age ranges of each reproductive group; complete arrows represent evidence of differentiation pathway found in this study; dotted arrows – no evidence found. Crosses suggest pathway is unlikely to occur. Ages shown correspond to ages of youngest individual, sexual maturity, sex change (if applicable), and maximum longevity in females and males. One immature male was found at eight years old.

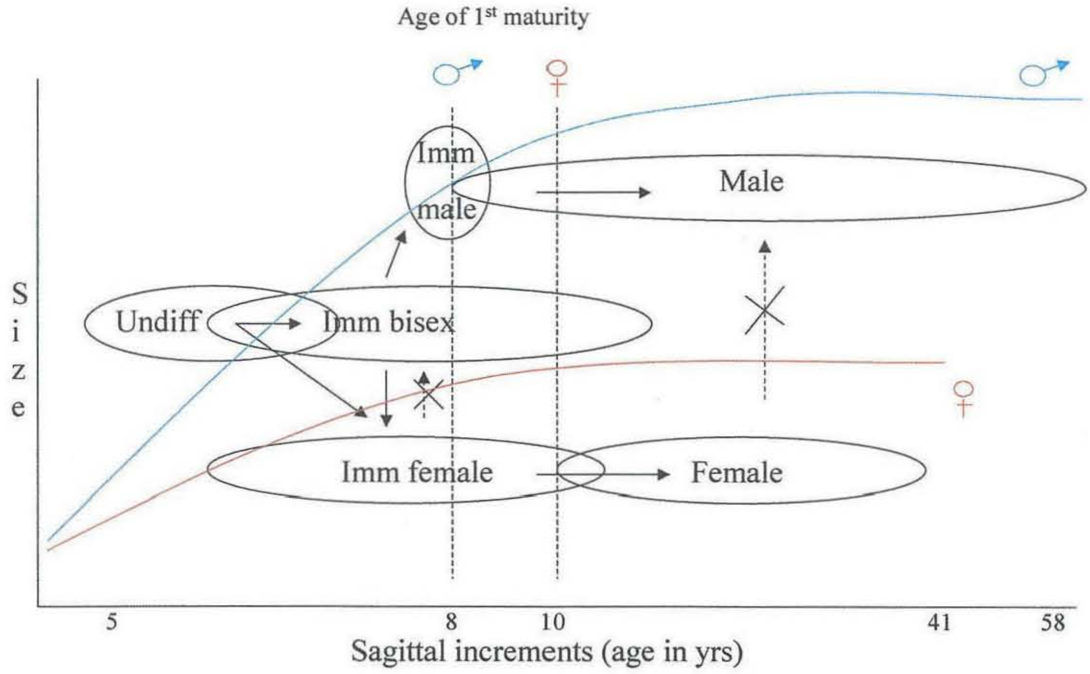


Figure 4.12. Possible sexual differentiation pathways for *C. bicolor.* See figure 4.11 for explanation of symbols.

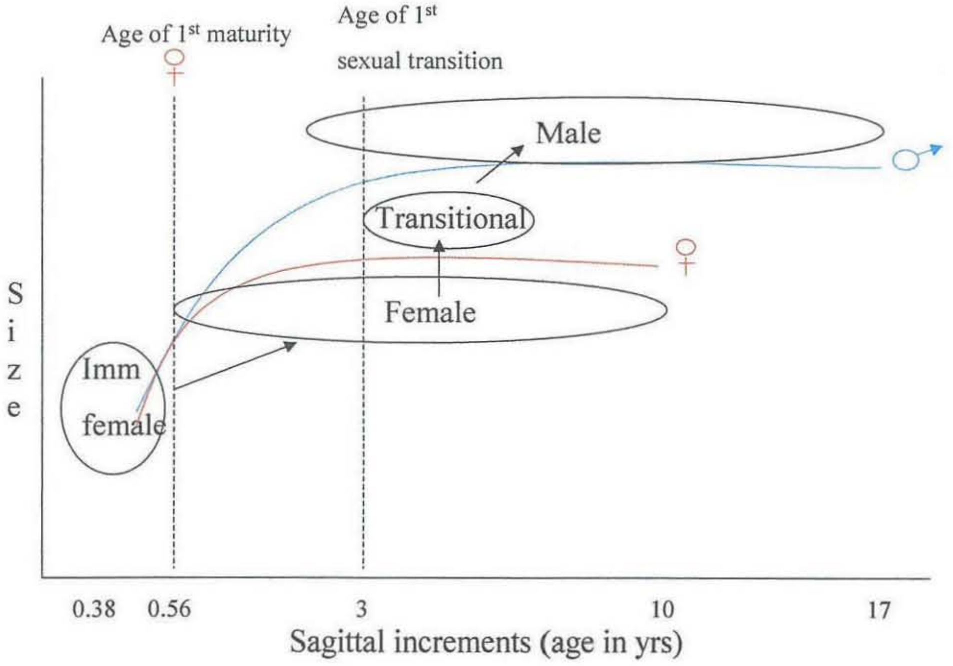


Figure 4.13. Possible sexual differentiation pathways for *C.loricula.* See figure 4.11 for explanation of symbols.

