Age, Growth, Reproduction and Population Biology of *Plectropomus spp* (Epinephelinae: Serranidae) on the Great Barrier Reef, Australia.

by

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Statement:

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Abstract

Size at age information provides an important basis for estimates of rates of growth, mortality and recruitment of fishes. Such estimates form the basis of fisheries stock assessment models necessary to estimate yields and monitor the responses of the populations to fishing pressure. The inshore coral trout *Plectropomus maculatus* and the common coral trout *P. leopardus* represent important commercial and recreational fishery resources on the Great Barrier Reef. The objectives of this thesis were to provide the information on age, growth and reproduction necessary to assess the effects of fishing on populations of *Plectropomus*.

In Chapter II, the age and growth of the inshore coral trout *P. maculatus* from the Central Great Barrier Reef region was determined by studies of annuli in whole and sectioned sagittae. The age and growth of the common coral trout *Plectropomus leopardus* from the Lizard Island area, Northern Great Barrier Reef is presented in Chapter IV. An alternating pattern of opaque (annulus) and translucent zones was clear in whole and sectioned otoliths of both species. A comparison of whole and sectioned otoliths of *P. leopardus* indicated that whole readings tended to underestimate age of older fish. The periodicity of formation of the annulus was validated through tetracycline labelling of mark-recaptured fishes in the wild and of captive fishes in aquaria. Results showed that for both species one annulus is formed per year during the winter and spring months. The von Bertalanffy growth curve for *P. maculatus* was $SL = 53.0 (1 - e^{-0.258 (t+1.0)})$, $r^2 = 0.766$. The oldest specimen examined was a 12 year old male of 58 cm SL. For *P. leopardus*, the von Bertalanffy model for

length (FL) was $L_t = 52.2 (1 - e^{-0.354 (t + 0.766)})$, $r^2 = 0.895$.. The oldest individual examined was 14 years of age. Line-fishing usually does not capture fishes smaller than 25 cm FL; thus, excluding most 0+ and 1+ year old fish and probably the slower growing 2+ year old fish. These first three years of life represent the period of fastest growth. Consequently, if the growth curve is fitted only to the line fishing data, the growth rate of the population is underestimated. Multiple regression was used to predict age of *P. leopardus* from otolith weight, fish length and fish weight. Otolith weight was the best predictor of age in the linear model and explained as much variation in age as did fish size in the von Bertalanffy growth curve.

In Chapters III and V, the reproductive biology of the inshore coral trout *P. maculatus* and the common coral trout *P. leopardus* respectively, were studied based on histological analyses of gonad material. Samples of *P. maculatus* were collected from inshore waters of the Central Great Barrier Reef. Samples of *P. leopardus* were collected from mid shelf reefs in the Central Great Barrier Reef, and mid shelf reefs and waters adjacent to Lizard Island in the Northern Great Barrier Reef. The mode of sexual development of both species was monandric protogynous hermaphroditism For the two species, a spawning period was observed from September through November, during which multiple spawning occurred. Sex change followed the usual protogynous mode with degeneration of ovarian germinal tissue accompanied by proliferation of male germinal tissue in the gonad. The sex/size and sex/age relationships indicated that sex-change can occur over a broad range of sizes and ages, but females were significantly smaller and younger than males. The stages of oocyte development for *P. maculatus* and *P. leopardus* are described in Appendix I.

In 1987 a zoning plan was established in the central section of the Great Barrier Reef Marine Park, Australia. Under this plan, fishing was excluded from some areas. Samples of *P. leopardus* were collected using line fishing at two reefs (Glow and Yankee) located in areas closed to fishing and in two reefs (Grub and Hopkinson) located in areas open to fishing. The four reefs were sampled in 1990 and 1991 two times per year, during June/July and September/October. The samples were compared to investigate the effects of this 3-4 year closure on the size, age and sex structure of coral trout populations (Chapter VI). There were no significant differences in mean size and age between protected reefs and unprotected reefs. However, the mean sizes and ages varied significantly between reefs. This result was due largely to variability between replicates, as the two open reefs were apparently not subject to the same fishing pressure.

In the two closed reefs, the population structure was dominated by the presence of a strong year class which settled in early 1984, indicating the occurrence of strong interannual fluctuations in recruitment. A similar pattern was not observed on the open reefs, with a corresponding mode not present at Grub reef and weak at Hopkinson reef. As Glow and Yankee reefs have been closed to fishing since 1987, and age of recruitment to the fishery is approximately 3 years, the individuals that settled onto Glow and Yankee in 1984 have been protected most of their lives. If the four reefs received a similar pulse of recruitment in 1984, fishing mortality has operated to largely decrease the abundance of this year class in the open reefs.

Sex-change occurred over a wide range of sizes and ages on the four reefs. The comparison of frequency of developmental stages between

reefs showed significant variation. The mean size and age observed for each sexual stage seemed to reflect closely the size and age structure of each population. When immature males were pooled with mature males for the sex-ratio calculations, the resulting sex-ratio was not significantly different among reefs. It appears that while the distribution of developmental stages in the populations was different, the same final female: male balance was being achieved. This type of result suggests that for the coral trout, sex-change results from a combination of a developmental process, in which individuals are more susceptible to sex-change as they grow larger and older, and a social process through behaviourally induced stimuli.

Differences in age structure were more obvious than differences in the size structure between closed and open reefs, suggesting that age structure may be far more useful than size structure for comparisons of fishing effects on long lived fishes such as epinepheline serranids. Comparisons of open and closed reefs based solely on mean sizes may fail to detect important differences.

This thesis has demonstrated the existence of annuli in the hard parts of tropical fish. Such age determinations can be carried out relatively cheaply and easily. The advent of routine age-determination may eventually see a trend toward age-based population dynamic studies of tropical reef fish.

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CHAPTER I: General Introduction

Fisheries Biology

Fishing is one of the oldest of human activities and has been practiced since Palaeolithic times by almost all ethnic groups in the world (Gulland, 1977). In modern times, advances in technology and increasing sizes of human populations have caused an overall increase in the rate of exploitation of natural resources, and fish communities are no exception. Until the first half of this century, it was only freshwater and anadromous fishes that were affected mostly, but the past 50 years have seen the collapse of many fisheries, caused by dramatic reductions in the size of fish populations due to over exploitation (Ricker, 1977).

The necessity of understanding the mechanisms regulating the abundance of fish populations in order to monitor exploited species was recognised almost a century ago, when a committee was established in the United Kingdom to investigate the depletion of fish stocks by trawl nets and beam trawls (Williams, 1977). Much of the current knowledge of fish population dynamics that has developed since then has been aimed at managing temperate species, in particular the commercially important species of the North Atlantic (Gulland, 1977).

Fisheries in the tropics remained primarily based on traditional fishing practices for much longer, with fishing techniques remaining largely as they had been for many centuries. In the Pacific Islands, for example, fisheries are of special importance for subsistence and cultural reasons, as much of the cultural tradition is preserved in relations with the marine ecosystem (Johannes, 1981). Traditional native fishermen have a rich knowledge of the biology and behaviour of fishes, acquired over many centuries of close interactions (see Johannes, 1981), but since such

information has remained largely ignored by western scientists, most of our present knowledge of tropical fisheries is based on fairly recent research. Volumes dealing with fisheries research and stock assessment specifically in the tropics began to appear in the 1980's (e. g. Saila and Roedel, 1979; Pauly and Murphy, 1982; Munro, 1983; Pauly, 1984; Longhurst and Pauly, 1987).

Partially through the support of international organisations, industrialised fishing was introduced to the tropics relatively recently, and as a result, many tropical fish stocks have already been modified (Longhurst and Pauly, 1987). Tropical fishes have been generally described as being short-lived, fast-growing species with high-mortality rates (Longhurst and Pauly, 1987). However, while this may be the case for many species, the generalisation does not apply for many commercially important species such as serranids and lutjanids (Manooch, 1987). Such species have low natural mortality, slow growth, long life, multiple reproductions, and distributions geographically restricted to areas of reef habitat (P.D.T., 1990). Such species may thus be especially sensitive to the effects of a mechanised, modern fishery. Furthermore, reefs are complex ecosystems where multi-gear fisheries are employed to exploit many species simultaneously (Munro, 1979; 1983; Munro and Williams, 1985). Tropical fisheries biology has now emerged as a well established field following the realisation that tropical fisheries cannot be managed based solely on North Sea solutions (Saila and Roedel, 1979; Longhurst and Pauly, 1987; P.D.T., 1990)

Age Determination

Size at age data form the basis of many traditional fisheries stock assessment techniques such as yield per recruit and virtual population analysis (Gulland, 1977). Size at age information provides the key for reliable estimates of rates of growth, mortality and, given a time-series of age structure data, recruitment. These estimates are necessary to estimate yield and monitor the responses of the populations to fishing pressure.

The age structure of a population can be estimated through length-frequency analysis, or from direct determination of age from periodic marks in calcified structures of individual fish. The first technique, however, is valid only for fast-growing, short-lived species, as its assumptions do not generally hold for slow growing, long-lived species (Pauly, 1981). With the recognition that many commercially exploited species of fish are much older than previously thought (Beamish and McFarlane, 1987), assessment of age of fish from their calcified structures stands as the most vital tool in growth and mortality studies for fisheries management.

Various calcified structures have been used in age determination, for example, scales, otoliths, vertebrae, spines, flat bones -cleithrum, opercula and branchiostegals- and fin rays (see Casselman, 1983 for review). The scale method has been used to estimate the age and growth of fish for almost a century (Carlander, 1987). More recently, studies have indicated that scales underestimate true age in old or slower growing fishes (Casselman, 1983; Beamish and McFarlane, 1987). Scale growth reflects the pattern of fish growth and scale age

may become inaccurate if growth is reduced during a prolonged period or when growth becomes asymptotic (Beamish and McFarlane, 1987). This also seems to be the case for most calcified structures (Casselman, 1983; 1990). In contrast, otolith growth has been shown to continue with age in several species of fishes, independent of fish size (Boehlert, 1985; Casselman, 1990; Beckman et al., 1991). In fact, it has been demonstrated that otoliths grow at a faster rate than the body during slow somatic growth and therefore they are excellent structures for recording the seasonal growth cycle and age of slow-growing and old fish, especially those approaching their asymptotic length (Casselman, 1990).

The discovery of daily growth increments in fish otoliths by Pannella (1971) is considered the most significant recent development in age determination (Beamish and MacFarlane, 1987). Since then, it has been demonstrated that for several species of fish, concentric rings, or unit increments, are laid down on a daily basis within the microstructure of otoliths (see Beamish and MacFarlane 1987 for review). The absence of marked seasonal changes in sea temperatures at low latitudes has led to a general belief that tropical fishes do not form annual rings in their calcified structures (Longhurst and Pauly, 1987). In fact, difficulties have been reported by some authors in identifying and interpreting banding patterns in otoliths of tropical fishes (e. g. Thompson and Munro, 1983). Analysis of otolith microstructure has been used extensively to age at least the younger stages of several coral reef fishes successfully (Brothers, 1980; Ralston and Miyamoto, 1981; Morales-Nin, 1989). However, this technique is time consuming and tedious when applied to long-lived species (see Longhurst and Pauly, 1987 and Beamish and McFarlane, 1987 for review). More recently, the presence of annual marks in otoliths has been validated for an increasing number of species

of tropical fishes (Samuel et al.; 1987, Fowler, 1990; Ferreira and Russ, 1992; Lou, 1992; Ferreira and Russ, 1993). Much of the emphasis in the development of the field of tropical fisheries biology mentioned previously has been based upon use of length-frequency data to generate estimates of growth, mortality and recruitment (Pauly and David, 1981; Pauly and Murphy, 1982). This emphasis has grown from the beliefs that tropical fish are difficult and expensive to age and generally fast-growing and long-lived. The recognition that age determination of tropical fishes is possible opens up the possibility of age-based population dynamics and fisheries yield models in the tropics.

Interpretation

Virtually all methods of age determination in fishes involve a certain degree of subjectivity. It is important to establish objective criteria for distinguishing growth checks in hard structures in order to obtain precise (repeatable) counts from the same individual, especially from different observers (Beamish and Fournier, 1981, Kimura and Lyons, 1991). It does not imply that the age estimates are accurate and relates only to the consistency among determinations.

Validation aims to establish that a technique is accurate. Regardless of the technique employed, validation is an essential step in age determination, stressed repeatedly by several authors as the use of inaccurate age estimates can cause serious errors in the understanding of fish populations and consequently in the management of valuable fisheries to sustainable levels (Casselman, 1983; Beamish and McFarlane, 1983; Manooch,1987). Validation of ages of fish can be

achieved by different methods (see Casselman, 1983 and Brothers, 1987 for reviews). The most powerful direct validation is obtained by examining calcified structures of fish of known age (i.e., stocked or captive fish) or fish of partly known age (i.e. mark-released-recaptured fishes). In the latter case, live fish are captured and dosed with the antibiotic tetracycline which is absorbed rapidly by vertebrates and deposited at sites which are calcifying actively at the time of injection (McFarlane and Beamish, 1987). After treatment, these fish are tagged externally for subsequent recognition and released. Upon recapture, the hard parts are dissected and treated in the usual manner. When viewed under fluorescent light, the site of tetracycline deposition is revealed as a thin yellow-green horizon identifying the growth surface at the time of application. This time-mark can be used to determine the periodicity of formation of the annuli observed after the marker, i.e., during the period at liberty between the first and second captures.

Otolith scaling

For several species of fishes, otolith growth has been described to continue with age, independent of fish size (Boehlert, 1985; Casselman, 1990; Beckman et al., 1991). Boehlert (1985) suggested the use of weight and otolith measurements as a non-subjective, cost-effective methodology for age determination, that would decrease variability among age estimates. The relationship between otolith weight and body size has not been investigated previously for the coral trout.

Groupers: the Epinephelinae of the Serranidae

The fishes of the serranid subfamily Epinephelinae, commonly known as groupers, are a high-valued and important fishery resource throughout the tropical and subtropical regions of the world. Groupers occupy the paramount position in food chains in these reef environments and play a major role in the structure of coral reef fish communities (various contributions in Polovina and Ralston, 1987).

The groupers of the genus *Plectropomus* spp (Oken) are widely distributed, occurring in shallow tropical and sub-tropical seas of the Indo-Pacific region. Five species of this genus are recorded from Australian waters (Randall and Hoese, 1986). On the Great Barrier Reef, there is a marked cross-shelf distribution for the most common species, with *P. maculatus* a near shore species, *P. leopardus* a mid-shelf species and *P. laevis* an outer shelf species (Williams and Russ, 1991).

Large reef fishes constitute more than 45% of the commercial and recreational line fishing catch on the Great Barrier Reef (Trainor, 1991). A total annual catch of about 4000 tonnes of line fish species is reported to be taken by the Queensland commercial fleet of 176 line-fishing vessels. Coral trout species contribute the largest single component of this annual catch (over 30 %) at about 1200 tonnes, valued at about A$10 million (Trainor, 1991). The recreational sector of this fishery, consisting of 19,000 small boats, is estimated to catch 2-3 times the commercial catch of reef fish (Hundloe, 1985; Craik, 1989).

Previous Studies of *Plectropomus* growth

On the Great Barrier Reef, Australia, information on age, growth and longevity is available for *Plectropomus leopardus* at Heron Island (Goeden, 1978) and in the Cairns region (McPherson et al., 1985, 1988). Goeden (1978) estimated the growth rate of *P. leopardus* at Heron Island based on length-frequency data. McPherson et al. (1988), determined age and growth of *P. leopardus* in the Cairns region by counts of annuli in whole otoliths. In New Caledonia, Loubens (1980) estimated age and growth for *P. leopardus* based on counts of annuli in broken and burnt whole otoliths. The periodicity of formation of annual rings was verified through observation of marginal increments in all studies using otoliths, but direct validation of the bands laid down in calcified structures has not yet been attempted for *P. leopardus*.

Growth Curves

Fish population models usually require a general description of the growth process by means of an appropriate mathematical function. The main criteria for choosing a growth curve are quality of fit and convenience, differing according to whether the need is for mathematical description of a detailed physiological growth process or for fishery management (Moreau, 1987). The von Bertalanffy (1938) growth model has been used most frequently since its application by Beverton and Holt (1957) to the yield per recruit problem (Kimura, 1980; Gallucci and Quinn, 1979). This model, however, has been the subject of some controversy (Knight, 1968; Roff, 1980). Among its principal problems are:

1- inability to represent sigmoidal growth in a linear dimension (Galluci and Quinn,1979);

2- lack of independence between the estimates of the parameters $L\infty$, K and t_0 (Knight,1968; Bailey, 1977; Leaman and Mulligan, 1992).

3- difficulties encountered in fitting the curve to data (Roff, 1980) due to unstable parameters (Schnute, 1981).

Many alternative growth curves have been proposed in the past (see Moreau, 1987) as well as the use of polynomial functions (Chen et al., 1992). Moreau (1987) suggested that particular attention should be paid to the statistical properties of growth models, bias in parameter evaluations, techniques for calculation of parameters and the quality of estimations of these parameters. Keeping in mind the possible biological inaccuracy of computations of $L\infty$ and t_0, Schnute and Fournier (1980) introduced the upper and lower limits of the observed length frequency distribution into the growth equation. In a similar way, Schnute (1981) proposed a general versatile growth model without using the concept of asymptotic size. This function provides statistically stable parameters whose range of values include not only the von Bertalanffy function, but Richards, Gompertz, Logistic, and Linear growth models as well (Schnute, 1981), thus allowing the user to choose the model that is most appropriate to describe the data.

Reproduction

With regard to the expression of sexuality, the class Pisces, with over 20,000 species, is the most diversified group of vertebrates. The superorder Teleostei is almost unique among all the vertebrate groups in

having sex change occurring as a natural phenomenon (Chan and Yeung, 1983). A possible explanation for the widespread occurrence of intersexuality among teleosts is the fact that in this group, unlike in the other vertebrates, the embryonic gonad lacks cortical and medullary organisation.

Among tropical fishes, hermaphroditism and sex succession constitute the normal mode of reproduction for many families (Thresher, 1984). Coral reef fishes display a great variety of social and mating systems, and this interspecific variability is matched by equally large intraspecific variability (Shapiro, 1991).

There are many theories explaining the origins of hermaphroditism. Ghiselin (1969), in a review of the evolution of hermaphroditism discussed three different models: the low-density model, the size-advantage model, and the gene-dispersal model. Sequential hermaphroditism, such as observed for the epinephelinae, was explained by the size-advantage model. A number of mathematical-genetic theories that have been developed since that support the size-advantage model (Warner, 1975; Warner et al., 1975; Leigh et al., 1976). According to this model, hermaphroditism evolves when an individual reproduces more efficiently first as one sex and then as the other. This is the case when one sex gains in fertility much more rapidly with size than the other. Nevertheless, the size-advantage model and its applicability to marine fishes has been subject to recent criticisms (see Shapiro, 1988 and Shapiro, 1989 for review). There are metabolic and physiological costs involved in the sex-change process (Chan and Yeung, 1983), and the possibility of increased fertility being accompanied by increased mortality, if both factors are equally related to size in the two sexes

(Warner, 1975). Chan and Yeung (1983) noted that an early differentiation of sex would result in the individual becoming a better adapted male or female. Therefore, gonochorism and decisive sex-determination are favoured when a highly specialised reproductive system is required for reproductive success, while hermaphroditism and its flexible environmental sex-control mechanisms, are favoured in the special socio-ecological settings of some fishes (Chan and Yeung, 1983).

All groupers (sub-family Epinephelinae of the Serranidae) that have been studied carefully are protogynous hermaphrodites (Shapiro, 1987). However hermaphroditism has been established definitively for only one species of the genus *Plectropomus*, *P. leopardus* (Goeden, 1978).

Robertson and Justines (1982) pointed out that not all species within a genus containing protogynous species are necessarily protogynous. Furthermore, as shown in a review by Sadovy and Shapiro (1987), a series of characteristics used as indicative of protogynous hermaphroditism require careful assessment and demonstration before conclusions can be reached on the mode of reproduction of a species.

The importance of determining the mode of reproduction of a species is enhanced by the fact that the management of a fishery is considerably complicated by sequential hermaphroditism (Shapiro, 1987; Bannerot et al., 1987). Since males in protogynous species tend to be larger, older and less numerous than females, fishing may remove more males than females. The responses of these populations to fishing pressure are largely unknown. Such questions enhance the importance of conducting

histological studies on any species that has not previously been well studied.

Social induction of sex change in Epinepheline serranids has been suggested as an alternative to genetically programmed sex-change, which would occur at a certain size or age (Shapiro, 1987). By integrating reproductive studies with information on the demography of a species it is possible to resolve some crucial questions of fisheries stock assessment and management:

- What is the age and size at first maturity?
- What is the average age and size of sex transition?
- What is the range of sizes and ages of females and males and the levels of variation expected in such estimates?

Marine Reserves and Reef Fishery Management

A widely recognised management strategy in the conservation of reef fish stocks is the implementation of Marine Fisheries Reserves, areas designed to protect reef fish stocks and habitat from all forms of exploitation (P.D.T. 1990; Williams and Russ, 1991). In modern times, the first marine protected area was established in Florida in 1930. Since then, protected marine areas have been implemented all over the world (P.D.T., 1990). In Australia, the first protected marine areas were established in 1981 in the Capricornia Section of the Great Barrier Reef Marine Park, under the first zoning plan to come into operation (Craik, 1989).

Evidence indicates that long term exclusion of fishing, increases the density, biomass, average size and fecundity of reef fishes (see P.D.T.,

1990 and Russ, 1991 for reviews). Furthermore, by enabling populations of reef fishes to maintain natural levels in some places, marine reserves have been suggested to enhance yields of fishes from adjacent areas (Alcala, 1981; Alcala and Russ, 1990; Russ, 1985).

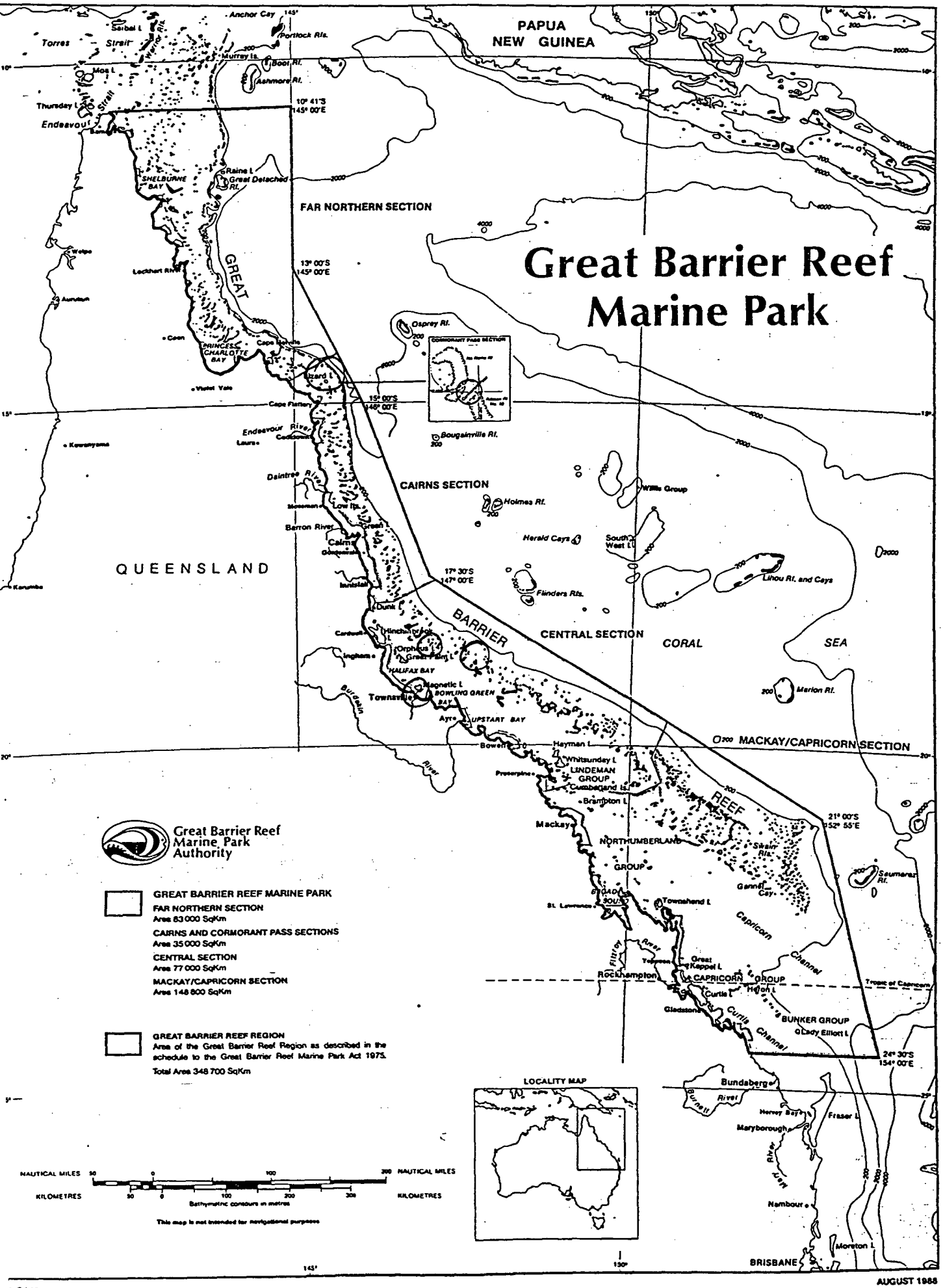
The spatial arrangement of many coral reefs as separate structural entities provides an excellent opportunity to test for the effects of different management alternatives (Hilborn and Walters, 1992). The importance of experimental investigations on the effects of fishing on coral reefs using reefs as replicate experimental units has been pointed out by various authors (Walters and Sainsbury, 1990; Russ, 1991; Hilborn and Walters, 1992). Yet, in spite of the high expectations placed on Marine Reserves, few direct tests exist on the effects of such protection on yields of marine resources (Alcala and Russ, 1990).

Because of its economic importance, the coral trout *Plectropomus leopardus* has been the subject of several studies investigating the effects of fishing which compared the abundance and size structure of populations from open (fished) and closed (unfished) reefs on the Great Barrier Reef (see Williams and Russ, 1991, for review). Most of these studies were conducted using underwater visual census (UVC) techniques (e. g. Craik, 1981; Ayling and Ayling, 1984, 1986; Ayling and Mapstone, 1991; but see also Beinessen, 1989a). No study, however, has yet investigated the effects of fishing on the age and sex structure of coral trout populations.

Contents of this thesis

In this thesis, the age, growth, reproduction and mortality of two species of coral trout, *Plectropomus maculatus* and *P. leopardus* are documented. Samples of *P. maculatus* were collected from inshore reefs off Townsville and waters adjacent to Orpheus Island, central Great Barrier Reef (Fig. 1.1). Samples of *P. leopardus* were collected from midshelf reefs off Townsville, Central Great Barrier Reef, and waters adjacent to Lizard Island, northern Great Barrier Reef (Fig. 1.1). Fishes were tagged and injected with tetracycline during a mark-recapture program at Orpheus and Lizard Islands (Davies, 1989; 1993) making it possible to validate the age determination of the two species. For *P. leopardus*, the information obtained was used to investigate the effects of fishing on this species, by comparing the age and sex structure of populations sampled from reefs open and closed to line fishing on the Central Great Barrier Reef.

Figure 1.1- Map showing the location of the study sites in the Great Barrier Reef, Australia. Map reproduced with permission of the Great Barrier Reef Marine Park Authority, Townsville



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CHAPTER II: Age, growth and mortality of the inshore coral trout *Plectropomus maculatus* (Bloch, 1790) from the Central Great Barrier Reef, Australia.

2.1 - Introduction

The inshore or bar-cheeked coral trout, *Plectropomus maculatus*, one of 7 species in the genus *Plectropomus*, is most abundant on near shore reefs and islands in turbid waters (Randall and Hoese, 1986). Although this species is not a major component of the commercial fishery, which targets mainly mid-shelf and outer-shelf reefs, the proximity of its habitat to the coast makes the inshore trout an important component of catches of recreational fisherman (Williams and Russ, 1991). The recreational fisheries in Queensland have been estimated to account for about 50 to 75% of the catch of reef fish from the Great Barrier Reef region, representing an estimated yield of between 3500 and 6500 mT/year (Hundloe, 1985; Craik, 1989).

Information on age, growth and longevity is not available for the inshore coral trout. The purpose of this study was to produce validated estimates of growth for *P. maculatus*. Such information is necessary for determining population dynamics, for making estimates of yield and for monitoring the responses of populations to fishing pressure.

In summary, the objectives in this chapter were:

1- To determine the presence of annuli in otoliths of *P. maculatus* and to validate their periodicity;

2- To determine the rate of growth of *P. maculatus* ;

3- To estimate the rate of mortality of *P. maculatus*.

2.2 - Materials and Methods

A total of 100 fishes were examined in this study. Filleted carcasses of *Plectropomus maculatus* were obtained from recreational line and spear fishermen. The fishes were collected from inshore reefs off Townsville, Queensland (Latitude 18° to 19° 30' S, Longitude 146° to 147° E), between 1989 and 1991. Individuals under the legal size (35 cm Total Length) were caught by divers using fence-nets at Orpheus Island, where some line fishing was conducted also. The Fork length (FL), defined as the length from the front of the snout to the caudal fork and Standard length (SL), defined as the length from the front of the upper lip to the posterior end of the vertebral column of each fish were measured in centimetres. A simple linear regression was used to describe the relationship between FL and SL.

Sagittae were removed, cleaned and stored dry. All otoliths were read whole and sectioned. To increase contrast between bands, whole otoliths were burned lightly on a hot plate at 180° C (Christensen, 1964). Both right and left sagitta were read whole under reflected light using a dissecting microscope at 16x magnification. The otoliths were placed with the concave side up in a black container filled with immersion oil. The left saggitae was prepared for reading by embedding in epoxy resin (Spurr, 1969) and sectioned transversely through the core of the sagitta using a low speed saw. Sections were mounted on glass slides with Crystal Bond 509 adhesive, ground on 600 and 1200 grade sand paper, polished with 0.3μ alumina micropolish and then examined under a dissecting microscope at 40x magnification with reflected light and a black background.

Two readers independently counted annuli of whole and sectioned otoliths. The results were accepted and used in the analysis when the two readers agreed. If the results were different, the readings were repeated, and a third reader was asked to perform the counting also. The results were then accepted if at least two of the readers obtained the same count.

Sections of sagittae from 11 fishes of 0, 1 and 2 years of age were ground further to 300-500 microns thickness with 0.9 μ Imperial Lapping film and finished with 0.3 μ alumina polish. These sections were observed under high power (1000x) with immersion oil, using a green filter over transmitted light. Daily growth increments, defined by Mugiya et al. (1981) as a discontinuous zone (narrow and dark under transmitted light) followed by an incremental zone (broad and translucent under transmitted light) were counted from the nucleus until the beginning of the first opaque zone assumed to be an annulus. This region was previously located under reflected light using lower magnification and its position marked with a scratch on the otolith surface. Increments were counted three times by the same reader after time intervals of 15 days. The three counts did not differ by more than 10% from each other and the results were averaged (Secor and Dean, 1989).

To determine time of annulus formation and to study growth, from August 1989 to April 1991, 51 fishes were caught in a trapping program at Orpheus Island fringing reef (Lat. 18° 35' S, Long. 146° 28' E), tagged with T-bar anchor tags and injected with tetracycline hydrochloride before being released (see Davies, 1989). The fishes were injected in the coelomic cavity under the pelvic fin with a dosage of 50 mg of tetracycline per kg of fish (McFarlane and Beamish, 1987), in a concentration of 50 mg

per ml of sterile saline solution. After periods of at least one year at large, 3 fish were recaptured, reinjected and kept in tanks at Orpheus Island Research Station (Fig. 2.1). Also, 5 fish were caught by line fishing at Orpheus Island, injected with tetracycline and kept in tanks for periods of 3 to 6 months to determine the time of annulus formation (Fig. 2.1). The fish in the tanks were submitted to natural periods of daylight and the water was pumped continuously from the sea, with a turnover rate of 5 times a day. After death of the fish, the otoliths of the fish treated with tetracycline were removed and placed in dark containers for later examination using a microscope with fluorescent light. A fiber optic light source was used in conjunction with the fluorescent light for the simultaneous observation of fluorescent tetracycline bands and annuli visible under reflected light. The distances between fluorescent bands, the otolith margin and the outer edge of adjacent annuli were measured in sectioned otoliths to determine the time of annulus formation. Measurements were taken from the outer edge of the opaque band because this region represents an interface zone, where the discontinuity between opaque/translucent zones provides a sharper contrast.

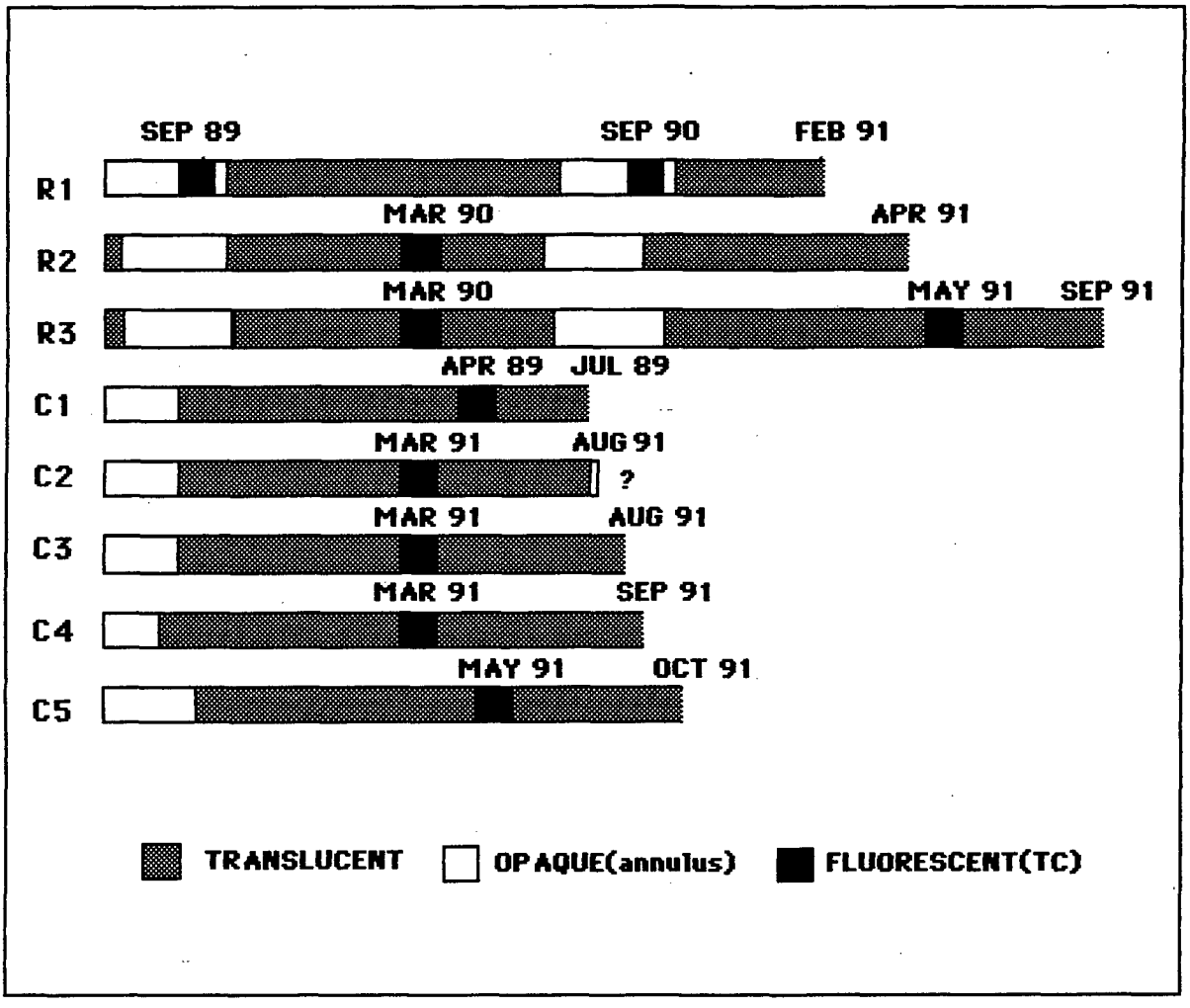


Fig 2. 1: Diagrammatic representation of the relative positions of fluorescent bands, opaque and translucent zones in sectioned otoliths of *P. maculatus*. Dates indicate time of tetracycline treatment and death of the fish. R= recaptured fish; C= captive fish.

Obs: (?) a hint of an opaque band was observed in the margin of sectioned otolith C2.

The Von Bertalanffy growth equation for length was fitted to length-at-age data obtained from otolith readings using both linear and non-linear techniques. The linear fit was obtained by a von Bertalanffy plot (Pauly, 1984). The parameter $L\infty$ was estimated as Lmax/0.95 (Pauly, 1984), and Lmax was the maximum length observed in a continuous size distribution (Munro, 1983). The non-linear fit was obtained using Systat (Wilkinson, 1989). Schnute's (1981) reformulation of the von Bertalanffy

growth function was also fitted to length-at-age data using a non-linear technique (Systat, Wilkinson, 1989).

An estimate of total mortality Z was obtained using the age-based catch-curve method of Beverton and Holt (1957) and Chapman and Robson (1960) (*in* Pauly, 1984). The natural logarithm of the number of fish in each age class (Nt) was plotted against their corresponding age (t) and Z was estimated from the descending slope (b) of the regression.

2. 3 - Results

Otolith morphology and readings

The sagittae of *P. maculatus* are the largest of the three pairs of otoliths. They are oval structures, laterally compressed, with a concave distal surface and a pointed rostrum (Fig. 2.2). A curved sulcus crosses their proximal surface longitudinally. The otolith presents a pattern of alternating translucent and opaque zones. The annuli found in the sectioned sagittae of *P. maculatus* were opaque bands with a milky appearance under reflected light on a black background (Fig. 2.2). Under transmitted light the annuli are darker than the adjacent translucent zones.

In sections of tetracycline-labelled otoliths, the fluorescent mark could be followed from the dorsal to the ventral ends along the proximal surface but it was incomplete along the distal surface (Fig. 2.3). This indicates

that the growth of the otolith of *P. maculatus* occurs mainly along its proximal surface.

The annuli were clear and distinct in the posterior part of the whole otolith, where they could be counted up to 5 years of age. Further annuli were difficult to distinguish in this region, and were easier to follow along the dorsal side, that slopes down at a 45° angle (Fig. 2.2.)

In the sections of otoliths, the annuli were more distinct and had more consistent growth patterns in the region from the core to the proximal surface of the sagitta along the ventral margin of the *sulcus acousticus* (Fig. 2.2). The first annulus was usually wider and less well defined than the subsequent ones in sections. Whole sagittae were useful to confirm the presence of this first annulus. The results of counts of at least two of the readers were in agreement in 96% of the cases and the results of these otoliths were included in the growth curve. No differences were detected between the number of annuli counted in left and right sagittae.

Validation of annulus formation

The presence of structures likely to represent daily growth increments was observed in the otoliths of *P. maculatus* (Fig. 2.4). Total increment counts between the nucleus and the beginning of the first opaque zone ranged from 180 to 225 daily growth increments (n=11; mean=201.18; se=4.381). This indicated that the formation of the first annulus occurred during the first year of life of the fish.

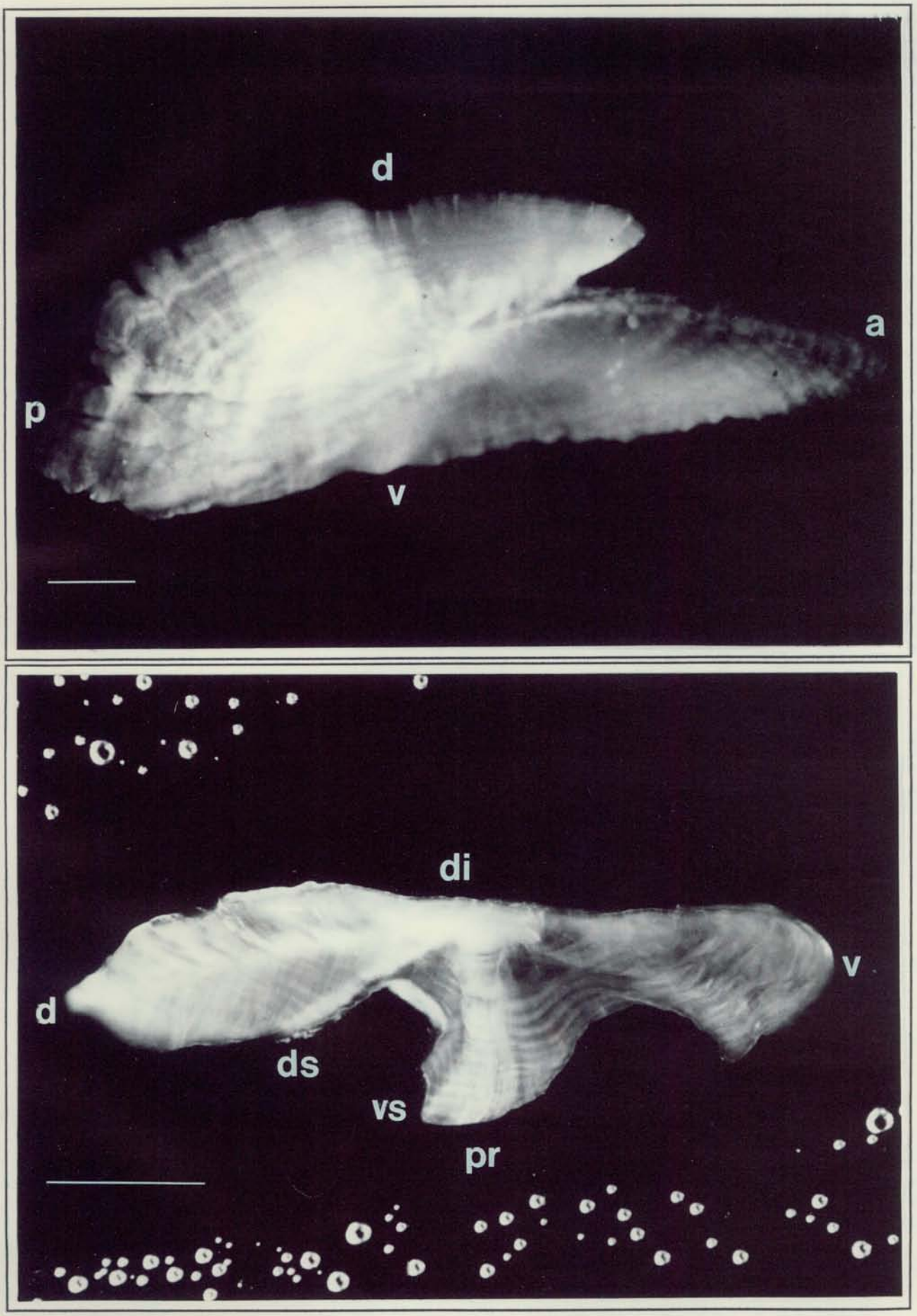


Figure 2.2: Whole and sectioned otolith of a 12 yr. old male *P. maculatus* under reflected light, showing the alternating pattern of opaque (white) and translucent (dark) zones. a- anterior, p- posterior region; d- dorsal, v- ventral side; pr- proximal, di- distal surface; vs- ventral, ds- dorsal side of sulcus acousticus. Scale bar= 1mm.

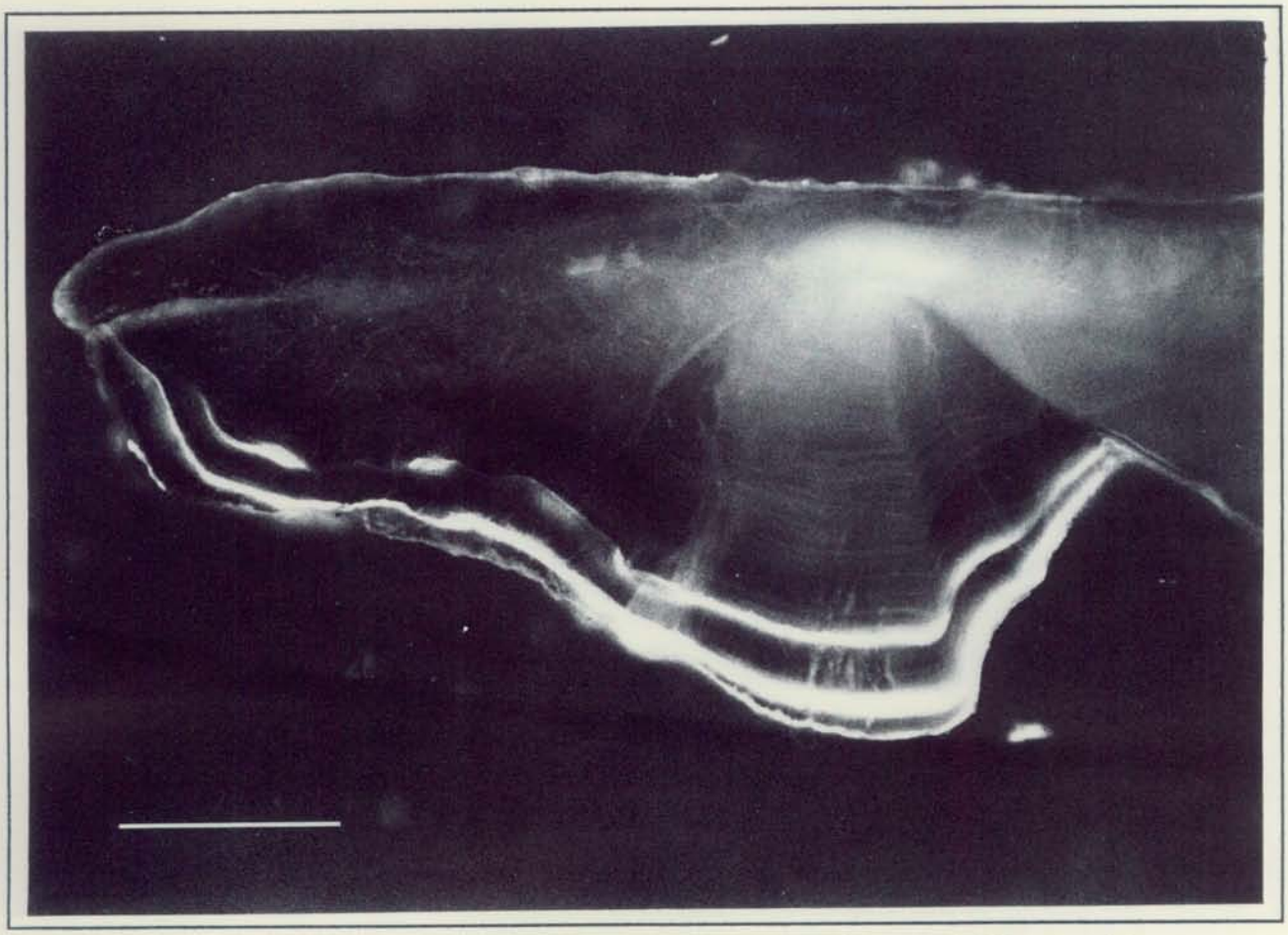


Figure 2. 3: Sectioned otolith of a *P. maculatus* (R3) double-injected with Tetracycline (1 year interval). Section viewed under fluorescent light. Scale bar= 0.25mm

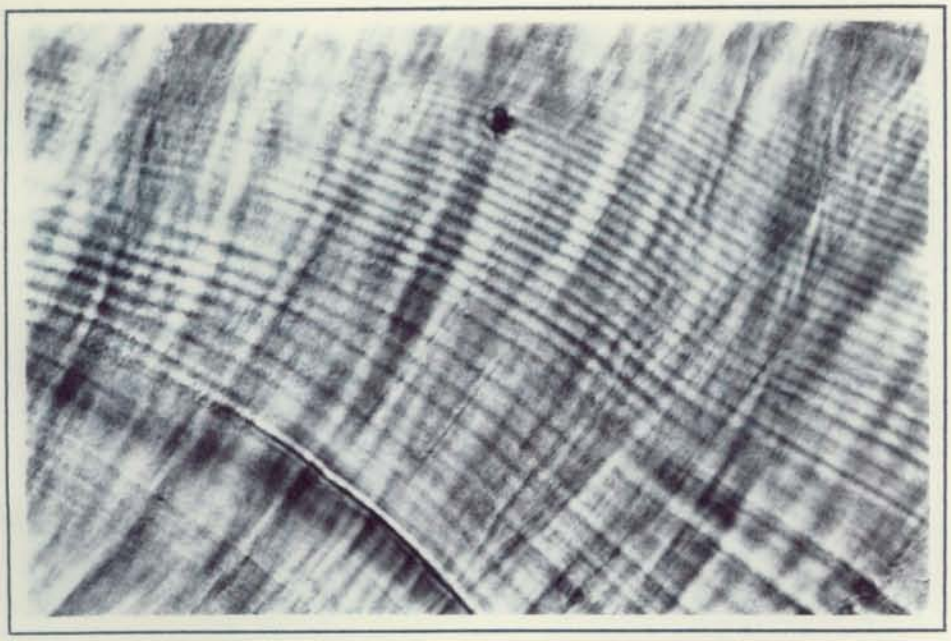


Figure 2. 4: Daily rings in sectioned otolith of *P. maculatus*. Mag. 400x.