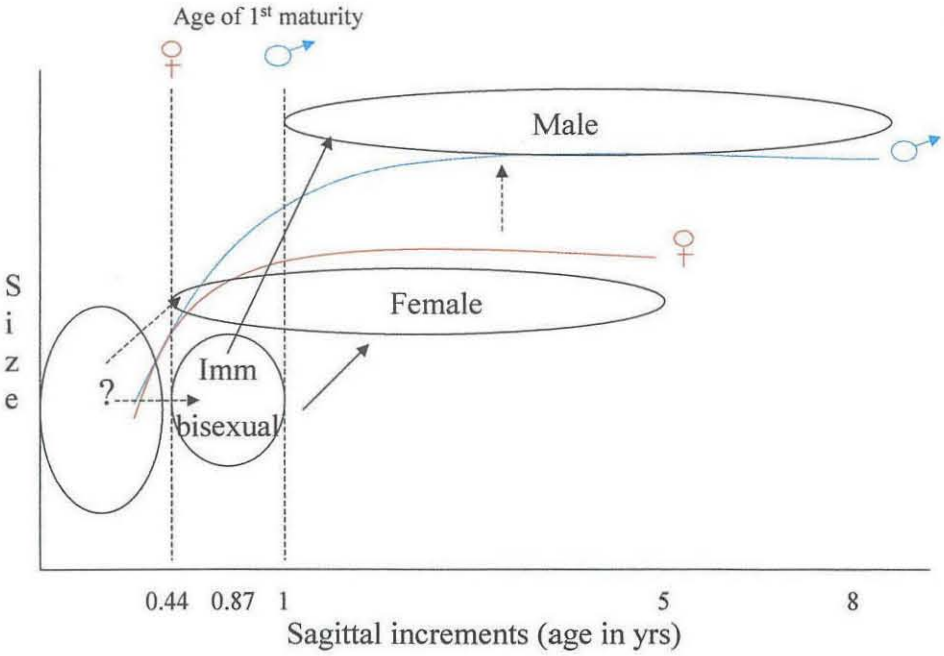
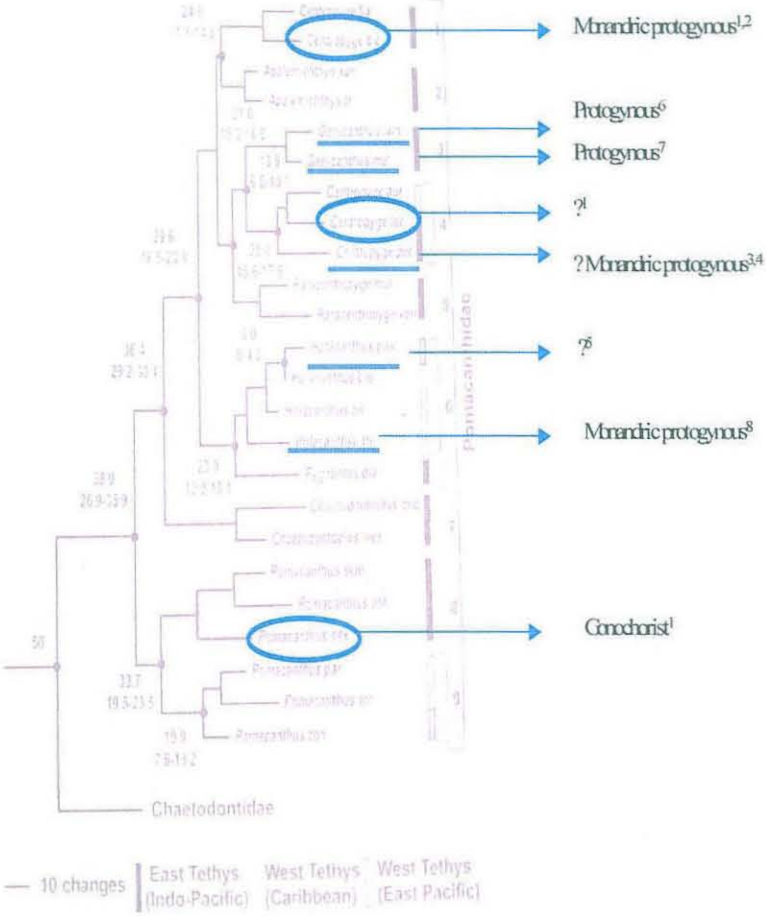


Figure 4. 14. Phylogenetic tree of 24 chosen species of Family Pomacathidae relative to the Family Chaetodontidae, showing species from this study (circled) and other species on which histological studies have been undertaken (underlined). Figure modified from Bellwood et al (2004). [1]This study; [2]Aldenhoven (1984); [3] Lutnesky (1996); [4]Lutnesky (1988); [5]Arellano-Martinez et al (1999); [6]Suzuki et al (1979) in Moyer (1990); [7]refs in Moyer (1990); [8]Hourigan and Kelley (1987).



CHAPTER 5. OTOLITH DYNAMICS OF POMACANTHUS SEXSTRIATUS AND CENTROPYGE BICOLOR

5.1. INTRODUCTION

Larger size-at-age of males compared to females is a common phenomenon in a wide range of coral reef fishes. It is most common in protogynous species (e.g., *Scarus niger, Chlorurus sordidus*: Choat et al. 1996; *Plectropomus maculatus*: Adams 2002) but is also being found increasingly in gonochoristic species (e.g., *Lutjanus adetii, L. quinquelineatus*: Newman et al. 1996; *Ctenochaetus striatus*: Laman Trip 2004). Sex-specific differences in size-at-age in protogynous species has been explained through two hypotheses: "transitional growth spurt hypothesis" and "juvenile growth hypothesis". Transitional growth spurt hypothesis involves an increase in somatic growth after sex change (Choat et al. 1996; Walker and McCormick 2004), while the juvenile growth hypothesis states variable size-at-age trajectories are established in juveniles resulting in faster growing individuals differentiating as males and slower individuals as females (Francis and Barlow 1993; Adams and Williams 2001).

To examine the mechanism of larger size of males compared to females at any given age in protogynous species or to conduct sex-specific growth comparisons in gonochoristic species, back-calculation methods are used to estimate sex-specific size at earlier ages before sexual maturation and/or sexual transition (Francis 1990 in Hare and Cowen 1995; Walker and McCormick 2004). Back-calculation uses sagittal otolith increments, which provide a record of otolith size-at-age and are used to estimate fish size-at-age (Francis 1990 in Hare and Cowen 1995). However two assumptions must be met before back-calculation can be used. Firstly sagittal increments must be validated as annual (or daily) rings, and secondly, that a constant proportional relationship between otolith growth and somatic growth exists (Hare and Cowen 1995). This second assumption has faced much discrepancy and needs to be evaluated further before any attempt of back-calculation can be made.