All fishes treated with tetracycline displayed clear fluorescent marks in their otoliths. The results obtained from 3 field-recaptured and 5 captive fishes showed that the annuli were formed once per year. The relative distances between fluorescent bands, otolith margin and annuli indicated that the formation of the annulus occurs during late winter and early spring months (Fig. 2.1). Fishes injected with tetracycline in March, April and May had fluorescent marks positioned in the middle of a translucent zone (Fig. 2.5) while a fish injected in September had the fluorescent mark positioned at the end of an opaque zone (annulus) (Fig. 2.6). Formation of opaque zones, however, could not be detected at the margin of the otoliths of captive fish killed in September or October, though a hint of an opaque zone was observed at the margin of the otolith of a fish killed in August (Fig. 2.1).

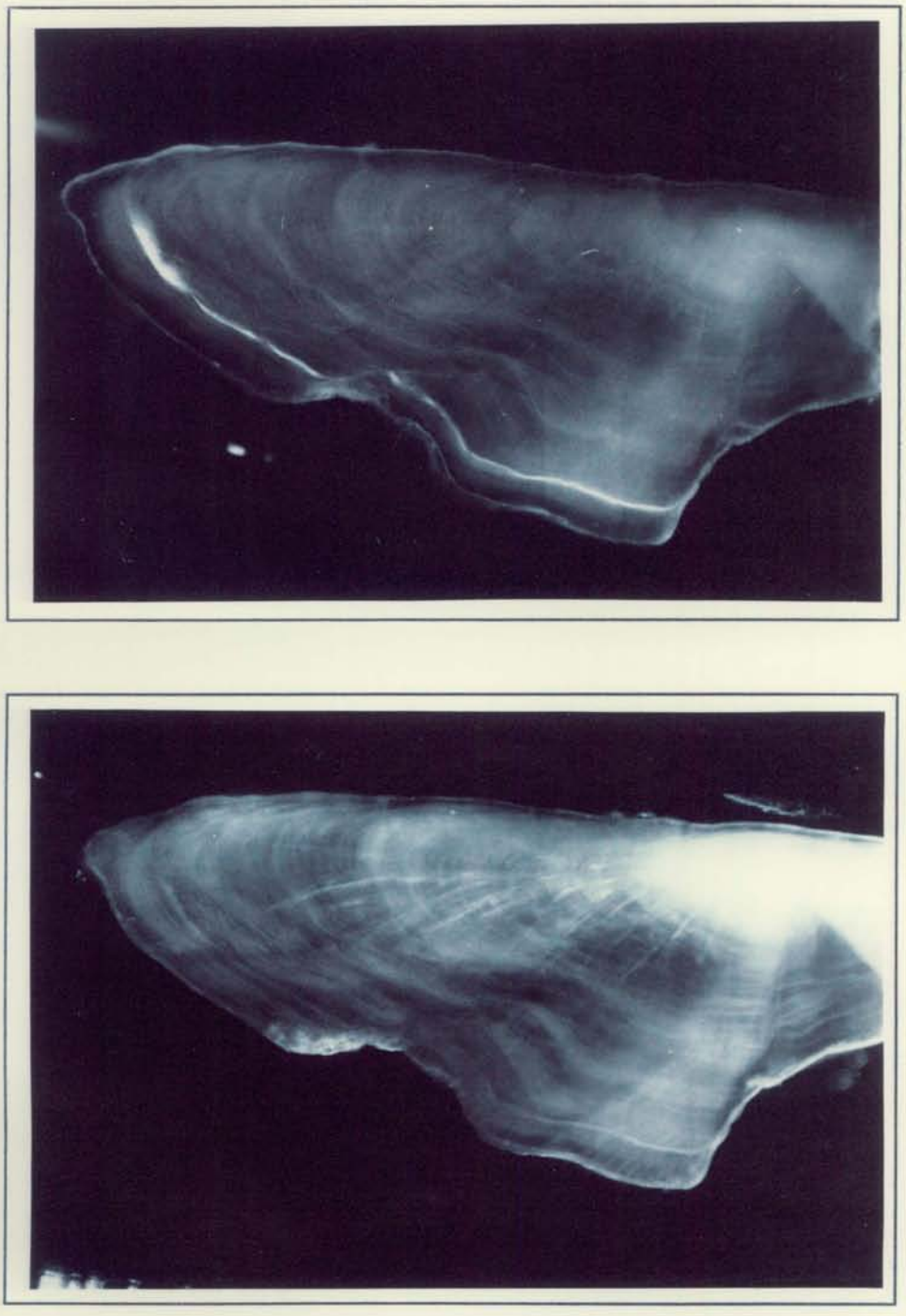


Figure 2.5: Sectioned otolith of a *P. maculatus* injected with tetracycline in March (R2, Fig. 2.1). Section viewed under fluorescent and reflected light. Note the tetracycline band positioned at the translucent zone.

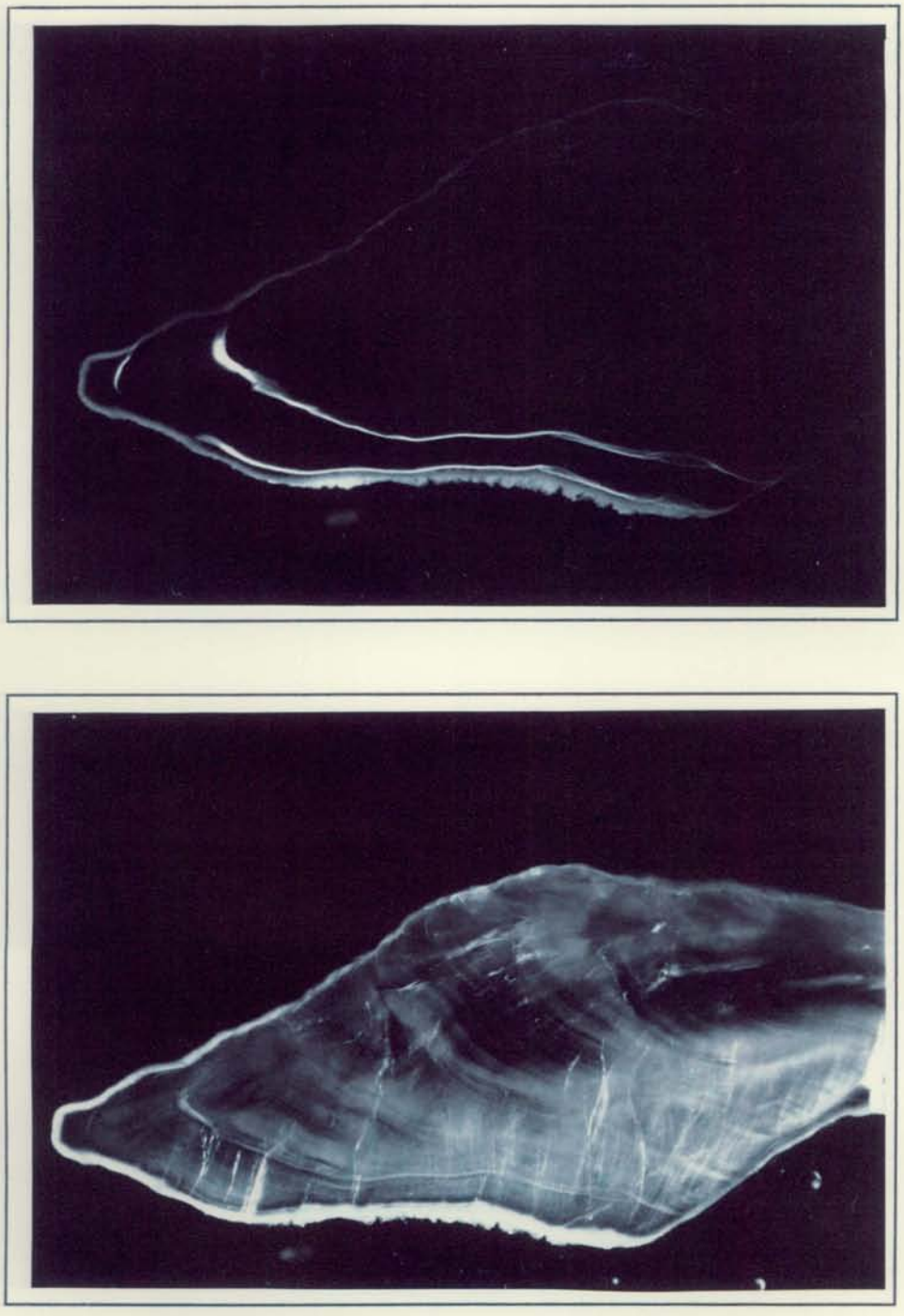


Figure 2. 6: Sectioned otolith of a *P. maculatus* injected with tetracycline in September 89 and again in September 90 (R1, Fig. 2.1). Section viewed under fluorescent and reflected light. Note the fluorescent band positioned at the annulus (opaque band).

Growth model

A wide range of size classes were represented in the sampled population (Fig. 2.7) and displayed a normal distribution (lKolmogorov-Smirnov test, P=0.233).

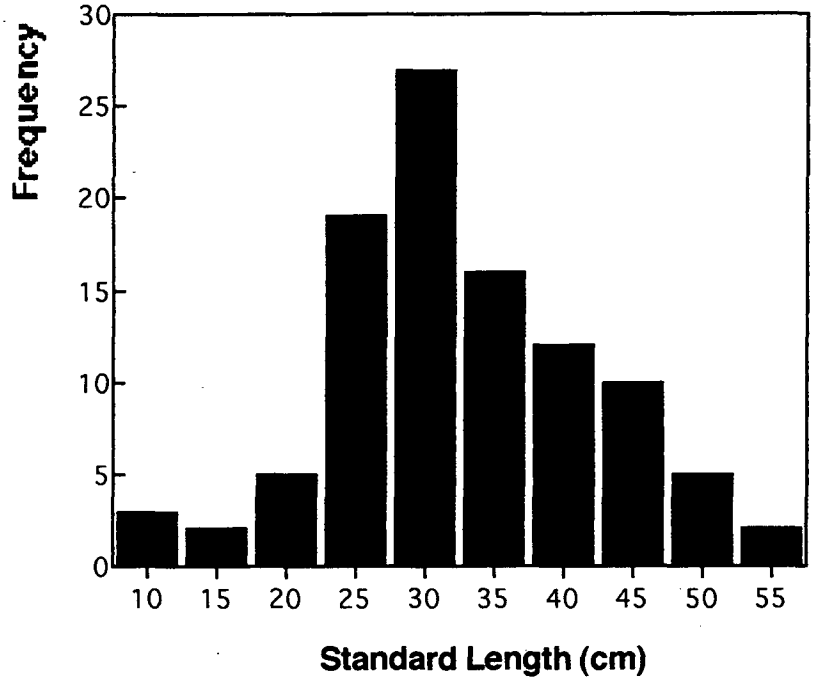


Figure 2.7: Length frequency distribution of sampled *P. maculatus*.

The Von Bertalanffy growth curve was used to describe the growth of *P. maculatus* from length-at-age data obtained from otolith counts (Fig. 2.8). The growth model fitted by length was:

Linear fit: (Lmax/0.95= 60.0cm SL)

$SL = 60\ (1-e^{-0.206\ (t+0.945)})$ $r2 = 0.687$

Non-linear fit:

$SL = 53\ (1-e^{-0.258\ (t+1.0)})$ $r2 = 0.766$

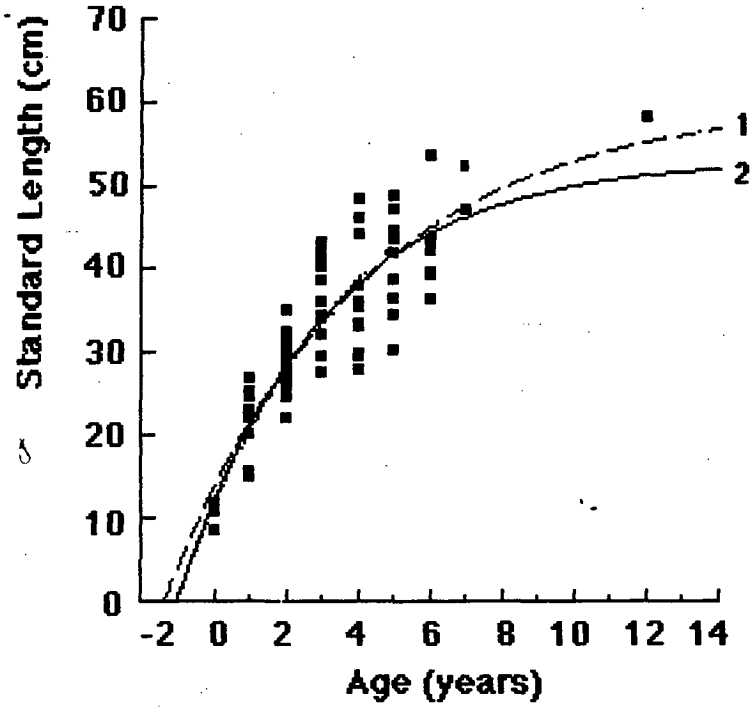


Figure 2. 8: von Bertalanffy growth curve for P. maculatus and observed length-at-age . curve 1: linear fit; curve2: non-linear fit.

When fitting Schnute's model to the data, the value of the parameter b obtained was very close to 1. In the boundary where b=1 the curve is reduced to a three parameter model that corresponds to the von Bertalanffy curve for length (Schnute, 1981), which indicates that the von Bertalanffy growth model describes the data well.

The relationship between the Fork length FL and the standard length SL was described by a linear regression in the form:

$SL = -0.4236 + 0.8565\ FL,\ r^2 = 0.997.$

Mortality

The total rate of mortality Z, estimated using ages of 2 to 12 years, was 0.39, representing an annual survivorship of approximately 68% (Fig. 2.9).

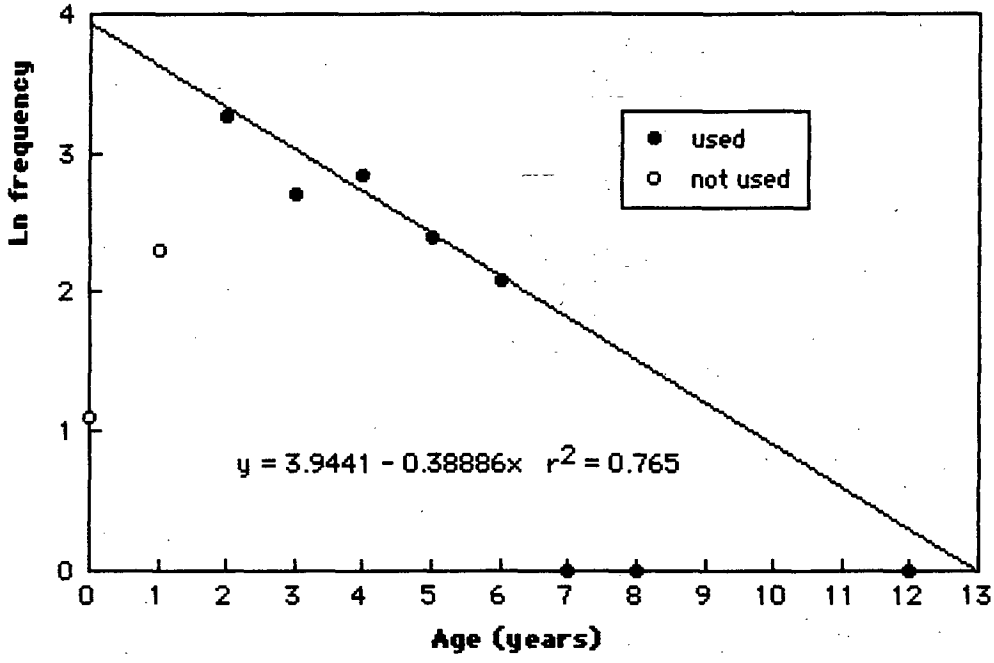


Figure 2. 9: Catch-curve for *P. maculatus* based on observed age.

With the exclusion of the 12 year age class (represented by a single individual) the calculated rate of total mortality estimated using ages of 2 to 8 was 0.57, representing an annual survivorship of approximately 55% (Fig. 2.10). Age 1 was excluded from both calculations because this age class was not fully represented in the sampled population.

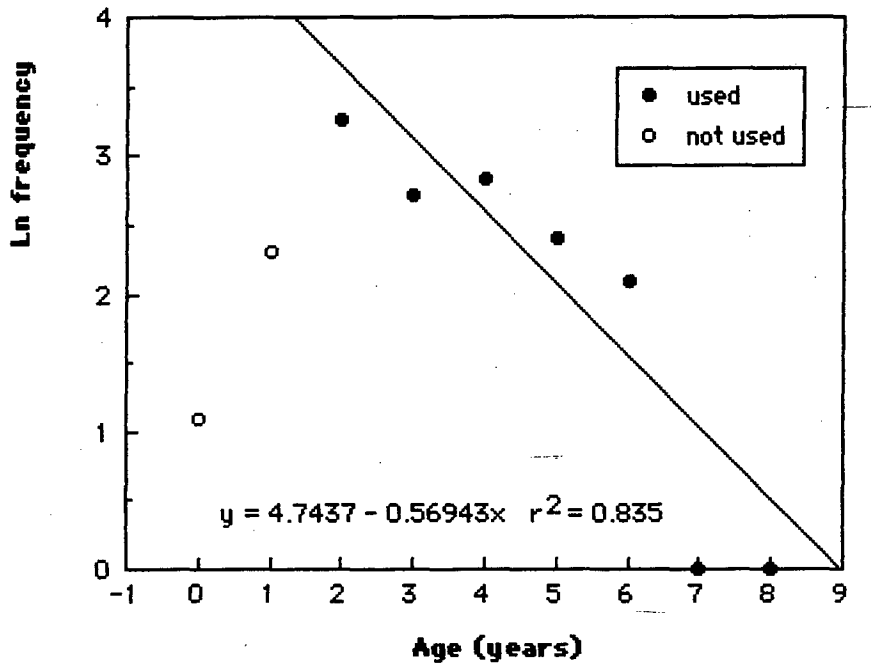


Figure 2. 10: Catch-curve for *P. maculatus* based on observed age.

2.4 - Discussion

Conventional methods of age determination have been suggested to have little utility for tropical species, where seasonal marks such as annuli are thought not to be generally present (Campana and Neilson, 1985). The presence of distinct annual bands in the otoliths of *Plectropomus maculatus*, validated in the present study, adds to the results of other recent works (Ferrel, 1989; Fowler, 1990; Lou, 1992) that have demonstrated the presence and usefulness of these periodic marks in the ageing of tropical fishes.

The growth of the otolith of *P. maculatus* occurs mainly on its proximal surface, a phenomenon observed for other species of fish also (Boehlert, 1985; Taubert and Tranquili, 1982). As a result of allometric growth, the region providing for more clear reading of banding patterns changes with age. Boehlert (1985) compared results of whole and sectioned otoliths and found that estimates of age from whole otoliths suffered from inaccuracies in older fish while sections suffered from inaccuracies in younger fish. To improve accuracy, both whole and sectioned otoliths were used to determine the age of *P.maculatus*.

The age and growth determined for *P. maculatus* from otolith counts indicate that the inshore coral trout is a relatively long-lived, slow-growing species. This appears to be the pattern worldwide for epinepheline serranids (Manooch; 1987). The linear technique used to estimate the parameters of the von Bertalanffy growth curve yielded slower growth estimates than the non-linear technique. When estimating the VB parameters linearly, a pre-set value of $L\infty$ is necessary. It has been suggested that $L\infty$ values generally lie around the maximum sizes observed in the population, and a rule of thumb widely used in fisheries is that $Lmax/0.95 \approx L\infty$ (Pauly, 1984). However, Mathews and Samuel (1990) observed that for long-lived fishes Lmax/0.95 overestimates $L\infty$. This seems to be the case for *P. maculatus*. In fact, when significant growth variability is present, overestimation is likely to occur if $L\infty$ is estimated from Lmax. Furthermore, there is often an inverse relationship between the rate parameter K and the asymptotic size $L\infty$ and this inverse relationship is generally accompanied by a direct relationship between K and the third parameter t_0. Thus, if $L\infty$ is overestimated, K and t_0 will be underestimated.

Galluci and Quinn (1979) and Vaughan and Kanciruk (1983) recommended the replacement of linear methods by non-inear fitting techniques. Age would be used as a true, non-stochastic, independent variable, and the estimation would focus upon the data and the model, not on its linearized forms. Galluci and Quinn (1979) presented geometric evidence that the three parameters of the VB model are not redundant and all three must be specified to identify a single solution curve. Vaughan and Kanciruk (1983) compared the traditional linear and non-linear solutions based upon Monte Carlo simulations with known growth parameters. They demonstrated that the iterative, non-linear method usually produced the most accurate and precise parameter estimates.

In a review of mortality rates of groupers (Epinephelinae of the Serranidae), Ralston (1987) concluded that they have a relatively limited productive capacity, and are vulnerable to overfishing. Estimations of mortality rates are essential to demographic analyses because losses from exploited populations largely control the yields that can be obtained from them (Gulland, 1971; Ricker, 1975, *in* Ralston, 1987). The estimate of Z obtained excluding the 12 year old age class is probably the more representative, considering the bias that would be caused by the fact that the 12 year old age class (represented by a single fish), if included, would be representing four separate age classes (9 to 12). The mortality rates presented here for *P. maculatus* are preliminary estimates, and a larger sample would be necessary to represent the population structure more effectively over the period of time considered. This estimate, however, is the first obtained for *P. maculatus*, and the use of a relatively small sample of aged fish in many cases is more appropriate than the use of

constructed age-length keys, which can result in seriously biased mortality estimates (Kimura, 1977).

McPherson et al. (1988) suggested that because most of their samples were collected by spear-fishing, larger fishes that are more common in deeper water could have been under-sampled (Williams and Russ, 1991). The sample used in the present study was collected mainly by spearfisherman also, and a positive water depth/fish age relationship may explain the fact that old individuals were relatively rare (Fig 2.8). Individuals over 38 cm SL, however, comprised 30% of the sample (Fig. 2.7) and the largest specimen examined, a 12 year old male, measured 58 cm SL (67 cm FL.), which is very close to the largest recorded specimen (70 cm FL, visual estimate, Randall and Hoese, 1986). Thus it is possible that the small number of old fishes observed here is a result of their small representation in the population. This situation would be expected in a population under fishing pressure and it was suggested by Williams and Russ (1991) that *P. maculatus* is a species likely to be particularly vulnerable to overfishing, because of its proximity to the coast.

The number of daily increments between annuli can be used as an aid for verification of annuli in otoliths (Victor and Brothers, 1981, Taubert and Tranquilli, 1982). The recruitment of a congeneric species, *Plectropomus leopardus*, was reported to occur mainly during January in the Northern Great Barrier Reef (Williams and Russ, 1991). If the same situation applies to *P. maculatus*, the average number of daily growth increments counted in otoliths indicates that the onset of formation of the opaque zone (annulus) occurs during winter, which is in agreement with the results obtained using tetracycline labelling. While the position of the fluorescent band indicated formation of the opaque zone still occurring

during early Spring, formation of opaque zones could not be detected at the margin of the otoliths of captive fish killed in September (Fig. 2.1). Further growth of a translucent edge is probably necessary to create enough contrast for the detection of the opaque (annulus) zone.

The formation of annuli partially coincided with the spawning season occurring during the Spring months (Ferreira, 1993; Chapter III). It is possible that both phenomena are related to similar cycles, instead of one being the cause of the other, since annuli were observed to form in otoliths of juveniles as well as adults. Temperature has been related frequently to annulus formation in fish otoliths (Simkiss, 1974; Longhurst and Pauly, 1987). Sea temperatures off Townsville reach their minimum during July (Kenny, 1974). The growth of tropical marine fishes can be influenced by seasonal changes in the environment (Longhurst and Pauly, 1987) and temperature could be the seasonal factor triggering changes in the mode of growth of the otolith of the inshore coral trout. Casselman (1990) suggested that, because otoliths are deposited by extracellular processes (Simkiss, 1974), the growth of fish otoliths may be more related to chemical processes that are directly affected by temperature than to metabolic processes that affect protein anabolism. The relationship between otolith growth and fish growth must be investigated to determine if the presence of annuli in the otoliths reflect seasonal growth oscillations in body size.

The presence of annual bands validated here in the otoliths of *P. maculatus*, shows the possibility of obtaining improved estimates of growth and mortality parameters for large, exploited species of reef fish using conventional methods of age determination.

CHAPTER III: Reproduction of the inshore coral trout *Plectropomus maculatus* (Bloch, 1790) from the Central Great Barrier Reef, Australia.

3.1 - Introduction

All groupers (sub-family Epinephelinae of the Serranidae) that have been studied carefully are protogynous hermaphrodites (Shapiro, 1987). However for only one species of the genus *Plectropomus*, *P. leopardus*, has a definitive study of the reproductive biology been made to establish hermaphroditism (Goeden, 1978).

The inshore or bar-cheeked coral trout, *Plectropomus maculatus* (Bloch), inhabits mainly turbid waters of near shore reefs and islands (Randall and Hoese, 1986). Due to its near shore habitat and thus easy access to fishermen, the inshore coral trout is a major target for recreational fisheries on the Queensland coast (Williams and Russ, 1991) and therefore a candidate for heavy exploitation. The importance of determining the mode of reproduction of a species is enhanced by the fact that the management of a fishery is considerably complicated by sequential hermaphroditism (Shapiro, 1987; Bannerot et al., 1987). Since males in protogynous species tend to be larger, older and less numerous than females, fishing may remove more males than females.

Information on the age and growth of this species is available for the Central Great Barrier Reef (Ferreira and Russ, 1992, Chapter II). Thus, it is possible to integrate a reproductive study with information on the demography of this species and resolve three crucial questions for fisheries management:

- What is the reproductive mode of the species?
- What is the age and size at first maturity?

- What is the average age and size of sex transition, the range of occurrence of females and males in length and age classes and the variation associated with these estimates.

In order to answer these questions, the reproductive biology of *P. maculatus* was described by histological analysis to determine the mode of reproduction, maturity stages and the events associated with sex-change.

3.2 - Materials and Methods

Histological analyses were conducted on the gonads of 68 specimens of inshore coral trout (1991: mid March, n= 45; late September, n= 8; mid October, n= 8; late November, n= 5 and early 1992: January, n=2). The fishes were collected from inshore reefs off Townsville and waters adjacent to Orpheus Island, Queensland, (Latitude 18° to 19° 30' S, Longitude 146° to 147° E). Samples were collected using spear-fishing (n=40) and line-fishing (n=28), at depths ranging from 2 to 25 meters.

The Fork length (FL) and Standard length (SL) of the fish were measured in centimetres. The gonads were removed, weighed and preserved in F.A.A.C (Formaldehyde 4%, Acetic Acid 5%, Calcium chloride 1.3%) for later sectioning (L. Winsor, pers. comm.). Observations on the macroscopic appearance of the gonads were conducted on fresh gonads. Middle portions of the gonads were embedded in paraffin and sectioned transversely at 5 μ thickness; then

stained with Mayer's haematoxylin-eosin. Evidence for protogyny was considered following the criteria of Sadovy and Shapiro (1987).

The nomenclature for description of the stages of oogenesis and spermiogenesis were adapted from Yamamoto *et al.* (1965) and Nagahama (1983), and the classification of individuals into gonadal developmental classes followed Moe (1969). The definition of transitional individuals adopted was that of Hastings (1981). These were individuals whose gonads showed regressing ovarian and developing testicular tissues but in which sex-transition had not yet proceeded to the point at which the dorsal sperm sinuses were formed and filled with spermatozoa. When the transition was complete to the point of formation of sperm sinuses, individuals were classified as males even if the gonad was still largely ovarian.

The size and age of sexual transition was taken from the range over which the length or age distribution of males overlapped with the length or age distribution of females:
overlap= (maximum female size or age) - (minimum male size or age)

The mean values of size and age in these ranges of overlap and their confidence limits were considered to represent the size or age at which sex change usually occurred in the population (Shapiro, 1987). The extent of overlap of the sexes was calculated as a percentage of the maximum size or age observed:
(range of overlap)÷(maximum size or age) x 100.

For these calculations and for the calculation of the sex ratio, transitional individuals were considered as males. Immature females were excluded

from the calculation of sex ratio, as they were not fully represented in the sampled population (Ferreira and Russ, 1992). The ages of the fish were determined from whole and sectioned otoliths (Chapter II).

3.3 - Results

Reproductive biology

The gonads of male and female *P. maculatus* are elongated, paired organs attached to the dorsal body wall via mesenteries. The left gonadal lobe is connected to the lateral body wall while the right lobe is attached by mesenteries to the intestine and other organs in the coelomic cavity. Ovaries consisted of lamellae filled with oocytes that extend from the gonadal wall into the central lumen (Fig. 3.1). The testis was formed of lobules extending from the dorsal and lateral gonadal wall into the vestigial lumen. Sperm sinuses were located in the dorsal part of the gonad and ran longitudinally through the gonadal wall (Fig. 3.2).

The mode of reproduction of *P. maculatus* is protogynous hermaphroditism. All male gonads had a remnant ovarian lumen and sperm sinuses in the gonadal wall (Fig. 3.2). Based on the presumed sequence of morphological development of the gonad, rather than on actual reproductive function, this characteristic indicates that the species is monandric, i. e., all males were derived from females (Reinboth, 1967).

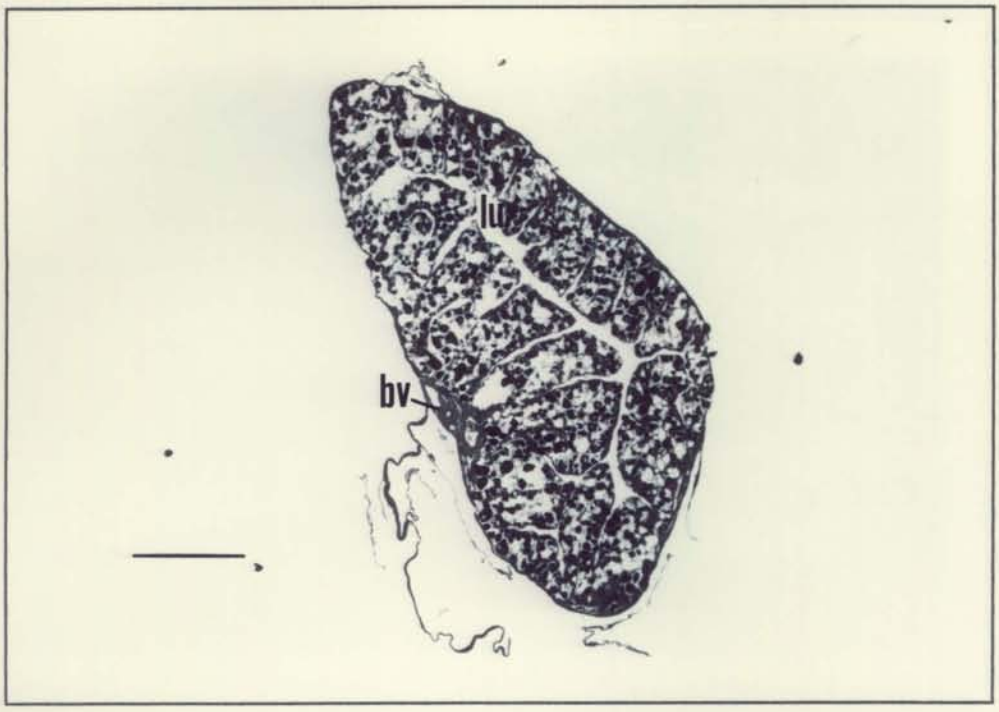


Fig 3.1: Section of the gonad of an immature female *P. maculatus*. **bv**, dorsal blood vessel; **lu**, lumen. 40x. Bar=500 μ

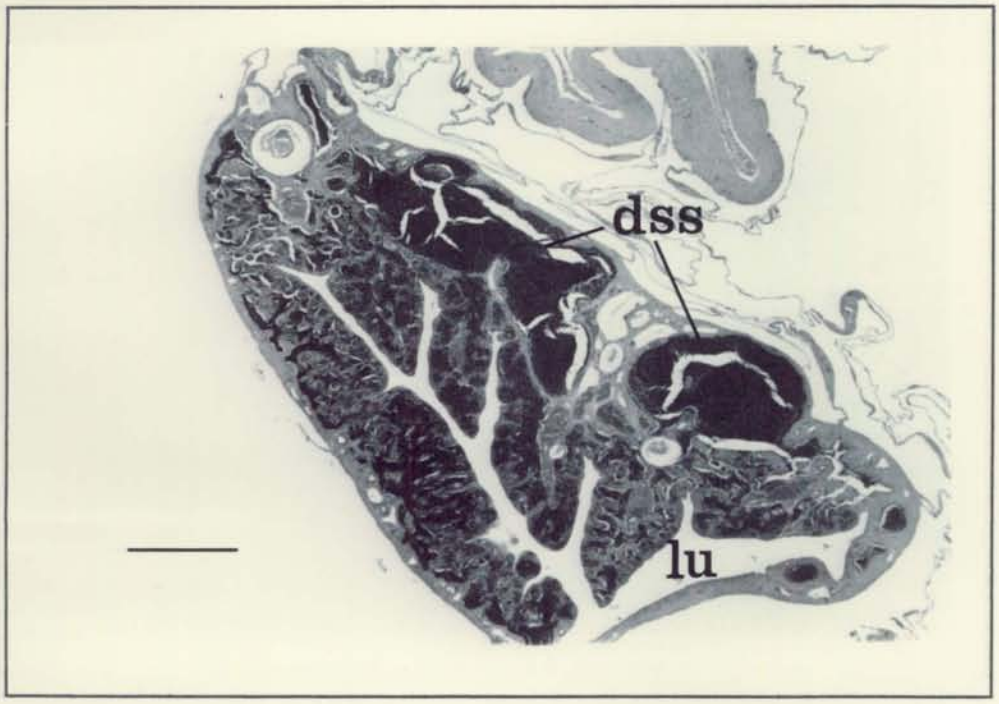


Fig. 3.2: Section of the gonad of a mature, ripe male *P. maculatus*. **lu**, lumen; **dss**, dorsal sperm sinus. 40x. Bar=500 μ.

Sex transition was observed in the ovaries of two females where the presence of degenerating yolky oocytes indicated the occurrence of recent spawning (Fig. 3.3). Crypts of spermatocytes proliferated throughout the lamellae as ovarian tissue was resorbed (Fig. 3.4). The intermingling of ovarian and testicular tissues (Fig. 3.5) characterises a germinal tissue of the undelimited or "Epinephelus" type (Smith, 1959).

The first oocytes to be reabsorbed were those containing yolk. Atretic mature oocytes are phagocytized by granulosa and thecal cells, and give origin to yellow-brown bodies (Fig. 3.6) (Sadovy and Shapiro, 1987). The presence of these yellow-brown bodies was a common feature in gonads of mature males and females. In post-spawning individuals, they could be seen macroscopically as brown dots along the gonads. Oocytes in previtellogenic stages suffered fragmentation and gradual reabsorption after transition (Fig. 3.5). Their presence was frequent in male gonads.

The ovarian lumen in males was not used to carry sperm. Instead, sperm sinuses were formed by the splitting of muscle layers of the ovarian capsule in the dorsal part of the gonad. After the formation of sperm sinuses, the gonad entered the male stage.

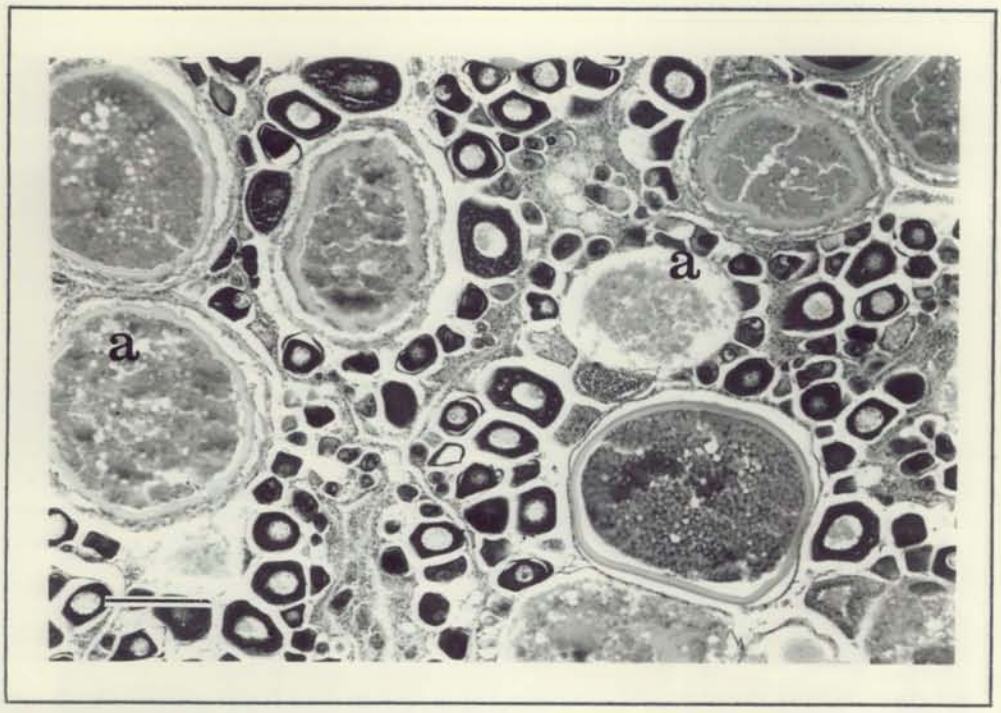


Fig. 3.3: Section of the gonad of a post-spawning female *P. maculatus*. **a**, atretic oocyte. 100x. Bar= 200 μ

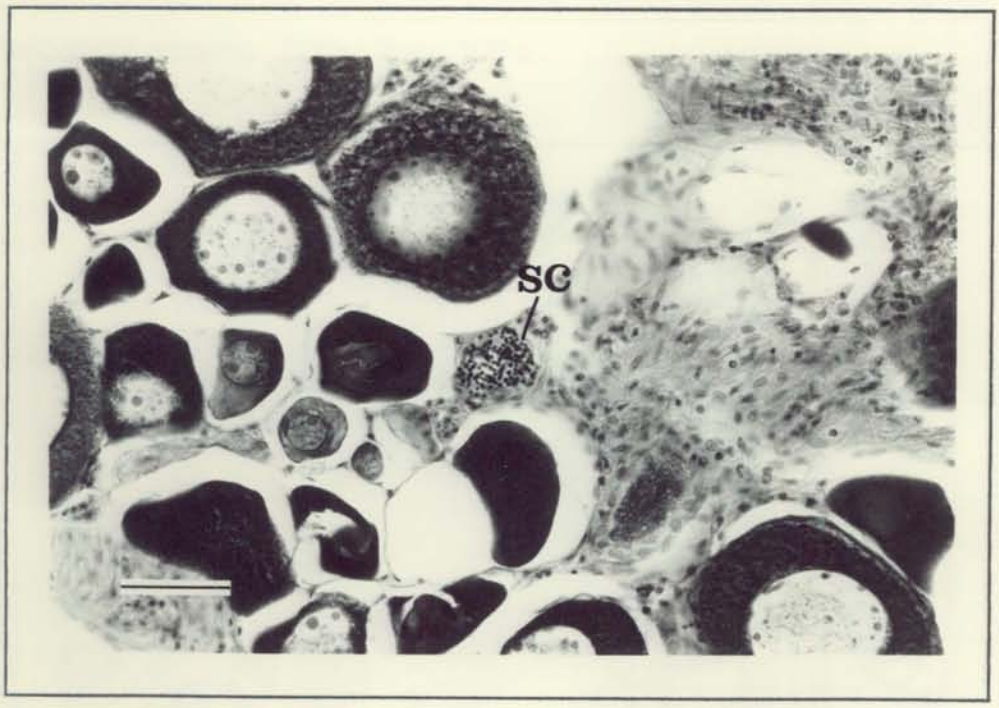


Fig. 3.4: Section of the gonad of a transitional *P. maculatus* showing crypt of secondary spermatocytes (**sc**) developing after spawning. Same individual of figure 3.3. 400x. Bar= 50 μ.

Gonads of newly transformed males (n=4) resembled an ovary in shape, and ovarian tissue dominated the lamellae. In spite of this, spermiogenesis was intense during the spawning season and the dorsal sinuses were filled with spermatozoa (Fig. 3.7). As the proliferation of testicular tissue continued, the testes assumed their characteristic lobular form. In males in which the gonads had reached this stage, large intralobular sinuses were formed during the spawning season. These central sinuses were formed by several crypts of spermatozoa that ruptured and joined within the testicular lobules (Fig. 3.2).

Gonadal Classes:

The following developmental stages of gonads, covering seasonal and ontogenetic changes, were observed in the specimens analysed:

Immature female (n=11): macroscopically, the ovaries in this stage were firm, pink-translucent and relatively small. In histological sections they were small in diameter and showed no evidence of prior spawning. The lamellae were filled with previtellogenic oocytes in early and late perinucleolus stages. Oocytes in the oogonium and chromatin nucleus stages were abundant also (Fig. 3.1).

Mature female (n=39):

-*Resting stage*: in this stage the ovary was usually pale pink-yellow and larger in diameter. The presence of yellow-brown bodies, possibly originated from atretic vitellogenic oocytes, was considered as evidence of prior spawning as these structures were not present in the gonads of immature females. The lamellae were filled with previtellogenic oocytes in early and late perinucleolus stages (Fig. 3.8).

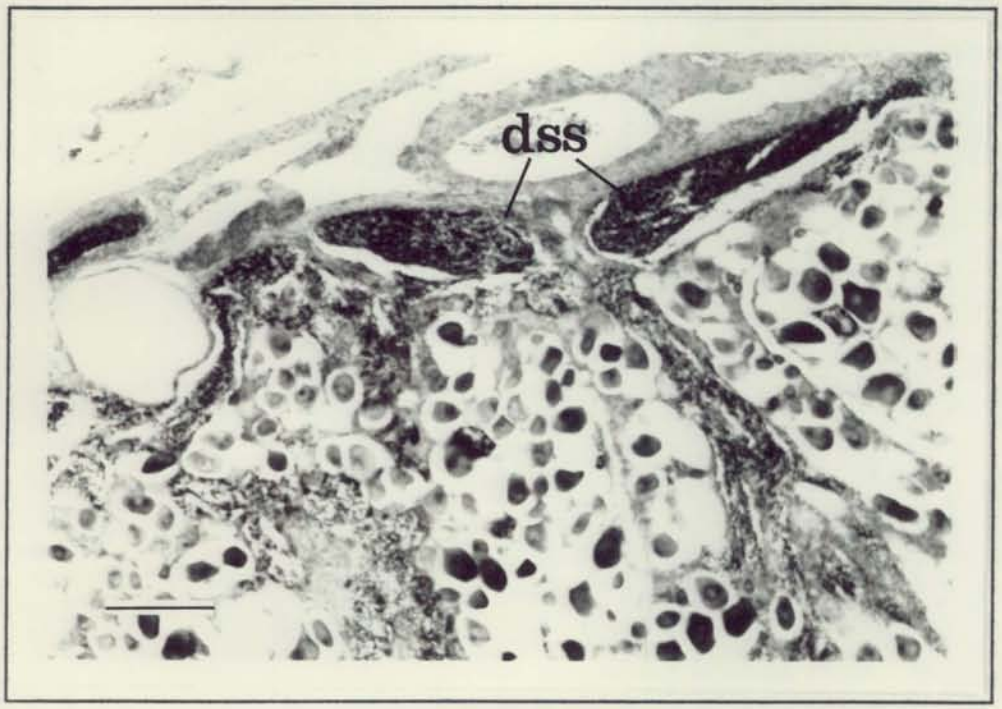


Fig. 3.7: Section of the gonad of a young male *P. maculatus*. **dss**, dorsal sperm sinus. 200x. Bar=100 μ.

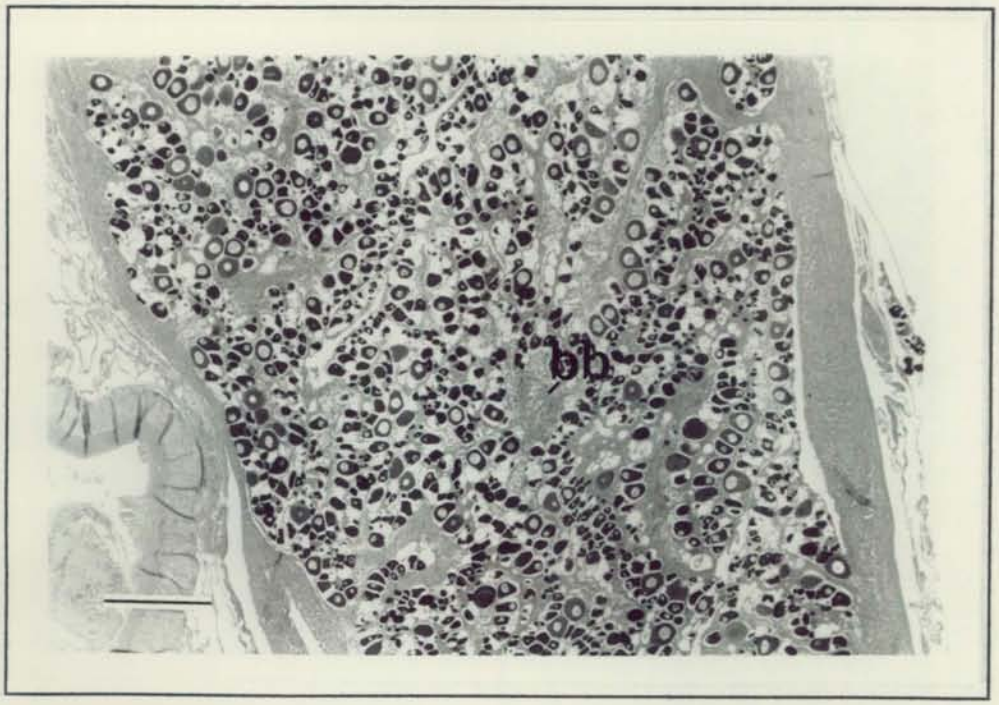


Fig. 3.8: Section of the gonad of a resting mature female *P. maculatus*. **bb**, brown-body. 40x. Bar= 500 μ

-*Ripe stage*: mature ovary in active vitellogenesis and in preparation for spawning. Macroscopically the ovary was large, yellow, and had a granular appearance due to the presence of mature oocytes that could be seen through the distended, translucent gonadal wall. Histologically, the gonad was large in diameter and contained within a thin, distended tunica. Oocytes in all stages of development, from early perinucleolus to ripe, could be identified but oocytes in late stages of vitellogenesis were dominant (Fig. 3.9).

-*Spent or Post-spawning stage*: a mature ovary that showed evidence of recent spawning. Macroscopically they were pale yellow, flaccid and bloody. In histological sections the lamellae were disrupted and disorganised, with several empty spaces that were presumably formerly occupied by ripe oocytes and extensive vascularization. Follicular cells, remnants of post-ovulatory follicles, were present throughout the gonad. Oocytes in the perinucleolus stage dominated, and oogonium and chromatin nucleus stage oocytes proliferated. Vitellogenic oocytes could be observed in several stages of atresia (Fig. 3.3).

Transitional (n=3): a transitional gonad in its earlier stages was characteristically a post-spawning ovary, with vitellogenic oocytes in atresia and proliferation of small crypts of spermatogonia and spermatocytes (Fig. 3.4) throughout the lamellae. The crypt of spermatocytes shown in Figure 3.4 was observed in the same gonad of Figure 3.3.

Young male (n=4): a post-transitional, newly transformed testis. There was active proliferation of testicular tissue and the dorsal sinus was formed. The gonad was largely ovarian, with numerous previtellogenic oocytes and it resembled a resting female gonad. The lamellae had not assumed the typical lobular form as in the mature testes. During the

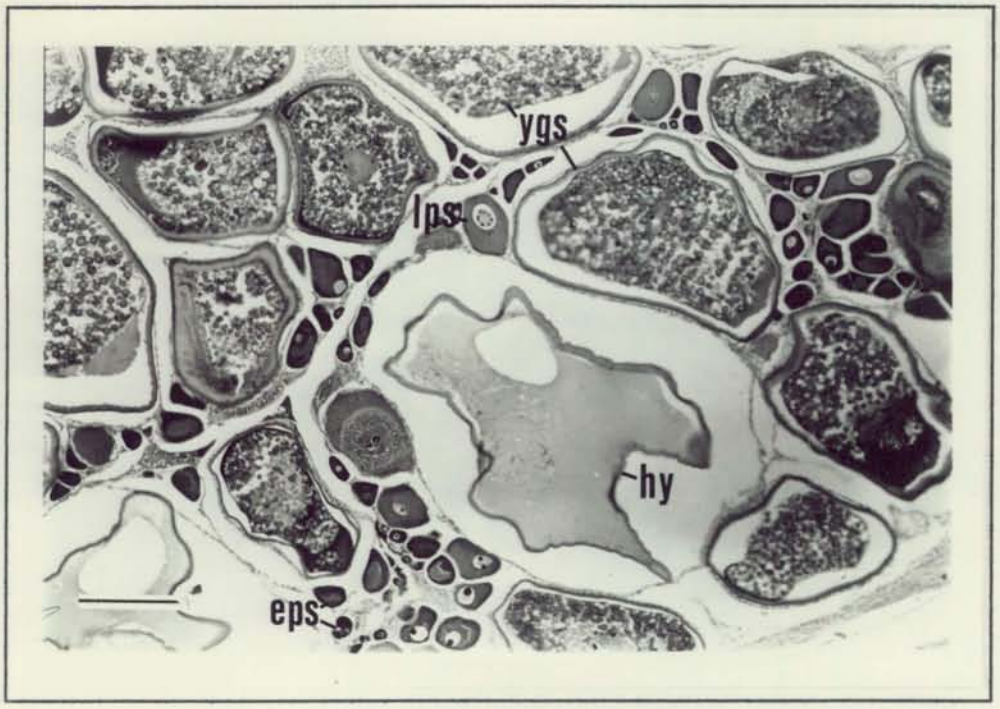


Fig. 3.9: Section of the gonad of a ripe mature female *P. maculatus*. **eps**, early perinucleolus stage oocyte; **lps**, late perinucleolus stage oocyte; **ygs**, yolk globule stage oocyte; **hy**, hydrated or ripe stage oocyte. 100x. Bar= 200μ

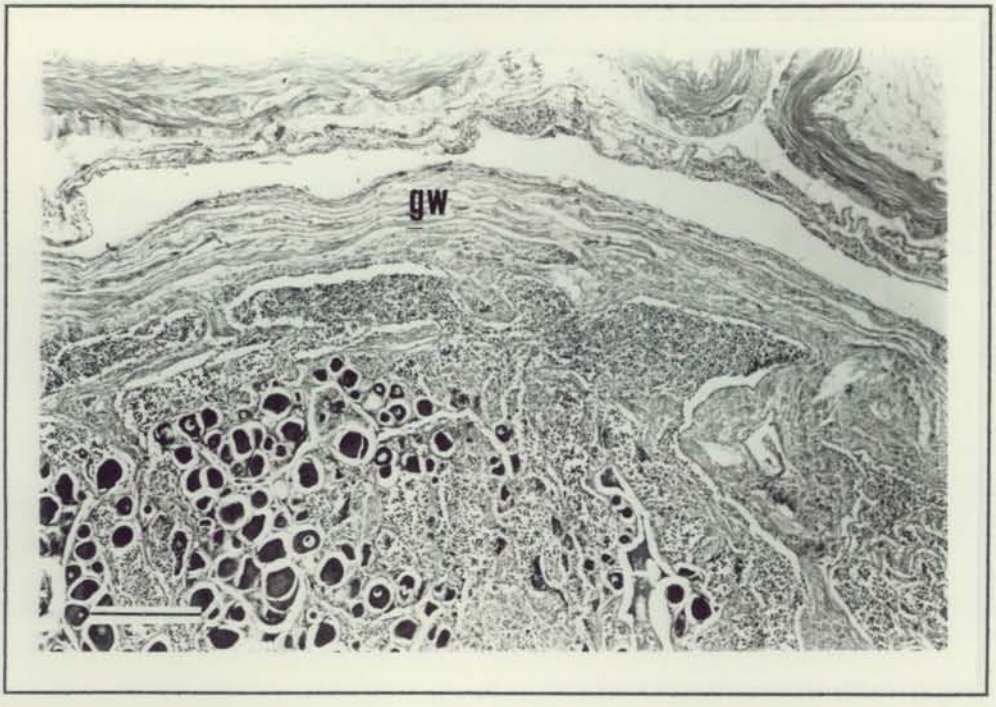


Fig. 3.10: Section of the gonad of a ripening male of *P. maculatus* showing intense spermiogenesis. 100x. Bar 200 μ.

spawning season, spermiogenesis was intense and the newly formed sinuses were filled with spermatozoa (Fig 3.7). The formation of central or intralobular sperm sinuses was not observed in this phase. The gonad of one of the young males appeared to be derived from an immature female ovary (Fig 3.7). No signs of previous spawning activity were present and the macroscopic appearance of the gonad was of an immature ovary.

Mature male (n=12):

-*Ripening stage*: testis dominated by the later stages of spermatogenesis and early stages of spermiogenesis. Most crypts contained secondary spermatocytes and spermatids. Spermatozoa filled the dorsal sinus (Fig. 3.10).

-*Ripe stage*: testis dominated by spermiogenesis. Most crypts contained spermatids and spermatozoa. Several crypts of spermatozoa ruptured and joined within the testicular lobules, forming large intralobular sinuses. The dorsal sinuses were filled with spermatozoa (Fig. 3.2).

-*Spent or Post-spawning stage*: in this stage, crypts of spermatogonia and primary spermatocytes were developing actively throughout the testis, occupying the empty spaces left by shed spermatozoa. Stromal tissue was well developed between crypts. Spermatozoa were still present in the dorsal and central sperm sinuses (Fig. 3.11).

-*Mature resting male*: testis dominated by stromal tissue and early stages of spermatogenesis. Spermatozoa were still present in the sperm sinuses and in the central spaces of the lobules that were reduced by invasion of stromal tissue (Fig. 3.12).

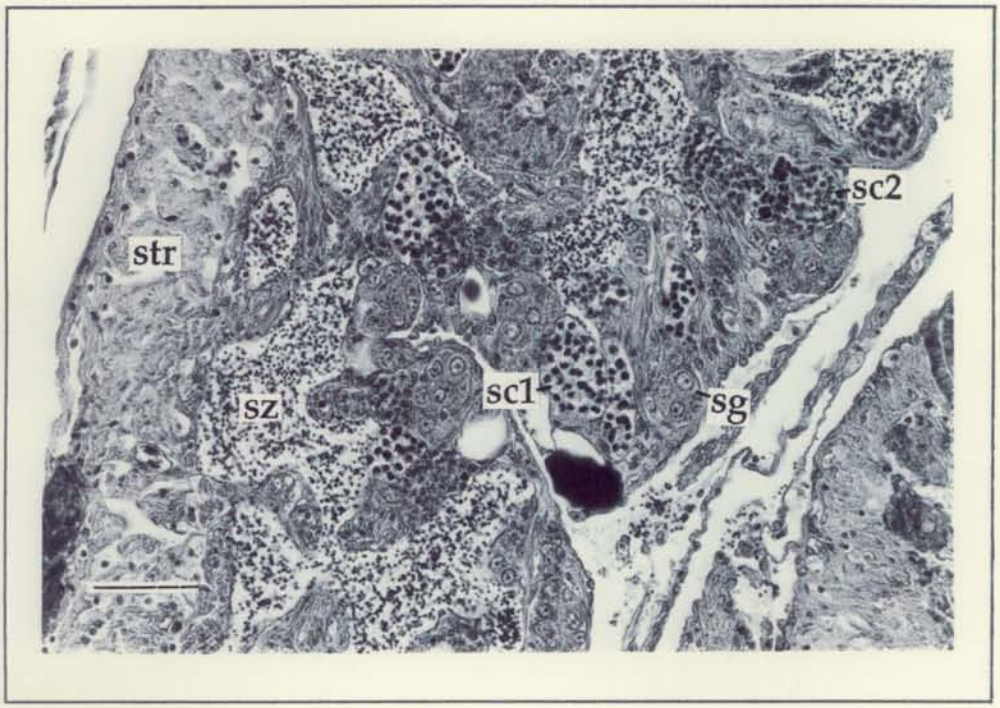


Fig. 3.11: Section of the gonad of a mature post-spawning male *P. maculatus*. Note intense proliferation of spermatogonia. **sg**, spermatogonia; **sc** spermatocyte; **sc1**, primary spermatocyte; **sc2**, secondary spermatocyte; **sz**, spermatozoa; **str**, stromal tissue1000x. Bar 20 μ.

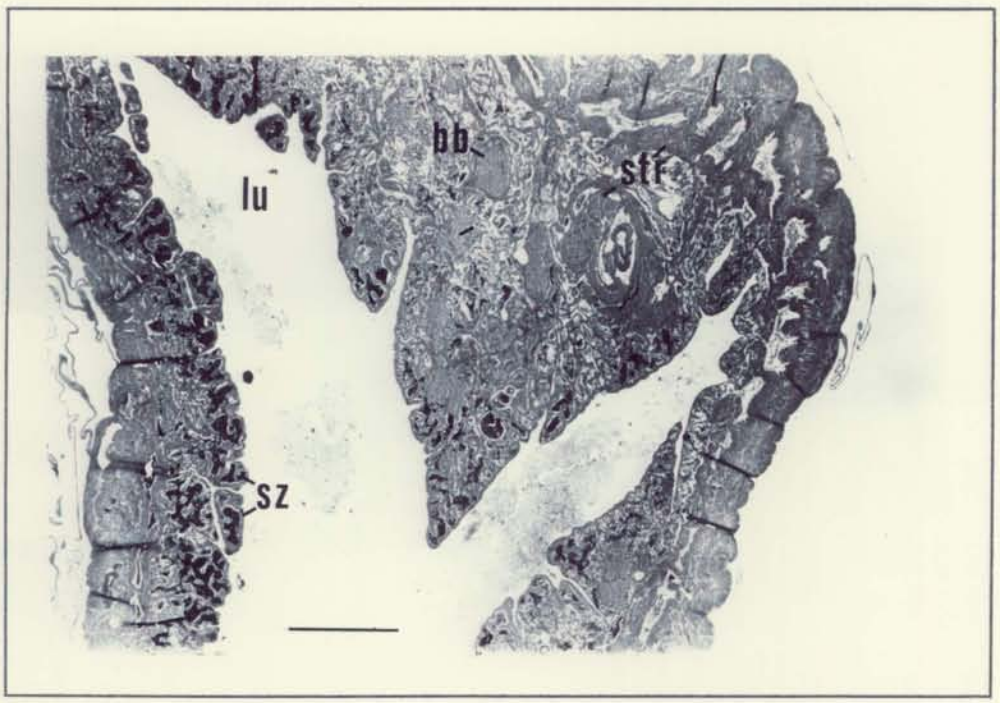


Fig. 3.12: Section of the gonad of a mature resting male *P. maculatus*. **lu**, lumen; **bb**, brown body; **str**, stromal tissue; **sz**, spermatozoa. 40x. Bar 500 μ.

Seasonality and periodicity of spawning

Spawning activity, as indicated by the presence of individuals in the ripe stage, was observed from September through November. During this time, multiple-spawning was indicated in females by the occurrence of asynchronous oocyte development. In males, spermatogenesis, spermiogenesis and the presence of partially spent areas occurring in the same testes (Fig. 3.11), indicated multiple-spawning. Spent females were observed in October and November and spent males in November and January. Transitional stages were observed in October. All mature males and females collected in March were in the resting stage.

Sexual maturation and sex transition

The size and age of first reproduction (50% of individuals reproductive) for females was 30.0 cm SL and 2 years of age, and the size and age at which all females were mature (100%) was 35.0 cm SL and 3 years (Fig. 3.13). The ranges over which males overlap with females and the ranges observed for transitional individuals are listed in Table 3.1. The mean values of the overlap, representing the size and age of sexual transition, were 35.38 cm SL and 4.17 years of age (Table 3.1). The extent of overlap of the sexes was 39% of SL and 42% of age.

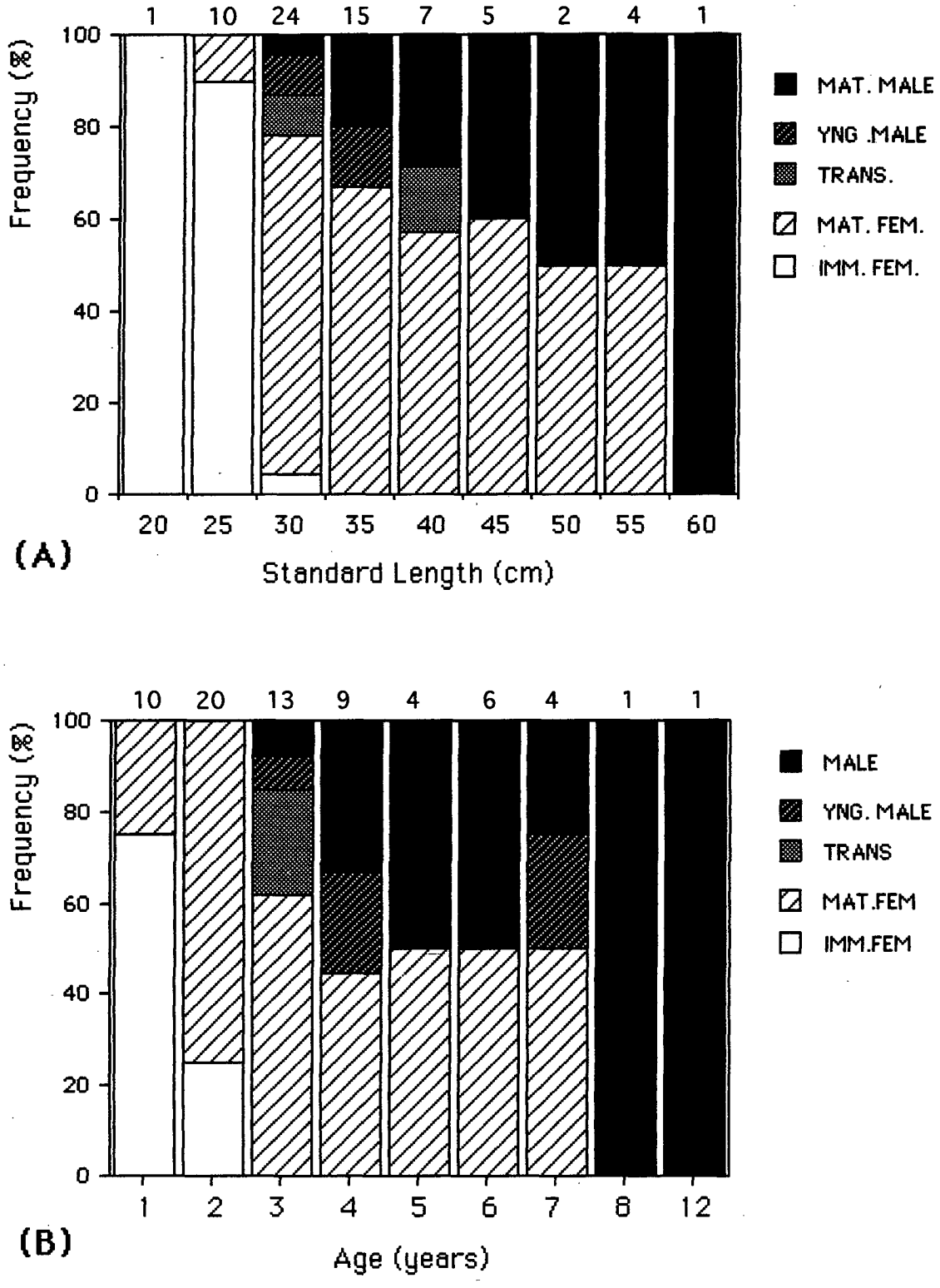
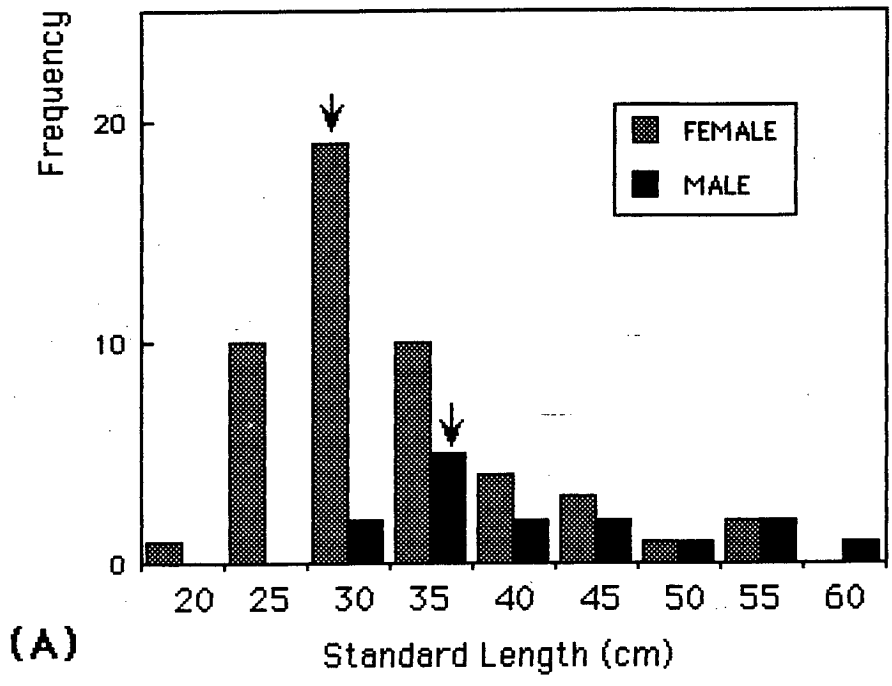


Figure 3.13: Percent frequency distribution of gonadal developmental stages of *P. maculatus* by size (A) and age (B). Size (SL) is represented by higher point of class interval.

TABLE 3.1: Extent of overlap of size distribution (SL) and age distribution (years) of males and females and range of transitional individuals of *P. maculatus*.

	FEMALES	MALES	OVERLAP	Transitionals
SL(cm) MIN.	15.5	28.5	28.5	28.5
SL(cm) MAX.	51.00	57.8	51.00	35.6
MEAN	30.37	37.71	35.38	31.2
SD. ERROR	1.062	2.058	0.986	2.219
(N)	49	19	44	3
AGE MIN.	1	3	3	3
AGE MAX.	7	12	7	3
MEAN	2.77	5.11	4.42	3
SD. ERROR	0.236	0.524	0.237	-
(N)	47	19	36	3

The sequence of protogynous sex-change results in differences in the modal size and age of females and males. Figure 3.14 shows the size and age distribution by sex. As expected, the modal size of females was smaller than that of males (30 cm SL vs 35 cm SL); and by age the mode for females was 2 years while for males it was 4 years. The sex ratio in the spawning population was biased towards females; the female : male ratio in the sample was 2.6:1.



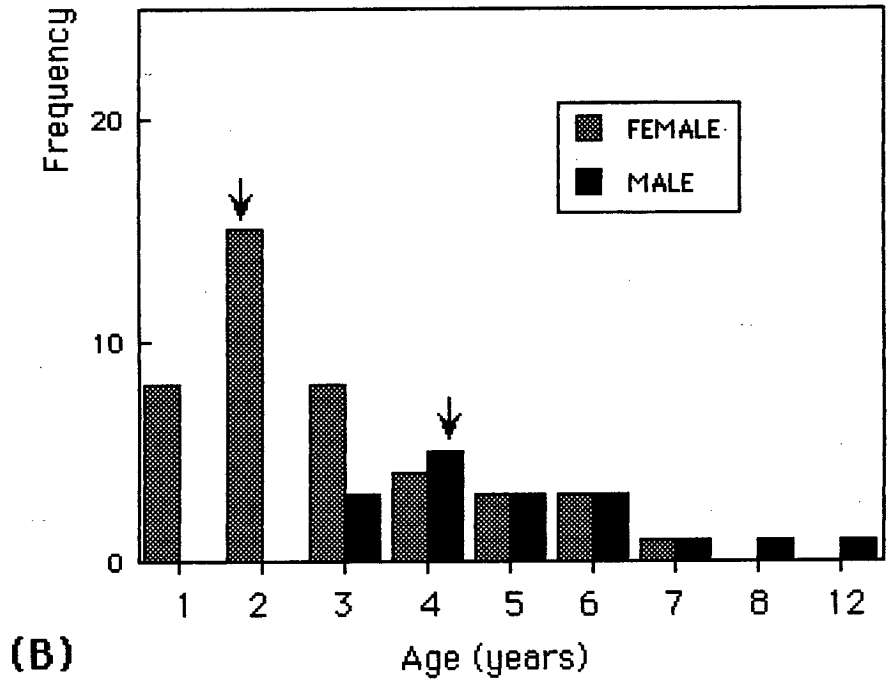


Figure 3.14: Frequency distribution of males and females of *Plectropomus maculatus* by size (A) and age (B). Size (SL) is represented by higher point of class interval. The arrows indicate the modal classes.

3.4 - Discussion

There is some tendency for species of groupers (sub-family Epinephelinae of the Serranidae) to spawn between early spring and summer in low latitudes (Shapiro, 1987). Consistent with this generalisation, gonadal stages of *P. maculatus* indicated spawning activity from September through November and a resting period until March. As no samples were examined from March to September, reproductive activity during this period remains unknown. Asynchronous oocyte development in females and continuous spermiogenesis in males was observed during the spawning period. Such features are commonly interpreted as a multiple-spawning indicators (Nagahama, 1983; Ebisawa, 1990). This is likely to be the case for *P. maculatus*, though multiple-spawning is not the only possible interpretation for asynchronous development of gametes. Oocytes not released during a spawn could be simply reabsorbed without entering a putative subsequent ripening phase leading to a second spawn, or, once ripe, oocytes could enter a latent stage allowing slower oocytes to develop for simultaneous release. Similar arguments could be made for males.

The production of yellow-brown bodies from vitellogenic oocytes, through atresia, is a well described occurrence and their presence in gonads has been used as evidence of previous vitellogenesis in mature females and in female-to-male sex change (Sadovy and Shapiro, 1987; Hastings, 1981). However, their presence can apparently result also from other processes not involving oocytic atresia. Similar bodies have been found in testes of gonochoristic species and simultaneous hermaphrodites (Sadovy and Shapiro, 1987). In the inshore coral trout,

yellow-brown bodies were observed in the testes of all males examined, including old males where the reabsorption of ovarian tissue was complete. This could indicate that brown bodies, once formed, are very long-lasting or even permanent structures. However, while post-spawning and resting mature females had numerous brown bodies, such structures were not so frequently observed in ripe, pre-spawning females. Furthermore, brown bodies were especially abundant in post-spawning and resting males, where they were usually located in the centre of the testicular lobes. Hence, it is also possible that their presence in males was associated with another kind of process like sperm degeneration, (Moe, 1969) or steroidogenic activity (see Nagahama, 1983, for references). Similarly, processes other than vitellogenesis could be involved in the formation of brown bodies in female ovaries. In this case, the degree of certainty about values of size and age at first reproduction would be affected.

The definition of "transitional" employed here was adapted from Hastings (1981) and Moe (1969), and described sex-changing individuals as not sexually active, i.e, in the act of change, not yet functional males but no longer females. In *P. maculatus*, sex-transition appeared to be initiated immediately after spawning, as observed for other species of Serranidae (Moe, 1969; Goeden, 1974; Sadovy and Shapiro, 1987). Young males, whose gonads were largely ovarian, showed spermiogenesis activity during the spawning season. Moe (1969) observed the same process for *Epinephelus morio*, a protogynous grouper, and suggested that an individual could spawn early in the season as a female and spawn later as a male.

Reproductively active males which were close in size and age to the low end of the size range of mature females were observed for *P. maculatus*.

Early sex-change can occur in two forms, as defined by Warner and Robertson (1978): pre-maturational, when sex-change occurs before the female ever functions as an adult; and early post-maturational, when the female functions as an adult for a very brief period before changing sex. Evidence of pre-maturational sex-change was found in one individual of *P. maculatus* in which the gonad showed no signs of previous spawning activity and resembled an immature ovary. Moe (1967) also observed early males of *E. morio*, and noted that for some individuals previous spawning activity during the female phase was not indicated.

The size or age classes in which transitional gonads occur can be taken to represent the range of sizes or ages of transitional individuals as well as the extent of overlap of sizes and ages of males and females (Shapiro, 1987). However, transitional gonads were relatively uncommon. If sex-transition occurs immediately after the spawning event, the seasonal character of sex-transition could be a factor contributing to the low number of transitional individuals observed, since only 31% of the sample was collected during the spawning season. The low number of transitional individuals observed here and the fact that this low rate seems to be the pattern for most Epinephelinae species (Shapiro, 1987), indicates that the extent of overlap of age and size distribution of males and females is more adequate to estimate the range of occurrence of sex-reversal than the range of sizes or ages of transitional individuals.

The overlapping of the sexes indicated that sex-change can take place over a broad range of sizes and ages for *P. maculatus*. This seems to be a common feature for many other species of Epinephelinae. Shapiro (1987) suggested that this kind of result is consistent with a mechanism for behavioural induction of sex change. Behavioural control, operating

independently within social subdivisions of the population, could result in more variability in the size or age at which particular females change sex. A developmental system in which sex change occurs at a certain age or size would result in all individuals changing sex at approximately the same size or age.

The presence of large females may be the consequence of a genetic inability to undergo sex-reversal (Brusle and Brusle, 1975) or lack of an environmental or social cue for sex-change (Sadovy and Shapiro, 1987). Moe (1969) found that not all females of *E. morio* transformed into males. A larger number of old individuals of *P. maculatus* would have to be analysed to assess this possibility, i.e., to verify the existence of these "primary females" (*sensu* Warner and Robertson, 1978) in the population.

Alternative reproductive strategies, such as protogynous hermaphroditism, are biological characteristics of reef fishes that pose problems for theoretical aspects of stock assessment. Bannerot et al. (1987) investigated the effects of protogynous hermaphroditic reproduction on the predictions from the standard Yield-per-Recruit (YPR) and Stock production models. They concluded that a definite risk exists in managing those populations strictly by YPR models at high fishing mortality. Information on the characteristics of sex-transition and range of sizes and ages of sex-change, such as presented here for *P. maculatus*, are essential in the assessment of the potential effects of fishing pressure on protogynous hermaphroditic populations. More information about behavioural aspects of reproduction and population compensation mechanisms for sperm limitation of the inshore coral trout are still necessary to assure effective management decisions.

Chapter IV: Age and Growth of the Coral Trout, *Plectropomus leopardus* (Lacepede 1802) from Lizard Island, Northern Great Barrier Reef.

4.1 - Introduction:

The common coral trout *Plectropomus leopardus* (Lacepede, 1802) is the most abundant species of the genus on the Great Barrier Reef (Randall and Hoese, 1986), and usually the primary target of recreational and commercial fishermen. With around 1200 tonnes caught annually, the Coral Trout is the largest single component in the annual catch of the Queensland commercial line-fishing fleet (Trainor, 1991).

Some information on age, growth and longevity is available for the common coral trout. On the Great Barrier Reef, Goeden (1978) estimated the growth rate of this species at Heron Island based on length-frequency data. McPherson et al. (1988) determined age and growth of the common coral trout in the Cairns region by counts of annuli in whole otoliths. Loubens (1980) estimated age and growth for *P. leopardus* from New Caledonia based on counts of annuli in whole otoliths. The periodicity of formation of annual rings in the latter two studies was verified through observation of marginal increments in otoliths. Direct validation has not yet been attempted for *P. leopardus*.

For several species of fishes, otolith growth has been described to be continuous (Boehlert, 1985; Casselman, 1990; Beckman et al., 1991). Boehlert (1985) suggested the use of weight and otolith measurements as a non-subjective, cost-effective methodology for age determination, that would decrease variability among age estimates.

The aims of this study were to obtain validated age-at-length information and to find the model that best described the growth of the common coral trout from Lizard Island, Northern Great Barrier Reef, Australia. The relationship between otolith weight and body size of the coral trout was studied to assess the usefulness of otolith dimensions in predicting age and to understand the mode of growth of the otolith.

4.2 - Materials and Methods:

Coral trout (n=310) were sampled in the Lizard Island area (Lat. 14° 40' S, Long. 154° 28' E) from March 1990 to February 1992. Fish were caught by recreational and commercial fishermen using hook-and-line (n=184) and by recreational spearfishermen (n=94). Individuals smaller than 20 cm total length are usually not vulnerable to line fishing, and were caught around Lizard Island by SCUBA divers using fence-nets (n=32). Fork length (FL, cm), Standard length (SL, cm) and Total weight (TW, g) were measured for each fish. Fork length is defined as the length from the front of the snout to the caudal fork, and Standard length is defined as the length from the front of the upper lip to the posterior end of the vertebral column. A simple linear regression of the form FL= a + b.SL was used to describe the relationship between FL and SL. The model $TW(g) = a.FL(cm)^{b}$ described the relationship between Fork length and Total weight, and was fitted by a non-linear regression.

Sagittae were removed, cleaned, weighed and stored dry. Left and right sagittae, when intact, were weighed to the nearest milligram. Otoliths

were prepared and read as described in Chapter II. To increase contrast between bands, whole otoliths were burned lightly on a hot plate at 180°C (Christensen, 1964). Both right and left sagitta were read whole under reflected light using a dissecting microscope at 16x magnification. The otoliths, with the concave side up, were placed in a black container filled with immersion oil. The left sagittae was prepared for reading by embedding in epoxy resin (Spurr, 1969) and sectioning transversely through the core with a Buehler Isomet low-speed saw. Sections were mounted on glass slides with crystal bond 509 adhesive, ground on 600 and 1200 grade sand paper, polished with 0.3μ alumina micropolish and then examined under a dissecting microscope at 40x magnification with reflected light and a black background.

Terminology for otolith readings followed definitions by Wilson et al. (1987). Two experienced readers independently counted opaque zones (annuli) in each whole and sectioned otolith of a random subsample (n=136) to assess the precision and accuracy of countings obtained through the two methods. The precision of age estimates was calculated using the Index Average Percent Error (IAPE) of Beamish and Fournier (1981). The results obtained from whole and sectioned otoliths were compared by plotting the difference between readings obtained from whole and sectioned otoliths (Section Age minus Whole Age) against Section Age. The results of this comparison indicated that whole otolith readings tended to be lower than readings from sectioned otoliths when more than 6 rings occurred in the otolith. Therefore, remaining otoliths were read whole first and, if the number of rings was higher than 6, or the whole otolith was considered unreadable, the otolith was sectioned and counts were repeated. Two readers independently counted annuli in each otolith. The results were

accepted and used in the analysis when the counts of the two readers agreed. If the counts differed, the readings were repeated once and if the counts still differed, the fish was excluded from the analysis.

Ages were assigned based on annulus counts and knowledge of the spawning season. The periodicity of annulus formation was determined with the use of tetracycline labelling. From August 1990 to February 1992, 80 fish were caught in a trapping program at Lizard Island fringing reef (Davies, 1989; in press), tagged with T-bar anchor tags and injected with tetracycline hydrochloride before being released. The fishes were injected in the coelomic cavity under the pelvic fin with a dosage of 50 mg of tetracycline per kg of fish (McFarlane and Beamish, 1987), in a concentration of 50 mg per ml of sterile saline solution. Five fish were recaptured after periods of at least one year at large. Two of those fish were reinjected at the time of recapture and kept in captivity for periods of 3 to 4 months. To determine the time of formation of the first annulus, five young-of-the year were captured with fence-nets. Three of these fish were injected with tetracycline at the time of capture, and all five fish were kept in captivity for periods of 3 to 17 months. The otoliths of the fish treated with tetracycline were removed, sectioned and observed under fluorescent light. To determine time of formation of the translucent and opaque zones, the distances between events for which time of occurrence was known (i. e.. between two tetracycline bands or between a tetracycline band and the margin of the otolith) were measured on otolith sections and plotted against the corresponding time interval. The relative positions of the translucent and opaque zones to these marks were then measured and plotted on the same scale. While this method does not provide real distances, it standardises the measurements allowing for comparison between fishes of different ages.

The relationship between otolith weight, fish size (length and weight) and age was analysed. Otolith weight was plotted against fish length (FL) for each age class separately. A multiple linear regression model was fitted in a step-wise manner to predict age from otolith weight and fish size and to predict otolith weight from age and fish size. The inclusion level for the independent variables was set at P=0.10. The assumptions of normality and homoscedascity were tested by plotting the residuals from the regression models.

The growth models were fitted to the data and their coefficients and standard errors estimated using standard non-linear optimisation methods (Wilkinson, 1989). As the plot of the length-at-age data indicated some form of asymptotic growth, Schnute's (1981) reformulation of the von Bertalanffy growth equation for length in which $a \neq 0$ was fitted to the data:

$$Lt = y1^{b} + (y2^{b} - y1^{b})\left\{\frac{1 - e^{-a(t-t1)}}{1 - e^{-a(t2-t1)}}\right\}^{\frac{1}{b}}$$

where Lt is length-at-age; t1 and t2 are ages fixed as 1 and 14 respectively ; y1 and y2 are estimated sizes at these ages; and a and b are the parameters which indicate if the appropriate growth curve lies closer to a three or two parameter sub-model. By limiting parameter values, the data were used directly in selecting the appropriate sub-model, namely the generalised von Bertalanffy, Richards, Gompertz, Logistic, or Linear growth models (Schnute, 1981). Subsequently, the original von Bertalanffy (1938) growth equation for length $Lt = L\infty(1 - e^{-K(t - to)})$ was fitted to the data. Lt is length-at-age;

L_∞ is the asymptotic length, K is the growth coefficient, t is age, and t_0 is the hypothetical age at which length is zero.

To evaluate the effects of use of gear selectivity (and consequently varying age composition) on the estimates of growth parameters, results obtained using only the sample collected by line and spear fishing were compared with that obtained using the same data complemented by the fence-net sample composed of younger fish.

4.3 - Results

Otolith morphology and readings

In the coral trout, the sagittae are the largest of the three pairs of otoliths. They are oval structures, laterally compressed, with a concave distal surface and a pointed rostrum. A curved sulcus crosses their proximal surface longitudinally. The otolith nucleus is usually opaque and followed by alternating translucent and opaque zones (annuli). The annuli in the coral trout otoliths were wide, and there was no sharp contrast between zones. Individual annuli were difficult to discern under transmitted light but clearly distinguishable under reflected light with a black background. Under reflected light, the opaque zones had a milky appearance and the translucent zones were dark (Fig. 4.1). The first two annuli were notably wider and less well defined than the subsequent ones in sectioned otoliths.

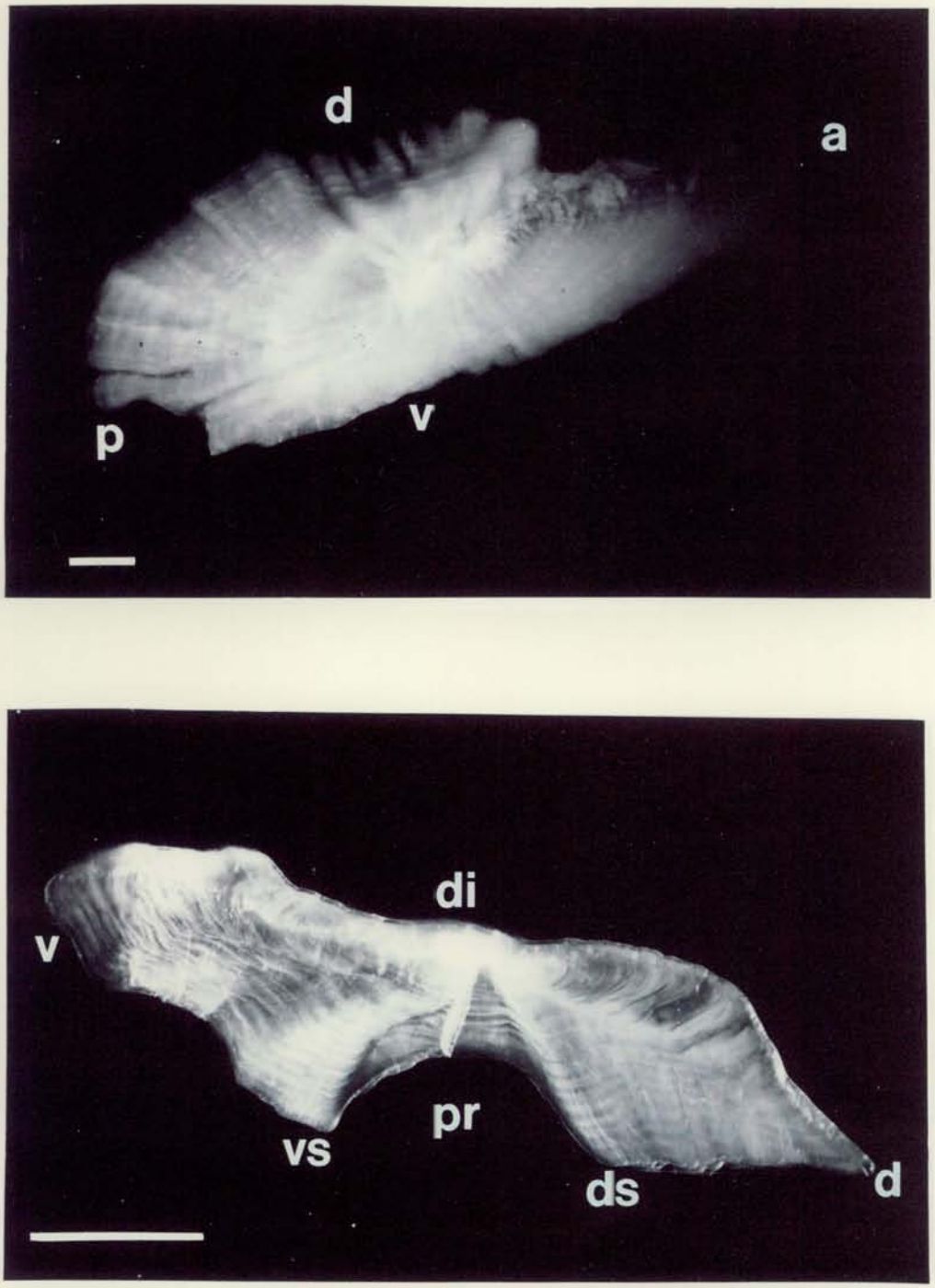


Figure 4. 1: Whole and sectioned otolith of a 12 year old *P. leopardus* under reflected light with a black background showing alternating pattern of translucent and opaque bands. a: anterior, p: posterior, d: dorsal, v: ventral, di: distal, pr: proximal, ds: dorsal sulcus, vs: ventral sulcus. Scale bar= 1mm

Whole sagittae were useful to confirm the presence of these first two annuli. In sectioned otoliths, the region from the nucleus to the proximal surface of the sagitta along the ventral margin of the *sulcus acousticus* was used for countings as the annuli in this region were more distinct and had more consistent growth patterns.

In whole otoliths, annuli were clearly distinguishable and easy to count along the dorsal side of the otolith, where up to 12 rings were counted in some otoliths. However, readings from whole otoliths tended to be lower than readings from sectioned otoliths when more than 6 rings were present and this tendency increased with the mean number of rings, particularly after 10 rings. (Fig. 4.2).

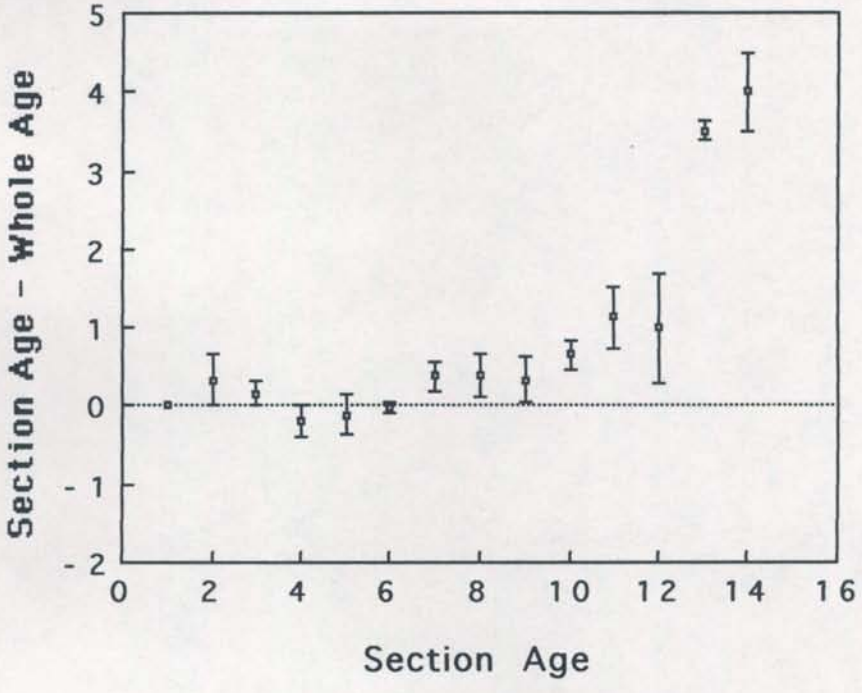


Figure 4. 2: Average difference between countings obtained from sectioned and whole otoliths (Section Age - Whole Age) plotted against Section Age. Error bars show standard error.