The otolith growth and somatic growth must be in constant proportion independent of age and growth rate (Hare and Cowen 1995). This assumption is not always followed. A disproportionate otolith growth –somatic growth relationship may be reflected either through a consistent increase in otolith size through life despite growth rate that is asymptotic or zero (“age effect”) or through fast growing individuals obtaining smaller otolith size (“growth effect”) (Secor and Dean 1992; Hare and Cowen 1995). Moreover, a recent age-based study by Munday et al. (2004) in a protogynous scarid, *Scarus frenatus* has suggested a growth effect that is sex-specific, whereby faster growing males have smaller otolith sizes at equivalent ages. Sex-specific growth effect marks an important area of study especially where relationship between sex- specific growth rates is involved.

This study therefore aims to observe the assumption that otolith growth and somatic growth are in constant proportion independent of age and growth rates by sex in two angelfishes, *P. sexstriatus* and *C. bicolor.* In both species, males are larger in size at any given age than females (Chapter 3: Growth and longevity) but differ in reproductive ontogeny, *P. sexstriatus* being a gonochorist and *C. bicolor* a monandric protogynous hermaphrodite (Chapter 4: Reproductive ontogeny). I used the approach used by Munday et al. (2004), which compares sex-specific size-at-age with the mean cumulative otolith radius-at-age plot. It predicts that if age by sex had an effect on the otolith-somatic growth relationship, an increasing cumulative otolith radius with age would result in both sexes, despite asymptotic growth (Chapter 3) (“sex-specific age effect”). It also predicts that if growth rate by sex had an effect on otolith-somatic growth relationship, cumulative otolith radius of slower growers (females) would be larger or similar at any given age than the faster growers (males) (“sex-specific growth effect”).

Therefore the two main questions of this study are:

1. Is there an sex-specific age-effect and/or
2. sex-specific growth effect in *P.sexstriatus* and *C. bicolor*?

5.2. METHODS

5.2.1. STUDY SPECIES

Pomacanthus sexstriatus has a gonochoristic reproductive ontogeny with a complex early mode of sexual differentiation. Males differentiate from immature bisexuals, while females may differentiate directly from immature female gonads or indirectly from immature bisexual gonads (Chapter 4: Reproductive ontogeny). *P. sexstriatus* has a lifespan of 58 years, however females live up to 41 years only (Chapter 3: Growth and longevity). Age of first maturity for males and females are eight and 10 years respectively (Chapter 4: Reproductive ontogeny).

Centropyge bicolor is a monandric protogynous species in which all males are secondarily derived from mature females through sexual transition. *C. bicolor* has a lifespan of 17 years, in which females live up to 10 years only. Females mature as early as 0.56 years, and males at 2 years. Age of first sex change is found at three years (Chapter 4: Reproductive ontogeny).

5.2.2.DATA COLLECTION

Field collection of samples, length at capture (fork length: FL), and aging of otoliths were undertaken as described in Chapter 3, section 3.2.2 – 3.2.5. Individual gonads were classified into reproductive categories through histological examination (as done in Chapter 4, section 4.2.3 – 4.2.4).

Measurements of cumulative and maximum otolith radius

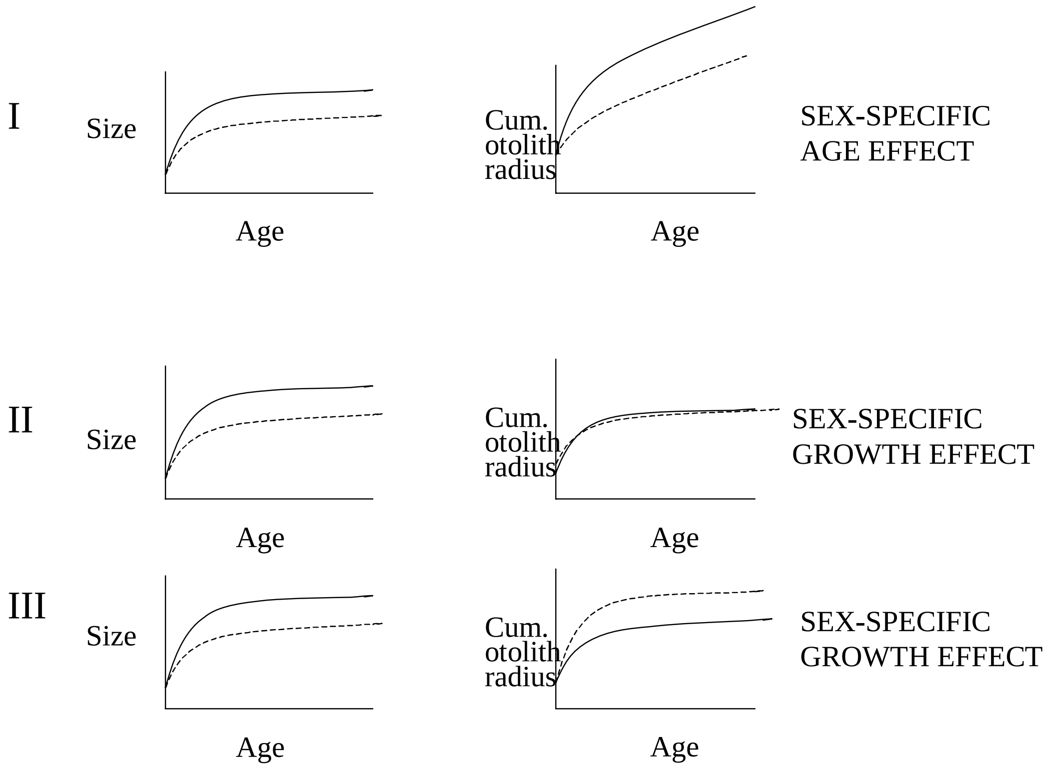
The “otolith radius in yearly or annual increments (cumulative otolith radius) is the distance from the core to the distal edge of the dark side of each annual ring along a constant angle” (Munday et al. 2004). The maximum otolith radius is therefore a measurement from the core to the outer edge of the sagittal otolith along the same constant angle. Images of chosen otoliths were captured using Image Grab software, which was connected to a 5x-dissecting microscope and a video camera. Images of *P.*