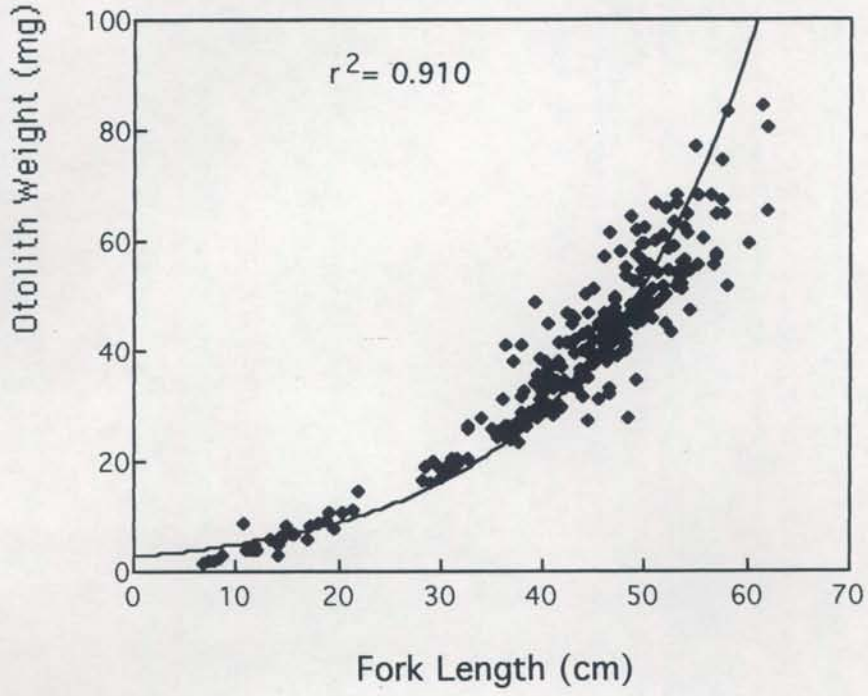
Tetracycline-labelled otoliths validated the periodicity of annuli in sectioned otoliths, indicating that whole otolith readings tend to underestimate age of fish >10 years old. A comparison between results of countings performed in whole and sectioned otoliths showed that, in the sub-sample analysed, the Index Average Percent Error (IAPE) of Beamish and Fournier (1981) was lower for countings performed on whole (6.7%) than for countings performed on sectioned otoliths (12.1 %). For the total sample, where readings from whole and sectioned otoliths were combined, the IAPE was reduced to 5.1%.

Otolith Growth

Otolith weight was directly related to age and an exponential function of fork length (Fig. 4.3). Within each age class, otolith weight was positively correlated to fork length for most classes (Table 4.1) indicating that there is a tendency for larger fishes to have larger otoliths than smaller fishes of the same age.

TABLE 4.1: Correlation between Otolith Weight (mg) and Fork Length (cm) for each age class of *P. leopardus*.

AGE	r^2	p<	df	AGE	r^2	p<	df
0	0.826	0.0001	18	**8**	0.481	0.0001	19
1	0.972	0.0001	10	**9**	0.405	0.0001	12
2	0.829	0.0001	27	**10**	0.120	NS	8
3	0.747	0.0001	19	**11**	0.937	0.0001	7
4	0.652	0.0001	18	**12**	0.526	NS	3
5	0.650	0.0001	30	**13**	0.993	0.05	2
6	0.489	0.0001	43	**14**	0.049	NS	2
7	0.514	0.0001	30				



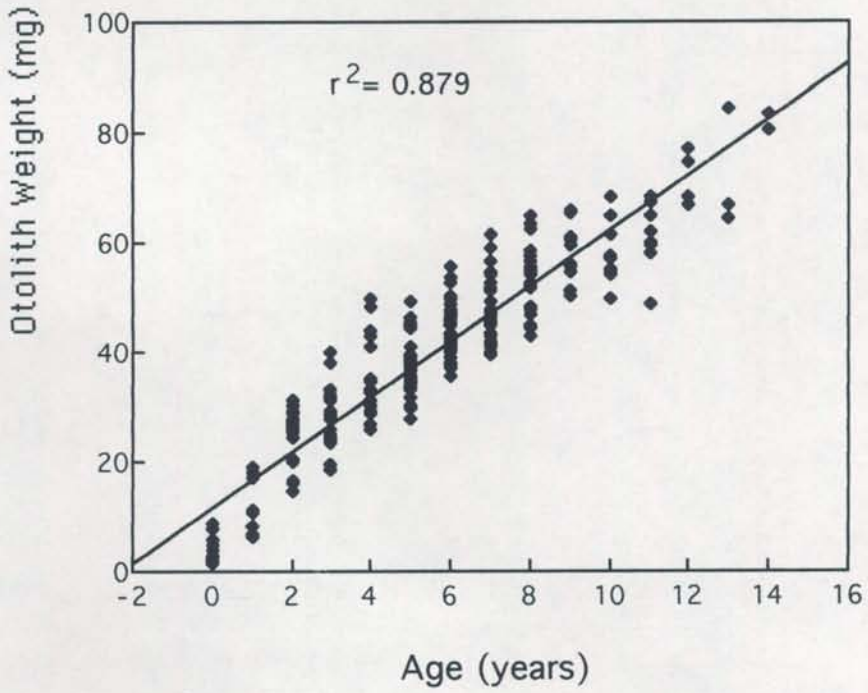


Figure 4.3 : Relationship between otolith weight and Fork Length (FL) and otolith weight and age for *P. leopardus* .

The weight of the otolith was a good predictor of age, and accounted alone for 89% of the variability in age of the coral trout (Table 4.2), with Fork Length accounting for 1.5% after that. Otolith weight was a function of age and fish size, as indicated by the results of the multiple regression fitting. The interaction between fish age and fish size (AGE-FL) accounted alone for 89% of the variability.

TABLE 4.2: Regression coefficients and statistics of multiple regression models [a] on age and otolith weight for coral trout.

Variable	Coefficient	S E	P	Partial r^2
MODEL 1 (n=262)				
Dependent: AGE				
Intercept	0.295			
OTOLITH WEIGHT	0.023	0.001	<0.0001	0.889
FORK LENGTH	-0.094	0.014	<0.0001	0.015
MODEL 2 (n=262)				
Dependent: OW				
Intercept	-38.80			
AGE-FL	0.536	0.021	<0.0001	0.892
FL	6.943	0.317	<0.0001	0.067

[a] Independent variables: **Model 1**: otolith weight (OW), fork length (FL), total weight (TW) and interaction factors OW-FL, OW-TW. **Model 2**: AGE, FL, TW and interaction factors AGE-FL and AGE-TW.

Validation of annulus formation

All fish treated with tetracycline displayed clear fluorescent marks in their otoliths (Fig. 4.4). The results obtained for recaptured and captive fish, ranging from 1 to 8 years old, indicated that the annuli are formed once per year (Fig. 4.5). The first annulus is formed in the otoliths of the juvenile coral trout during their first year of life (Fig. 4.6).

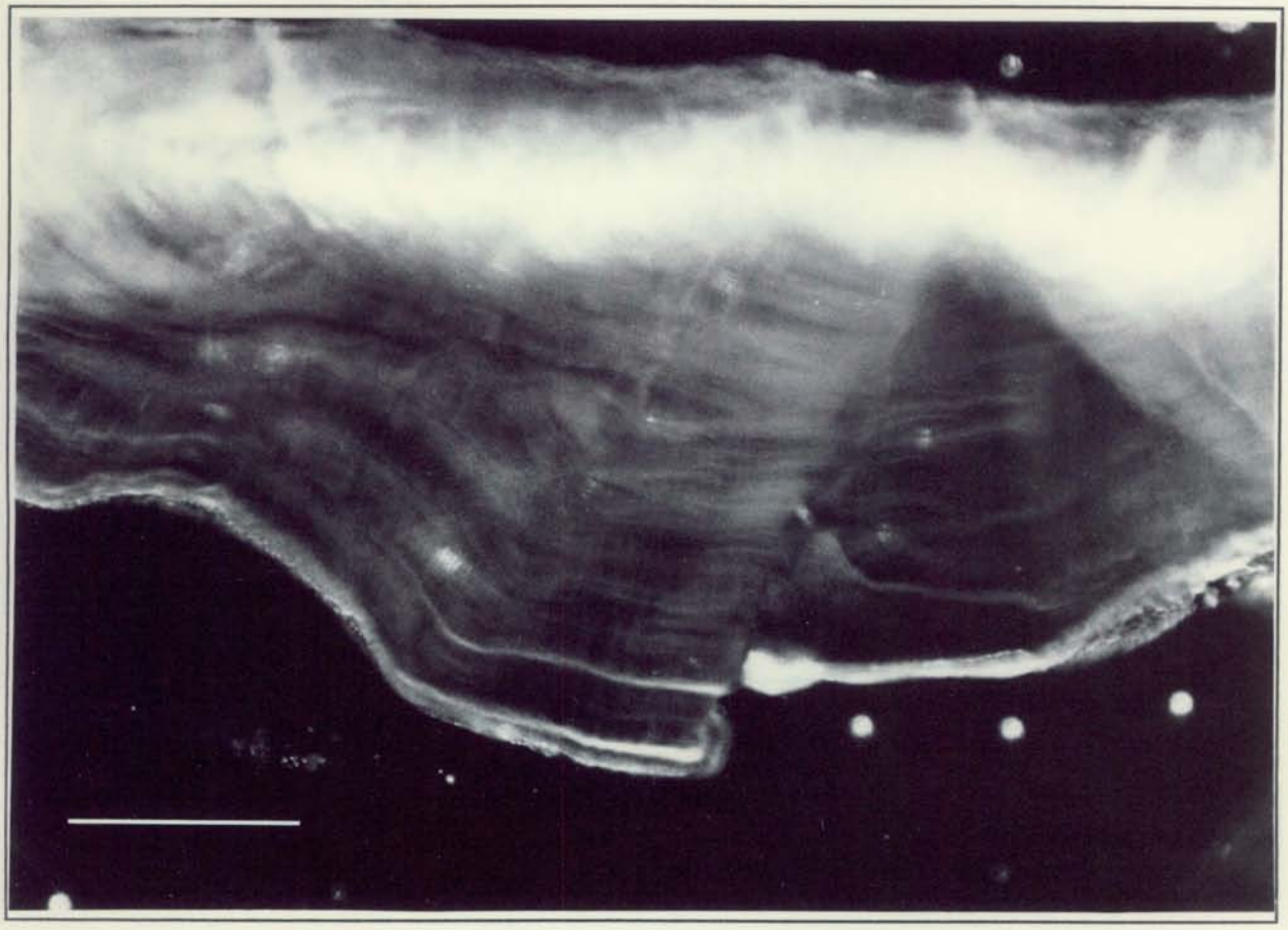


Figure 4.4: Sectioned otolith of an individual of *P. leopardus* (nº 1752) injected in March/ 90, recaptured and reinjected in March/ 91, died in July/ 91. Tetracycline bands positioned in translucent zones, with one opaque zone (ring) between. Scale Bar = 0.25 mm

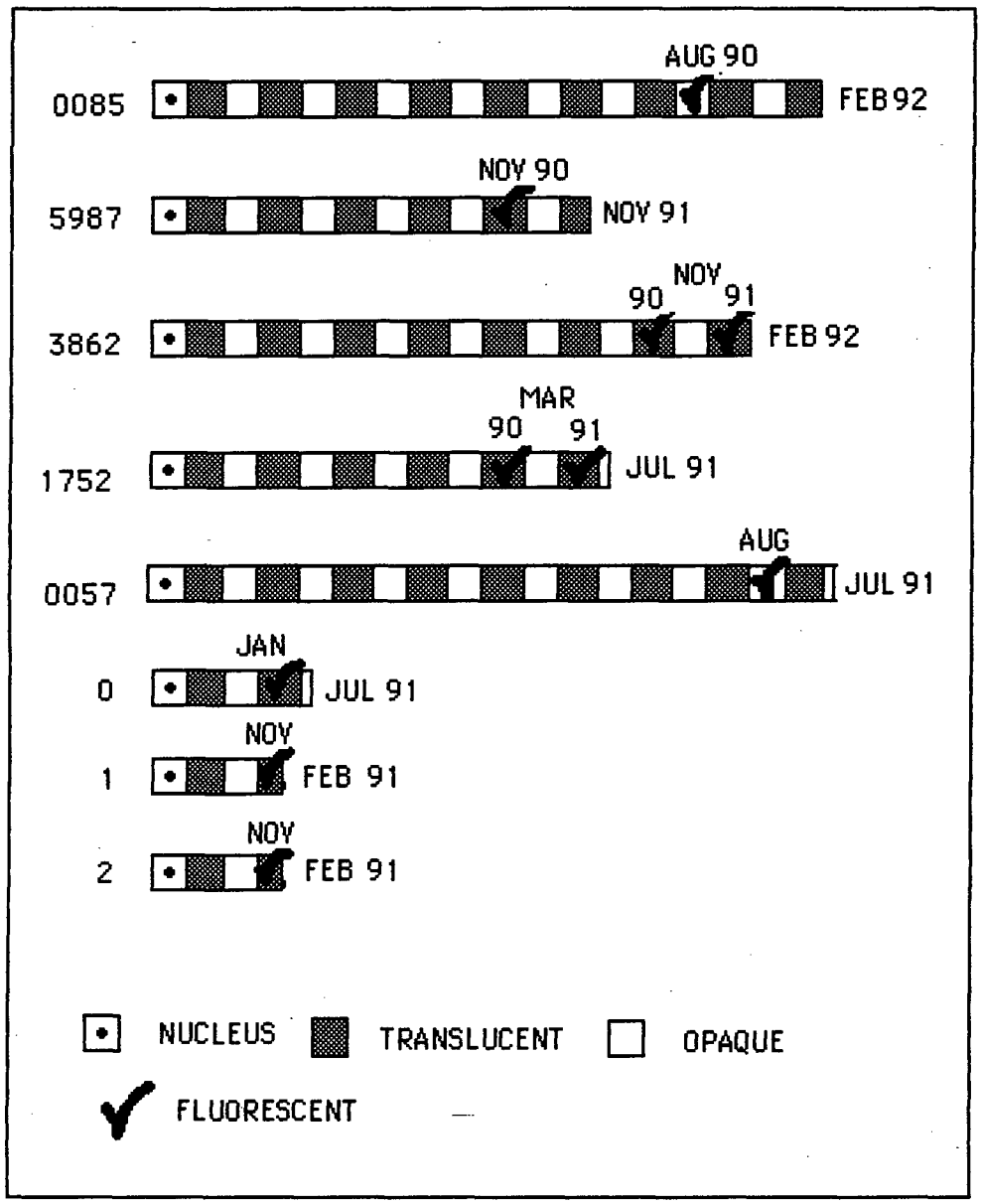


Figure 4.5: Diagrammatic representation of otoliths of mark-released-recaptured coral trout and young-of-the year coral trout treated with tetracycline showing the positions of the fluorescent bands in the otoliths. Bars represent the whole radius of the otolith section, measured from the nucleus to the proximal surface of the sagitta along the ventral margin of the sulcus acousticus. The number of opaque zones correspond to age for each fish. The dates on the top of the bars indicate time of tetracycline treatment and the dates on the end of the bars indicate time of death.

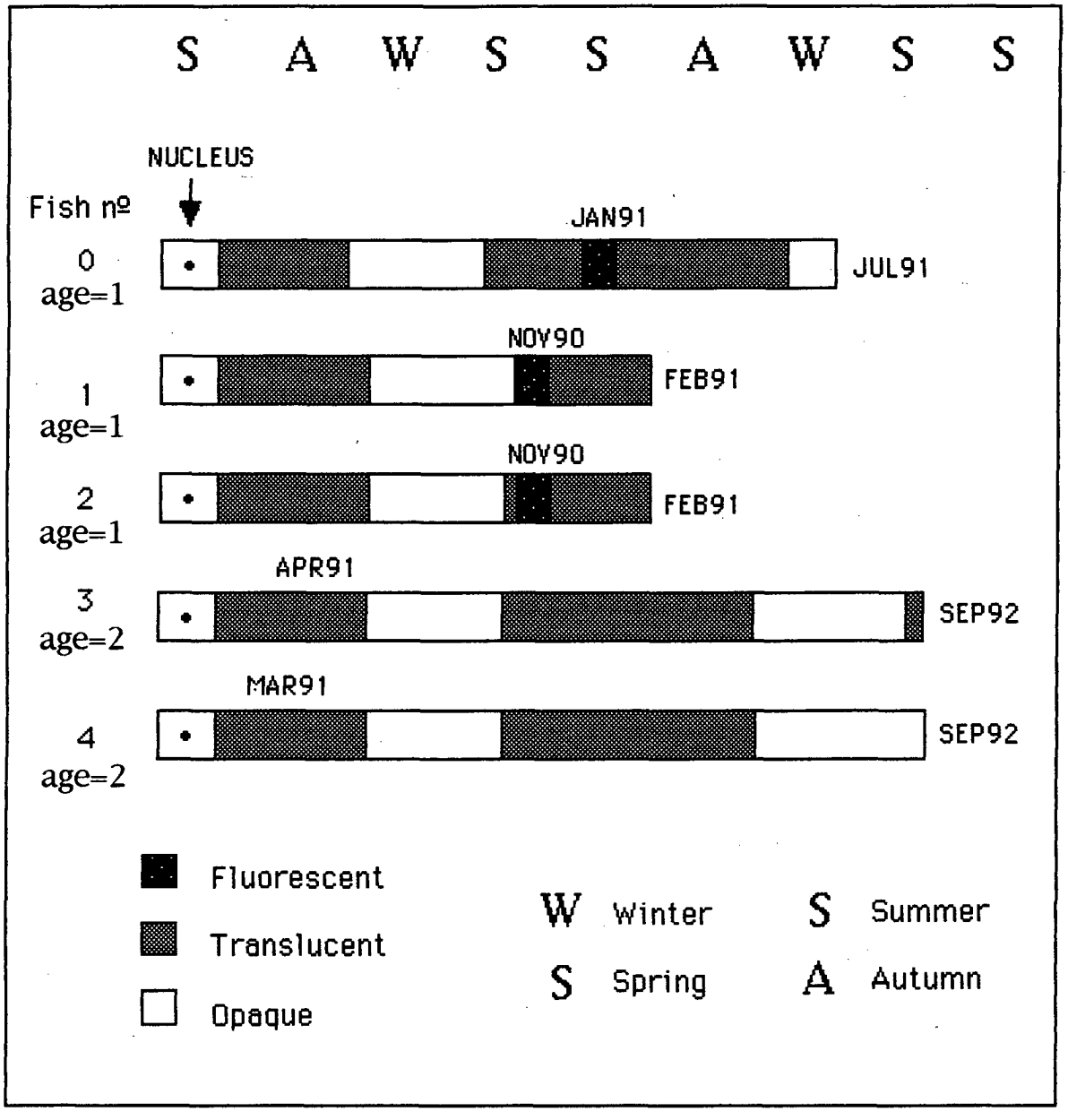


Figure 4. 6: Diagrammatic representation of otoliths of young-of-the year *P. leopardus* kept in captivity, showing relative positions of the fluorescent bands, otolith margin and translucent and opaque zones. Bars represent the whole radius of the otolith section, measured from the nucleus to the proximal surface of the sagitta along the ventral margin of the sulcus acousticus. The dates on the top of the bars indicate time of tetracycline treatment or capture and the dates on the end of the bars indicate time of death.

The relative positions of the fluorescent bands in relation to the otolith margin and the translucent and opaque zones (annuli) indicated that the formation of the annulus occurred mainly during Winter and early Spring (Figs. 4.6 and 4.7).

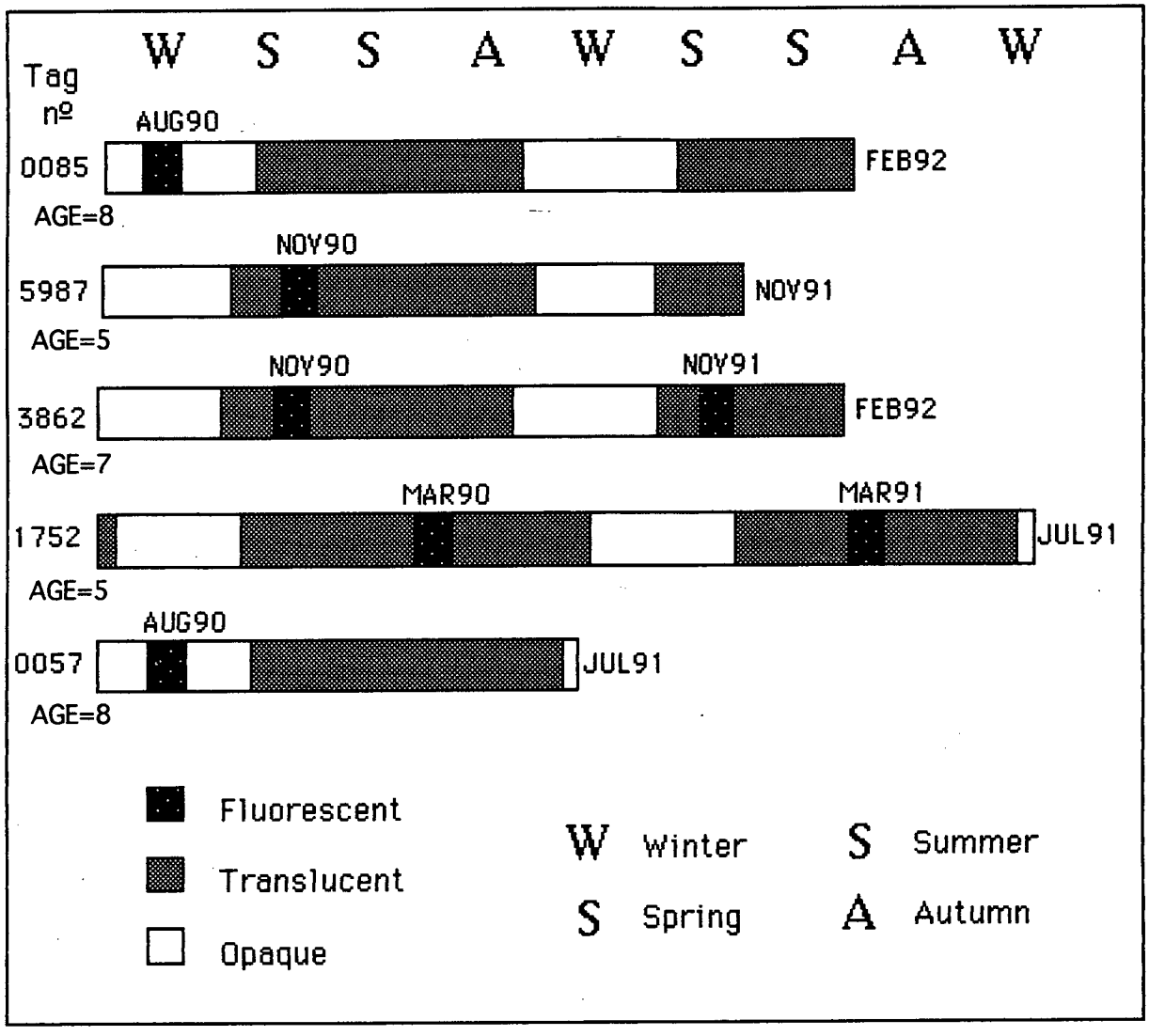


Fig. 4.7: Diagrammatic representation of otoliths of mark-released-recaptured *P. leopardus* treated with tetracycline showing relative positions of the fluorescent bands, otolith margin and translucent and opaque zones. Bars represent only the distal part of the radius of the otolith section, measured from the nucleus to the proximal surface of the sagitta along the ventral margin of the sulcus acousticus The dates on the top of the bars indicate time of tetracycline treatment and the dates on the end of the bars indicate time of recapture.

Growth model

The samples obtained from line-fishing and spear-fishing were selective towards individuals larger than 25 cm FL. Consequently, the 0+ age class was not represented in this sample and the 1 year class was represented only by 4 individuals (Fig. 4.8). The collection of fish using fence-nets was conducted to collect individuals from the smaller size classes. This sample collected by use of fence-nets was composed totally of individuals of the 0+ and 1+ year classes (Fig. 4.8).

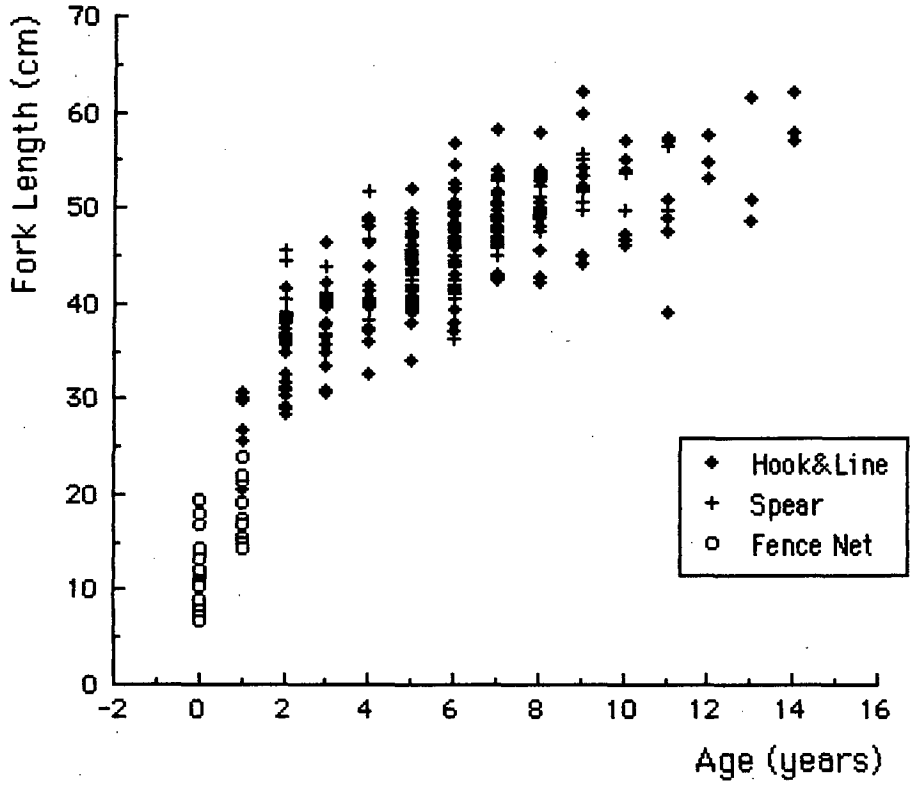


Figure 4. 8: Length-at-age data for *P. leopardus* from Lizard Island captured by each sampling gear used in this study.

Table 4.3 lists the results obtained when fitting the growth model to the data including all age classes and to the data including only ages≥2+.

TABLE 4.3: Von Bertalanffy growth parameters and respective standard errors (se), correlation coefficients (r^2) and degrees of freedom (df) for the growth curve fitted to all data and to the data for fish ≥2 year-old only.

	L_∞ (se)	K (se)	t_o (se)	r^2	df
V.B. all ages	52.20 (0.768)	0.354 (0.024)	-0.766 (0.097)	0.895	310
V.B. age ≥2+	61.29 (3.483)	0.132 (0.030)	-4.660 (1.024)	0.622	272

When fitting Schnute's model to both sets of data, the value of the parameter b obtained was very close to 1. In the boundary where b=1 the curve is reduced to a three parameter model that corresponds to the von Bertalanffy curve for length (Schnute, 1981). The resulting growth model for all age classes, in the form of a von Bertalanffy model, was:
$L_t = 52.2(1 - e^{-0.354(t+0.766)})$ $r^2=0.895$ (Fig. 4.9).

The results obtained when fitting the growth curve to all data and to the data for fish ≥2+ year-old only were quite different (Table 4.3). From age 2 onwards, the growth rate is much slower than the one estimated using all age classes, as indicated by the growth coefficient K. Consequently the estimated L_∞ is a larger size and the estimated t_o is a very large, negative value.

The resulting growth model was:

$Lt = 61.29(1 - e^{-0.132(t+4.66)})$ $r^2=0.622$ (Fig. 4.10).

No systematic trend in the residuals was observed (normality test $p>0.1$) (Figs. 4.9 and 4.10).

The relationship between the Fork length FL and the standard length SL was

$SL = -0.308 + 0.852 \times FL$, $r^2 = 0.994$,

and the relationship between the Fork length FL and the Total Weight TW was

$TW = 0.0031 \times FL^{3.416}$, $r^2 = 0.892$

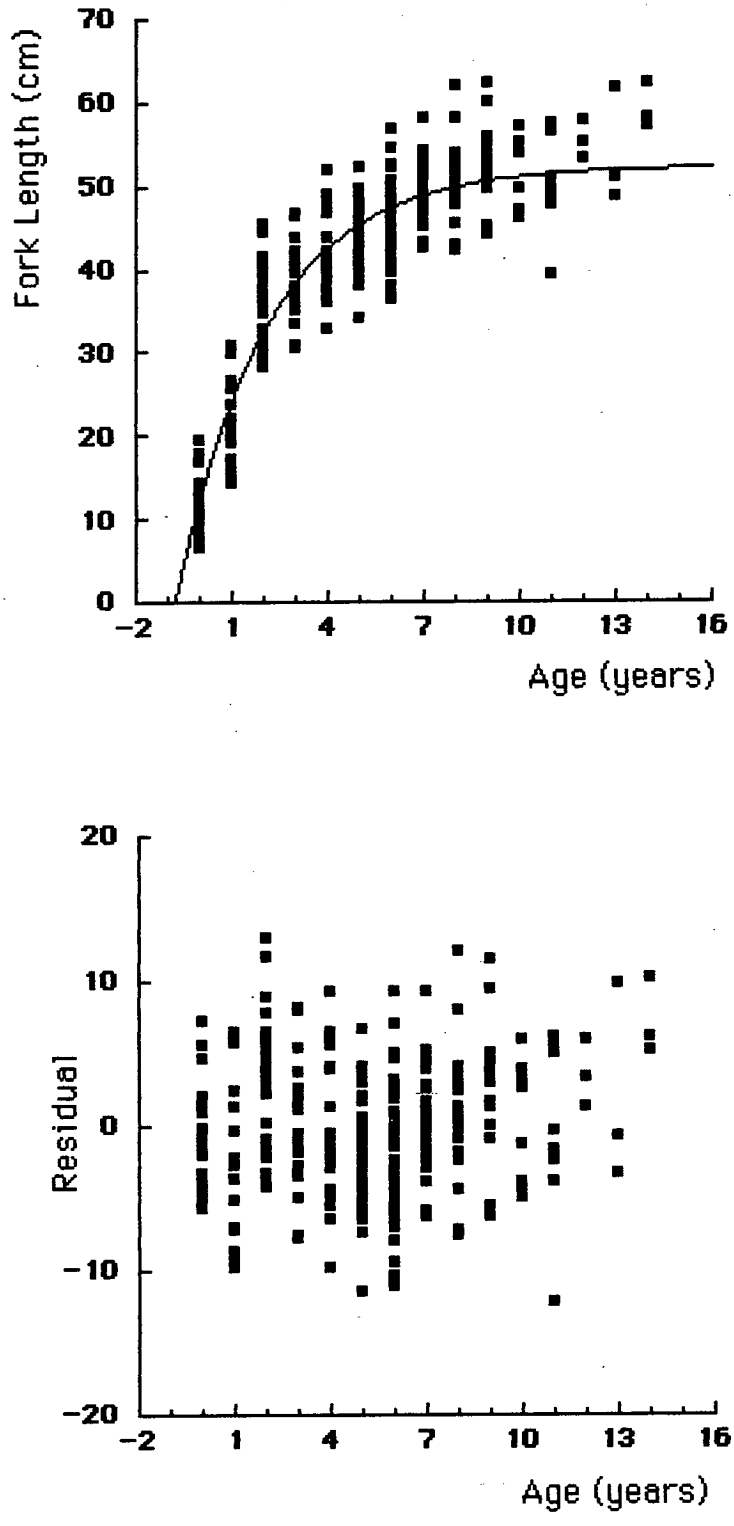


Figure 4. 9: Von Bertalanffy growth curve fitted to length-at-age data of all age classes of *P. leopardus* and plot of residuals.

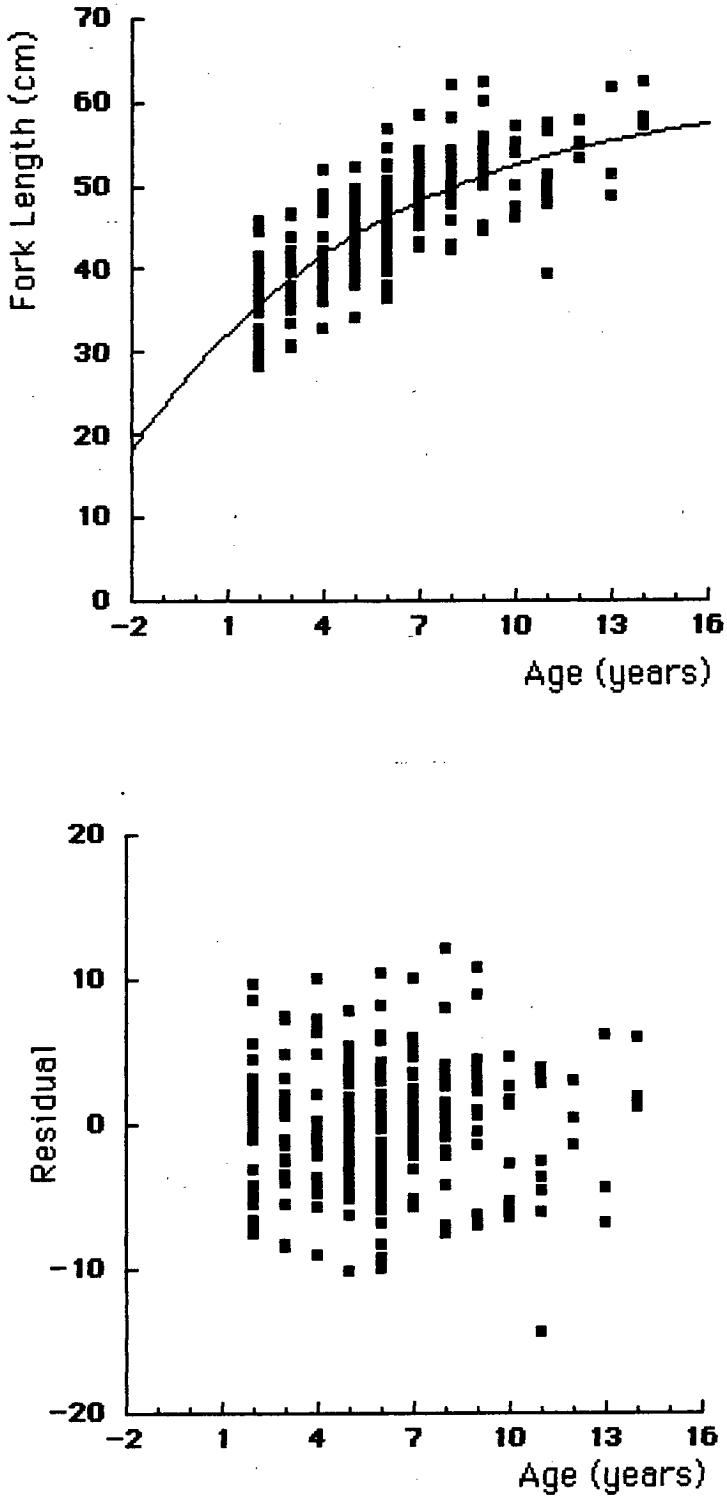


Figure 4. 10: Von Bertalanffy growth curve fitted to length-at-age data of ≥ 2+ years old *P. leopardus* and plot of residuals.

4.4 - Discussion

While some comparisons between readings of whole and sectioned otoliths have indicated good agreement (Boehlert, 1985; Maceina and Betsill, 1987) others have suggested that whole otoliths give underestimates of true age and that this problem becomes worse with fish age (Boehlert, 1985; Hoyer et al., 1985). This is due mainly to the fact that sagitta growth is asymmetrical in many species (Irie, 1960). Growth appears to be linear only up to a certain age or size, after which additions occur mainly on the interior proximal surface, along the sulcus region (Boehlert, 1985; Brothers, 1980, 1987; Beamish and McFarlane, 1987). This seemed to be the case for the coral trout, as comparison of results of whole and sectioned otoliths indicated that lateral views did not reveal many of the outer annual growth zones in older individuals. However, whole otoliths require much less time for analysis than sectioned ones, and seem to provide more precise readings. Therefore, it is useful to determine the limit of reliability of whole readings and thus incorporate the two techniques, as in this study.

Like the inshore coral trout *Plectropomus maculatus* (Chapter II) the common coral trout *P. leopardus* is a relatively long-lived, slow-growing species. The results obtained here differ somewhat from those of previous studies. Goeden (1978), using the Petersen method, identified assumed age cohorts up to age 5+ for *P. leopardus*. However, the limitations of the use of length-frequency data to estimate age of long-lived fish are well known (Manooch, 1987; Ferreira and Vooren, 1991). McPherson et al. (1988), using counts of annuli in whole otoliths, were able to age fish up to 7 years old. The longevity was probably

underestimated in their study as countings were performed only on whole otoliths. More recently, Brown et al. (1992) analysed whole and sectioned otoliths of coral trout from the same area as McPherson et al. (1988) and were able to count up to 14 rings. Loubens (1980) counted annuli from burnt and broken otoliths and estimated a maximum longevity for *P. leopardus* of 19 years in New Caledonia. These higher estimates of longevity suggest that coral trout at Lizard Island could also attain older ages. In this case, the absence of older fishes in the present sample could be related to fishing pressure.

The growth of the otolith was continuous with age but was related also to somatic growth. A similar pattern has been observed for other species of fish (Beckman et al., 1991). Otolith weight was a not a better predictor of age than length as both accounted separately for the same percentage of variability in age (89%).

Optically different zones in calcified structures are the result of changes in the relative amounts of both organic and inorganic material in the calcified tissue (Casselman, 1974). Translucent zones in otoliths contain relatively more calcium while opaque zones contain more protein (Irie, 1960; Mugyia, 1984; Casselman, 1974). Though the physiological basis for the formation of optically different zones in calcified structures has not been directly established, their presence has been commonly associated with varying growth rates, influenced by temperature, photoperiod, feeding rate or the reproductive cycle (see Casselman, 1983 and Longhurst and Pauly, 1987 for review). Mosegaard et al. (1988) examined the effect of temperature, fish size and somatic growth rate on otolith growth rate, and suggested that

metabolic activity, not necessarily somatic growth rate, governs otolith growth.

In the otoliths of *P. leopardus*, the opaque zone (annulus) was formed during the winter and spring months while the translucent zone was formed during summer and autumn. The formation of the opaque zone has been associated with slower somatic growth for some species (Irie, 1960; Mugyia, 1984) and to faster somatic growth for others (Pannella, 1974; Victor and Brothers, 1982). On a daily basis, it has been demonstrated that the translucent zone, or accretion zone, is formed during the phase of more active otolith growth, and the opaque or discontinuous zone is formed during growth stagnation (Watabe et al., 1982; Mugyia et al., 1981). Thus, if the formation of the opaque zone in the coral trout otoliths is associated with a period of reduced metabolic activity, an external determining factor could be temperature, as the lowest values for water temperature around Lizard Island are observed during Winter and early Spring (Lizard Island Research Station unpubl. data). Annulus formation occurred in otoliths of juveniles and adults of coral trout during the same period, suggesting that reproduction is not a determining factor. The presence of annuli in otoliths of juvenile fish seems to be common for many species of fishes (Beckman et al., 1991; Ferreira and Russ, 1992)

Since the otolith is an acellular product, its growth might be under different physiological controls than somatic growth (Casselman, 1990). This would explain the formation in otoliths of an opaque, protein dominant region during slower growth, while the opposite, i.e., a calcium-dominant region, is formed during slower growth in other

calcified structures of fish, like the cleithrum and vertebrae (Casselman, 1974, 1990; Ferreira and Vooren, 1991).

The main criteria for choosing a growth curve are quality of fit and convenience, differing according to whether the need is for a mathematical description of a detailed physiological growth process or for fishery stock assessment and management (Moreau, 1987). The results obtained here indicated clearly that the von Bertalanffy model adequately described the growth of the coral trout. Schnute's model was useful due to its flexibility and the stability of its parameters.

As most fishing gears are selective towards a certain size (Ricker, 1969), and smaller sizes are not usually available, it is common that growth curves are fitted to truncated data representing only part of the population. For the coral trout, because of gear selectivity and legal size-restrictions (legal minimum= 35 cm TL), only fish of 2+ years were captured commonly by line and spearfishing. However, these first three years of life represent the period of fastest growth, after which the growth pattern changes considerably. As a result, a dramatic change in the estimates of the growth parameters was obtained when the growth curve was fitted only to the age classes recruited to the fishery. The VB parameters K and L_∞ have an inverse relationship over a given longevity (Knight, 1968), so a reduced estimate of K results in a higher estimate of L_∞. This was accompanied by a direct relationship between K and the third parameter t_0, which took a negative and large value. The effects of different age ranges on estimated von Bertalanffy growth parameters have been recognized for many years (Knight 1968, Hirschhorn 1974) and greatly compromise comparisons of growth rates between populations (Mulligan and Leaman 1992).

Furthermore, one effect of size-dependent mortality is the selective removal of fast-growing individuals (Ricker, 1969; Miranda et al., 1987). Thus, it is likely that the average size of the youngest age-groups recruited to the fishery will be biased towards the largest, fast-growing individuals. If this is the case for age class 2+ and 3+, for example, the underestimation of K would be enhanced further, as well as overestimation of L_∞ (Mulligan and Leaman, 1992).

Recent research has suggested the possibility of different growth processes within a population with associated selective fishing mortality (Parma and Deriso, 1990) and natural mortality (Leaman and Mulligan, 1992). The large variability in size at a given age observed for the coral trout suggests the occurrence of individual variability in growth. The reliability of methods of growth estimation like length-frequency analysis and growth increments from marking-recapture techniques, is greatly affected by this kind of variation (Sainsbury, 1980), further enhancing the importance of obtaining validated length-at-age estimates for exploited fishes. The results of selective mortality are a direct effect of growth variability on the dynamics of abundance, and failure to consider the effects of different growth potentials can result in gross overestimation of optimal fishing levels (Parma and Deriso, 1990).

The absence of marked seasonal changes at low latitudes lead to the general belief that tropical fishes do not form annual rings in their calcified structures (Panella, 1974). Consequently, most of the studies of age in tropical fishes have concentrated on daily rings. This technique, however, is very time consuming, especially when applied to older ages (see Longhurst and Pauly, 1987 and Beamish and

McFarlane, 1987 for review). However, the presence of annual marks in otoliths has been validated for an increasing number of species of tropical fishes (Samuel et al., 1987; Fowler, 1990; Ferreira and Russ, 1992; Lou, 1992) showing the potential of this technique to be used routinely in tropical fishery stock assessment and management.

CHAPTER V: Reproduction of the common coral trout *Plectropomus leopardus* (Perciformes: Serranidae) from the Central and Northern Great Barrier Reef, Australia.

5.1- Introduction

The common coral trout *Plectropomus leopardus* (Lacepede 1802) is the most commercially exploited species of Serranidae on the Great Barrier Reef, with around 1200 tonnes caught annually by the commercial fishery(Trainor, 1991).

In spite of its importance, the reproductive biology of the common coral trout has been studied only for a population on the Southern Great Barrier Reef (Goeden, 1978). Like all epinepheline serranids that have been studied until now (Shapiro, 1987), the coral trout is a protogynous hermaphrodite (Goeden, 1978). Management of a fishery is considerably complicated by sequential hermaphroditism, as the selective removal of males is likely to occur (Shapiro, 1987; Bannerot et al.,1987). The responses of these populations to fishing pressure are largely unknown. Mechanisms of social induction of sex change have been suggested for epinepheline serranids in favour of genetically programmed mechanisms, in which sex-change would occur at a certain size or age (Shapiro, 1982, 1987). For the coral trout, however, no information on population structure is available at the moment to answer these questions.

In the present work, the reproductive biology of *P. leopardus* was studied from data collected in two areas of the Great Barrier Reef. Gonads were analysed to determine the mode of reproduction and spawning season. The sex, size and age structure of these populations were analysed in order to determine the age and size of first reproduction, sex-ratio and the range of occurrence of sex-change.

5.2 - Materials and Methods

Samples were collected from two geographically distinct sites; mid shelf reefs off Townsville (Lat.18° to 19° 30' S, Long.146° to 147° E) and mid shelf reefs and waters adjacent to Lizard Island, Northern Great Barrier Reef (Lat. 14° 40' S, Long. 154° 28' E), from 1990 to 1992 (Table 5.1). Fishes were captured using spear-fishing or line-fishing during collecting trips. Additional samples in the form of carcasses kept frozen after filleting, were obtained from local commercial and recreational fishermen.

TABLE 5.1: Number of fish collected per month from Townsville and Lizard Island reefs from 1990 to 1992

	TOWNSVILLE			LIZARD		
MONTH	1990	1991	1992	1990	1991	1992
JAN	-	-	-	-	-	10
FEB	-	-	-	-	8	14
MAR	-	6	-	5	4	-
APR	9	2	-	-	20	-
MAY	3	2	-	-	-	-
JUN	-	25	-	-	-	-
JUL	21	10	-	5	61	-
AUG	5	-	35	36	5	-
SEP	-	10	-	-	2	-
OCT	18	28	83	-	20	-
NOV	16	-	-	6	161	-
DEC	-	-	-	-	73	-
TOTAL	60	83	118	52	354	24

The Fork length (FL) and Standard length (SL) of the fish were measured in centimetres. The gonads were removed, weighed and staged macroscopically. Sex determination could be done macroscopically only if gonads were active. In this case, individuals were classified as ripe females or males and the information used to determine periodicity of spawning. The gonadosomatic index (GSI) was calculated as the ratio of gonad fresh weight to total weight of the fish. As total weight was not available for the commercial samples, estimated values were obtained through the relationship between Fork length and Total weight (Ferreira and Russ, 1992).

The amount of fat deposited in the mesenteries was estimated for the Lizard Island sample following a relative scale from 0 to 1, with 6 categories which indicated the proportion of fat covering the viscera (0=no visible fat; 0.2= thin threads of fat; 0.4; 0.6; 0.8 increasing amounts of fat, and 1.0= fat completely covering the viscera). This scale was chosen after observing the seasonal variation in the relative amounts of mesenteric fat for a year, and estimations were always made by the same observer.

Gonads from 230 fish from Lizard Island and 131 from Townsville were preserved in F.A.A.C (Formaldehyde 4%, Acetic Acid 5%, Calcium chloride 1.3%) for later sectioning (L. Winsor, pers. comm.). Middle portions of the two gonadal lobes were embedded in paraffin and sectioned transversely at 5 microns thickness and stained with Mayer's haematoxylin-eosin.

The ages of fish examined histologically were determined from otoliths as described in Chapter IV.

Gonadal stages:

The nomenclature for description of the stages of oogenesis and spermiogenesis followed Yamamoto *et al.* (1965). Classification of males and females into ontogenetic stages (stagium) and developmental stages (stadium) followed the adaptation by Ferreira (1993) from Moe (1969). Developmental stages of ovaries were determined according to the most advanced oocyte stage present in the gonad (Ebisawa, 1990) (see Appendix II for oocyte stages).

Immature female: ovaries that showed no evidence of prior spawning. The ovary is small in diameter and encased by a relatively thick gonadal wall. The lamellae is well packed and filled with previtellogenic oocytes in early and late perinucleolus stages. Gonia and chromatin nucleus stage oocytes are abundant .

Mature female:

-*Resting*: The ovary is larger in diameter than those of immature females and encased by a thinner, more distended gonadal wall. The lamellae is filled with previtellogenic oocytes in early and late perinucleolus stages. Gonia and chromatin nucleus stage oocytes are present but not as abundant as observed in immature females. The presence of yellow-brown bodies is common.

-*Ripening*: oocytes in early stages of vitellogenesis, from yolk vesicle stage to primary yolk globule stage.

-*Ripe*: oocytes in late stages of vitellogenesis from tertiary yolk globule stages to hydrating stages.

-*Spent*: lamellae disrupted and disorganised, with extensive vascularization. Vitellogenic oocytes in atresia. Follicular cells, remnants

of post-ovulatory follicles, present throughout the gonad. Proliferation of oogonium and chromatin nucleus stage oocytes.

Transitional: transitional individuals were defined as having gonads that showed proliferating testicular tissue in the presence of degenerating ovarian tissue, but in which sex-transition had not yet proceeded to the point at which the dorsal sperm sinuses were formed and filled with spermatozoa (Hastings 1981).

Young male: post-transitional, newly transformed testis. Ovarian tissue dominating the lamellae that had not assumed the typical lobular form of the mature testes. Sperm crypts occur in all stages of development. Dorsal sperm sinuses are formed and filled with spermatozoa.

Mature male:

-*Resting*: testis dominated by stromal tissue and early stages of spermatogenesis (spermatogonia and primary spermatocytes).

-*Ripening*: later stages of spermatogenesis (secondary spermatocytes and spermatides) and spermiogenesis; spermatozoa starting to fill the dorsal sinus.

-*Ripe*: testis dominated by spermiogenesis. Most crypts containing spermatids and spermatozoa. Crypts of spermatozoa ruptured and joined within the testicular lobules, forming large intralobular or "central" sperm sinuses. Dorsal sinuses filled with spermatozoa.

-*Spent*: active development of crypts of spermatogonia and primary spermatocytes throughout the testis. Stromal tissue well developed between crypts.

To estimate the percentage of remaining female tissue in transitional and male gonads, the whole gonad was observed under low magnification

(40x), the percentage area occupied by oocytes estimated twice independently, and the results averaged.

Size and Age structure:

Only individuals of age ≥2 years old were included in the comparisons between Lizard and Townsville, as younger individuals were only represented in the Lizard Island sample where fishing gear other than hook and line was employed (see Chapter IV).

The size and age range in which individuals changed sex was estimated from the zone in which size and age distributions of females overlapped with size and age distributions of transitional, young or mature males. The range of overlap was calculated as a percentage of the total range of sizes and ages observed. To compare if sex-change occurred at the same time for the two locations, the mean size and age of individuals within the overlap range were compared using analysis of variance (Shapiro, 1984).

For the calculation of sex ratio, only reproductively active, i.e., mature individuals, were included. The age and size of first spawning for females was determined as the age or size class in which 50% of females were mature.

Statistics:

One, two and three-way analyses of variance including post-hoc tests (Tukey-Kramer) were used for comparisons. The assumptions of normality and homoscedascity were examined and data were transformed if needed

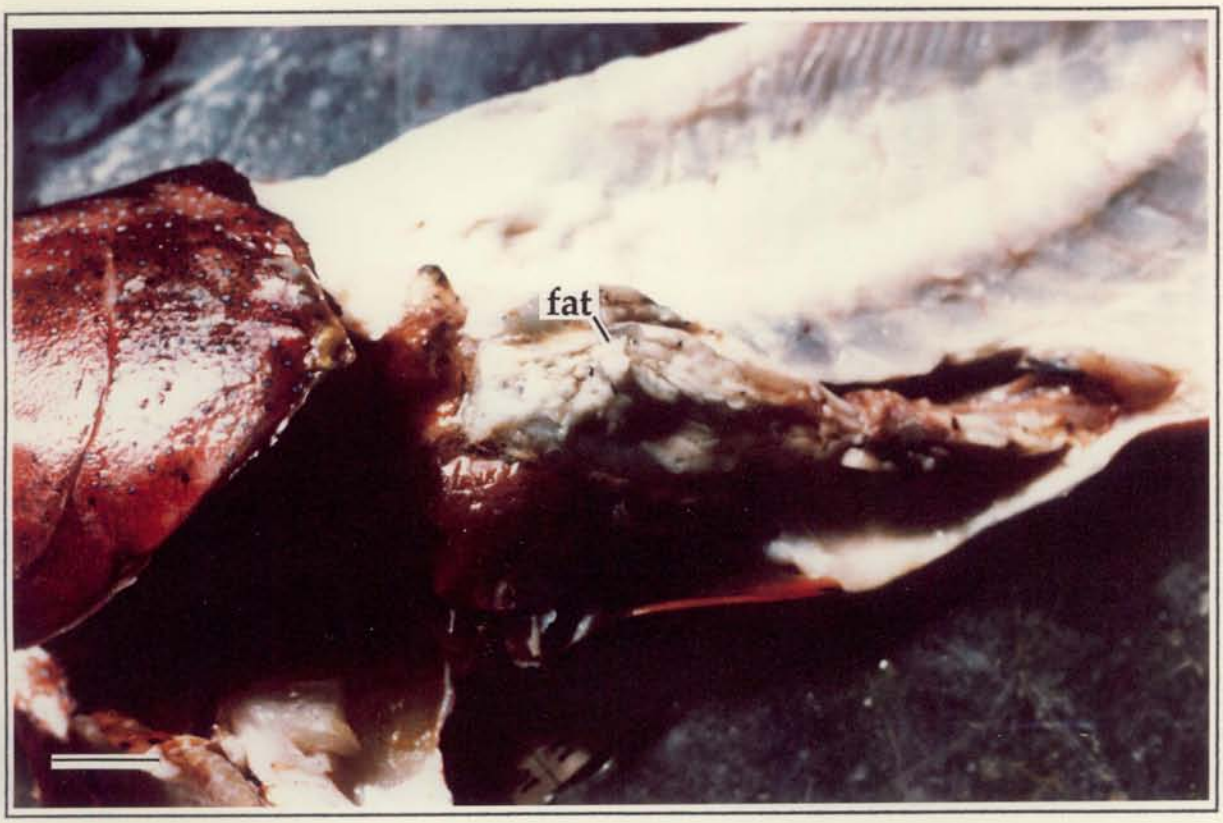
(transformed data indicated in tables). Level of significance used was $p<0.05$. Spearman-Rank correlation was used to analyse the relationship between gonad weight and fat (Lizard Island data only) and between gonad weight and age and size of fish. A Chi-square test was used to compare sex-ratios of samples collected with line-fishing and spear-fishing. The overlap of distributions of size and age of males and females were compared by t-test.

4.3 - RESULTS

Reproductive biology

The gonads of male and female *P. leopardus* are composed of two elongated lobes, usually unequal in size which are joined posteriorly into the common duct. The gonad is formed by a germinal epithelium extending from the dorsal and lateral gonad walls into the central lumen. The gonadal wall is formed by smooth muscle and connective tissue covered by a peritoneal layer. The gonad is attached dorsally to a complex net of mesenteries, ligaments, arteries and veins. The left gonadal lobe is connected by these mesenteries to the dorsal body wall and to the right gonadal lobe, which is also attached by the same mesenteries to the intestine and other organs in the coelomic cavity. Deposition of fat occurrs along the mesenteries (Fig. 5.1) in quantities that varied from no visible fat to a thick layer that covered all the viscera. Sex determination could be done macroscopically only if gonads were active. Resting gonads were not very reduced in length but were greatly reduced in diameter. During this stage, the gonads were difficult to distinguish, especially the right lobe which became covered by the viscera.

A



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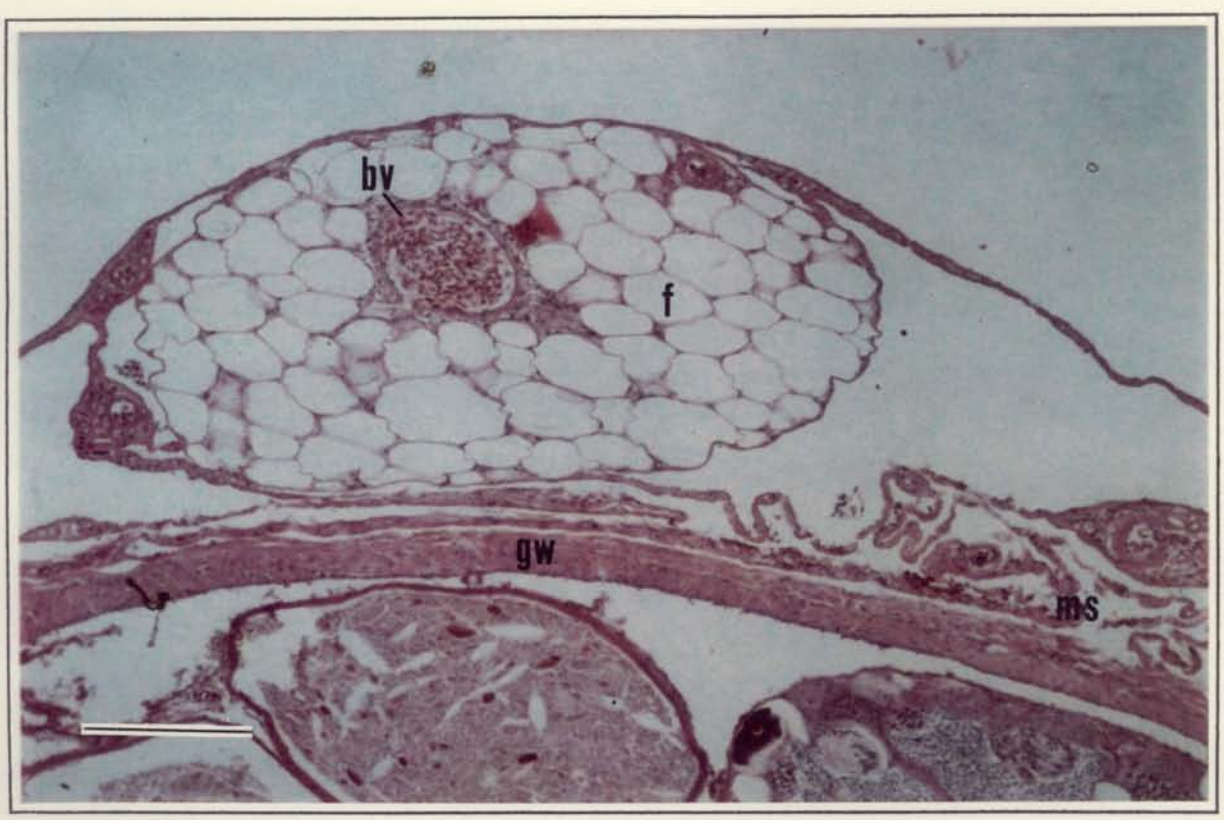
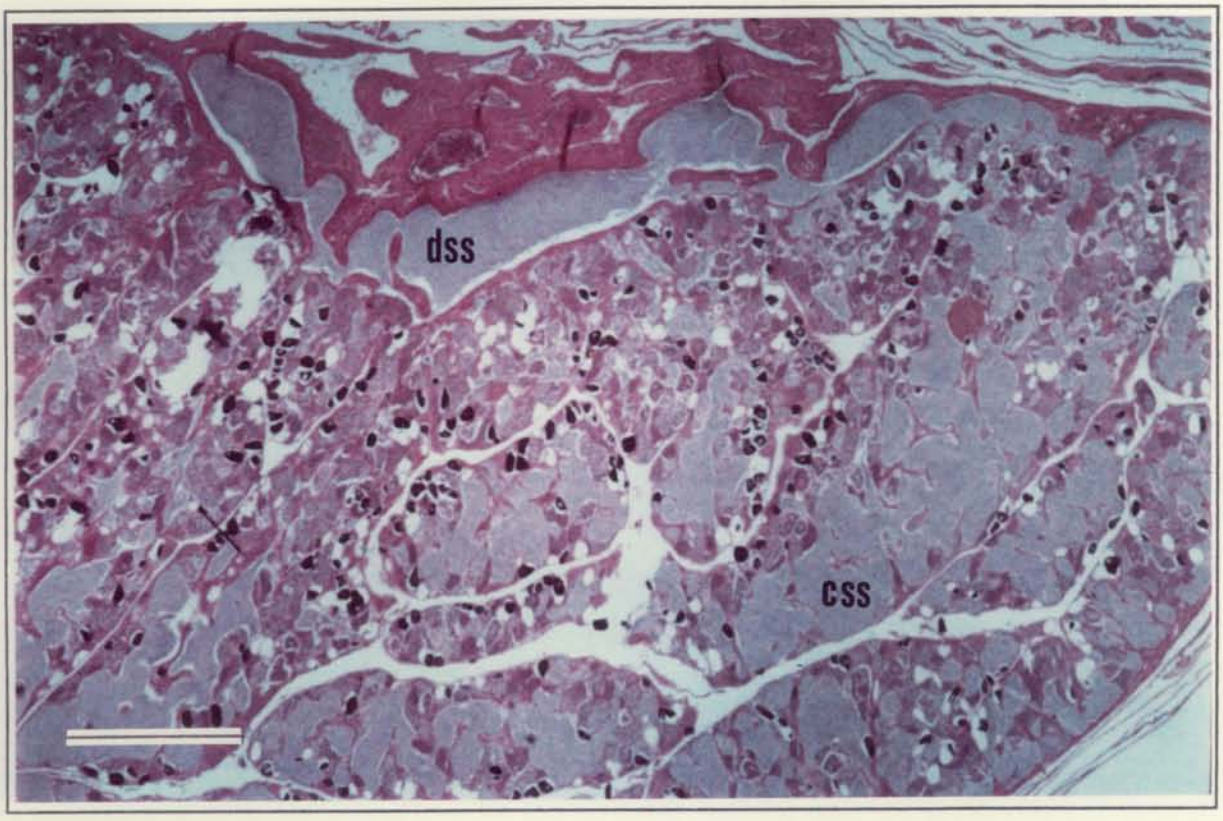


Figure 5.1: **A**- Mesenteric fat deposits partially covering the viscera of a coral trout (fat= 0.6). Scale bar= 2 cm. **B**- Mesenteric fat attached to gonad of male coral trout. Scale bar= 200µ. **gw**= gonad wall; **bv**= blood vessel; **ms**= mesenteries; **f**= fat cell.

A



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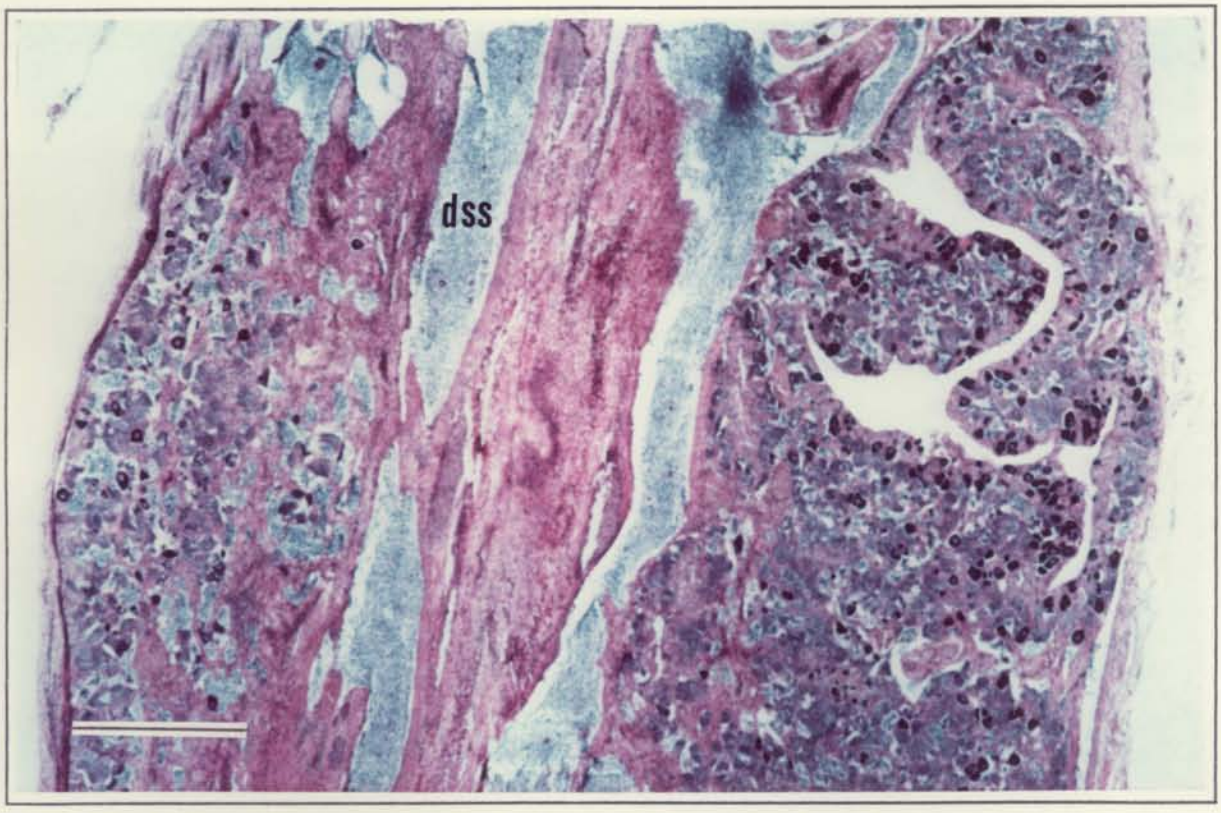


Figure 5.2: **A-** Transverse section of a testis of a ripe mature male coral trout, showing sperm sinuses in the centre of lobes joining dorsal sperm sinuses. Scale bar= 500μ **B-** Longitudinal section of a testis of a ripe mature male. Scale bar= 500μ. **dss**= dorsal sperm sinus; **css**= central sperm sinus.

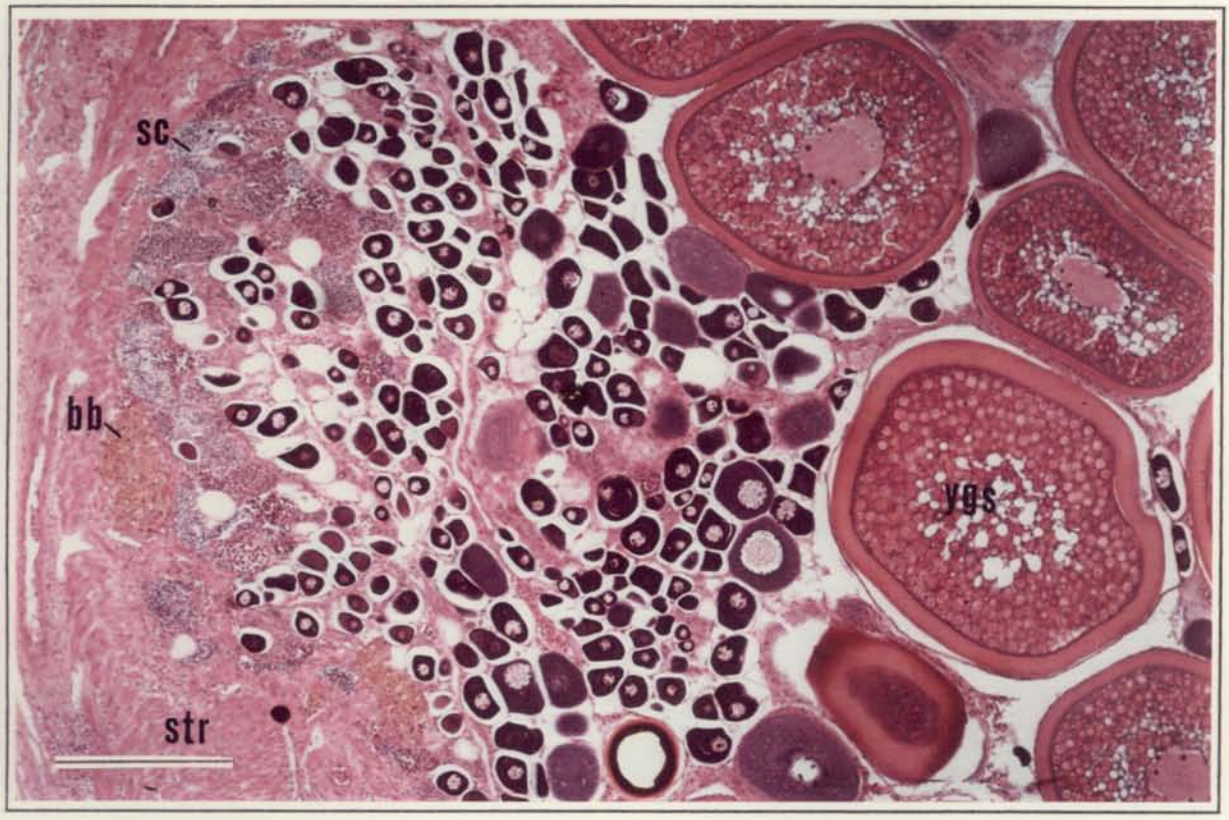
As first described by Goeden (1978), the mode of reproduction of *P. leopardus* is protogynous hermaphroditism. The germinal tissue is of the undelimited or *Epinephelus* type (Smith, 1959), with intermingling of ovarian and testicular tissues. All male gonads examined had a remnant ovarian lumen and sperm sinuses located in the dorsal part of the gonadal wall. The ovarian lumen in males was not used to carry sperm. Sperm is carried in dorsal sinuses, formed by the splitting of muscle layers of the ovarian capsule. Within the testicular lobules, central sinuses are formed by the rupture of crypts of spermatozoa, and join the dorsal sperm sinuses (Fig. 5.2).

Sex transition:

The presence of a few precocious sperm crypts (*sensu* Smith, 1965) was observed in the ovaries of some resting females from the two locations. Transition was clearly indicated only when proliferation of sperm crypts was more advanced and accompanied by fragmentation and reabsorption of previtellogenic oocytes. Transitional gonads (n=22) typically had a large number of germ cells, presumably spermatogonia. Proliferation of sperm crypts was concentrated in the dorsal part of the gonad, apparently in close association with the stromal tissue. Stromal cells were conspicuous during this phase and seemed to be undergoing proliferation.

During the spawning season, sperm crypts were observed in the ovaries of some mature ripe females in the Townsville region (n=5) (Fig. 5.3; A and B are the same individual). These sperm crypts were in all stages of development, from primary spermatocytes to spermatozoa. Vitellogenic oocytes were in the final stages of development, and showed no signs of

A



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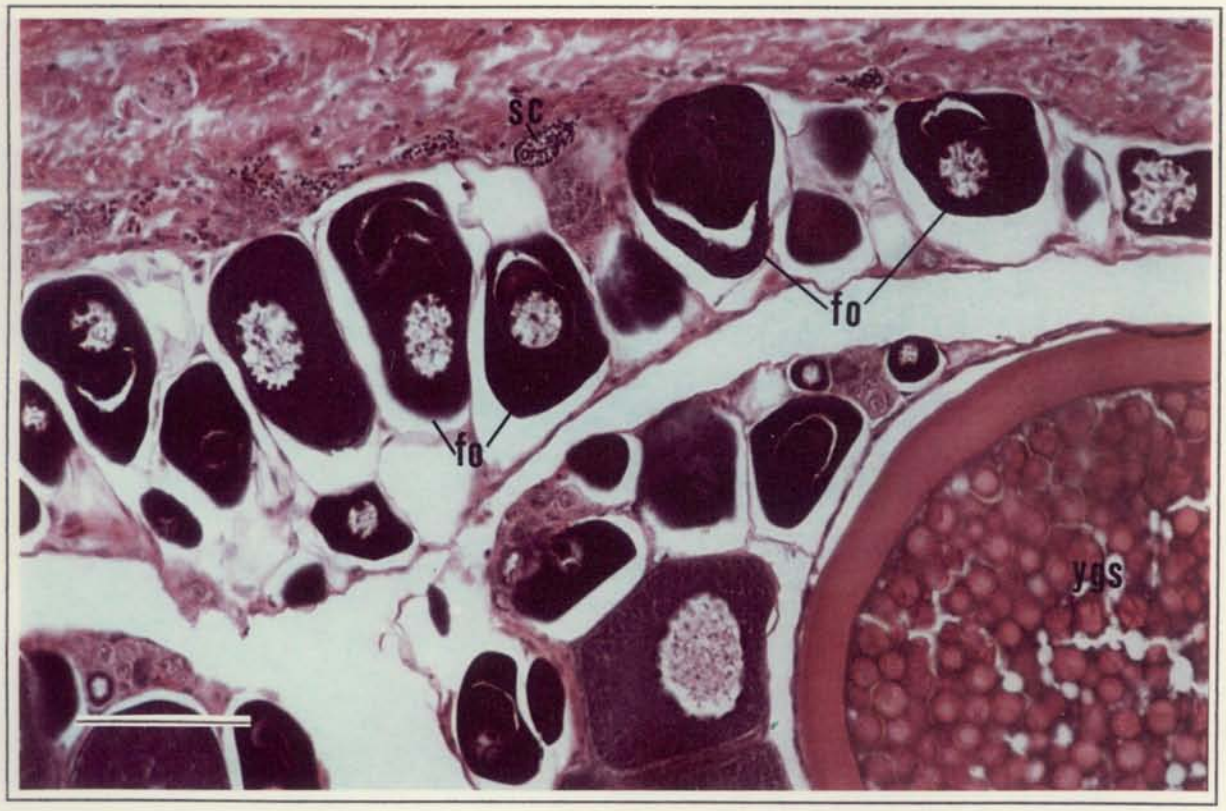


Figure 5.3: **A-** Developing sperm crypts in ovary of ripe female. Scale bar= 200μ **B-** Developing sperm crypts in ovary of ripe female and fragmenting previtellogenic oocytes. Scale bar= 50 μ. **ygs**= yolk globule stage oocyte; **sc**= sperm crypt; **str**= stromal tissue, **bb**= brown-body, **fo**= fragmenting previtellogenic oocytes.

degeneration, though fragmenting previtellogenic oocytes were observed. (Fig. 5.3). No sperm sinuses were formed, indicating that in spite of the presence of spermatozoa, these individuals probably would not spawn simultaneously as males and females, so they were classified as mature females. Developing sperm crypts were observed also in ovaries of females had spawned recently (Fig. 5.4) (n=3). In these gonads, development of sperm crypts occurred simultaneously to reabsorption of vitellogenic oocytes. These individuals were classified as transitionals. In two other individuals the development of sperm tissue was more advanced and the dorsal sperm sinuses were formed, so the individuals were classified as young males (Fig. 5.5). A few atretic yolk oocytes were still present in these gonads suggesting that the transition process had occurred over a short period. Transition was observed also in gonads that were characteristically immature ovaries (Fig. 5.6). In these cases, only previtellogenic oocytes were observed and the proliferating sperm tissue occupied a higher percentage of the gonad.

Proliferation of sperm tissue was accompanied by degeneration and reabsorption of the ovarian tissue. The percentage of ovarian tissue in gonads of transitional, young and mature males was compared by a one-way analyses of variance.

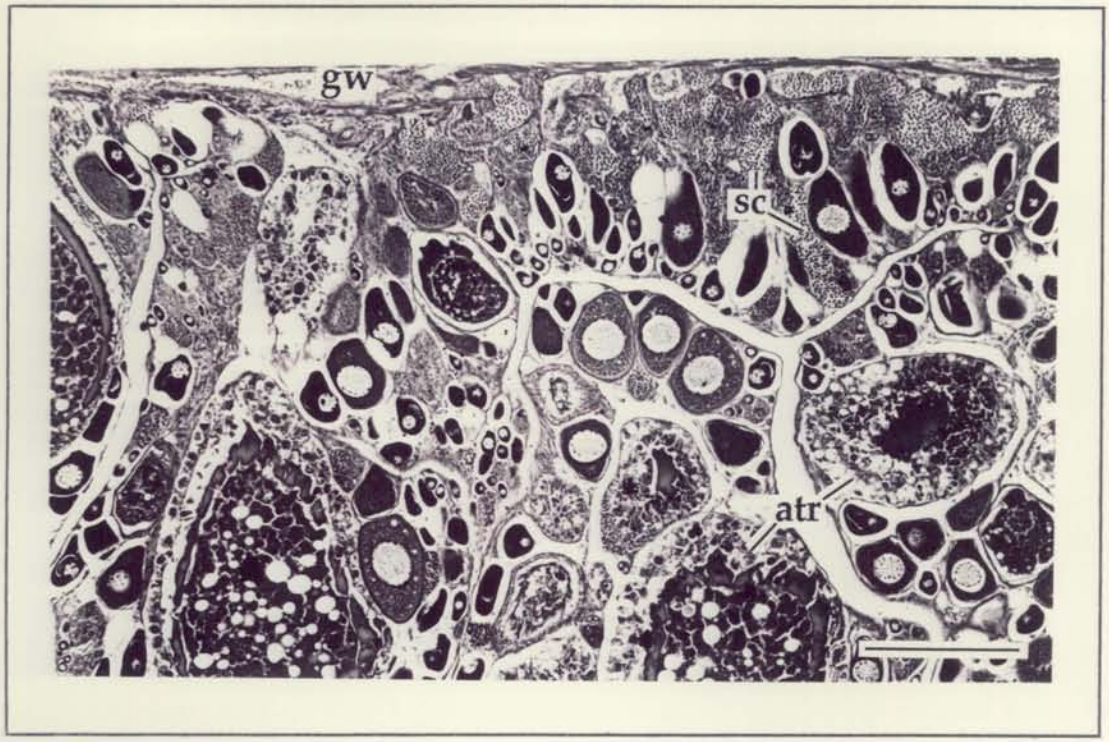


Figure 5.4: Developing sperm crypts in ovary of spent female. Scale bar= 125µ. **gw**= gonadal wall; **atr**= atretic vitellogenic oocytes; **sc**= sperm crypts.

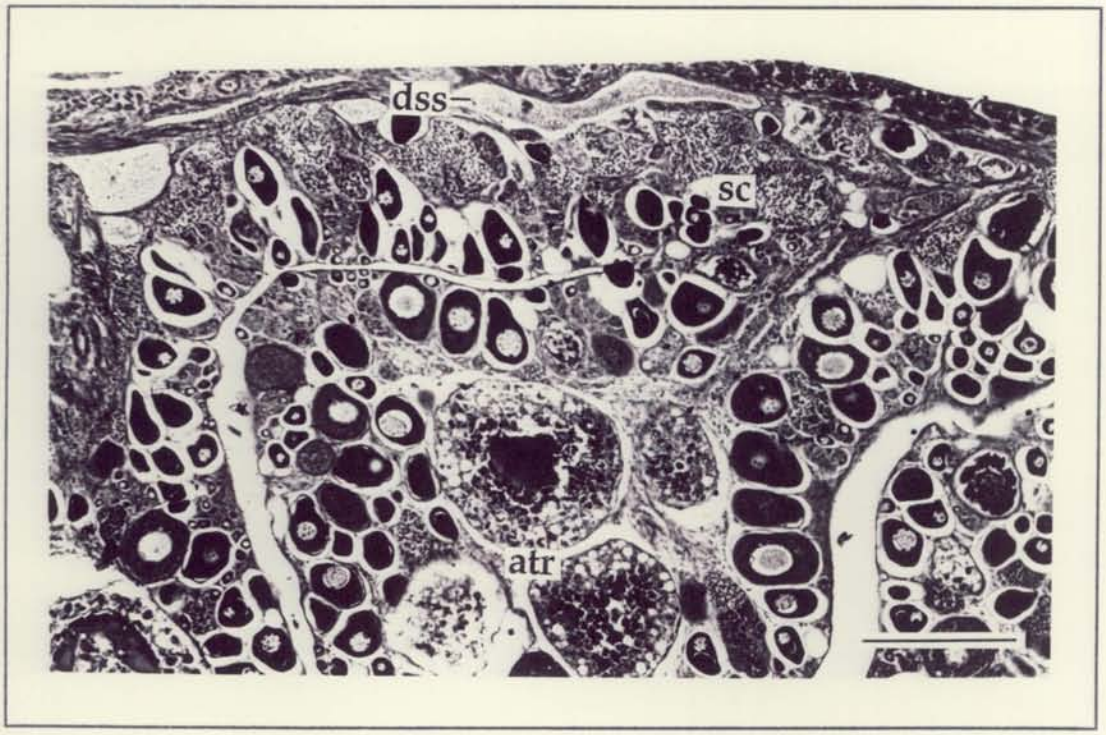


Figure 5.5: Gonad in early immature male stage. Dorsal sperm sinus formed and filled with spermatozoa and atretic vitellogenic oocytes still present. Scale bar= 125µ. **dss**= dorsal sperm sinus; **sc**= sperm crypts; **atr**= atretic vitellogenic oocytes.

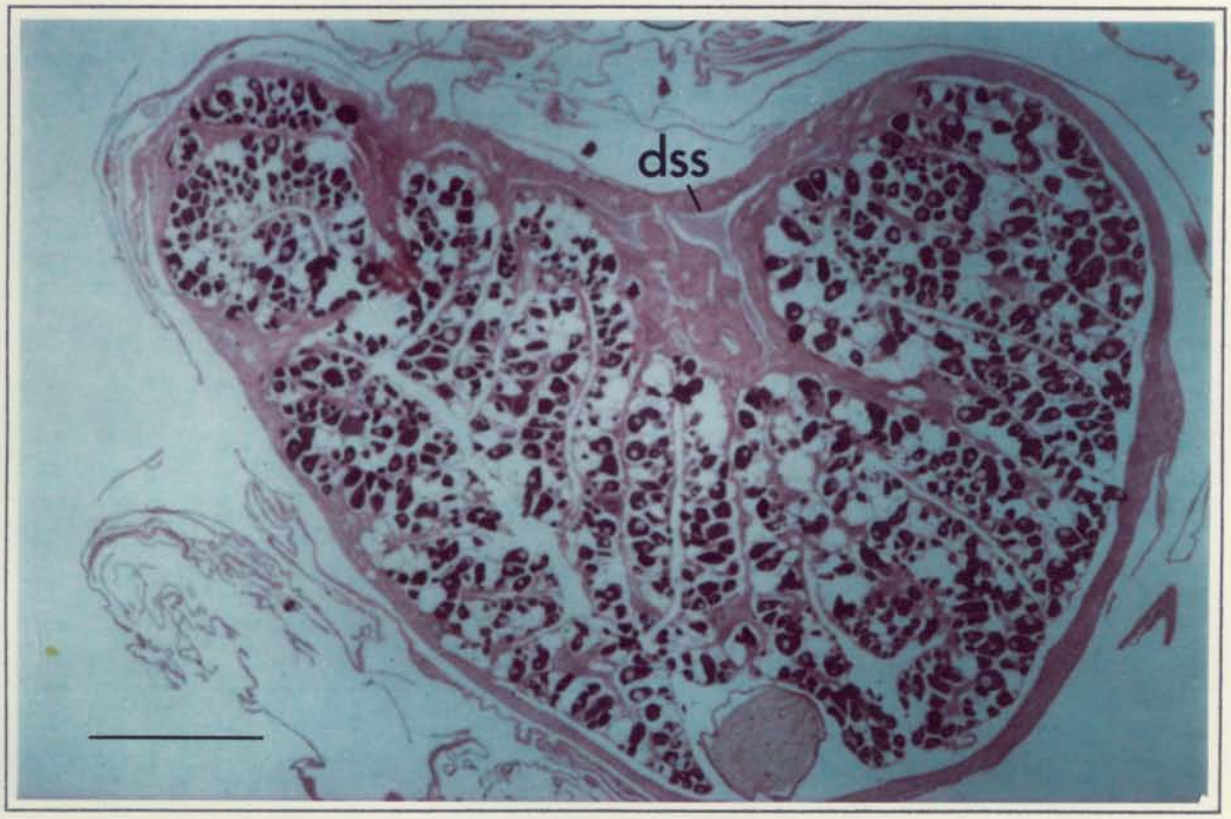


Figure 5.6: Gonad of immature male after prematurational sex change. Lamellae filled with early perinucleolus oocytes, dorsal sperm sinus formed and filled with spermatozoa. Scale bar= 500μ. **dss**= dorsal sperm sinus.

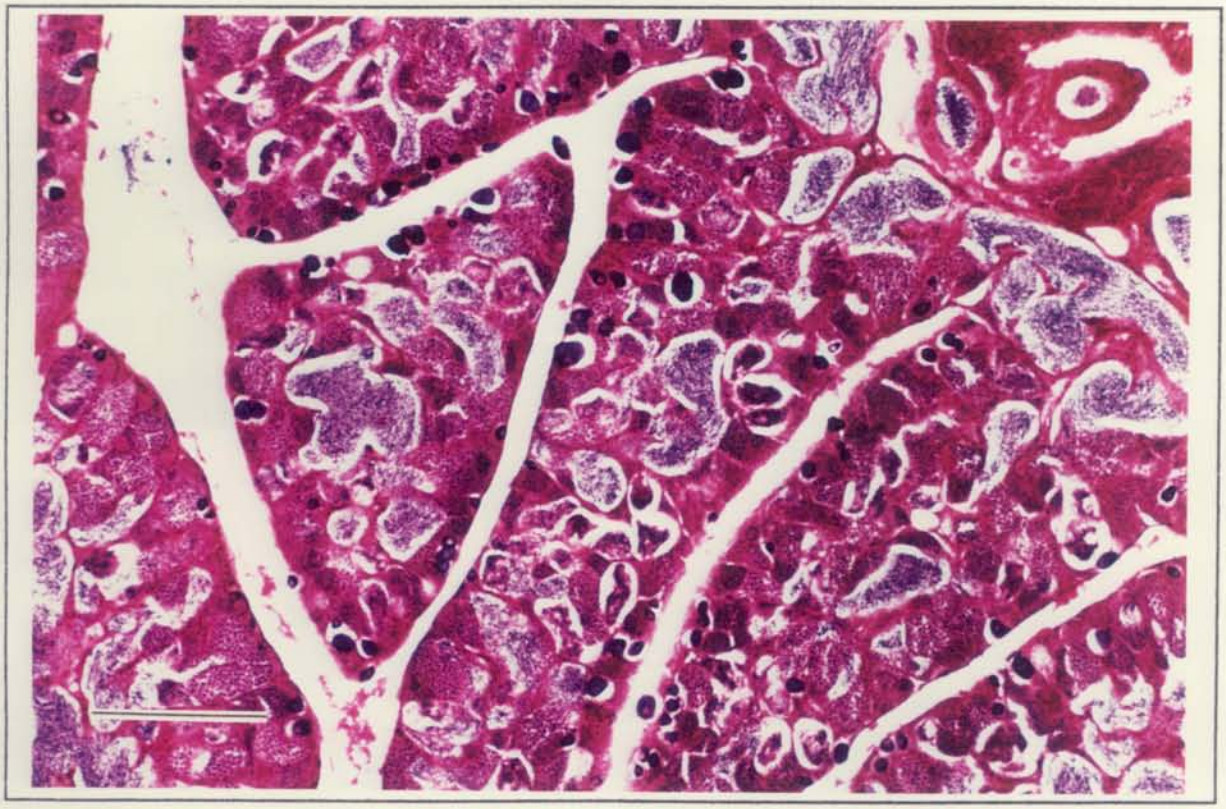


Figure 5.8: Transverse section of testis of mature male resting showing remnant previtellogenic oocytes located in the periphery of testicular lobes. Scale bar= 200μ.

There was a significant reduction in the ovarian tissue as the gonad developed from transitional to the mature male stage ($p<0.001$) (Fig. 5.7).

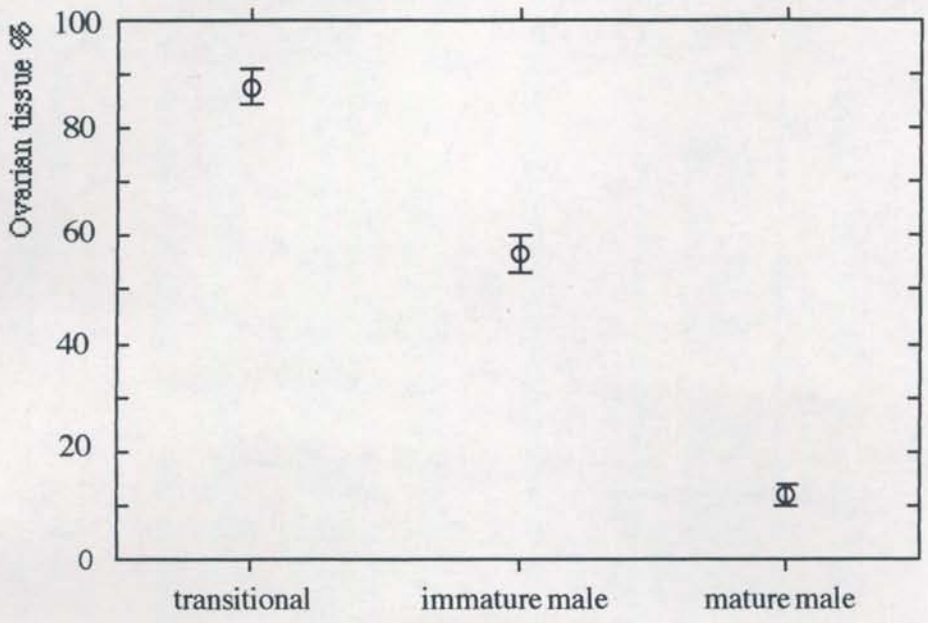


Figure 5.7 - Percentage of ovarian tissue in gonads of transitional, young and mature males. Error bars show standard error.

Gonads of young males were still largely ovarian, but significantly less than transitional gonads. In testes of mature males, ovarian tissue was further reduced and the presence of oocytes was usually restricted to the periphery of the testicular lobes (Fig. 5.8).

The presence of yellow-brown bodies was not clearly associated with sex-transition as they were common in gonads of resting and spent males and females. In mature males, they were usually located in the centre of the testicular lobes. Brown bodies were especially conspicuous in post-spawning individuals, and could be seen macroscopically as brown dots along the gonads.

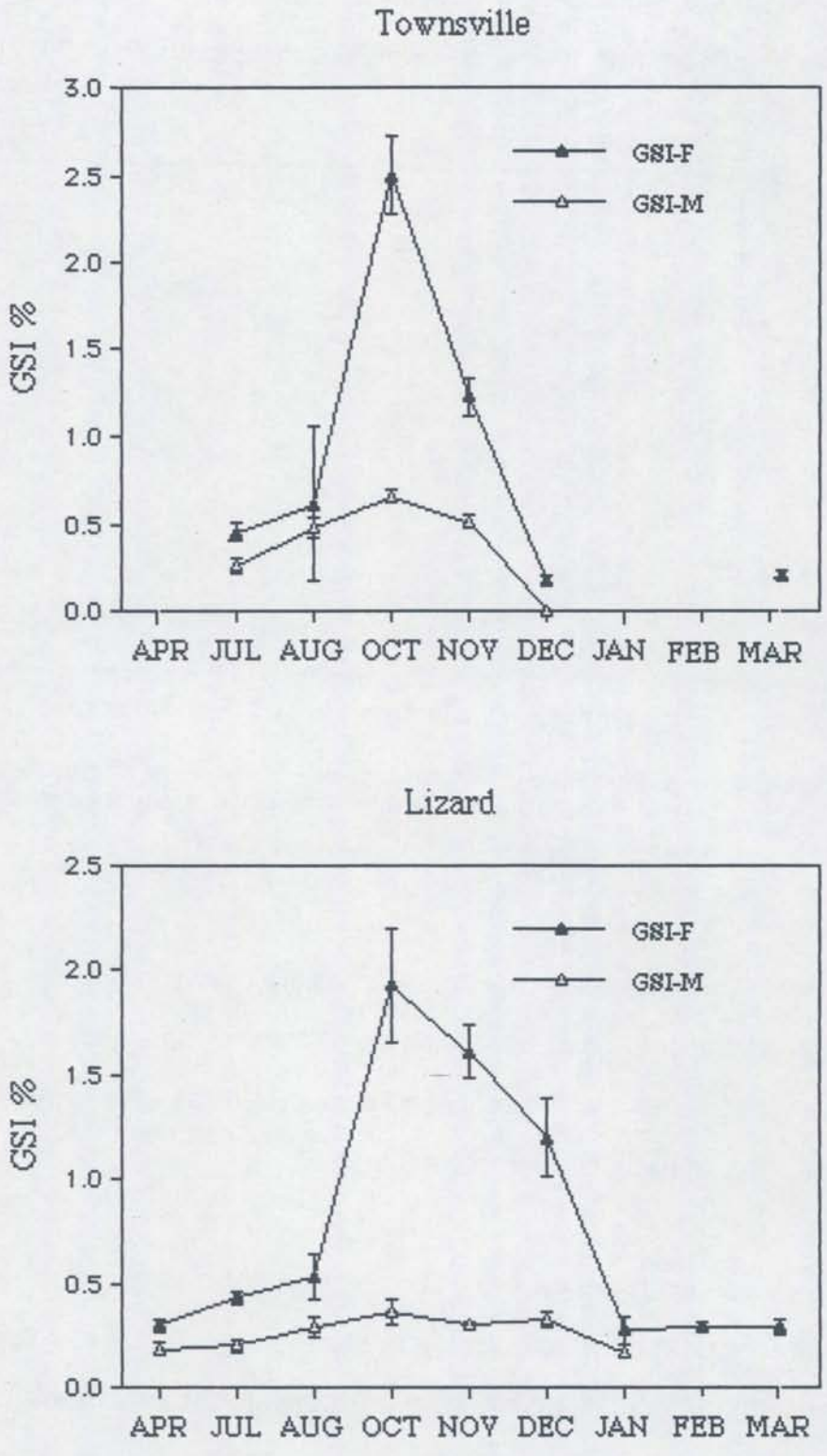


Figure 5.9: Monthly variations of gonadosomatic index (GSI) of mature males and females from Townsville and Lizard Island. Error bars represent standard error.

Seasonality and periodicity of spawning

For both the Lizard Island and Townsville regions, spawning was indicated for the period from September through December by gonadal stages and the gonado-somatic index (GSI).