sexstriatus were captured at 3x magnification, and *C. bicolor* at 5x magnification. These images were opened in Paint software and horizontal lines were drawn and saved accordingly. Using digital image analysis software called UTHSCSA Image Tool Version 3.0, an angle to the horizontal on the dorsal side of the sulcus, was chosen for each species, in which annual increments were clear in most or all chosen otoliths. Accordingly they were set at 30^0 and 50^0 to the horizontal for *P. sexstriatus* and *C. bicolor* respectively. The angle and respective annual rings along the chosen angles were marked for each otolith using Paint, so that measurements would be fixed (Figure 5.1). The following measurements were taken using Image Tool: cumulative otolith radius of individuals in older categories (over 20 years in *P. sexstriatus*: (7 males, 6 females) and over 4 years in *C. bicolor* (24 males; 14 females)). This enabled comparison of sex-specific patterns of otolith growth over the life of each species.

5.2.3. DATA ANALYSIS

To examine whether otolith-somatic growth are constantly proportional independent of age and sex specific growth rates, the relationship between (i) sex-specific size and age fitted onto the von Bertalanffy growth function (as done in Chapter 3, section 3.2.7.2) and (ii) sex-specific cumulative otolith radius with age were examined (following the method by Munday et al. 2004). Cumulative otolith radius is expected to increase continuously in both sexes despite sex-specific asymptotic growth with a sex-specific age effect (Figure 5.1 I). Moreover, cumulative otolith radius of slower growers (females) is expected to be larger or similar at any given age than the faster growers (males) if there is a sex-specific growth effect (Figure 5.1 II & III). Repeated-measures analysis of variance (RM-ANOVA) was used to statistically compare cumulative otolith radius at respective ages for both males and females (α = 0.05) (Zar 1999). RM-ANOVA was done up to 31 years for *P. sexstriatus* and up to 9 years for *C. bicolor*, due to firstly, shorter lifespan in females, and secondly, limited data for statistical analysis in the older females. SPSS 11.0 was used for this analysis.

Figure 5.1. Diagram illustrating expected relationship between size at age and cumulative otolith radius at age plots with a sex-specific age and growth effect (according to Munday et al. 2004). Full lines represent male growth, dotted lines represent female growth.



5.3. RESULTS

P.sexstriatus

Sex-specific size-at age trajectories revealed larger size with age of males compared with females (Figure 5.2A; Chapter 3). Divergence of sex-specific growth initiates at around eight years of age, so that at 15 years a female is about 200mm FL and a male is around 270 mm FL. Growth asymptotes after 20 years in males, and earlier in females, after 10 years. Mean size-at age is significantly different between sexes between 10-41 years (Chapter 3). Prior to this difference, immature bisexuals are almost consistently larger than immature females, both of which are larger than undifferentiated individuals.

Examination of the sex-specific cumulative otolith radius with age showed no difference in otolith growth between sexes in the early years up to 10 years of age (Figure 5.3A). Beyond 15 years otolith growth starts to diverge so that otolith growth in males accelerates resulting in faster growing otoliths than females. This difference in cumulative otolith radius between sexes, however, was not statistically different up to 31 years of age (Table 5.1: Sex; $F_{[1,2]}$ = 0.388, P = 0.597). After 31 years, otolith growth in males seemed to accelerate up to 58 years compared to minimal increase in females up to its maximum observed lifespan of 41 years. This period between 31-41 yrs of age could not be tested statistically due to insufficient data. These data provide evidence of a sex-specific growth effect early in its life, of which is reduced later in its life. That is, faster growing males have similar otolith growth as slower growing females up to the age of 31, after which growth rate is possibly reflected in otolith growth in both sexes. The two plots also show strong evidence of an age-effect. Even though growth asymptotes in both sexes (after 20 years in males; 10 years in females), the otoliths continue growing.

C. bicolor

Unlike *P. sexstriatus*, growth rate between sexes in *C. bicolor* was reflected in sex-specific otolith growth (Figure 5.2B & 5.3B). Sex-specific growth revealed a significantly larger mean size of males compared to females between ages 2-10 years, but was not as distinct as that in *P. sexstriatus* (Chapter 3). Males were first recruited into the sampled population at an age of 2 years, after which growth in both sexes tends to asymptote. Prior to this, the population consisted only of females, which matured at the age of 0.56 years. Two transitional individuals were found between 3-5 years of age. Sex-specific cumulative otolith size-at age plots showed sex-specific differences in otolith size throughout the life history, so that at a given age, the cumulative otolith radius in males was always larger than that of females. Moreover, this difference was seen before the age of sex change (3 years). This difference in otolith size between sex was statistically different up to 9 years (Table 5.1: Sex; $F_{[1,15]}$ = 5.83, P = 0.029), after which otolith growth in males continues to increase in a consistent manner. Moreover, there was strong evidence of an age-effect through the consistent increase in otolith size with age in both sexes.

Figure 5.2. Sex-specific von Bertalanffy growth function (VBGF) for (A) *P. sexstriatus* and (B) *C. bicolor*. Trajectories were constrained to a size at settlement of 24.6mm. Undiff = undifferentiated; Bisex = bisexual; imm = immature; M = male; F = female. Reproductive category symbols: empty = immature; full symbols = mature.

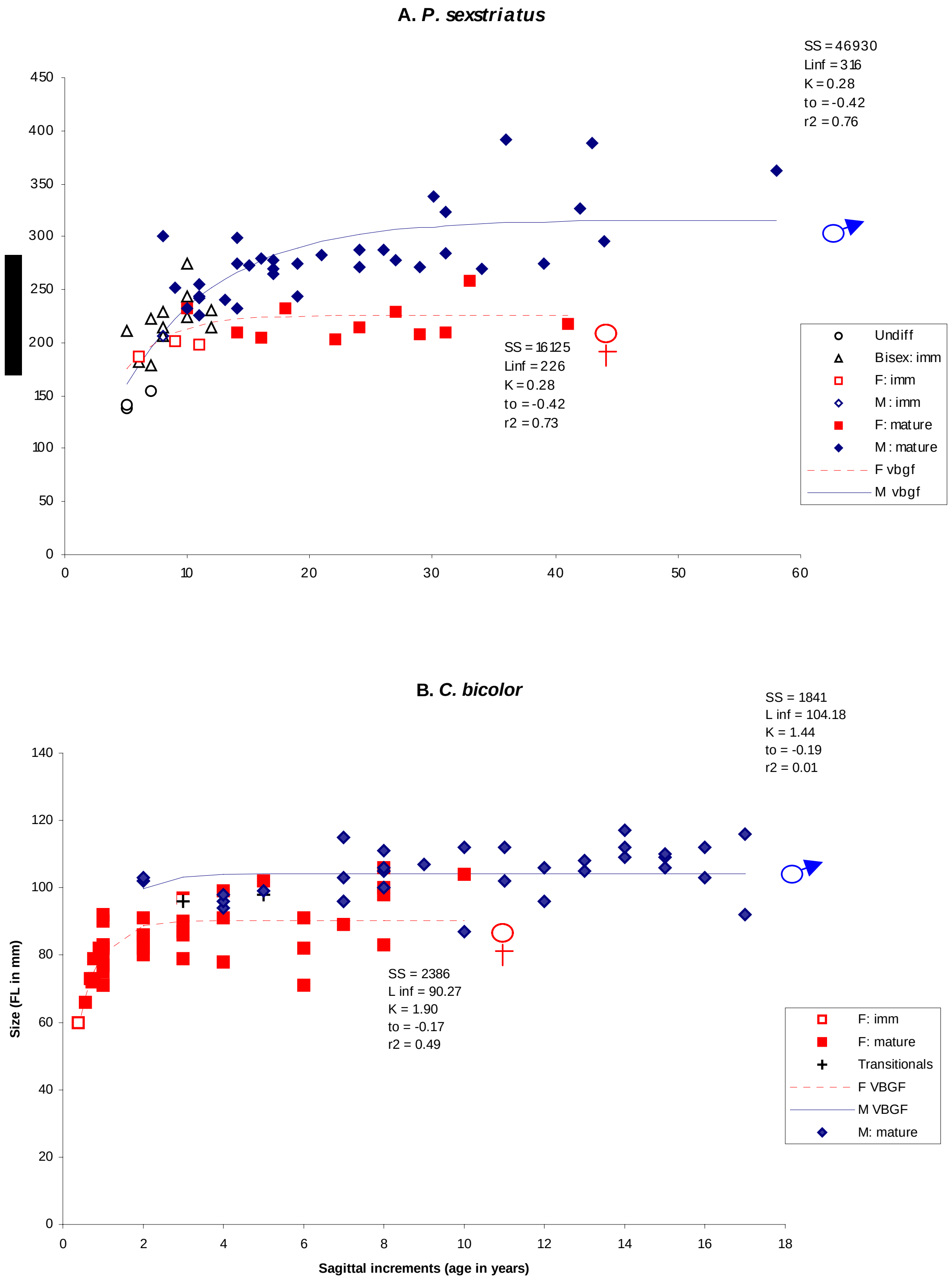
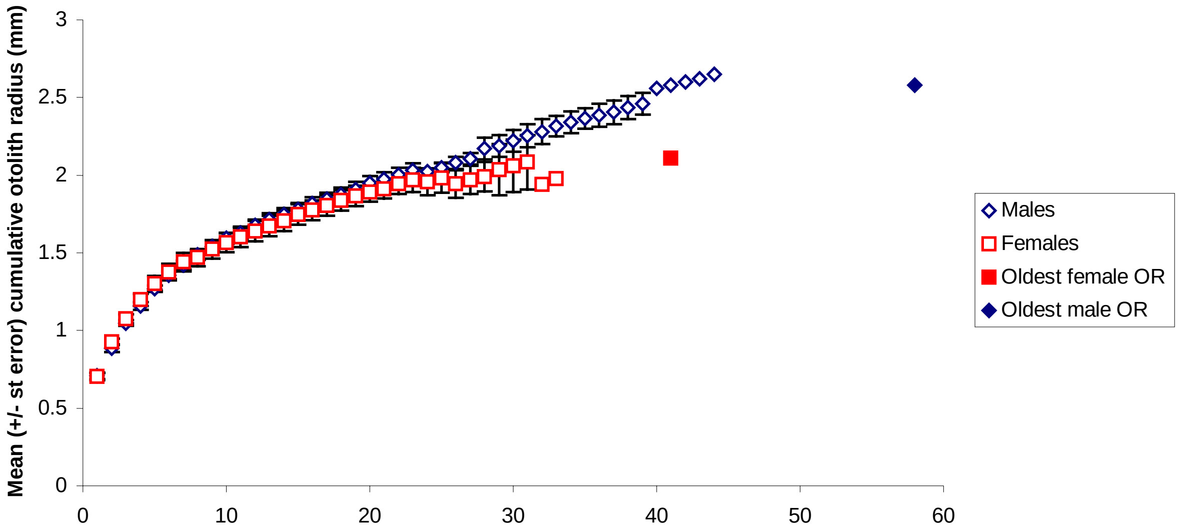


Figure 5.3. Sex-specific mean (+/- s.error) cumulative otolith radius (OR) with age for **(A)** *P. sexstriatus*, showing maximum OR for oldest male and female, and for **(B)** *C. bicolor*. Points without standard error represent single measurements.