There were no significant differences between GSI values observed for Townsville and Lizard Island Reefs. GSI values of females were significantly higher than those of males, and both varied equally between months in the two locations (Table 5.2) (Fig. 5.9). There was a significant increase from August to the peak in October, with values remaining high until November and dropping significantly by December (Tukey-Kramer, $p<0.05$). A significant interaction between sex and month indicated that differences in GSIs between males and females were dependent on month. During the spawning months, the increase in the GSI values of females is up to four times greater than the increase observed for males.

Table 5.2: Three-way analysis of variance examining the effects of location, sex and month on the variation of GSI[(a)] values of mature males and females.

Source	df	SS	MS	F-value	P-value
LOCATION	1	0.001	0.001	0.857	0.3552
SEX	1	0.024	0.024	19.722	0.0001
DATE	8	0.064	0.008	6.544	0.0001
LOCATION * SEX	1	0.001	0.001	0.463	0.4965
LOCATION * DATE	3	0.003	0.001	0.716	0.5429
SEX * DATE	6	0.017	0.003	2.350	0.0305
LOCATION * SEX * DATE	3	0.001	0.000	0.353	0.7868
Residual	402	0.493	0.001		

(a) Data arcsin square root transformed prior to analysis

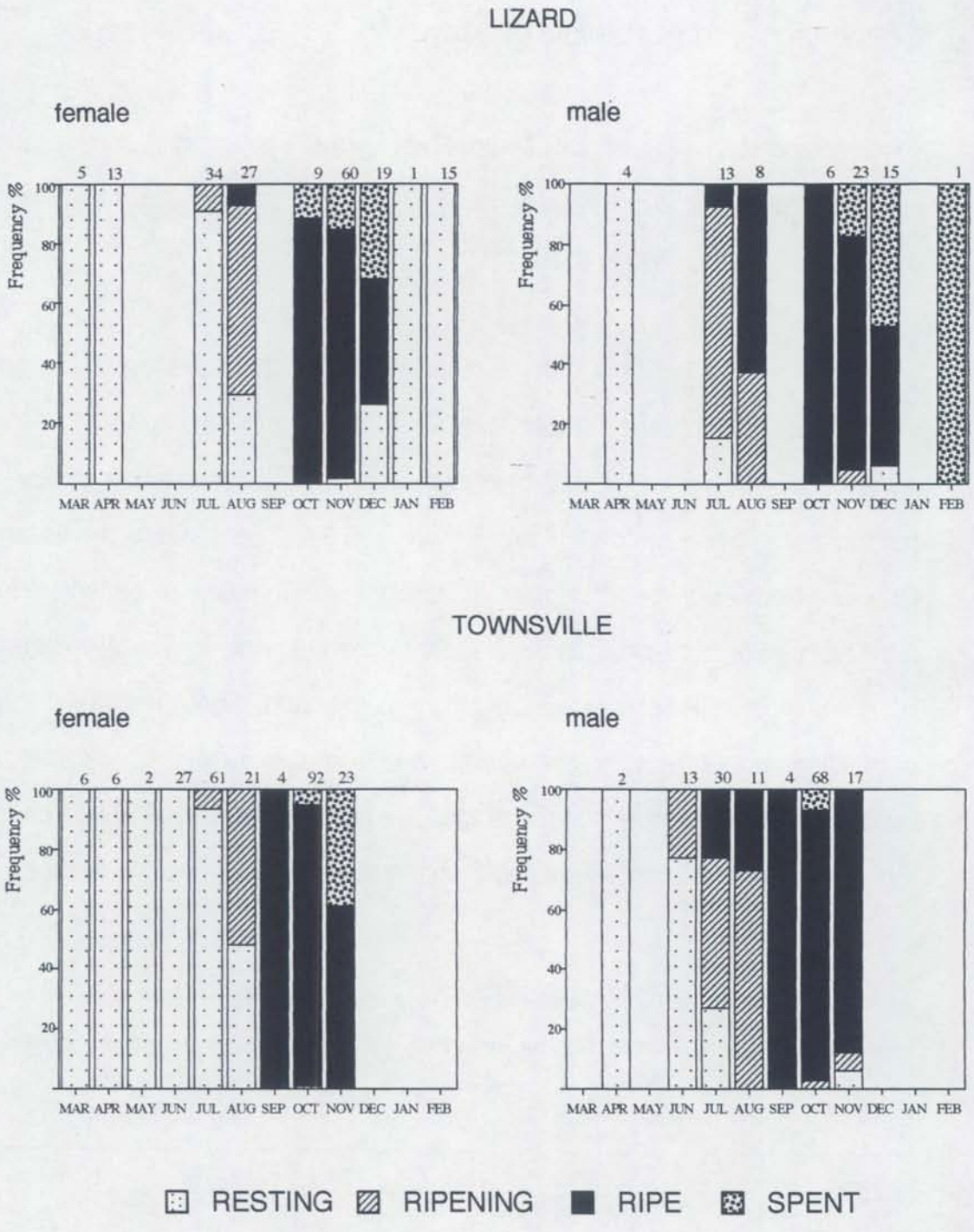


Figure 5.10- Monthly distribution of percentages of gonadal stages for males and females of coral trout from the Lizard Island and Townsville regions.

Ripe females were observed from August through December at Lizard Is. and from September through November at Townsville (no data for December). Ripe males were present from July through December at Lizard and from July through November at Townsville (no data for December) (Fig. 5.10).

In testes of ripe males, spermatogenesis and spermiogenesis occurred simultaneously, indicating continuous spawning activity (Fig. 5. 11). Ripe females characteristically had lamellae packed with oocytes in the tertiary yolk globule stage, but oocytes in earlier stages of development were always present (Fig. 5.12), indicating multiple spawning throughout the season (Nagahama, 1983; Ebisawa, 1990). Oocytes in final stages of maturation (hydrated oocytes) were present in 40% of the ripe female gonads observed in the period from September through December. In those oocytes, the lipid droplets and yolk globules had coalesced and the overall size of the oocyte increased due to hydration. Hydrated oocytes were present in gonads of females caught during all moon phases. In the gonads of females caught in the morning and early afternoon, they were present in numbers varying from just a few to more than half of the late vitellogenic oocytes in the gonad. Females in which all the hydrated eggs had been emptied into the lumen ("running-ripe"), however, were observed only during late afternoon. The ovaries of these females were flaccid and histologically were characteristically spent gonads, with disorganised lamellae and post ovulatory follicles (POF) (Figs. 5.13 and 5.14). Post ovulatory follicles are probably very short-lived as they were only observed in the ovaries of running-ripe females. Vitellogenic oocytes in several stages were also present and no hydrated oocytes were left in the gonad.

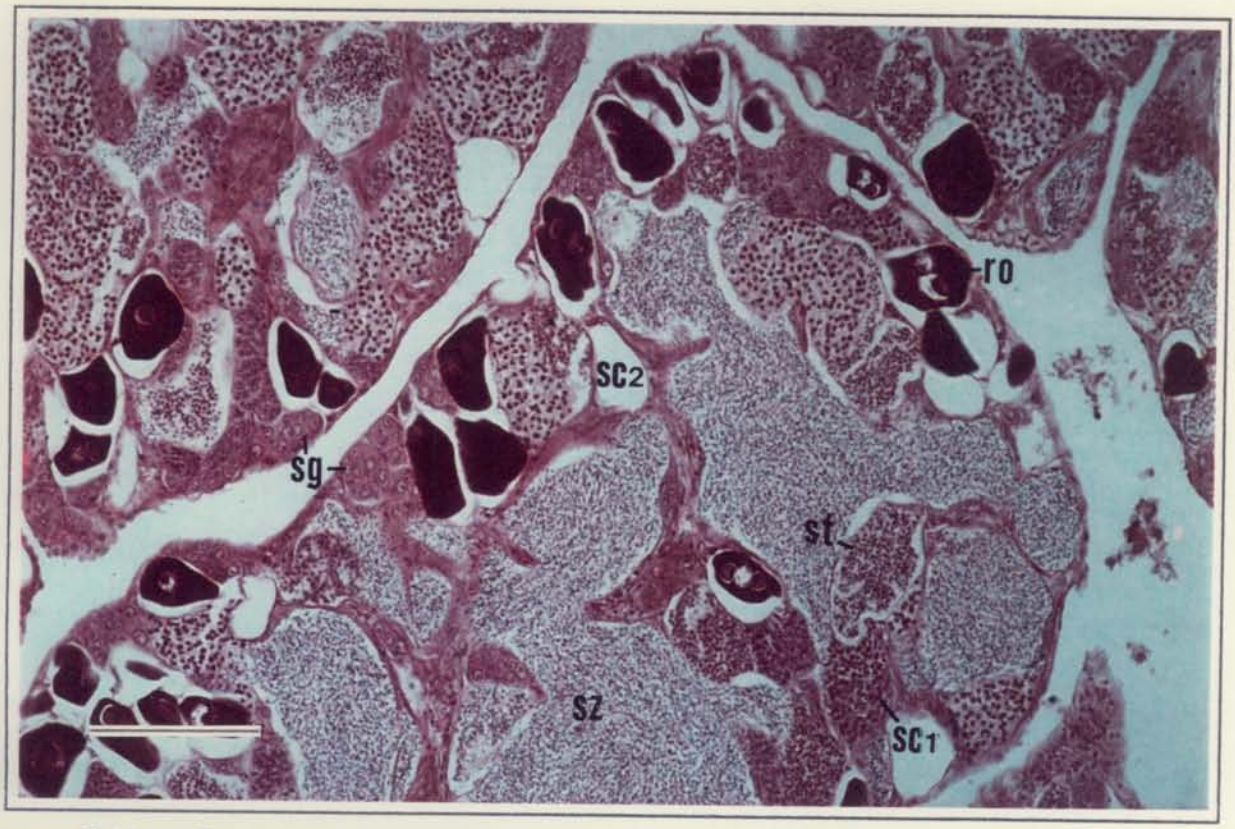


Figure 5.11: Testis of ripe male showing simultaneous occurrence of stages of spermiogenesis and spermatogenesis. Scale bar= 100μ. **sg**= spermatogonia; **sc1**= primary spermatocyte; **sc2**= secondary spermatocyte; **st**= spermatid; **sz**= spermatozoa; **ro**= remnant oocyte..

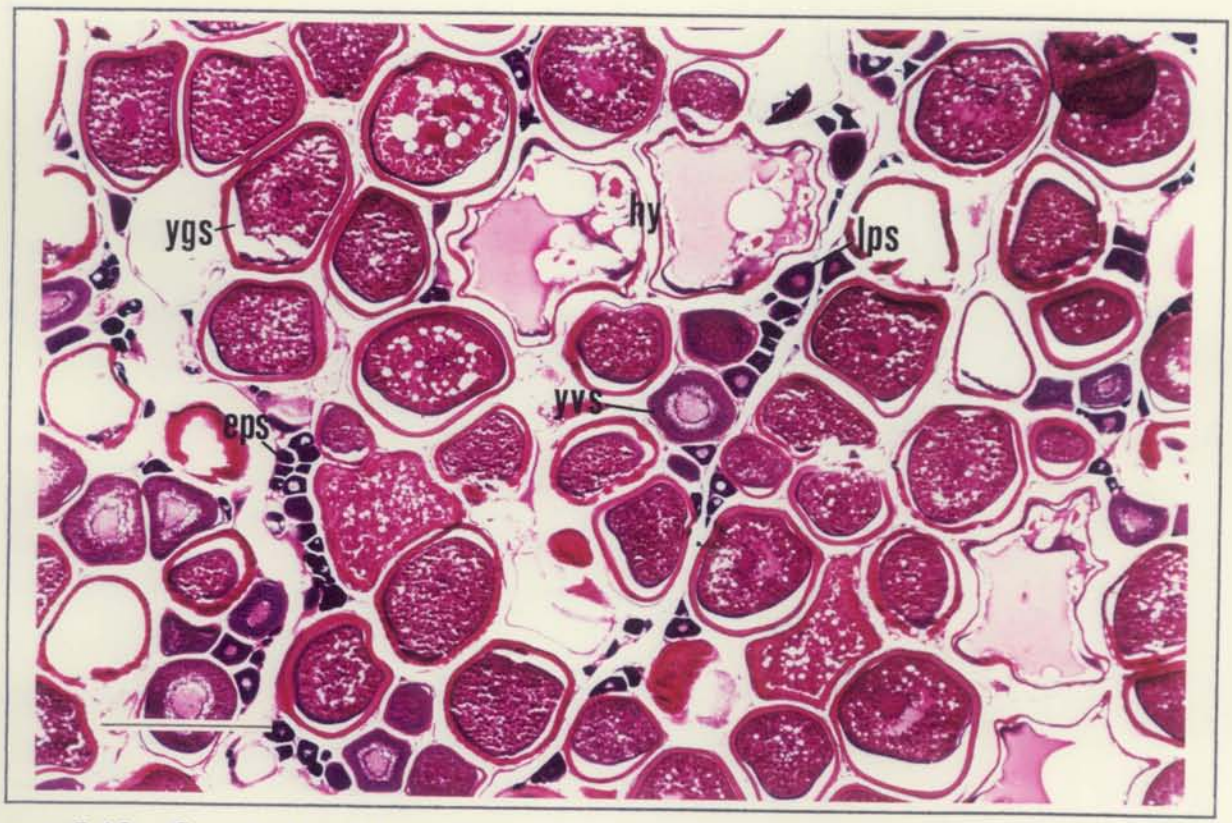


Figure 5.12: Ovary of ripe female showing asynchronous oocyte development. Scale bar= 500μ. **eps**= early perinucleolus stage; **lps**= late perinucleolus stage; **yvs**= yolk vesicle stage; **ygs**= yolk globule stage; **hy**= hydrated stage.

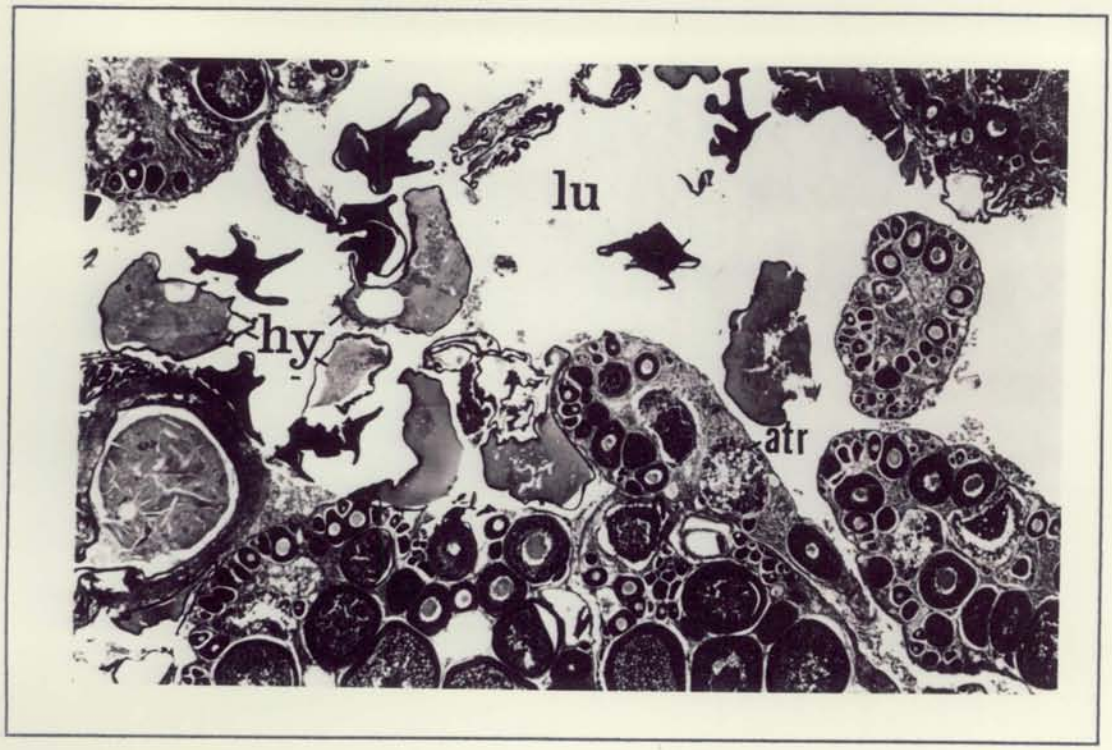


Figure 5.13: Hydrated oocytes in the lumen of "running-ripe" female. Scale bar= 125μ. **lu**= lumen; **hy**= hydrated oocytes; **atr**= atretic vitellogenic oocytes.

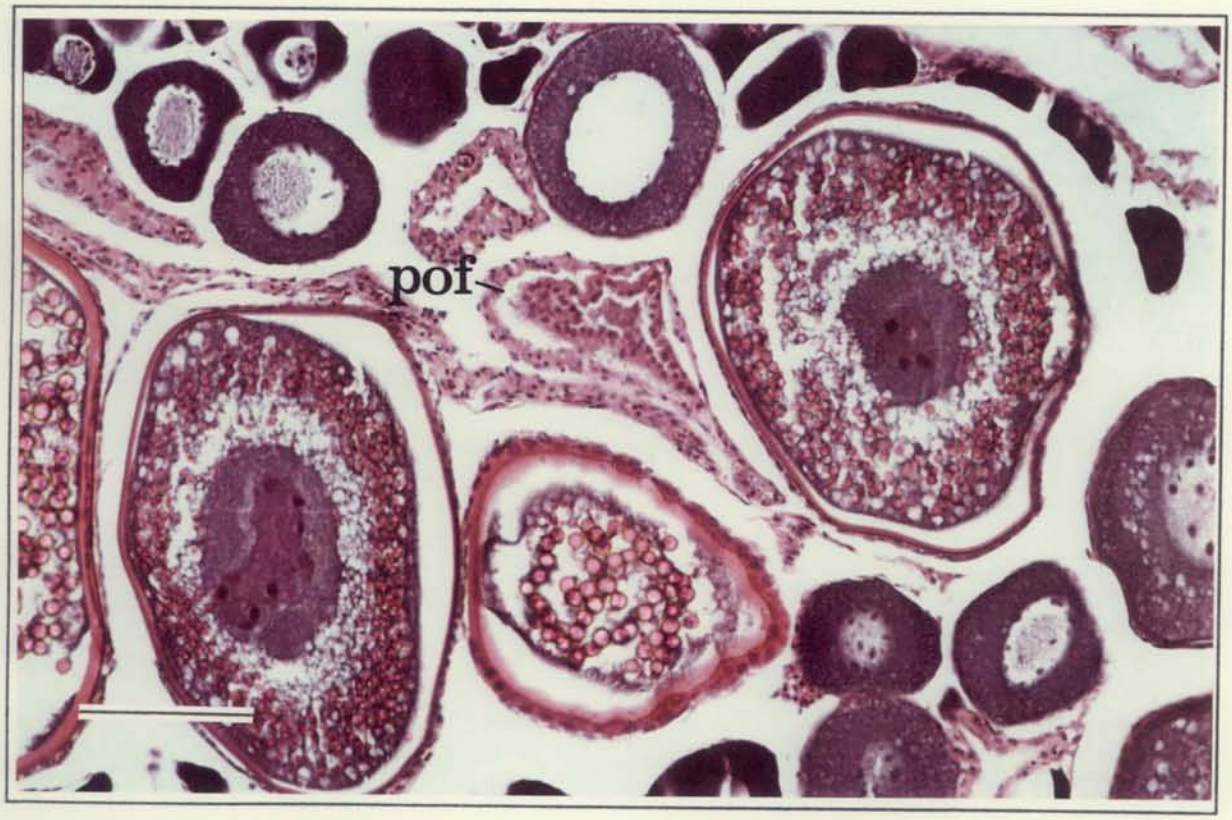


Figure 5.14: Post-ovulatorium follicle (**pof**) in ovary of spent female. Scale bar= 100μ.

A small spawning aggregation, consistent with that described by Samoilys and Squire (1993), was observed using scuba-diving at North Point, Lizard Island, on the 5th of November of 1991, approximately half an hour before sunset. Three females and one male were captured at sunset. Two of the females were running-ripe, with the ovarian lumen full of eggs, and the other one had hydrated oocytes. Histologically, the male was ripe but only a small amount of milt could be obtained.

The amount of fat deposited in the mesenteries of mature males and females from Lizard Island varied seasonally (Table 5.3). There was no significant difference between males and females, and both sexes showed a significant variation between months. From April onwards, there was a significant increase in the amount of fat until the peak in August (Tukey-Kramer, $p<0.05$). By October, the amount of mesenteric fat observed had dropped to almost zero in most individuals, and remained low until December, with a slight increase by February (Fig. 5. 15).

Table 5.3: Two-way analysis of variance examining the effects of sex and month on the amount of fat deposited in the mesenteries of mature males and females from Lizard Island.

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	
DATE	8	25.404	3.175	29.129	.0001	* * *
SEX	1	.105	.105	.959	.3281	
DATE * SEX	7	1.160	.166	1.520	.1597	
Residual	305	33.250	.109			

Dependent: arcsin(sqr(fat))

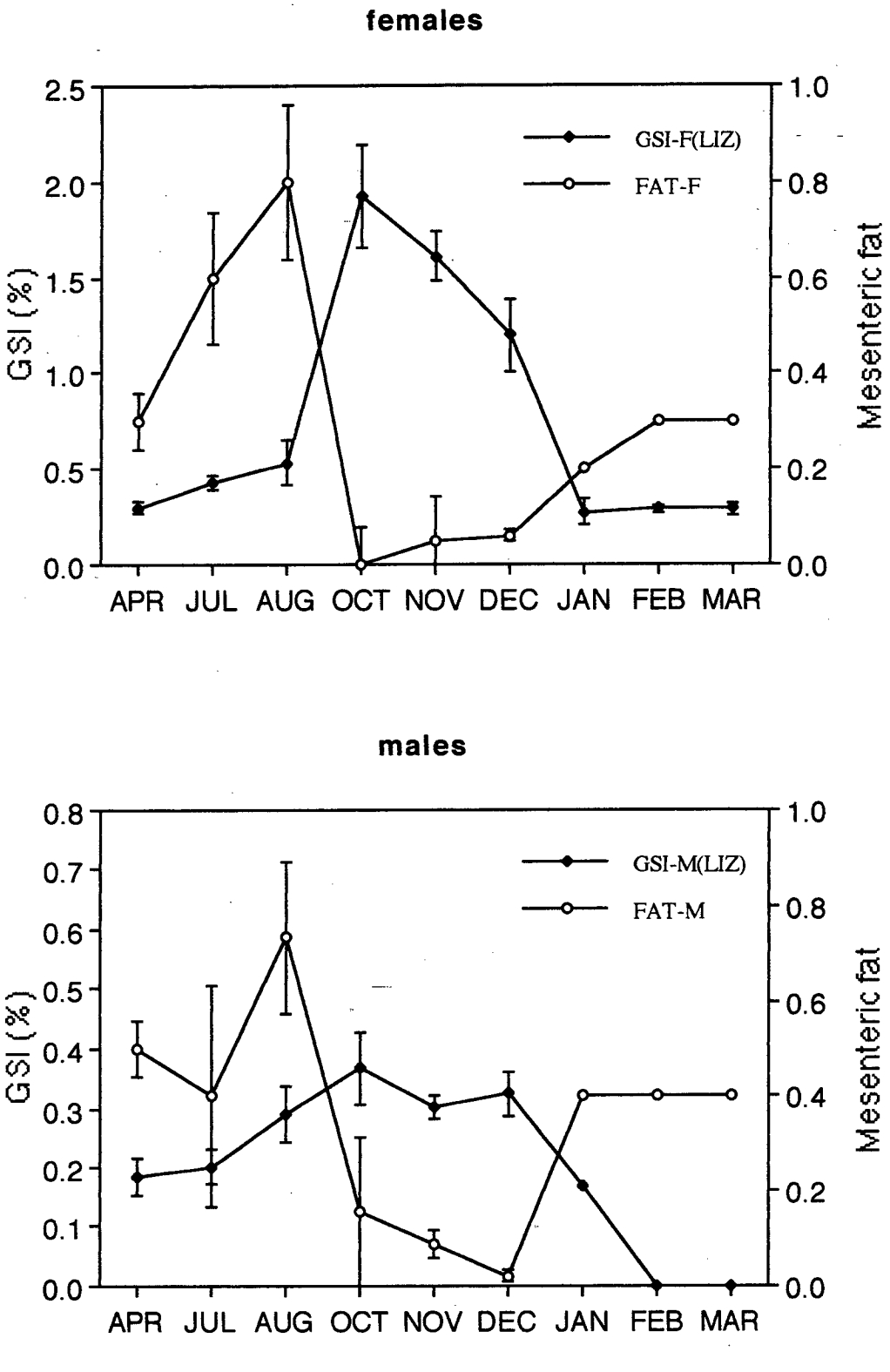


Figure 5.15- Monthly variation of the gonadosomatic index (GSI) and amount of fat deposited in the mesenteries of mature males and females from Lizard Island. Error bars represent standard error.

The variation in the amount of mesenteric fat was antiphasic to the variation observed in the GSI for males and females (Fig. 5. 15), and the amount of fat was inversely correlated with gonad weight for mature females (Spearman rank Rs= -0.361, $p<0.001$).

Population structure:

The sex-ratio (mature females : mature males) of the sample collected with spear-fishing was statistically not different from the sex-ratio of the sample collected with line-fishing (Chi-square, $p=0.133$), so the samples were pooled.

The proportion of each developmental stage for each location is shown in Table 5.4. Sex-ratio (mature females : mature males) in both samples was biased towards females.

Table 5.4: Frequencies of developmental stages and sex-ratio (mature females:mature males) for the Lizard Island and Townsville samples

	Immat. female	Mature female	Trans.	Young male	Mature male	sex-ratio fem : male
LIZARD	8 (3.5%)	141 (61%)	10 (4%)	10 (4%)	61 (26.5%)	2.31:1
TSV	12 (10%)	61 (51%)	12 (10%)	12 (10%)	22 (19%)	2.77:1

The Townsville sample had a smaller proportion of mature males, but a higher proportion of transitional and young male stages. If these individuals were considered as males, the sex-ratio would be: Lizard Is.=1.74:1; Townsville=1.33:1.

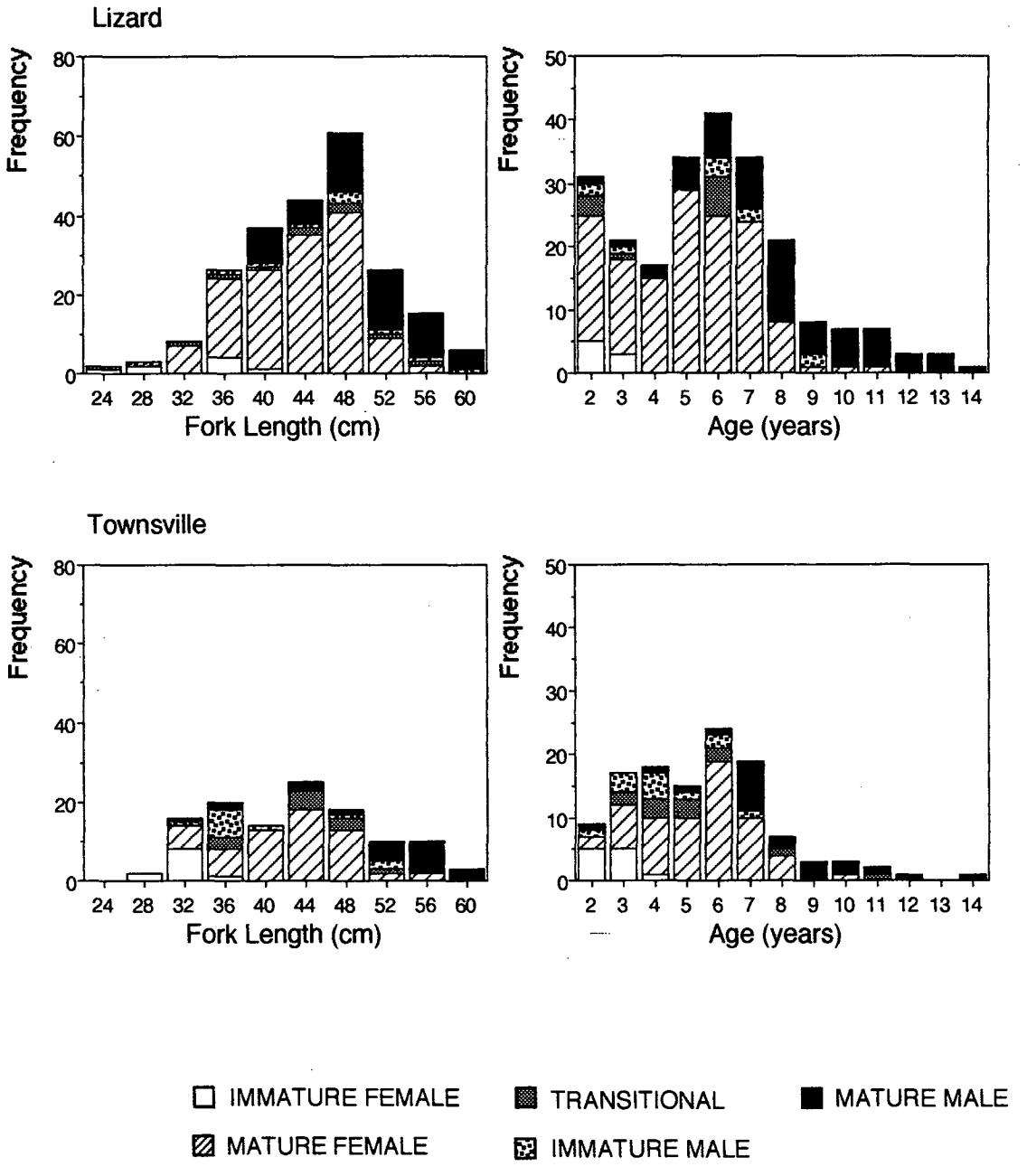


Figure 5.16- Size and age distributions of developmental stages of the coral trout *Plectropomus leopardus* for the Lizard Island and Townsville regions.

Size distributions of mature males and females overlapped over most of the range of sizes observed (Fig 5.16). The mean sizes and ages of the Lizard Is. and Townsville samples were not significantly different (Tables 5.5 and 5.6). There were significant differences among ontogenetic stages (immature and mature females, transitional individuals and young and mature males), but no interaction between location and stage, indicating that the mean sizes and ages of each stage were not different for each location.

TABLE 5.5 Two-way ANOVA comparing mean sizes of the Lizard Island and Townsville samples.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
LOCATION	1	94.364	94.364	2.620	0.1064
STAGE	4	5370.695	1342.674	37.285	0.0001
LOCATION * STAGE	4	217.141	54.285	1.507	0.1996
Residual	336	12099.851	36.011		

Dependent: FL (CM)

TABLE 5.6 Two-way ANOVA comparing mean ages of the Lizard Is. and Townsville samples.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
LOCATION	1	0.006	0.006	0.180	0.6716
STAGE	4	3.959	0.990	32.179	0.0001
LOCATION * STAGE	4	0.181	0.045	1.468	0.2115
Residual	339	10.427	0.031		

Dependent: LOG (AGE)

Sizes and ages of mature females, transitional and young males were not significantly different, while immature females and mature males were significantly smaller and larger respectively than all other stages. Size and age of individuals within the zone where frequency distribution of males overlapped with females were not significantly different between locations (t-test, size: p=0.192; age: p=0.190).

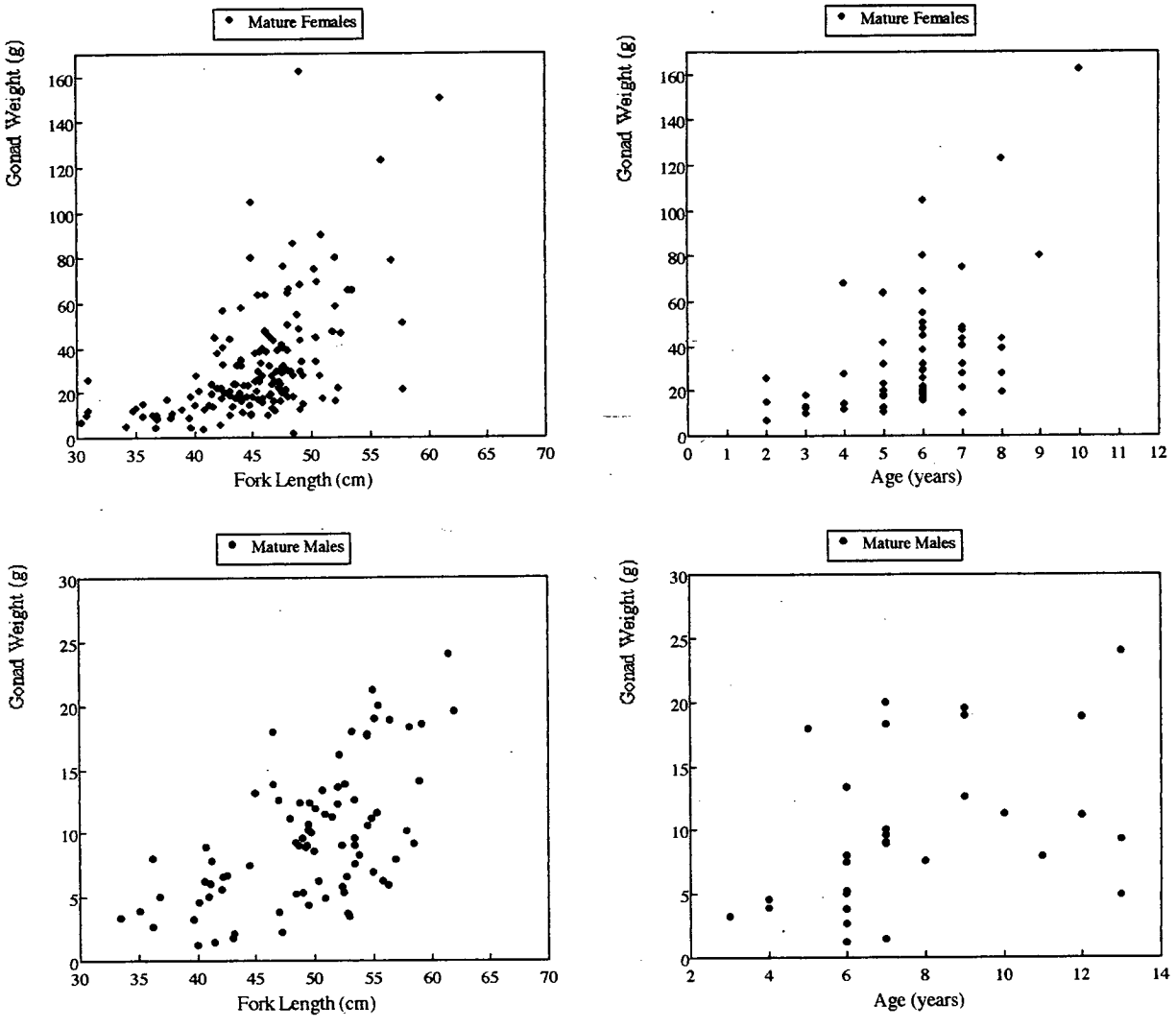


Figure 5.17- Relationship between gonad weight (g) and size (fork length) and age of ripe females and males of coral trout. Data from Lizard Island and Townsville regions pooled.

Maturation:

During the spawning season some immature females were observed undergoing limited vitellogenesis, as indicated by the presence of yolk oocytes in early stages. After the spawning season, it is possible that signs of previous vitellogenesis remained, in the form of scattered yolk globules or brown-bodies, making it difficult to separate these immature gonads from mature resting gonads. Therefore, for the calculation of size and age of first maturity, only immature individuals classified during the spawning season were included.

The size class of first reproduction (50% of individuals reproductive) for females was size classes 32 cm FL (between 30 and 34 cm FL) for the two locations (Fig. 5.16). The age of first reproduction for females was 3 years old for the Townsville sample and 2 years old for the Lizard Is. sample (Fig. 5.16).

Gonad weight was positively related to age and size for mature males and females (Spearman-Rank, $p<0.0001$). Some females were mature at 30 cm FL but gonad weight started to increase only after 40 cm FL (Fig. 5.17).

Transitional gonads that resembled immature ovaries (Fig. 5.6) were observed during and outside the spawning season. Individuals observed during the spawning season (n=7) were aged between 2 and 3 years (mean= 2.7, se= 0.184) and measured between 26.9 and 36.2 cm (mean= 33.1, se= 1.37), indicating the possibility of prematurational sex-change.

4.5- DISCUSSION

Spawning Season

The spawning period observed for *P. leopardus* between early spring and summer in the Central and Northern regions of the Great Barrier Reef coincides with the spawning period observed by Goeden (1978) for the Southern region and with the spawning period observed by Samoilys and Squire (1993) for the Cairns region. Spawning season during this period has also been observed for the congeneric species *P. maculatus* from the Townsville region (Ferreira 1993, Chapter III). The sampling design employed here did not allow for effective comparisons between locations regarding exact time of spawning, and it is possible that latitudinal differences exist for coral trout populations of the Great Barrier Reef in terms of time of the beginning and end of the spawning season. Nevertheless, it seems reasonable to infer that the spawning season for the coral trout on the Great Barrier Reef occurs generally in the same period, i.e., from early Spring to early Summer.

Multiple-spawning during this period was indicated by asynchronous oocyte development in females and continuous spermiogenesis in males (Nagahama, 1983; Ebisawa, 1990). Males mature earlier in the season and remain active for longer and have lower GSI values than females. As males in protogynous species tend to spawn more than females (Shapiro, 1984) it is likely that the strategy employed by coral trout males is a limited but continuous production of sperm throughout the season.

In fishes, oocytes in the tertiary yolk globule stage are maintained within the ovary for a variable period of time, following completion of vitellogenesis, until a series of endocrine events stimulates their final maturation and ovulation (Liley and Stacey, 1983). Hydration of oocytes is known to occur just a few hours before ovulation for some species (Clarke, 1987), however final oocyte maturation and ovulation are not always associated (Nagahama 1983). Failure to observe hydrated oocytes in mature female gonads during the spawning season lead Smith (1965) and Moe (1969) to conclude that ovulation quickly followed maturation for *Cephalopholis fulva* and *Epinephelus morio* respectively. Goeden (1978) did not observe any hydrated oocytes within the ovarian lamellae *P. leopardus* and similarly concluded that those were rapidly ovulated. In contrast, hydrated oocytes were present in 40% of the gonads of ripe female *P. leopardus* observed during the spawning season in the Townsville and Lizard Is. regions. The absence of hydrated stages within the ovarian lamellae of *P. leopardus* reported by Goeden (1978) is probably related to the small sample size, as only 34 ripe females, collected during day-time, were examined.

Samoilys and Squire (1993) monitored spawning aggregations of coral trout on the northern Great Barrier Reef and observed that spawning rushes were restricted to a 22 minute period around sunset. In the present study, although hydrated oocytes were observed in females caught during the morning and early afternoon, running-ripe females were only caught in the late afternoon, suggesting that hydration can occur as early as 7 to 8 hours before ovulation.

Samoilys and Squire (1993) also described that fish density in the spawning aggregations peaked during the new moon. This lunar

periodicity has been ascribed to increased egg survival, through quick dispersion by strong tidal flows (Johannes 1978), or with the necessity to synchronise spawning activity (Colin et al. 1987). Females with hydrated oocytes within the lamellae were observed during all moon phases. Hence, it seems that although spawning activity may peak at a certain moon phase (Samoilys and Squire, 1993), spawning also occurs throughout most of the spawning season.

An inverse relationship between fat and gonad weight was observed for the coral trout, indicating that these deposits of mesenteric fat are probably being used in the processing of gonad products. A similar pattern has been observed for the Baltic Herring (Rajasilta, 1992). Male GSIs were much lower than female GSIs, but no differences were observed in the amounts of fat stored. It is possible that males and females have similar energy requirements, as males remain reproductively active for longer periods in the season and may be involved in more spawning episodes than females.

Protogynous hermaphroditism

As reviewed by Sadovy and Shapiro (1987) a series of characteristics that have been used as indicative of protogynous hermaphroditism require careful assessment before concluding on the mode of reproduction of a species. The presence of a vestigial lumen and dorsal sperm sinuses in male gonads is not necessarily an indicator of functional hermaphroditism, as their presence, as well as remnant ovarian tissue, can result from juvenile hermaphroditism or bisexuality (Sadovy and Shapiro 1987, Ebisawa 1990). Therefore, only the occurrence of developing sperm

crypts in the presence of degenerating mature, ripe female tissue is conclusive evidence of functional protogynous hermaphroditism. Such evidence was found for coral trout, where crypts of spermatocytes, spermatids and spermatozoa were observed in spent female gonads.

Classification of transitional individuals is also dependent on the basic structure of the gonad. In the *Epinephelus* type gonad (*sensu* Smith, 1965), where the female and male tissues are intermingled, the development of precocious sperm crypts in immature and mature female ovaries seems to be a widespread phenomena (Smith, 1965; Moe, 1969). Developing sperm crypts were observed in the ovaries of some female coral trout, but their development did not seem to interfere with the spawning process, as no degeneration of the vitellogenic oocytes was observed. Smith (1965) also observed sperm crypts in ovaries of ripe females of *Cephalopholis fulva* and *Petrometopon cruentatus* and concluded that they did not interfered with spawning.

It is not clear if the development of sperm tissue in gonads of ripe females will proceed into sex-change following the spawning season. However, for several species of Serranidae, it has been suggested that sex-transition is initiated immediately after spawning (Smith, 1965 Moe, 1969; Shapiro 1984; Sadovy and Shapiro, 1987). In fact, sex transition was observed occurring in gonads of female coral trout which showed signs of recent spawning. As the coral trout is a multiple spawner, it is possible that development of sperm tissue is initiated in a ripe female after an early spawning event, and continues while the ovary is preparing for the next. The presence of fragmenting previtellogenic oocytes in the ovaries of these ripe females seems to substantiate this hypothesis. Actual sex

transition, with degeneration and reabsorption of vitellogenic oocytes, may take place when the female is totally spent.

The process of sex-transition can apparently be completed within the same spawning season, as indicated by the presence of degenerating yolk oocytes in the gonads of young males. However, transitional individuals with gonads largely ovarian and no sperm sinuses were observed outside the spawning season. So, the sex-transition process in the coral trout can either take a variable length of time to be completed, or it can be initiated year-round. The reasons for this variability may be related to the factors influencing sex-change.

Population Structure and Mechanisms Determining Sex-Change

The control of sex is known to be primarily genetic, however mechanisms of sex determination in fishes are primitive and labile (Chan and Yeung 1983). The precise genetic basis for sexuality in hermaphroditic fishes is not well understood (Price, 1984). Sex phenotype is probably a consequence of the interaction between the genetic constitution of an organism and its environment, although the extent of environmental influences probably varies from one species to another (Chan and Yeung 1983). In theory, sex-reversal may be induced by developmental or environmental (physical or social) causes (Sadovy and Shapiro, 1987). Social induction of sex-change is known or claimed for many species of fish, but behaviourally induced sex-change has not yet been successfully proven for groupers (Shapiro 1987).

Size and age of mature females were significantly lower than those for males in both geographic samples. This expected consequence of protogynous hermaphroditism has been interpreted as an indicative that sex change is a developmental process, initiated endogenously when females attain a certain size and age (Smith 1965, Moe 1969). Alternatively, Shapiro and Lubbock (1980) formulated a model suggesting that this characteristic population structure could equally be explained if sex change was controlled mainly by social processes, where a decline in the level of male-female interactions would cause female to male sex change.

The sex-ratio data indicated a slightly higher proportion of females in the Townsville sample than in the Lizard Is. sample. However, there were proportionally more transitional and young male stages in the Townsville sample. Development of sperm crypts in the ovaries of ripe females was observed only in the Townsville sample. It is possible that the reefs off Townsville are subject to a greater fishing pressure than the reefs around Lizard Island, due to proximity to populated areas (Craik et al., 1989). If so, it is possible that the selective removal of larger individuals (presumably mostly males), is triggering earlier sex-change as a form of a compensatory mechanism.

The coral trout, like other species of groupers (Shapiro, 1987), is known to aggregate at specific sites during their spawning season (Johannes, 1978; Samoilys and Squire, 1993), and it has been suggested that social interactions occurring during these aggregations would be important for the determination of the distribution of sexes in such populations (Shapiro, 1987; Gilmore and Jones 1992; Samoilys and Squire, 1993).

Chan and Yeung (1983) referred to a hypothetical scheme combining developmental and environmental factors. Under this model, sex change would be determined by an inherited responsiveness of the germ cells to hormonal stimulation, the gonadal endocrine interactions, and the function of the hypothalamic-hypophysial-gonadal axis. Chan and Yeung (1983) suggested that under external stimuli, the central nervous system may act through the pituitary in sex control and maturation of the germinal elements in the gonad. The pituitary is involved in germ cell maturation and formation of associated endocrine tissues, and a possible route by which it may affect sex-change is through somatic elements of the gonad (Chan and Yeung, 1983). Development of sperm crypts in transitional coral trout usually occurs in the dorsal part of the gonad, in apparent association with stromal tissue. Brown-bodies, formed after the spawning season, have been described as steroidogenic tissues (Nagahama, 1983); that would indicate a possible role in sex change and also explain their resilient presence in mature male gonads.

Distribution of size and age of male and female coral trout overlapped over a wide range of sizes and ages. Several factors could be contributing to the occurrence of such extensive overlap. Among those are the occurrence of prematurational sex-change, presence of "primary females" (*sensu* Warner and Robertson, 1978) that never change sex, and variation in the size at sex change among sub-populations that have been pooled. All of these alternatives are likely to apply in the case of the coral trout. Pre-maturational sex change was indicated by the occurrence of young fish in the transitional, young and mature males stages. Histological observation of gonads indicated that some of those individuals did not seem to have spawned as females before changing sex. The occurrence of females that never change sex is possible, as large and old females

were observed in the samples analysed. However, this species seems to be able to attain older ages, as observed by Loubens (1980) in New Caledonia. Collection of individuals in the upper limits of ages would be necessary to test this hypothesis. Finally, both Townsville and Lizard Island samples were likely to contain elements from different sub populations within the two locations. In this case, if sex-change is behaviourally induced and different mechanisms are operating in each sub population, great variability in size and age at sex change would be expected in the pooled sample.

While it is not clear how sex change is determined for the coral trout, the variability observed in the size and age in which sex change occurs and in the process of transition itself, suggests that behavioural processes could be involved. If so, factors such as recruitment variability and fishing mortality are likely to influence the social structure of the spawning population, and therefore the distribution of sexes in coral trout populations.

Such a strategy would allow the development of social structures that would optimise egg production and the spread of successful genes (Gilmore and Jones, 1992). A major question arises regarding the effect of fishing in relation to such social structures. Even if sex change is stimulated by social conditions assessed during spawning how fast and effectively can the population adjust if its structure is being changed continuously?

Gilmore and Jones (1992) pointed out that an undisturbed population of groupers would contain large numbers of older, sexually active and highly fecund females. Dominant males would fertilise an extraordinary number

of ova passing their genotypes to entire generations of offspring. Removal of dominant males by natural mortality could be compensated by female sex change, but would this replacement be effective under constant fishing pressure?

Considering the difficulties in answering those questions, Gilmore and Jones (1992) proposed closure to fishing during the spawning season to protect grouper populations in Florida. The P.D.T. (1990) argued for fisheries reserves, totally protected areas that would have the important advantage of protecting the genetic variability of the populations.

In conclusion, the coral trout *Plectropomus leopardus* is a protogynous hermaphroditic species in which sex change is probably governed by both developmental and behavioural processes. Population sex structure is a result of the interaction between factors such as recruitment variability and social structure of the spawning population. More information on behavioural aspects of the coral trout reproduction is still necessary to understand the precise mechanisms operating in these populations. At the present point, management decisions should include measures to preserve populations in their natural state.

CHAPTER VI: Population structure of the coral trout *Plectropomus leopardus* on fished and unfished reefs off Townsville, Central Great Barrier Reef, Australia.

6.1- Introduction:

Fishing is one of the most important human exploitative activities on coral reefs (Munro, 1983; Munro and Williams, 1985; Russ, 1991). The impact of fisheries on populations and communities of coral reef fishes has been reason for concern, as it is often suggested that fishing may have a greater impact upon fish populations and communities of coral reefs than upon those of temperate seas (Russ, 1991). Large predatory species are especially affected by overfishing, due to life history characteristics such as slow growth, high longevity, low rates of natural mortality and limited adult mobility (P.D.T., 1990; Russ, 1991).

Fishing is known to cause selective removal of larger (and presumably older) individuals, thus reducing their proportion in the population. While evidence for effects of fishing on the size structure of populations coral reef fishes is good (Munro, 1983, P.D.T., 1990), evidence for the effects of fishing on age structure are rare, which is basically a consequence of the perceived difficulties in ageing tropical fishes (Manooch, 1987). On the Great Barrier Reef, for example, information on age structure from a number of reefs exists for only one species, the damselfish *Pomacentrus molucensis* (Doherty and Fowler, 1993).

Sequential hermaphroditism is common among coral reef fishes (Thresher, 1984). Bannerot et al. (1987) modelled the resilience of protogynous populations to exploitation and concluded that a definite risk existed in managing these stocks by traditional Yield-per-Recruit models under high fishing pressure. The effects of selective removal of larger individuals (presumably mostly males) on the sex-ratio of a population, however, will

depend on the mechanisms controlling sex-reversal. For protogynous populations, for example, if female to male sex-change is determined by size or age, a decline in the proportion of males will be expected. Such effects have been reported by Thompson and Munro (1983) when comparing populations of serranids subject to different levels of fishing pressure in the Caribbean. In contrast, no fishing related effects were detected by Reeson (1983) on populations of scarids. Social induction of sex-change is known or claimed for many species of fish (Shapiro 1987). If this is the case, selective removal of larger individuals would induce female to male sex-change, compensating for the effects of fishing on the sex-ratio. Consequently, a reduction in the average size and age of sex-change would be expected.

A widely recognized management strategy in the conservation of reefs is the implementation of Marine Fisheries Reserves, areas designed to protect stocks of reef fish and habitats from all forms of exploitation (P.D.T. 1990; Williams and Russ, 1991). In modern times, the first marine protected area was established in Florida in 1930. Since then, protected marine areas have been implemented all over the world (P.D.T. 1990). In Australia, the first protected marine areas were established in the Capricornia Section of the Great Barrier Reef Marine Park in 1981, under the first zoning plan to come into operation (Craik, 1989).

Evidence indicates that long term spatial closure to fishing increases the density, biomass, average size and fecundity of reef fishes (see P.D.T., 1990 and Russ, 1991, Russ et al. in press, for reviews, but see de Martini, in press). Furthermore, by enabling populations of reef fishes to attain or maintain natural levels, marine reserves have been suggested to help to maintain or even enhance yields of fishes from areas adjacent to the reserves (Russ, 1985; Alcala and Russ, 1990).

The spatial structure of coral reefs provides an excellent opportunity to test for the effects of different management alternatives (Hilborn and Walters, 1992). The importance of experimental investigations on the effects of fishing on coral reefs using reefs as replicate experimental units has been pointed out by various authors (Walters and Sainsbury, 1990, Russ, 1991, Hilborn and Walters, 1992). Yet, in spite of the high expectations placed on Marine Reserves, few direct tests exist on the effects of such protection on yields of marine resources (Alcala and Russ, 1990).

The coral trout *Plectropomus leopardus* is a long-lived, protogynous hermaphroditic fish which represents a very important fishery resource over the whole Great Barrier Reef, Australia. Because of its importance, the coral trout has been the subject of many studies investigating the effects of fishing. These studies compared the abundance and size structure of populations from open and closed reefs on the Great Barrier Reef (see Williams and Russ, 1991, for review). Most of these studies were conducted using underwater visual census (UVC) techniques. Increased average size of the coral trout on reefs closed to fishing was detected in most cases (Craik, 1981; Ayling and Ayling, 1984, 1986; Ayling and Mapstone, 1991). Beinssen (1989a) used UVC, line fishing and mark-release-recapture techniques to investigate the effects of a 3.5 year closure on Boult reef and detected a significant increase in average size of coral trout. The same reef was subsequently opened to fishing and after 18 months a significant decrease in the average size of coral trout was detected (Beinssen, 1989b). No study, however, has investigated the effects of fishing on the age and sex structure of coral trout populations. The age and growth of *Plectropomus leopardus* has been recently validated (Ferreira and Russ, 1993; Chapter IV), making it

possible to effectively use age as an indicator of changes in population structure under different levels of fishing pressure and through time.

In 1987 a zoning plan was established in the central section of the Great Barrier Reef Marine Park, Australia, dividing the area into zones which allowed different activities. Under this plan, fishing was excluded from some areas. In the present work, samples taken from reefs in the central section of the Great Barrier Reef located in areas closed to fishing (National Park Zones) since 1987, are compared with samples taken from reefs located in areas open to fishing (General Use Zones). The effects of this 3-4 year closure on the size, age and sex structure of coral trout populations are investigated.

6.2- Material and Methods

Four mid-shelf reefs off Townsville, Central Great Barrier Reef (Fig. 6.1), were chosen as the sample reefs for this experiment. Two reefs, Grub and Hopkinson, were located in General Use Zones, and were open to line and spear-fishing, while the other two, Glow and Yankee, were located in National Park Zones, closed to line fishing since September 1987. The 4 reefs were sampled two times per year, during June-July and September-October, in 1990 and 1991 (Table 6.1). During each sampling trip, a crew of four line fisherman fished one reef per day (during the day light hours) for a period of approximately four hours. The same vessel was used for each trip. The fishing crew was relatively consistent in composition, with overall fishing ability maintained as consistent between trips as possible.

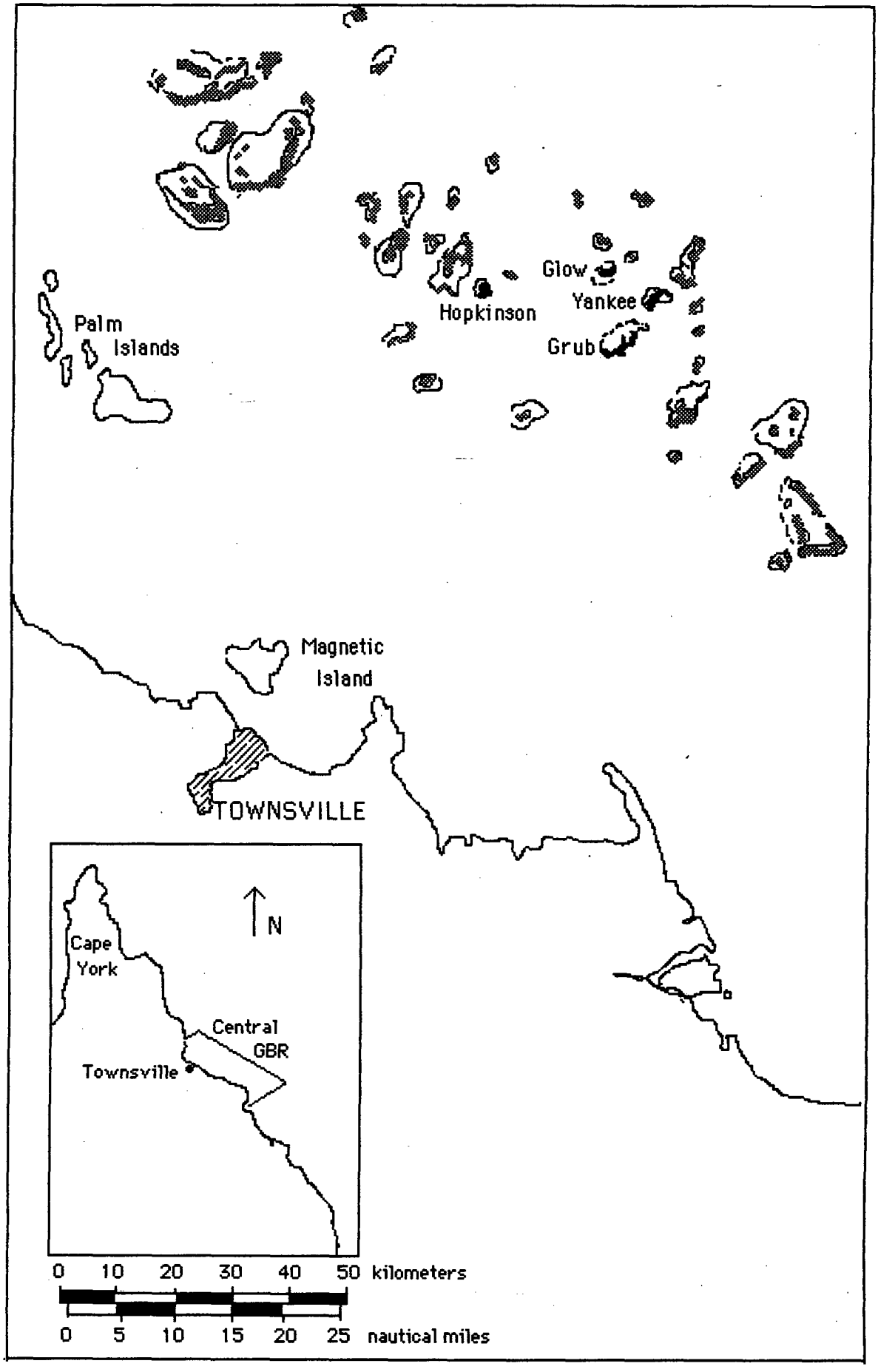


Figure 6.1 : Map showing the location of the sampled reefs: Glow and Yankee (closed to fishing) and Grub and Hopkinson (open to fishing).

	CLOSED		OPEN	
	GLOW	YANKEE	GRUB	HOPKINSON
JUN/JUL 1990	51	18	9	14
SEP/OCT 1990	49	42	11	17
JUN/JUL 1991	74	54	14	30
SEP/OCT 1991	23	11	15	15
TOTAL	197	125	49	76

Table 6.1: Dates and number of coral trout collected in each one day trip.

The results of CPUE obtained during the present experiment will be presented elswhere (Russ and Laycock, in prep.)

The fishes were measured and weighed and had their otoliths and gonads removed. The gonads were preserved in FAAC on board and sectioned and stained using standard techniques described in Ferreira and Russ 1992, 1993 and Chapter III and V. Each gonad was classified into one of the following gonadal developmental stages: immature female, mature female, transitional, young male and mature male (see Ferreira, 1993 and also Chapter III and V, for description of stages).

To determine the age of each fish, the otoliths were read whole or sectioned following the methodology described in Chapter IV. The number of opaque zones or rings were counted from the center to the margin of each otolith. Coral trout recruitment occurs in the first months of the year (Doherty et al., 1993), so the birth date was assigned as the 1st of January. Opaque zones are formed once a year, from July to November (Chapter IV), and are counted only when there is further deposition of a translucent zone, i.e., from December onwards. Therefore, the number of rings corresponded to the real age of the fishes.

Statistical Analysis:

Nested analyses of variance were used to compare mean age and size of coral trout between closed and open reefs (=status). Factorial analyses of variance and Kruskall-Wallis tests were used to compare mean size and age of coral trout on the four reefs, independent of the reef status. Multiple comparisons were performed using post-hoc tests (Tukey-Kramer, level of significance $p<0.05$), and pair-wise comparisons using Kolmogorov-Smirnov tests. Schnute's growth function (1981) was used to fit length-at-age data of coral trout for each reef using standard non-linear optimisation methods (Wilkinson, 1989). Schnute's model includes the von Bertalanffy, Richards, Gompertz, Logistic, and Linear growth models, which correspond simply to limiting parameter values. Analysis of covariance was used to test for differences in size-at-age between reefs. Chi-square contingency tables were used to compare the frequency of sexes between reefs. The assumptions of normality and homoscedascity were examined and data were transformed if needed (transformed data indicated in tables). Level of significance used was $p<0.05$.

6.3- Results:

There were no significant differences in mean size and age between protected reefs and unprotected reefs (status). However, the mean sizes and ages varied significantly between reefs within status level (Table 6.2).

TABLE 6.2: Nested analysis of variance comparing mean size and age of protected and unprotected reefs (status).

Source	df	SS	MS	F-Value	P-Value
STATUS	1	346.124	346.124	0.766	0.4737
REEF (STATUS)	2	903.323	451.662	10.898	0.0001
Residual	585	24245.827	41.446		

Dependent: FL (CM)

Source	df	SS	MS	F-Value	P-Value
STATUS	1	0.611	0.611	4.195	0.1771
REEF (STATUS)	2	0.291	0.146	9.378	0.0001
Residual	413	6.418	0.016		

Dependent: LOG AGE

Pos-hoc tests showed that mean size and mean age were larger for Glow than for all other reefs, while mean ages for Grub were smaller than for all other reefs. The mean sizes were not significantly different for Yankee, Hopkinson and Grub and the mean ages were not significantly different for Yankee and Hopkinson. (Figs. 6.2 and 6.3)

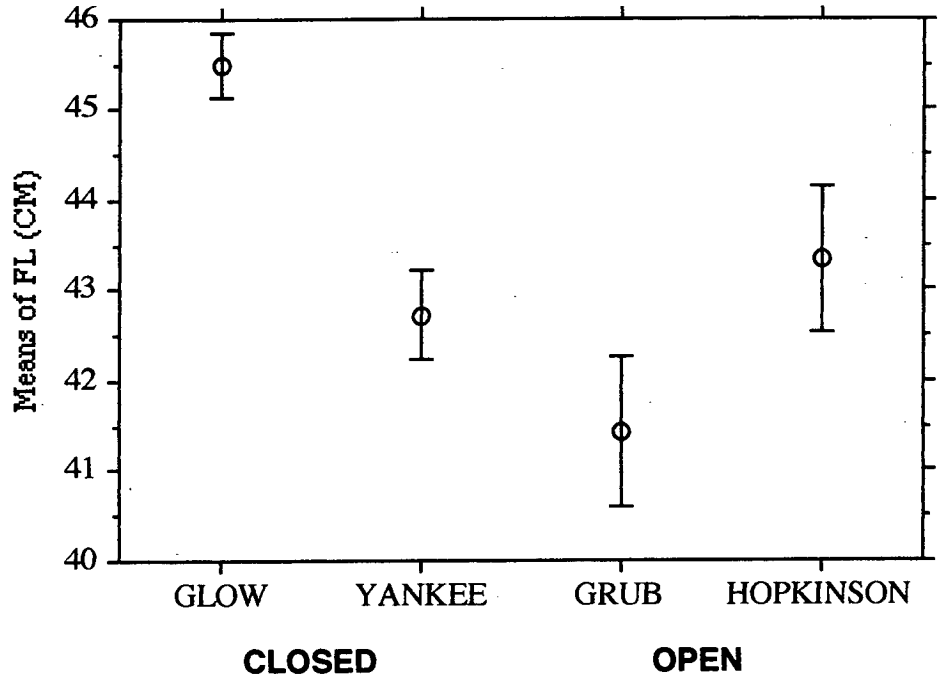


Figure 6.2 : Mean size (FL) for each reef and standard error bars.

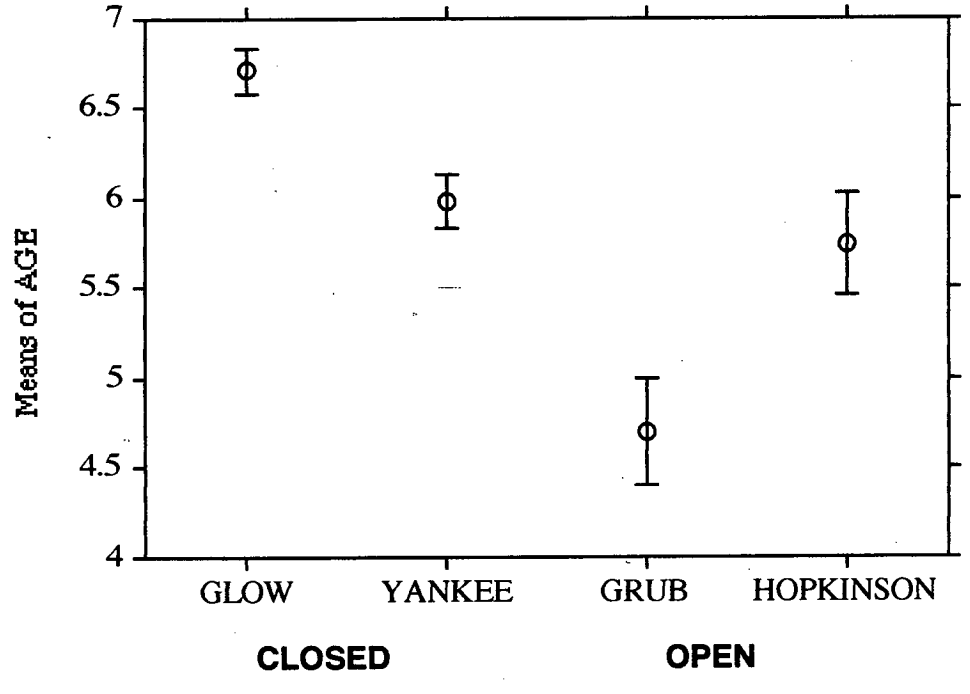


Figure 6.3: Mean age for each reef and standard error bars.

All samples were normally distributed except the age samples for Glow and Yankee, which were leptokurtic. Bartlett tests indicated that these samples were also heteroscedastic, but this test is affected badly by nonnormality

(Zar, 1984). Even under such circumstances, ANOVA is expected to be robust, as long as the sample sizes are large and nearly equal (Zar, 1984). Otherwise, the probability of type I error is increased (Zar, 1984). Kruskall-Wallis tests and indicated that there were significant differences in size and age structure between reefs (p=0.0001). Pair-wise Kolmogorov-Smirnov tests were then employed (Table 6.3). These tests failed to detect differences in three cases : Glow x Yankee- size and age, and Grub x Hopkinson- age. Kolmogorov-Smirnov tests are less powerful than ANOVAS, and are known to fail in detecting differences of up 50% in the abundance of each class in size distributions of coral trout (Crimp, 1984), but it is also possible that the poor performance of the ANOVA was the result of violating the assumptions of this analysis (i.e., leptokurtosis, heteroscedascity).

TABLE 6.3: Results of one-way anova (post-hoc tests, p<0.05) and Kolmogorov-Smirnov tests (KS) comparing pair-wise the mean size (FL) and age of each reef.

	GLOW	YANKEE	GRUB
YANKEE	Anova: FL: * AGE: * **KS: FL (p=0.07) NS** **AGE (p=0.121) NS**		
GRUB	Anova: FL: * AGE: * **KS: FL (p=0.023)*** **AGE (p=0.001)****	Anova: FL: NS AGE: * **KS: FL (p=0.111) NS** **AGE(p=0.014)***	
HOPKINSON	Anova: FL: * AGE: * **KS: FL (p=0.045)*** **AGE (p=0.015)***	Anova: FL: NS AGE: NS **KS: FL(p=0.209) NS** **AGE(p=0.128) NS**	Anova: FL: NS AGE: * **KS: FL (p=0.149) NS** **AGE (p=0.074) NS**

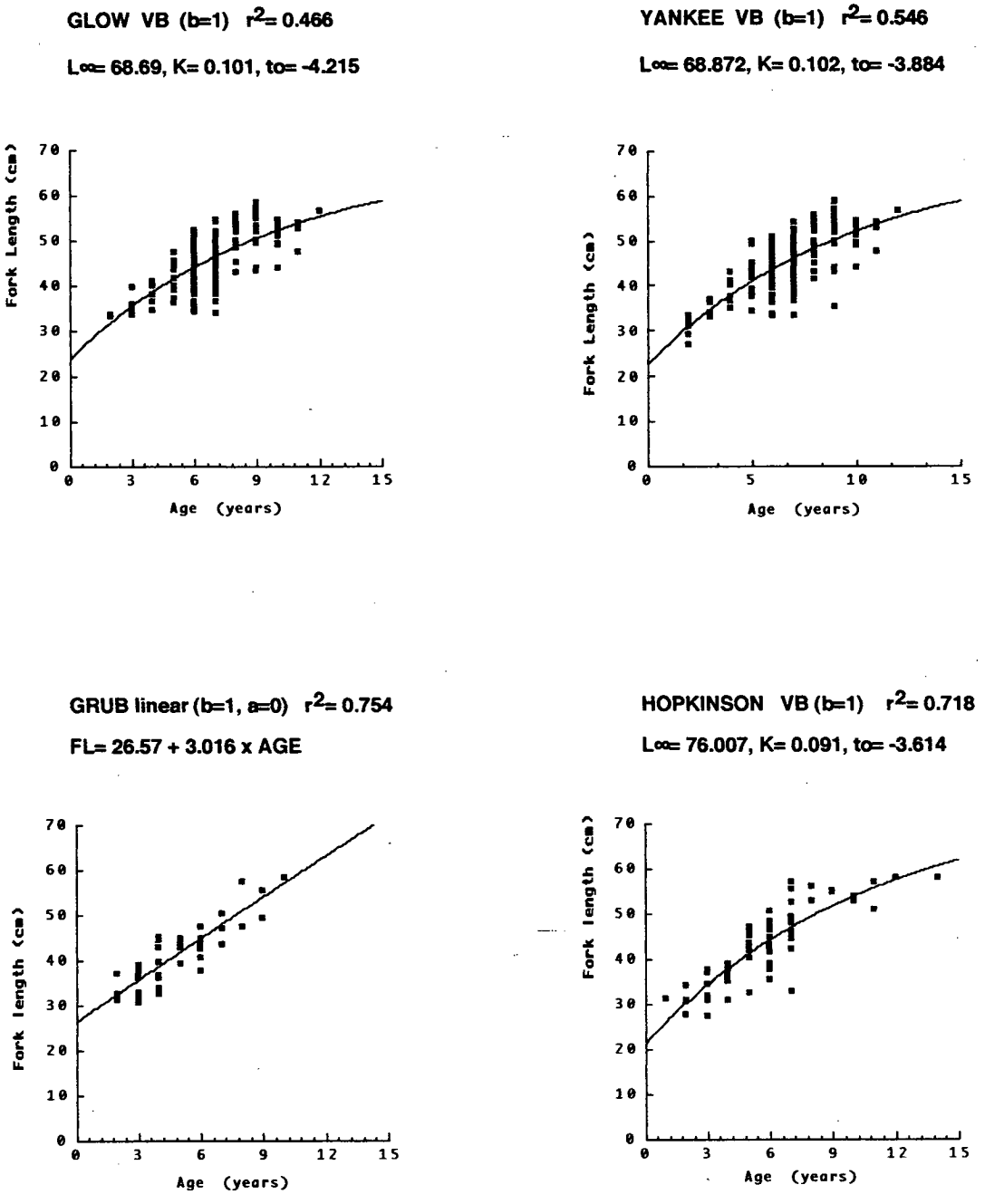


FIGURE 6.4: Size-at-age data and estimated growth curve for the coral trout *Plectropomus leopardus* from each sampled reef.

Growth:

Schnute's growth function was fitted to size at age data for each reef. The submodel corresponding to the von Bertalanffy formula (**b**=1) described data from all reefs well (Fig. 6.4). The estimated **A** (corresponding to the von Bertalanffy **K**) for Grub, however, approached zero, indicating that the data could be described also by a linear regression model.

Estimates of growth parameters are highly affected by different ranges of size-at-age data (Chapter IV). Therefore, for comparison of growth between reefs, the age range was limited to age classes occurring at all four reefs (2 to 10 years), and Schnute's growth function was fitted to these truncated data. For Hopkinson, Grub and Glow, estimates of **A** approached zero (Table 6.4) indicating linear growth. As the estimate of A for Yankee was also low, simple linear models were fitted to the data from all four reefs for comparative purposes (Table 6.3). Analysis of the sum of squares indicated that linear models were more appropriate to describe the growth data for all reefs with the exception of Yankee, for which an asymptotic model was more appropriate. The linear regressions obtained for each reef were compared using analysis of covariance. No significant differences were observed (P=0.276), indicating that the mean size-at-age, and therefore growth, did not vary significantly between the four reefs .

TABLE 6.4: Schnute's parameter A and r^2 values for non-linear and linear models for fish of ages 2 to 10.

	CLOSED		OPEN	
	GLOW	**YANKEE**	**GRUB**	**HOPKINSON**
A	0.080	0.102	0.004	-0.040
Non-linear r^2	0.450	0.546	0.754	0.669
Linear r^2	0.445	0.448	0.754	0.666

Analysing the age and size distributions of each reef:

Age distributions: Glow and Yankee had very strong modes in the year classes 6 and 7 (Fig. 6.5). Separating age distribution by year (Fig. 6.6), it is clear that these modes represent a strong year class, that is 6 year olds in 1990 and 7 year olds in 1991. This result rules out the possibility of selection towards one year class by fishing gear or bias in the age determination. This strong year class was not evident on the unprotected reefs (Fig. 6.6). At Hopkinson, year class 6 formed a small mode in 1990, but the pattern was not consistent, as year class 7 is not strong in 1991. In Grub, younger ages were proportionally more abundant, with the mode in the 3 year old class for two consecutive years.

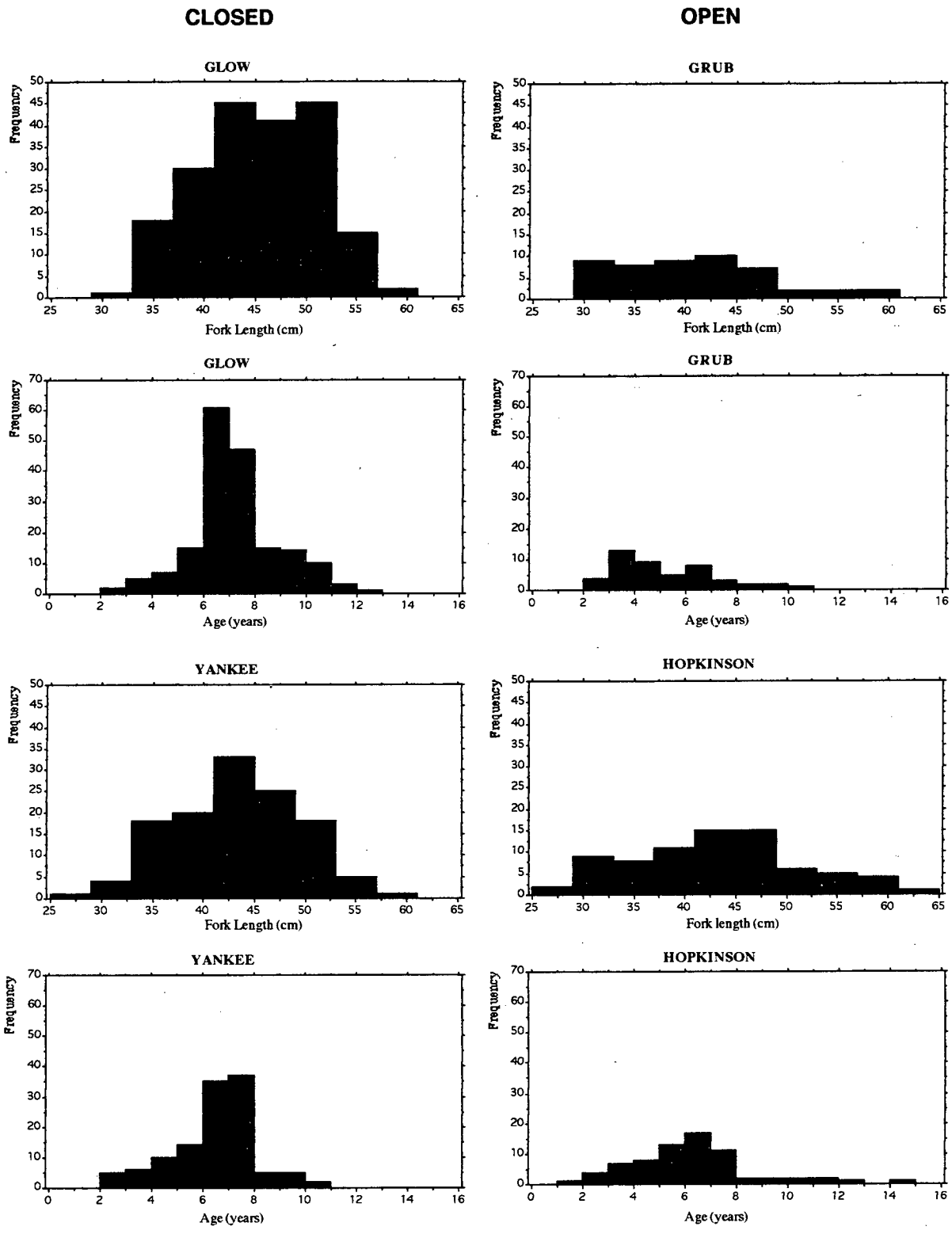


FIGURE 6.5: Size and age frequency distribution of coral trout for each reef, 1990 and 1991 data combined. Data are presented in the same scale for comparative purposes.

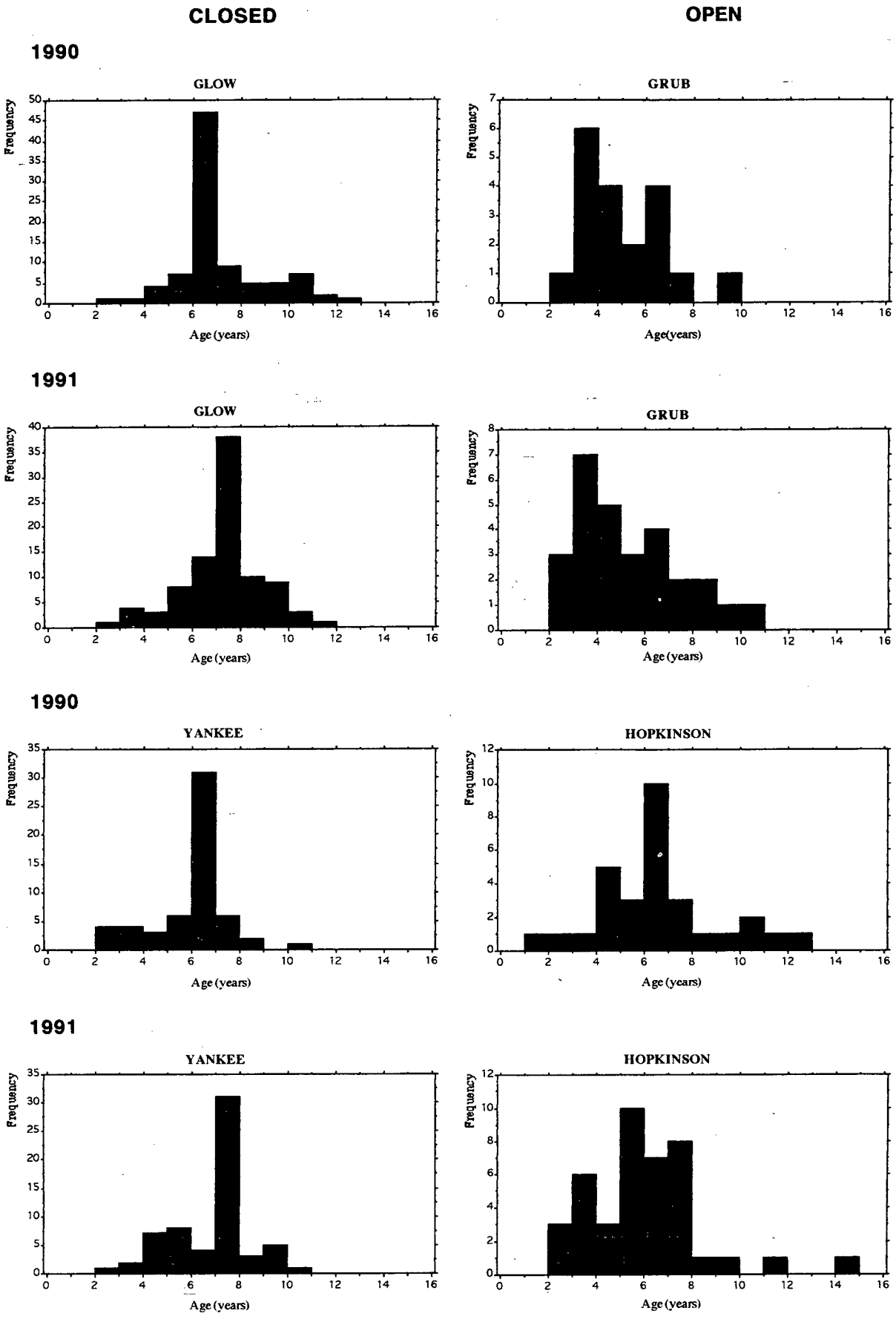


Figure 6.6: Age distribution of coral trout for each reef in each sampling year.

The 6+ year old class of 1990 and the 7+ year old class of 1991 settled onto the reefs at the begining of 1984 (Fig. 6.7). As Glow and Yankee have been closed to fishing since 1987, and age of recruitment to the fishery is approximately 3 yr. of age (Chapter IV), the individuals settling onto Glow and Yankee in 1984 have been protected most of their lives.

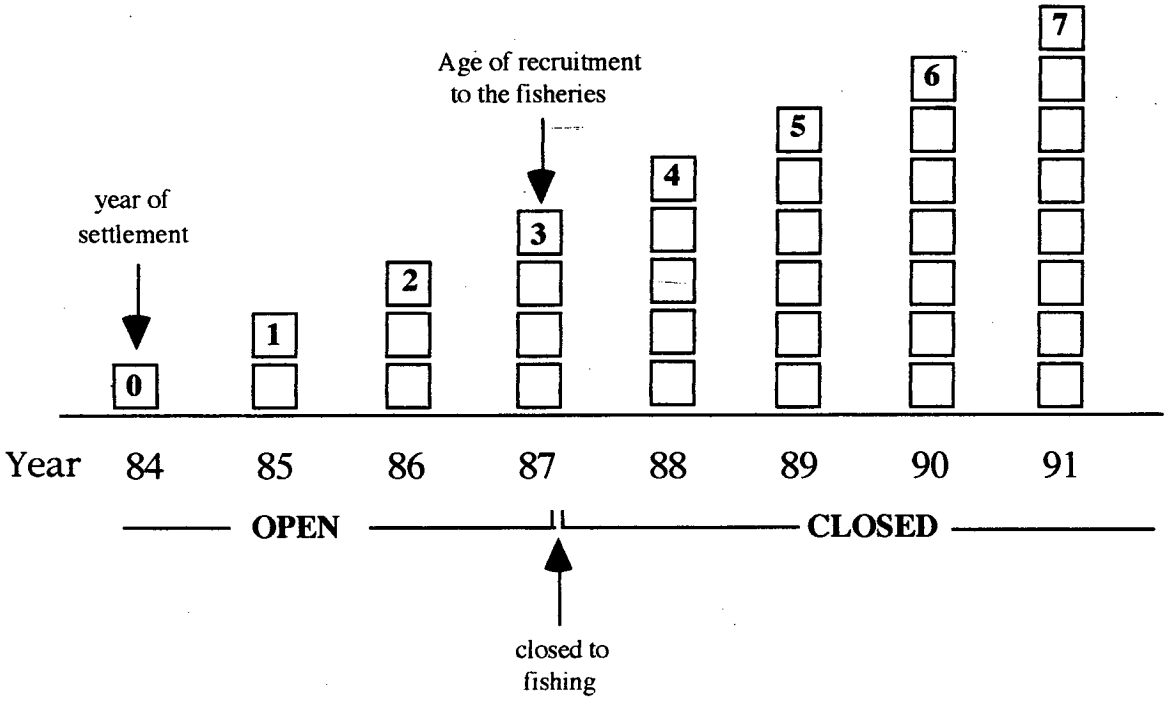
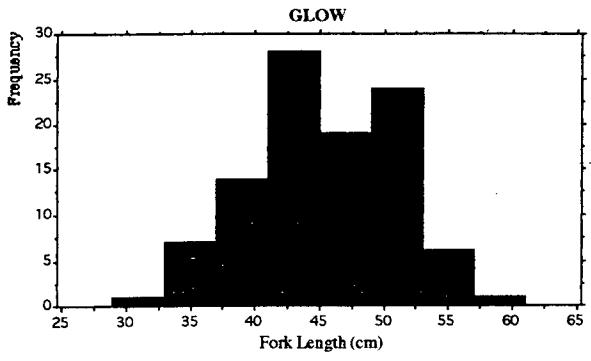


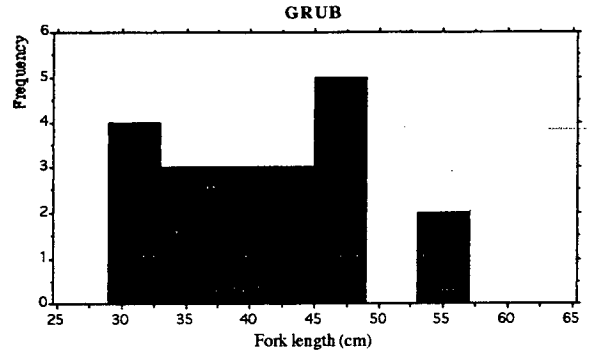
Figure 6.7: Diagram representing the strong year class from the settlement stage at the beginning of 1984 until 1991.

Modal progression was not particularly evident in the size distributions (Fig. 6.8).

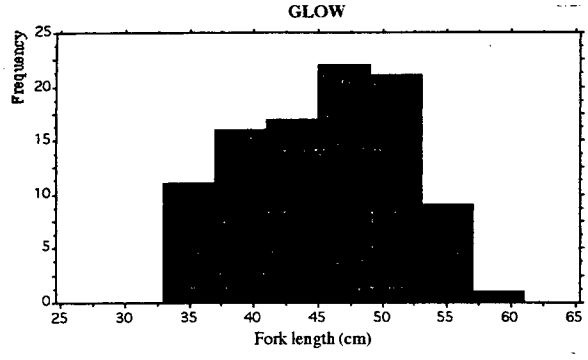
CLOSED OPEN

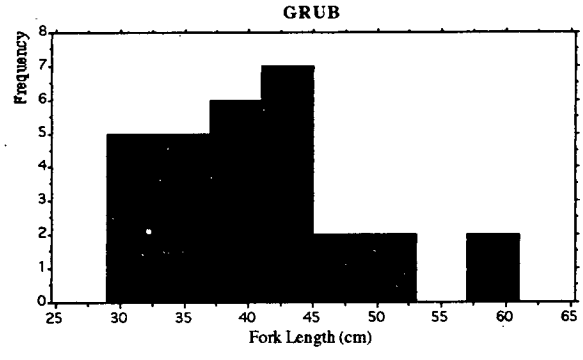
1990



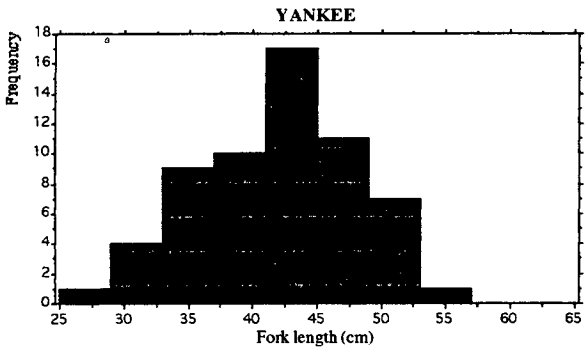


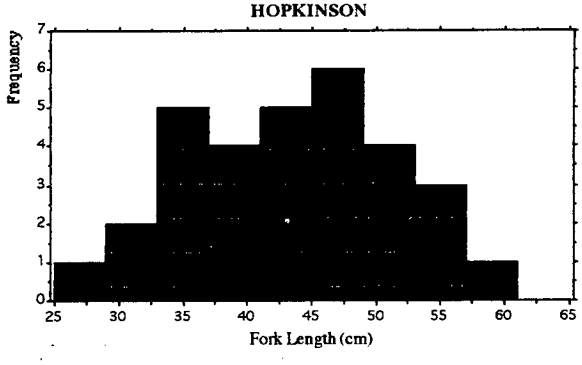
1991



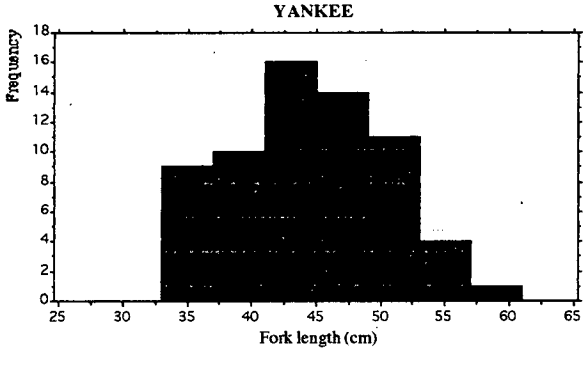


1990





1991



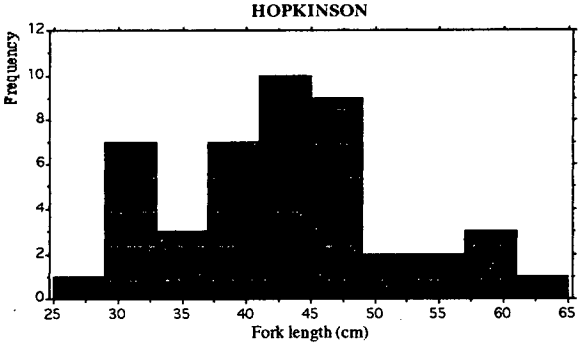


Figure 6.8: Size distribution of coral trout for each reef in each sampling year.

Sex structure:

The distribution of developmental stages by size and age (Fig. 6.11) indicated that sex-change occurs over a wide range of sizes and ages on the four reefs. The frequencies of developmental stages observed for each reef (Table 6.5) were compared using Chi-square. The frequencies were significantly different between all reefs ($p<0.05$), with the exception of the frequencies observed for Yankee and Hopkinson, as indicated by post-hoc testing ($p=0.245$). For the calculation of sex-ratio, frequencies of young males were pooled with frequencies of mature males, as individuals in both categories were sexually potential males. The resulting sex-ratios (Table 6.5) were not significantly different among reefs ($p=0.086$).

TABLE 6.5: Frequency (%) of each developmental stage in the four reefs and sex-ratio (mature females : young and mature males)

	Imm. Female	Mat. Female	Trans.	Young Male	Mat. Male	SEX RATIO
GLOW	1	80	8	8	38	**1.7:1**
	(1%)	(59%)	(6%)	(6%)	(28%)	
YANKEE	4	40	16	11	33	**0.91:1**
	(4%)	(38%)	(15%)	(11%)	(32%)	
GRUB	7	15	10	7	5	**1.25:1**
	(16%)	(34%)	(23%)	(16%)	(11%)	
HOPKINSON	5	36	9	4	15	**1.9:1**
	(7%)	(52%)	(13%)	(6%)	(22%)	

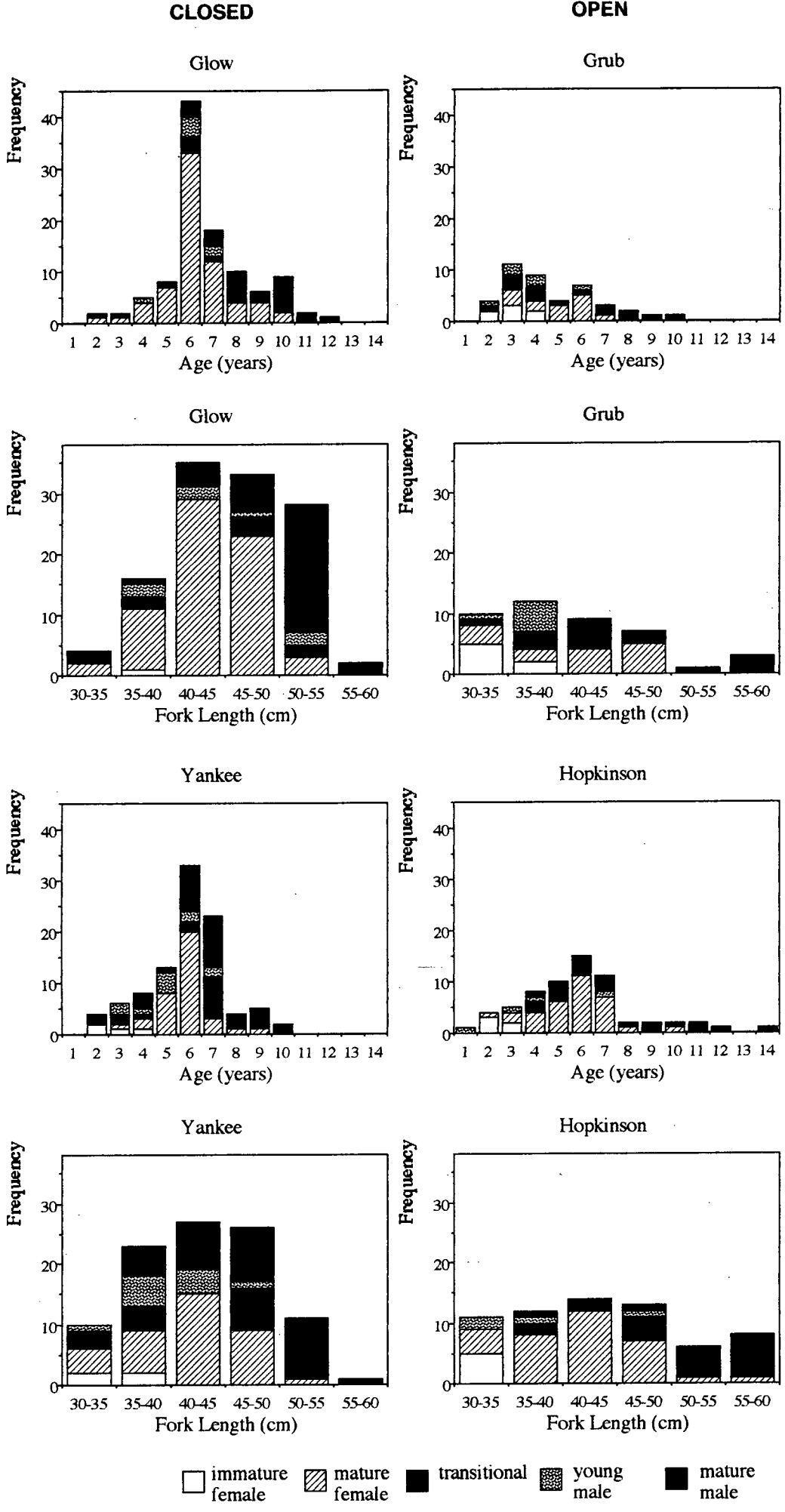


Figure 6.9: Distribution of developmental stages of the coral trout *Plectropomus leopardus* at each reef by age (years) and size (fork length).