A. *P. sexstriatus*



B. *C. bicolor*

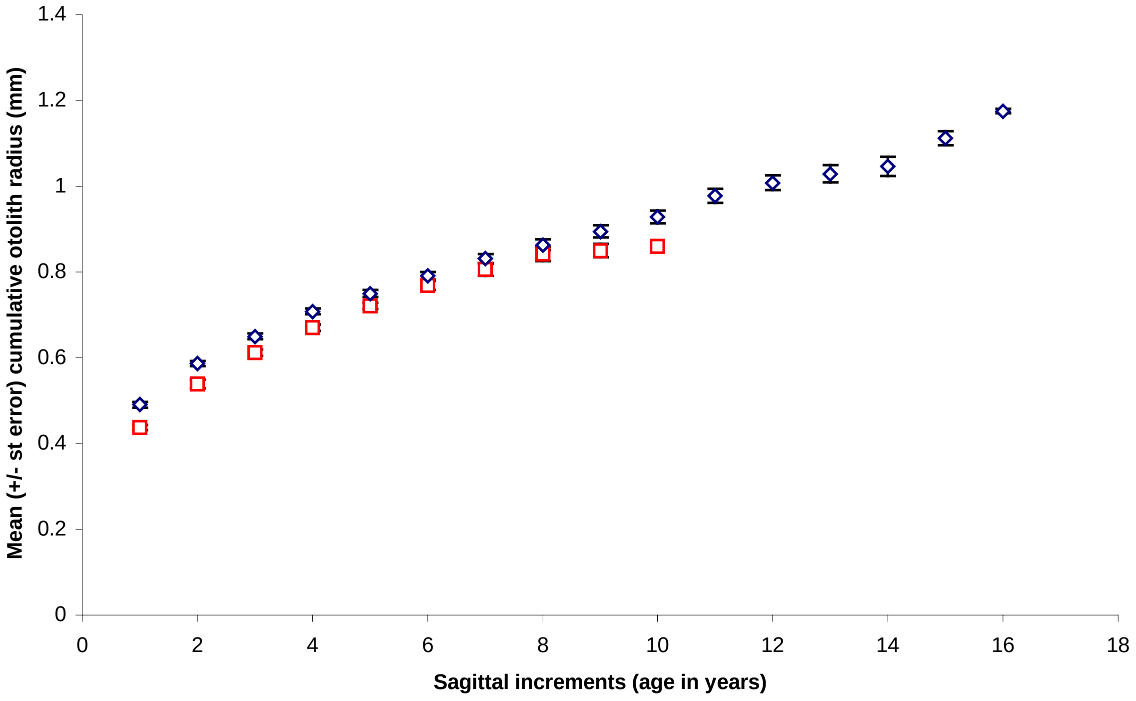


Table 5.1. Results of Repeated-measures analysis of variance (RM-ANOVA) comparing cumulative otolith radius between males and females for *P. sexstriatus* (0-31 years) and *C. bicolor* (0-9 years). * denotes P values <0.05

Source of variation	Mean square	df	F	P
P. sexstriatus				
Between subjects				
Sex	0.234	1	0.388	0.597
Error	0.604	2		
Within subjects				
Age	0.578	30	221.70	0.000*
Age x sex	0.003	30	1.26	0.220
Error	0.003	60		
C. bicolor				
Between subjects				
Sex	0.071	1	5.83	0.029*
Error	0.012	15		
Within subjects				
Age	0.18	8	509.63	0.000*
Age x sex	1.43E-04	8	0.408	0.914
Error	3.51E-04	120		

5.4. DISCUSSION

Age-based examination of the assumptions underlying back-calculation: that is evidence of constant proportions in otolith-somatic growth independent of age and growth rate by sex in the two study species, *Pomacanthus sexstriatus* and *Centropyge bicolor* provided evidence of complex otolith dynamics. Age effects by sex were consistent in both species, so that the mean cumulative otolith radius was consistently increasing in both sexes throughout life. Despite asymptotic growth rates in both males and females, sex-specific growth effect was found only in *P. sexstriatus* but not in *C. bicolor*. Moreover, sex-specific growth effect was evident only in the early life history of *P. sexstriatus*.

Back-calculation of sex-specific growth challenged

Back-calculation of sex-specific growth trajectories early in life is not as practical as previously suggested (Secor and Dean 1992). There is increasing evidence that otolith growth does not record somatic growth by sex (This study; Munday et al. 2004), implying a need to find either other methods or to take into account of both growth and age effects in back-calculation models. While age effect is expected in most species due to an ongoing deposition of calcium carbonate ($CaCO_3$) within each sagittal increment so that the increment growth of an otolith continues throughout its lifespan (Secor and Dean 1989), sex- specific growth effects is a new finding. Sex-specific growth effects was suggested by Secor and Dean (1989) regarding work done by Wilson in 1984 and Templeman and Squires in 1956 (cited in Secor and Dean 1989), who both observed the effect of sex on the otolith-somatic relationship. This was recently confirmed in one other study on the scarids: *Scarus frenatus* and *Chlorurus sordidus* by Munday et al (2004), where otoliths of the faster growing males were either similar or smaller in cumulative otolith radius at any given age than female otoliths. Combined with results of this current study, it is evident that the effect of growth rate on the otolith-somatic growth relationship may vary between sexes in some species but not in others, implying a need to explore growth effects at the species level. Moreover, sex-specific growth effects may vary ontogenetically, depending on which stage of a species' life history is observed. This brings forth more challenges for sex-specific growth trajectories early in life history. In species where otolith growth records somatic growth by sex throughout all of its life history as seen in *C. bicolor,* back-calculation techniques will almost always have to take into account, age effects.

Predictions of mechanisms explaining larger male size at given age

That otolith-somatic growth is in constant proportion, with respect to growth by sex in *C. bicolor* from the age of one and before the age of sexual transition, providing evidence of larger male size with age, via the mechanism of "juvenile growth hypothesis". In this case, females that change sex to males may have always had faster growth rates than those that remain as females (Francis and Barlow 1993; Adams and Williams 2001). This prediction coincides with the social system of *C. bicolor* which has a strict haremic social system, in which one large male dominates 2-5 females (Sakai et al. 2003b). Individuals that are faster growers will become males and dominate the remaining females. This may also explain early sex change in the presence of the dominant male (Aldenhoven 1984), the relatively faster growing female may take advantage of its size and prepare for dominance once the current male is removed or dies. Previous studies on asymptotic species have hypothesized that the type of growth mechanism underlying the occurrence of larger male size is linked to rapid growth in the juvenile stage (Laman Trip 2004). Further investigation on other species with highly asymptotic growth curves is required to examine this hypothesis over a wider range of species.

Alternative method to investigate the mechanism of larger male size

Predictions to explain the larger male size at any given age in *P. sexstriatus* are not possible using the back-calculation technique. Another method to investigate the mechanism explaining larger male size at age, is through direct comparison of bootstrapped reparameterisation estimates of mean size-at-age (eg. Laman Trip 2004) before the age of sexual maturity and/or sex change and the growth rate between sexes. Using this method, minimum sampling of at least 10 individuals within each age is needed in order for the bootsrapping method to precise estimates (Ackerman pers com). This method however is limited to species in which male and female differentiation occurs early in life, as in acanthurids (Laman Trip 2004). In contrast, *P. sexstriatus* becomes more complicated due to its initial bisexual gonad ontogeny, in which male and female differentiation occur as late as 8 years and 10 years of age respectively. There is therefore limited means of comparison of growth rates within the first 15% of life, where 85% of growth is occurring. This is a new issue for future research.

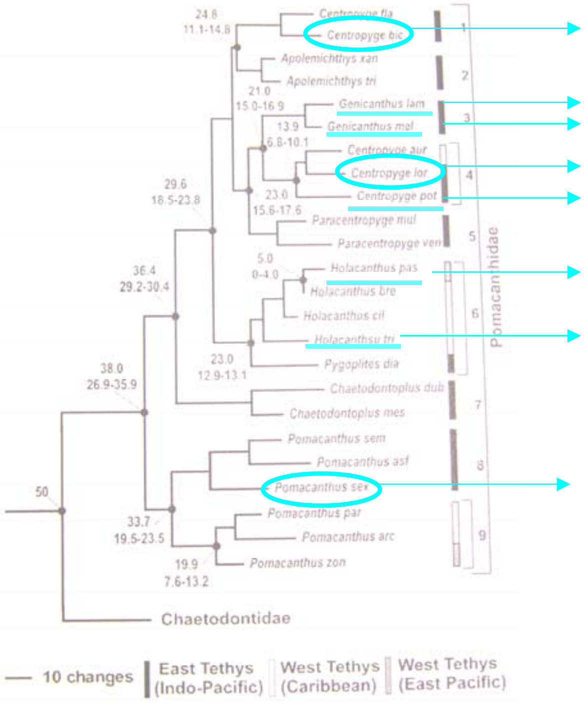
In conclusion, the phenomena that slowly growing individuals (in this case, females) have relatively larger otoliths may not be as widespread as previously thought (Reznick et al. 1989). Instead, it will differ according to species, and ontogenetically. It is of critical importance that the assumption of constant proportion in the otolith-somatic growth relationship with respect to sex-specific growth is tested, especially in studies that involve examination of the relationship between sexes of back-calculated size at age estimates.

CHAPTER 6. GENERAL CONCLUSIONS

General summary of key findings

This study reveals the complexity of the demography and reproductive ontogeny within the family Pomacanthidae. Firstly, pomacanthids have distinctive asymptotic growth where most growth occurs early in life . An interesting pattern of differential growth and longevity between sexes was consistent within the three study species, so that males were relatively larger at a given age, and were always longer lived. This pattern was consistent despite longer lifespan in the larger species (Chapter 3, Figure 6.1). Secondly, there is evidence of highly complex reproductive ontogeny including both protogyny and gonochorism. *Pomacanthus sexstriatus* displayed an unusual pattern of male recruitment involving prolonged periods of differentiation from immature bisexuals. *Centropyge bicolor* displayed a protogynous ontogeny similar to the more commonly studied scarids. These results suggested that the *Pomacanthus* reproductive ontogeny is an ancestral condition, and protogyny is a derived condition (Chapter 4, Figure 6.1). Thirdly, an attempt to investigate the assumption underlying back-calculation raised new challenges. Sex-specific age effects were consistent in both *P. sexstriatus* and *C. bicolor,* however sex-specific growth effects were found only in *P. sexstriatus*. This meant that otolith growth did not record somatic growth in *P. sexstriatus,* so that it would not be possible to generate back-calculated sex-specific size-at-age estimates to investigate the mechanism underlying larger male size (Chapter 5, Figure 6.1). These preliminary results raise key issues that need to be considered in the study of the more vulnerable endemic *Centropyge* and *Pomacanthus* species that are more at risk of local depletion in the Global Marine Aquarium Trade (Chapter 2), and may be used as the basis for the study of other reef fishes, especially fast growing species.

Figure 6.1. Findings on reproductive ontogeny, longevity and otolith dynamics with respect to the phylogeny of 24 chosen species of Family Pomacathidae, showing study species (circled) and other species on which histological studies have been undertaken (underlined). Figure modified from Bellwood et al (2004). [1]This study; [2]Aldenhoven (1984); [3]Lutnesky (1996); [4]Lutnesky (1988); [5]Arellano-Martinez et al (1999); [6]Suzuki et al (1979) in Moyer (1990); [7]refs in Moyer (1990); [8]Hourigan and Kelley (1987). Maximum longevity of both male (M) and female (F) is recorded.



Reproductive ontogeny	**Maximum longevity (M/F)**	**Age effect by sex**	**Growth effect by sex**
Monandric protogynous[1,2]	17/10 yrs[1]	Age effect[1]	No Growth effect[1]
Protogynous[6]			
Protogynous[7]			
?[1]	8/5 yrs[1]		
? Monandric protogynous[3,4]			
?[5]			
Monandric protogynous[8]			
Gonochorist[1]	58/41 yrs [1]	Age effect[1]	Growth effect[1]

Important aspects of this study

Asymptotic growth is characterized by rapid initial rates of growth limited to the first 10-15% of its lifespan, so that adult size is achieved early in life. This implies high level of pressure associated with small size within this period. This form of growth is often associated with a gonochoristic reproductive ontogeny (Newman et al. 1996; Laman Trip 2004) with few examples of hermaphroditism possibly due to physiological constraints associated with fast growth early in life (Laman Trip 2004). Results of this study demonstrated however that while asymptotic growth was consistent among pomacanthid species, gonochorism was a basal condition, and protogynous hermaphroditism a derived condition, suggesting an evolutionary basis for hermaphroditism in species that display asymptotic growth. This may suggest a highly adaptive characteristic of the family, which over time has been able to overcome constraints associated with fast growth.

A consistent pattern of differential growth and longevity between sexes, whereby males were always relatively larger and longer-lived demonstrated a characteristic feature within the family that occurred over a range of longevities and reproductive ontogenies. While differential growth is a common phenomenon among reef fishes, higher maximum longevity in males is a new finding. One may predict a sexual ontogeny in which all females change sex to males at a certain maximum age, however evidence from this study suggests otherwise. Sexual maturation was established early within the period of ascending growth, in males of the gonochoristic *P. sexstriatus*, but in females of the protogynous *C. bicolor* and *C. loricula* without affecting the relative longevity between sexes. This contradicts predictions of life history trait offs between early sexual maturation and longevity, which predicts reduced longevity in individuals that mature early. The significance of this differential growth is not clear from this study, but it suggests a response that is clearly sex-specific, irrespective of lifespan and reproductive ontogeny.

Age-based histological analysis demonstrated highly complex gonad ontogeny where it is critical to firstly examine all stages of gonad development from juvenile through to adult stage and secondly, to interpret reproductive ontogeny based on functional or mature gonad stages. An age-based analysis is particularly important in fast growing species, where growths of both sexes differ and size is not related to age.

While this study was not able to examine the mechanism underlying larger male size at a given age, it illustrated the complications that may arise in attempting to generate back-calculated sex-specific mean size-at age estimates: a disproportionate relationship between otolith-somatic growth. A constant proportion between otolith growth and somatic growth with respect to growth by sex in *C. bicolor* only, enabled predictions of fast juvenile growth underlying larger male sizes. This coincides with findings on other fast growing acanthurids where the direction of variation in growth occurs both ways; that is where males are relatively larger than females (male-biased) and where females are relatively larger (female-biased) at a given age (Laman Trip 2004). Laman Trip (2004) predicted that the mechanism explaining sexual size dimorphism in asymptotic species is related to fast juvenile growth.

Implications for fisheries management

Size selectivity in the aquarium fisheries for small to intermediate fish fit for aquarium display or home aquaria within size ranges 2-15 cm, differs from conventional fisheries which select for larger fish (Chung and Woo 1999; Sadovy and Vincent 2002). This study confirms that the commercial harvest occurs within the ascending part of the growth trajectory in *P. sexstriatus,* where i) the majority of growth and ii) the potential for divergence of growth trajectories between sexes occur. This is predicted to affect asymptotic mean sizes of both sexes (Laman Trip 2004). Moreover, harvest before the age of sexual maturation in this species can contribute to reduction of recruitment to the spawning stock. In comparison, *C. bicolor* and *C. loricula* are harvested at all size ranges, in which both the juvenile and breeding stock is reduced, and at the extreme, threatened with local extinction (Wood 2001). Vulnerable life history characteristics, combined with highly complex demographic and reproductive features challenges current management schemes to find optimal sizes and quantities for exploitation.

Future directions

There is a need to investigate the mechanism underlying larger male size relative to females in pomacanthids. A more complete sampling regime(10 individuals per age group), will aid in better investigation of sex-specific growth trajectories. An investigation of this hypothesis, which predicts a mechanism of fast juvenile growth in species with asymptotic growth, also needs confirmation. Phylogenetic analysis of all species within the family Pomacanthidae is essential for an evolutionary interpretation. Finally, urgent attention is needed in investigation of the demography in endemics with relatively long life spans such as in the genus *Pomacanthus* which are collected by commercial fishers at juvenile stages, and *Centropyge*, which constitutes the majority of endemics, and are exploited at all sizes. In particular, comparison of reproductive and demographical parameters between endemics in isolated oceanic reef systems and those inhabiting continental masses would prove intriguing area for future research.

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