The mean size of mature females was not significantly different between reefs (one-way ANOVA, p=0.208). The mean age of mature females was significantly different between reefs (log age, p=0.0077; age, p=0.0068), with mature females from Glow significantly older than mature females from Grub (post-hoc, p<0.05, Fig. 6.10).

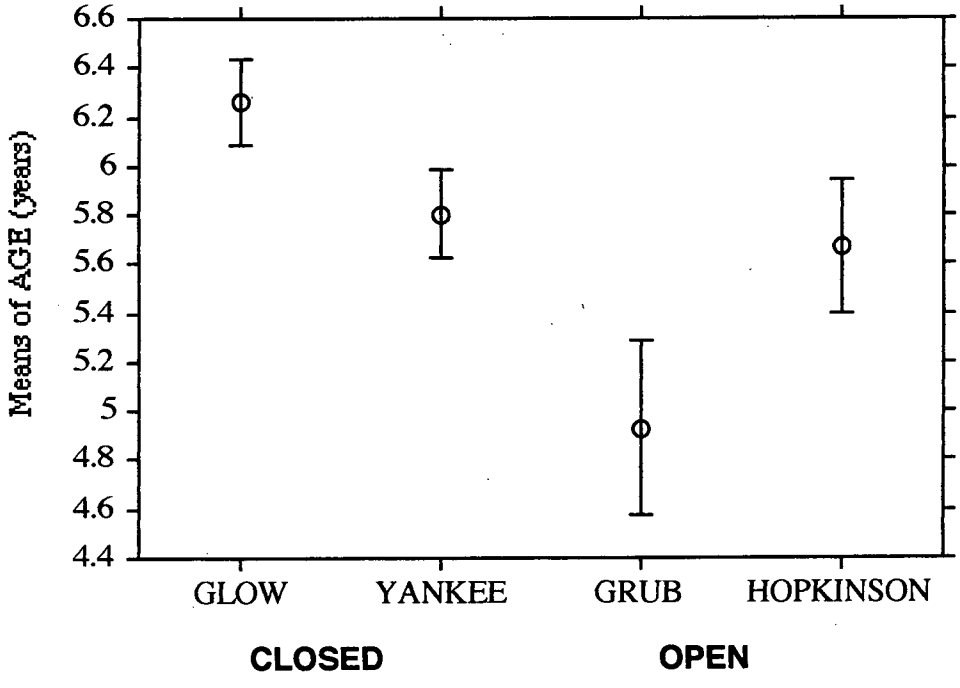
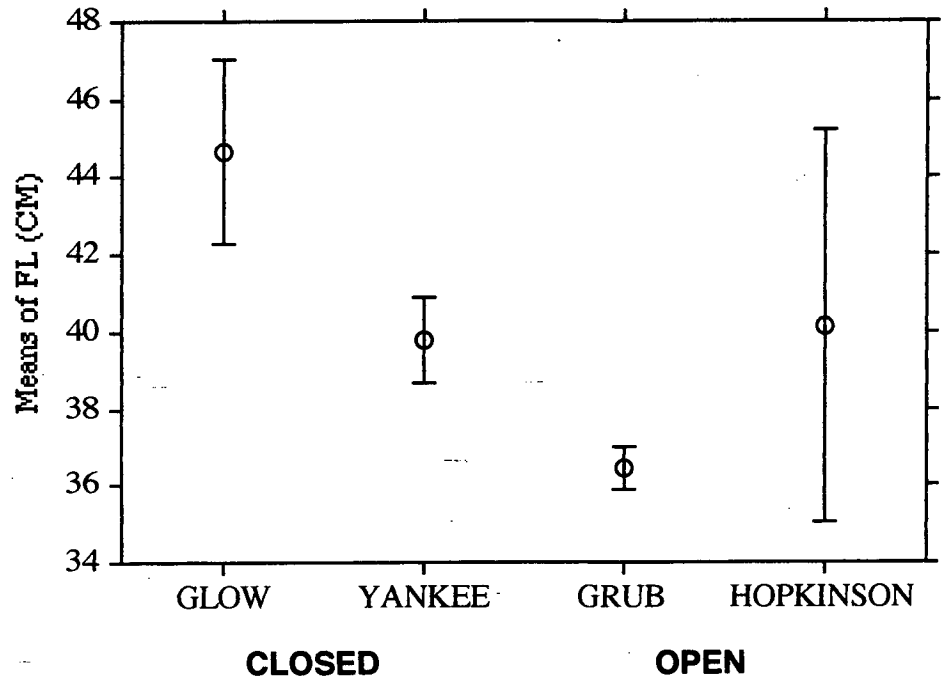


Figure 6.10: Mean age of mature females for each reef and standard error bars.

Age and size of transitionals were not significantly different between reefs (FL: p=0.2426, log AGE: p=0.1123).

Younger males from Glow had significantly larger sizes and ages than young males from Grub (FL: p=0.0414; log AGE: p=0.0311, Fig. 6.11).

A



B

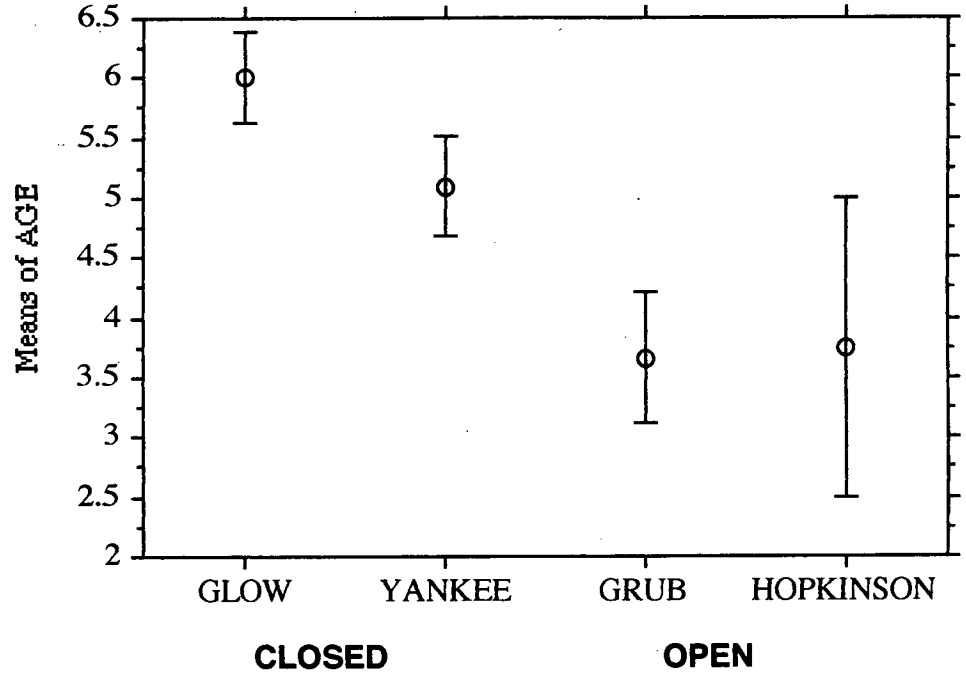


Figure 6.11: Mean size (A) and age (B) of immature males for each reef and standard error bars.

Age of mature males was not significantly different between reefs (p=0.2235). However, size varied significantly between reefs (p=0.0025), with mature

males at Yankee significantly smaller than mature males at Hopkinson (post-hoc $p<0.05$) (FIG. 6.12).

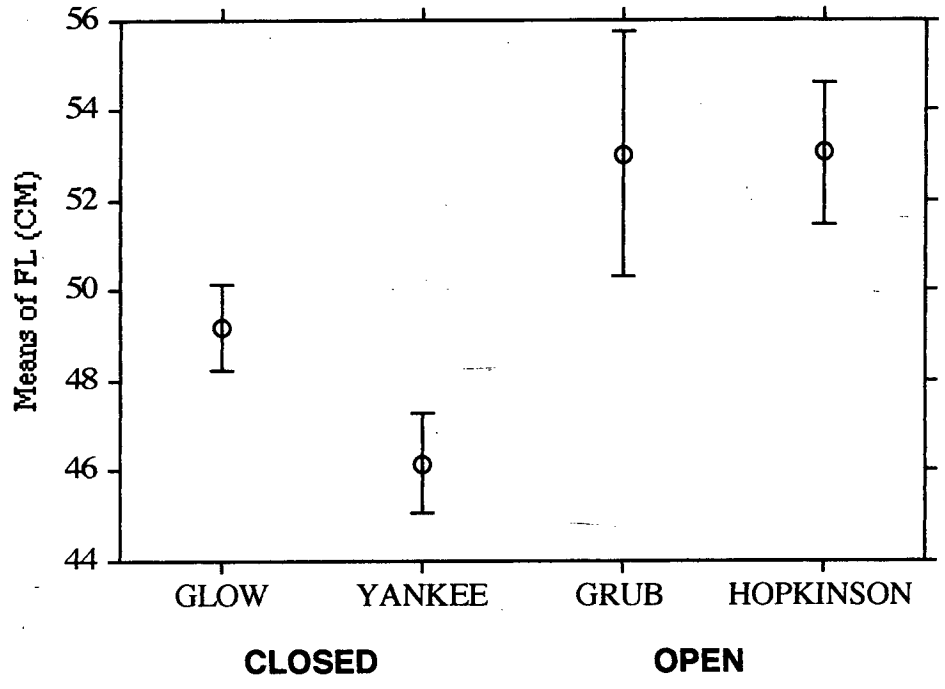


Figure 6.12: Mean size (FL) of mature males for each reef and standard error bars.

6.4: Discussion:

Expected effects of fishing are a reduction in the size and age range and average size and age of the population (Russ, 1991). In addition, line fishing might select for the larger and older individuals in a population (Ricker 1969, Miranda et al. 1987), what would exacerbate this tendency. Significant differences between size and age structures on closed and open reefs, however, will depend largely on the duration of closure in relation to the species longevity and fishing mortality. In the present study, given the short

period of time for which the reefs had been closed (3-4 years) in relation to the longevity of the coral trout (14+ years), great differences in the size and age structure were not likely to be detected. The failure to detect a significant difference between either mean size or age of coral trout on open and closed reefs, however, was largely due to variability between replicates. If in the present study, only Glow (closed) and Grub (open) had been compared, the result would reveal a classic effects of fishing scenario, with a larger range of sizes and ages and significantly larger mean sizes and ages observed on the reef closed to fishing. In contrast, if only Yankee (closed) and Hopkinson (open) had been compared, no effect of fishing would have been detected on the population structure. These results emphasize the importance of replicate reefs when analysing the effects of fishing on coral reef populations. More replicates (i.e. more reefs per treatment group) would increase the degrees of freedom and thus the power of the nested ANOVA.

One possible reason for the differences between the two open reefs is the fact that they are apparently not subject to the same fishing pressure. Grub is renowned for its excellent anchorage (Townsville Coast Guard, pers. comm.), and therefore is a favorite site for recreational and commercial fishing vessels. Aerial surveys conducted by the Great Barrier Reef Marine Park Authority between 1989 and 1992 (GBRMPA, data base, 1992), indicated that Grub is frequented by boats 2.2 times more than Hopkinson and that fishing vessels are sighted 3 times more frequently at Grub than at Hopkinson. Such factors should be taken into account in designing future sampling and experimental programs on the effects of fishing on the Great Barrier Reef.

Nevertheless, there was a major and consistent difference between the open and closed reefs analysed. For the two closed reefs, the population structure

was dominated by the presence of a strong year class which settled in early 1984. A similar pattern was not observed on the open reefs, with a corresponding mode not present at Grub and weak one at Hopkinson. Occurrence of strong year-classes is a well documented phenomenon in commercial catches of temperate species (Hjort, 1914; Rothschild, 1986; Sissenwine, 1984). For temperate species, year-class strength has been linked to early life history processes since the begining of this century (Hjort, 1914). However, for populations of coral reef fish, the importance of recruitment as a major driving force in the temporal variability of abundance has been recognized only recently (Williams, 1980, Doherty, 1981; Doherty and Williams, 1988 a, b).

There is evidence for the possibility of strong recruitment pulses of reef fishes occurring concurrently on midshelf reefs off Townsville which are separated by distances of up to 10-30 km (Doherty and Williams, 1988 a, b; Williams, 1991). The age structure data for the two closed reefs provides circumstantial evidence in support of pulses of recruitment being synchronous on reefs at least 10 km apart (Fig. 6.1). Assuming that the four reefs have received a similar pulse of recruitment in 1984, it is apparent that fishing mortality has operated to largely decrease the abundance of this year class. On the closed reefs, this strong year class was protected from fishing for almost its entire life and as a result had its strength maintained. In contrast, on the open reefs, the same year class has probably been supporting the fisheries in a disproportionate way in relation to the other age classes, having its abundance consequently reduced. An alternative hypothesis is that the settlement pulse occurred only on the two closed reefs due to some process independent of fishing.

A common question regarding the effects of fishing on protogynous hermaphrodite fishes is how the sex-structure of the population would respond to fishing mortality. If sex-change is determined by age and size, and selective removal of larger and older individuals occurs, this should result in a decrease in the proportion of males in the population. However, if sex-change is behaviourally induced, the population is expected to compensate to some extent for the selective removal of males by female to male sex-change, i.e., by changing sex at smaller ages and sizes.

The mean size and age observed for each stage also seemed to follow the size and age structure of each population. Mature females and young males were larger and older at Glow than at Grub, as individuals from Glow were on average larger and older than at Grub. Age and size of transitionals did not differ significantly between reefs, but with the high variability in the age and size of sex transition that characterizes the coral trout, and the small numbers of transitional individuals observed, this is not surprising. Mature males from Yankee were smaller than those at Hopkinson. This is possibly a consequence of the age distribution and consequent size distribution. Yankee had proportionally more individuals of 6 and 7 years of age, and not many in the older age classes, so most males would be in these classes, dragging the mean towards smaller sizes. At Hopkinson, the age frequency was more evenly distributed, without strong modal classes for years 6 and 7 and a wider range of age classes.

The comparison of frequency of developmental stages between reefs showed significant variation. When transitional and immature males were pooled with mature males for the sex-ratio calculations the resulting sex-ratio was not significantly different among reefs. The two unprotected reefs had a smaller proportion of males, but that seemed to be compensated for by the proportion

of transitionals and young males. It appears that while the distribution of developmental stages in the populations was different, the same final female: male balance was being achieved.

This type of result suggests that behavioural mechanisms are probably contributing to the determination of the distribution of sexes in the populations of coral trout. It is possible that for the coral trout, sex-change results from a combination of a developmental process, in which individuals are more susceptible to sex-change as they grow larger and older, and a social process through behaviourally induced stimuli. Genetic variability would widen the range in which sex-change can occur and phenotypic plasticity would allow individuals to respond to different social structures. Manipulative experiments are probably necessary to detect the exact mechanisms determining the distribution of sexes in coral trout populations.

Estimations of mortality rates are essential to fishery management, and yet few studies have made estimates of the rate of total mortality of coral reef fishes (Russ, 1991). However, an important assumption of the most commonly employed methods to estimate mortality (Beverton and Holt, 1956), is that all age groups have been recruited with the same abundance (Pauly, 1984). The present data represents a clear example of the problems that can result from the presence of strong recruitment pulses when calculating the total mortality rate Z from age or length structured catch curves. Because of a strong year class, estimates of Z calculated from the slopes of the catch curves would suggest very high mortality for the two closed reefs, compared to much lower mortality on the open reefs. Mortality estimates obviously can not be drawn from the present data with any confidence or from similar cases where significant recruitment fluctuation is retained in the age structure.

For the coral trout, the results observed here suggest the occurrence of strong interannual fluctuations in recruitment retained in the age structure. Similar evidence has been presented for only one other species of coral reef fish, the damselfish *Pomacentrus moluccensis* (Doherty and Fowler, 1993). With recruitment as a major factor driving the patterns of abundance, recovery of coral trout populations after closure may be largely dependent on a good pulse of recruitment. Thus recoveries of populations after closure to fishing are likely to be "events" rather than gradual "processes", with recovery potentially being rapid or slow, depending on the timing of closure with respect to occurrence of a very large year class. Furthermore, in the event of a strong recruitment pulse, population parameters will be highly influenced by this strong year class. Management effects can be greatly confounded by the occurrence of strong recruitment episodes, as in the classic Thompson-Burkenroad debate (Hilborn and Walters, 1992), making it essential to consider such processes when managing stocks of commercially exploited species, such as the coral trout.

One very practical aspect of these results for management of coral trout populations should be noted. A very large settlement of coral trout in any particular year (detected by, say, visual surveys of newly settled juveniles) is likely to be followed 3 years later by a large recruitment to the fishery. If stocks ever got to the stage where concern existed for low levels of spawning stock biomass, a management agency would have a 3 year lead time to close a larger than average number of reefs to allow a pulse build up of spawning stock biomass. Aditional reefs to be closed may be selected on the basis of oceanographic data which suggests that they are likely to be good sources of larvae in the future. It could also be argued that closure of a smaller percentage of reefs, with closures timed to maximize build up of spawning

stock biomass, may be more beneficial than closures of a greater percentage of reefs, but with closures timed poorly with respect to recruitment pulses.

Differences in age structure were more obvious than differences in the size structure between open and closed reefs. As the coral trout is a relatively slow-growing fish, differences in the size structure of a population will take longer to show up than differences in the age structures. Additionally, due to variability in growth, recruitment fluctuations may also pass unnoticed if size-structure data alone are examined. The results presented here indicate that age structure may be far more useful than size structure for comparisons of fishing effects on long- lived fishes such as epinepheline serranids. Comparisons of open and closed reefs based solely on mean sizes may fail to detect important differences.

Larvae from reef populations are likely to disperse over tens or hundreds of kilometers (Doherty and Williams, 1988 a, b), with colonization rates largely independent of local spawning effort. Local densities are believed to reflect the balance between settlement and survivorship, with balance achieved by nonequilibrial mechanisms (Doherty, 1991). Marine reserves are an efficient strategy in maintaning high abundances of reef fishes (Alcala and Russ, 1990). To assure effectiveness of closures, though, it is important to understand the processes determining differences in abundance. It seems clear that studies of the effects of closures to fishing on long-lived species should consider looking at age structure, must replicate reefs and must take into account strong recruitment pulses which may mask fishing effects. Additionally, when estimating mortality, the effects of strong recruitment pulses must be considered.

In conclusion, the results of this experiment have shown:

1- no significant differences between the mean size and age of coral trout in open and closed reefs, a result that could have been a consequence of variability among replicates, as one of the open reefs (Hopkinson) was apparently subject to less fishing pressure than the other (Grub). In addition, the duration of closure (3-4 years) was short relative to the longevity of coral trout;

2- Lack of difference in the overall sex-ratio despite observed differences in the sex-structure, suggesting social induction of sex-change;

3- The occurrence of a strong recruitment pulse, that may be extremely important in determining variation of abundance of coral trout, and therefore very relevant to the fisheries;

4- That age structure is more useful than size structure in detecting effects of fishing, and therefore that age determination must be a routine component in the management of coral trout populations.

CHAPTER VII: GENERAL DISCUSSION AND SOME DIRECTIONS FOR FUTURE RESEARCH

The use of periodic marks in otoliths to determine the age of tropical marine fishes

The formation of optically different zones in calcified structures of fish is usually associated with variations in growth rate, determined by temperature, photoperiod, feeding rate or reproductive cycle (Casselman 1983). When compared to temperate species, fishes in the tropics are not subject to large variations in temperature or photoperiod. This lack of marked environmental seasonality has lead to the widespread belief that marine tropical fishes would not be subject to growth oscillations (Longhurst and Pauly, 1987). Difficulties experienced by some authors in interpreting bands in calcified structures of tropical fishes has often reinforced this view (Thompson and Munro, 1983; Radtke, 1987; Ralston and Williams, 1988). However, Pauly and Ingles (1981) have shown that significant growth oscillations are frequently encountered in tropical fishes.

With the discovery by Pannella (1971) that tropical fishes could be aged by means of counting daily microstructural checks in otoliths, an important alternative was offered for ageing those tropical fishes which do not display annual rings (Ralston and Miyamoto, 1981; Ralston, 1985; Ralston and Williams, 1988). Since then, the technique of counting daily rings has been used extensively, and it is possible that in some cases, the presence of annual bands has been overlooked for tropical fishes, based on the preconception that they would not occur (Longhurst and Pauly, 1987). Age determination by means of daily rings is time consuming, with the degree of difficulty increasing with age (Brothers, 1987). Not surprisingly, estimation of growth rates of tropical fishes using otoliths has been perceived as a "difficult and persistent problem" (Ralston and Williams, 1988).

In this thesis, the presence of annual bands in otoliths was validated for two species of coral reef fish, *Plectropomus maculatus* and *Plectropomus leopardus*. These results add to other studies which have validated the presence of annual marks in otoliths of a number of species of tropical fishes (Samuel et al., 1987; Fowler, 1989; 1992; Lou,1992), and indicate the potential of this technique to be used routinely in stock assessment and management in the tropics. This is an important step toward the understanding of the dynamics of populations of coral reef fishes, as accurate estimates of age and longevity are of great significance to estimates of growth and mortality.

Beamish and McFarlane (1987) stressed the importance of accuracy in age determination. For both species, comparison of counts performed on whole and sectioned otoliths indicated that whole otoliths provided accurate readings only up to a certain age. However, as whole otoliths require much less time for analysis than sectioned ones, an useful strategy is to determine the limit of reliability of whole readings and thus incorporate the two techniques.

The growth of the otolith of *Plectropomus maculatus* and *Plectropomus leopardus* was continuous with age, a characteristic that poses obvious problems for the use of otoliths for back-calculation. Sizes of younger ages have to be determined directly, through the sampling of young fish.

Growth

The von Bertalanffy (1938) growth formula was appropriate to describe the growth of the two species of *Plectropomus* studied. Neverthless, a few

problems inherent to the interpretation of the parameter estimates of the von Bertalanffy model must be considered. The parameter L_∞, for example, is often assumed to be close to the maximum length (Pauly, 1984), and some authors may be surprised when deviations are observed. However, this would be the case only if every fish grew exactly according to a single growth curve, i.e., if there was no variation among individuals resulting from environmental or genetic influences (Francis, 1988; Hampton, 1991). Different age ranges can greatly affect the estimates of growth parameters (Knight 1968, Hirschhorn 1974; Mulligan and Leaman, 1992). Lack of length at age data for small sizes usually results in larger estimates of L_∞, accompanied by small values of K and large, negative values of t_0 (see Chapter IV). For *P. leopardus*, it was shown that the exclusion of younger ages resulted in a larger estimate of L_∞. Such a value was closer to the "expected" value of L_∞, i.e., closer to the maximum length. As most fisheries data lack observations at small sizes (Mulligan and Leaman 1992), it is possible that overestimated values of L_∞ have strengthened the view that this parameter should lie close to Lmax.

The large variability in size at a given age observed for *Plectropomus maculatus* and *Plectropomus leopardus* indicate the occurrence of individual variability in growth. Sainsbury (1980) demonstrated that when growth parameters vary between individuals, the reliability of methods of growth estimation like length-frequency analysis and growth increment analysis from marking-recapture techniques, is affected greatly. When using length at age data, however, this is not a serious problem (Sainsbury, 1980). For the two species of *Plectropomus*, it was assumed that individual variation in growth parameters resulted in similar coefficients of variation for all ages (Hilborn and Walters, 1992).

However, in most fish populations, each year class is subject to differential mortality in relation to size at some stage of their lifes (Ricker, 1969). Size-selective mortality, and therefore growth-rate related mortality, may be natural, caused by physiological processes, or be due to fishing mortality, when catchability varies according to size (Ricker, 1969; Parma and Deriso, 1990; Mulligan and Leaman, 1992). When size-selective sources of mortality are present, resulting patterns of size distribution at age may affect the perception of the underlying growth process, if the survival dynamics are not taken into account (Deriso and Parma, 1988).

It is very likely that size and growth-rate related mortality processes occur in coral trout populations (see Chapter IV). Demographic models incorporating growth variability and selective mortality are available (Parma and Deriso, 1990; Mulligan and Leaman, 1992). These models are beneficial because they can improve the representation of associated processes, such as mortality and fecundity (Parma and Deriso, 1990). However, large sample sizes and a time series of data are necessary in order to effectively assess the magnitude of such processes in a population. For commercially important species, this is a step to consider if management is to be conducted as efficiently as possible. In yield-per-recruit analysis, it is assumed that mean weights at age are constant, independent of the fishing mortality rate. Failure to consider the effects of different growth potentials can result in gross overestimation of optimal fishing levels (Parma and Deriso, 1990). Considering the importance of sustaining the fishery for coral trout, it seems that this species is an obvious candidate for this kind of approach in the future.

Reproduction

In this thesis, it was shown that *P. maculatus* and *P. leopardus* are protogynous hermaphrodites. An important question is how is sex change determined; as a developmental process, initiated endogenously when females attain a certain size and age, or as a social process, through male-female interactions? Sex-change is obviously related to size and age, as all individuals are born females and change to males later in life, resulting in populations where females are on average smaller and younger than males. Nevertheless, the overlapping of the sexes observed for *P. maculatus* and *P. leopardus* indicated that there was no specific age or size of sex-change for these species.

Comparison of samples of *P. leopardus* from reefs open and closed to fishing indicated that higher proportions of females seemed to be compensated for by higher proportions of transitional and young male stages. So it is possible that the selective removal of males can trigger sex-change as a form of compensatory mechanism.

While it is currently not clear how sex change is determined for the coral trout, it seems that sex-change results from a combination of a developmental process, in which individuals are more susceptible to sex-change as they grow larger and older, and a social process, through behaviourally induced stimuli. Genetic variability would widen the range over which sex-change could occur and phenotypic plasticity would allow individuals to respond to different social structures.

More information on behavioural aspects of coral trout reproduction is still necessary to understand the precise mechanisms of sex-change operating

in these populations. Manipulative experiments are probably necessary to detect the exact mechanisms determining the distribution of sexes in coral trout populations.

Effects of fishing

Expected effects of fishing such as reduction in the size and age range and average size and age of the population (Russ, 1991) will depend largely on the duration of closure of fishing in relation to species longevity and fishing mortality. Another important factor to be considered is variability between replicate experimental units. As demonstrated in the present work, open reefs can be subject to different levels of fishing pressure, according to specific characteristics of the reef, such as assess to good anchorage or proximity to the coast. This result emphasizes the importance of replicating reefs when investigating the effects of closures to fishing on long-lived species such as the coral trout.

An important outcome in the present work was the observation of strong year classes of *P. leopardus*, resulting from interannual fluctuations in recruitment. For temperate species, the importance of recruitment variability in the dynamics of abundance of exploited fish populations has long been recognized (Hjort, 1914; Rothschild, 1986; Sissenwine, 1984). A plethora of evidence exists for interannual variability in recruitment of coral reef fishes (see Doherty and Williams, 1988a; b; Doherty, 1991 for review). However, evidence of recruitment variability based upon age-structure evidence is available for only one non-exploited species, the damselfish *Pomacentrus moluccensis* (Doherty and Fowler, 1993).

It is important to be able to identify the occurrence of strong recruitment pulses as they will have a great influence on population parameters. Problems can arise, for instance, when calculating the total mortality rate Z from age or length structured catch curves, as the occurrence of large recruitment fluctuations violates the underlying assumptions of such methods.

If recruitment is the major natural factor driving the patterns of abundance (e. g. Doherty and Williams, 1988a; b), recoveries of populations after closure to fishing are dependent on the timing of closure with respect to occurrence of a large recruitment pulse. It seems that for the coral trout, fishing mortality operates to largely decrease the abundance of strong year classes on open reefs. If the occurrence of a strong recruitment episode is not detected, management effects can be greatly confounded (Hilborn and Walters, 1992), making it essential to consider such processes when managing stocks of commercially exploited species, such as the coral trout.

As coral trout are a relatively slow-growing fishes, differences in their age structures were more obvious than differences in their size structures, indicating that age structure is more useful than size structure for comparisons of fishing effects. Additionally, due to variability in growth, recruitment fluctuations may also pass unnoticed if size-structure data alone are examined. Comparisons of open and closed reefs based solely on mean sizes may fail to detect important differences. It is common for mean length at age data to be presented instead of the actual individual length at age data, masking the occurrence of variability in growth. As pointed out by Hilborn and Walters (1992), given the levels of variability in length at age that are common in fish populations, the simple presentation of a length versus age plot (rather than a mean length

versus age plot), would in many cases suffice to discourage the use of length as a substitute for age.

Production of strong-year classes, longevity, fecundity and population size have probably evolved as adaptations to environmental fluctuations (McFarlane and Beamish, 1992). While it is difficult to predict the occurrence of very strong year classes (Doherty, 1991; McFarlane and Beamish, 1992), determining their relative importance as compensatory mechanisms is essential to evaluate responses of exploited populations, as interactions between environmental conditions and fisheries can destabilize an exploited population (Fogarty et al., 1991). Marine reserves are an efficient strategy in maintaining high abundances of reef fishes (Alcala and Russ, 1990). However, to increase the chances of success of such management strategies, it is necessary to understand the processes determining this abundance. Therefore, studies of the effects of closures to fishing on long-lived species must look at age structure to detect the occurrence of strong recruitment pulses.

The spatial structure of coral reefs provides an excellent opportunity for experimental investigations (Hilborn and Walters, 1992). By monitoring the progression of a strong year class of coral trout through time, both in open and closed reefs, much insight can be gained about the interaction of factors such as age, size, growth, natural and fishing mortality and sex change in the structure of the reef fish populations.

Age-Based Population Dynamic Studies of Tropical Fish

Much of the justification for the development of length-based stock assessment techniques for the tropics (e. g. Munro, 1980; Pauly, 1980;

Pauly and David, 1981; Pauly and Morgan, 1987; Sparre et al., 1991; Gayanilo et al, 1992) was based on the presumed difficulty and expense of age-determination of tropical fishes. This thesis and other studies (e. g. Samuel et al., 1987; Fowler, 1989; 1990; Lou, 1992) have validated the presence of annuli which are relatively easy to read in whole and sectioned otoliths. Such findings open up the possibilities of routine, age-based stock assessment in the tropics.

In addition, this thesis has provided examples of three aspects of the biology of tropical fishes which may reduce somewhat the widespread utility of length-based stock assessment in the tropics. These three aspects are:

1- A large number of tropical fish may be far more long-lived than has previously been assumed (e. g. this study; Samuel et al., 1987; Fowler, 1989; 1990; Lou, 1992);

2- A great deal of natural variability occurs in size for a given age;

3- Considerable year to year variability in recruitment is probably very widespread in tropical stocks (Doherty and Fowler, 1993).

The first two points pose considerable problems for length-based estimates of growth rates, and the third poses problems for length-based (and age-based) catch curve estimates of mortality. The advent of routine age-determination may eventually see a trend toward age-based population dynamics studies of tropical fish.

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APPENDIX I: Oocyte stages of the inshore coral trout *Plectropomus maculatus* and the common coral trout *P. leopardus*.

Reproductive aspects of *P. maculatus* and *P. leopardus* were studied based on histological analyses of gonad material (Chapters II and IV). No differences in the mode of reproduction and the morphology of the reproductive system were observed between these two species. Both species present asynchronous oocyte development, with many stages of development occurring simultaneously in gonads of ripe females. Here, the stages of oocyte development most commonly observed in ovaries of females of *P. maculatus* and *P. leopardus* are described.

The stages of oocyte development were adapted from Yamamoto et al. (1965). Each stage was defined cytologically by size, appearance of the nucleus and the nucleolus and the type and localisation of cytoplasmic inclusions. Measurements refer to the range of sizes most frequently observed.

I- Pre-vitellogenic Growth:

1- *Gonium stage*: Small round cells, measuring around 10 μ in diameter. Cells in this stage are weakly basophilic, and usually occur in nests of three or more cells (Fig. A).

2- *Chromatin Nucleolus Stage*: Elongated cells, measuring between 25 to 50 μ maximum diameter. Oocytes have a narrow, basophilic cytoplasm and a conspicuous nucleolus. (Fig. A).

3- *Early Perinucleolus Stage*: Oocytes in this stage measure between 60 and 80 μ in diameter. The nucleus is larger in size and has numerous nucleoli located around its periphery. The cytoplasm is strongly basophilic, more so than in all of the other stages. Lampbrush chromosomes are formed in this phase (diplotene stage of meiosis) and are visible in the nucleus until the final stage of maturation, at which time the the meiotic divisions are continuing (Nagahama, 1983). (Fig. B)

4- *Late Perinucleolus Stage*: Differs from the previous stage by the general enlargement of the oocyte. Oocytes measure between 85 and 140 μ in diameter. Cytoplasm is less basophilic and therefore is stained lightly by the haematoxylin. A small basophilic juxtanuclear mass, termed "yolk nucleus" (Nagahama, 1983) is visible during this stage. (Fig. C).

II- Vitellogenesis:

5- *Yolk Vesicle Stage*: Yolk vesicles appear in the cytoplasm. The oocytes increase in size as the vesicles grow in size and number. Oocytes measure between 150 and 200 μ in diameter. The zona radiata is evident in this phase, measuring around 2.5 μ in thickness. (Fig. D).

6- *Early Yolk Globule Stage*: Yolk globules, formed by the fusion of small yolk vesicles (Nagahama, 1983), appear in the mid cortical zone of the oocyte. Oocytes measure between 150 and 200 μ in diameter. (Fig. E)

7- *Late Yolk Globule Stage*: Yolk globules, measuring up to 10 μ in diameter, increase in size and number, and most of the cytoplasm becomes occupied by these yolk globules. The oocytes in this phase measure between 300 and 450 μ. The zona radiata increases to 20 μ in thickness, displaying conspicuous striations (Fig. F).

8- *Ripe or hydrated stage*: The yolk globules fuse with each other forming a single, clear mass of yolk. The overall size of the oocyte is increased by

hydration to 500 to 550 μ in diameter. The zona radiata becomes reduced in thickness to 5 μ (Fig. G).

- *Post-ovulatory follicles*: Vitellogenic oocytes are surrounded by a follicular layer consisting of an outer thecal layer and an inner granulosa layer (Nagahama, 1983). Before ovulation, the follicle layer detaches from the oocyte and a wide space is formed between the follicle cell and the egg membrane (Nagahama, 1983). After ovulation, the follicle cells remain in the ovary constituting the post-ovulatory follicles (POF). These structures were observed only in the ovaries of *Plectropomus leopardus* captured shortly after ovulation (Fig. H).

Following pages: Plates 1 to 4: Transverse sections of ovaries of coral trout *Plectropomus leopardus*, stained with Haematoxylin-eosin. Abbreviations: **og**, oogonia; **cns**, chromatin nucleus stage; **n**, nucleus; **nu**, nucleolus; **lc**, lampbrush chromosomes; **yn**, yolk nucleus; **yv**, yolk vesicles; **yg**, yolk globule; **zr**, zona radiata; **th**, thecal layer, **gr**, granulosa cells; **pof**, post-ovulatory follicles.

Plate 1: **A** - Nest of oogonia and chromatin nucleus stage oocyte. Scale bar= 20 μ . **B** - Early perinucleolus stage oocyte. Scale bar= 20 μ

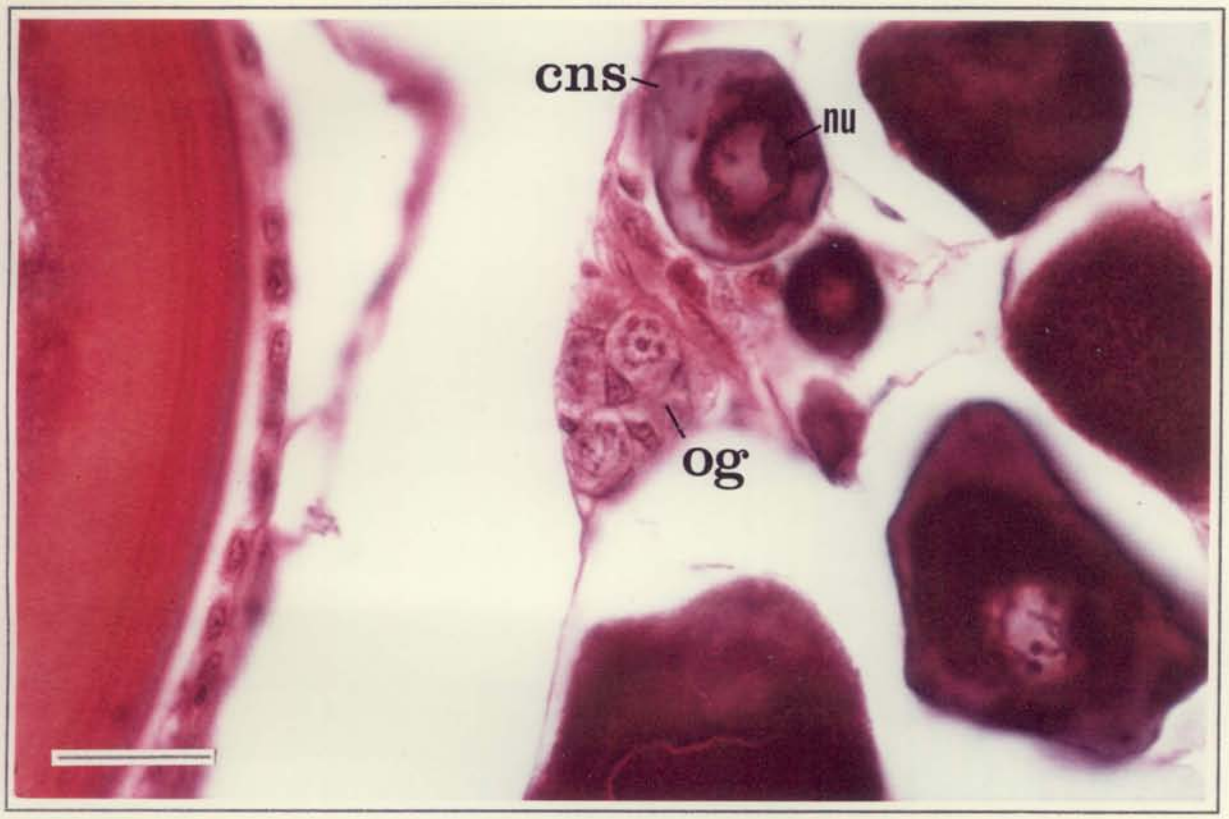
Plate 2: **C** - Late perinucleolus stage oocyte Scale bar= 20 μ. **D** - Yolk vesicle stage oocyte. Scale bar= 40 μ.

Plate 3: **E** - Early yolk globule stage oocyte Scale bar= 40 μ. **F** - Late yolk globule stage oocyte. Scale bar= 80 μ.

Plate 4: **G** - Hydrated stage oocyte Scale bar= 100 μ. **H** - Post-ovulatory follicle. Scale bar= 40 μ.

PLATE 1

A



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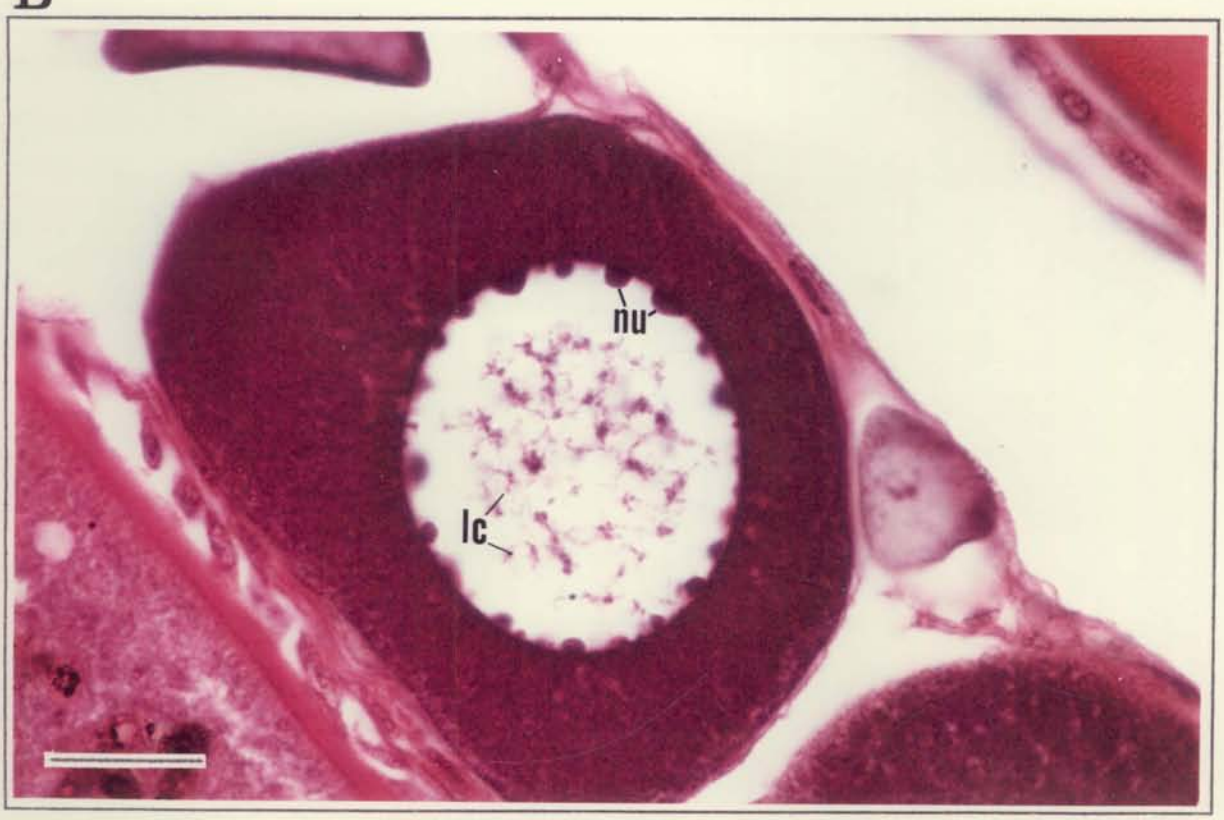
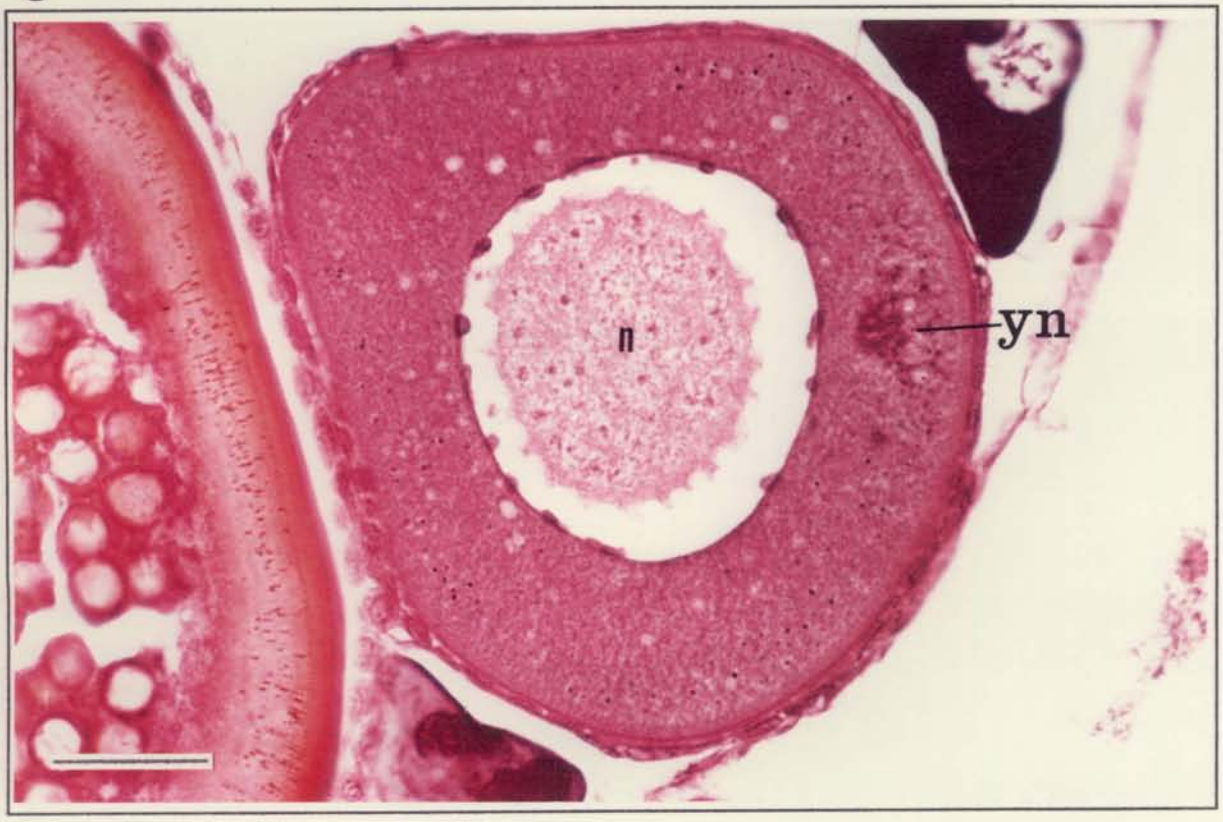


PLATE 2

C



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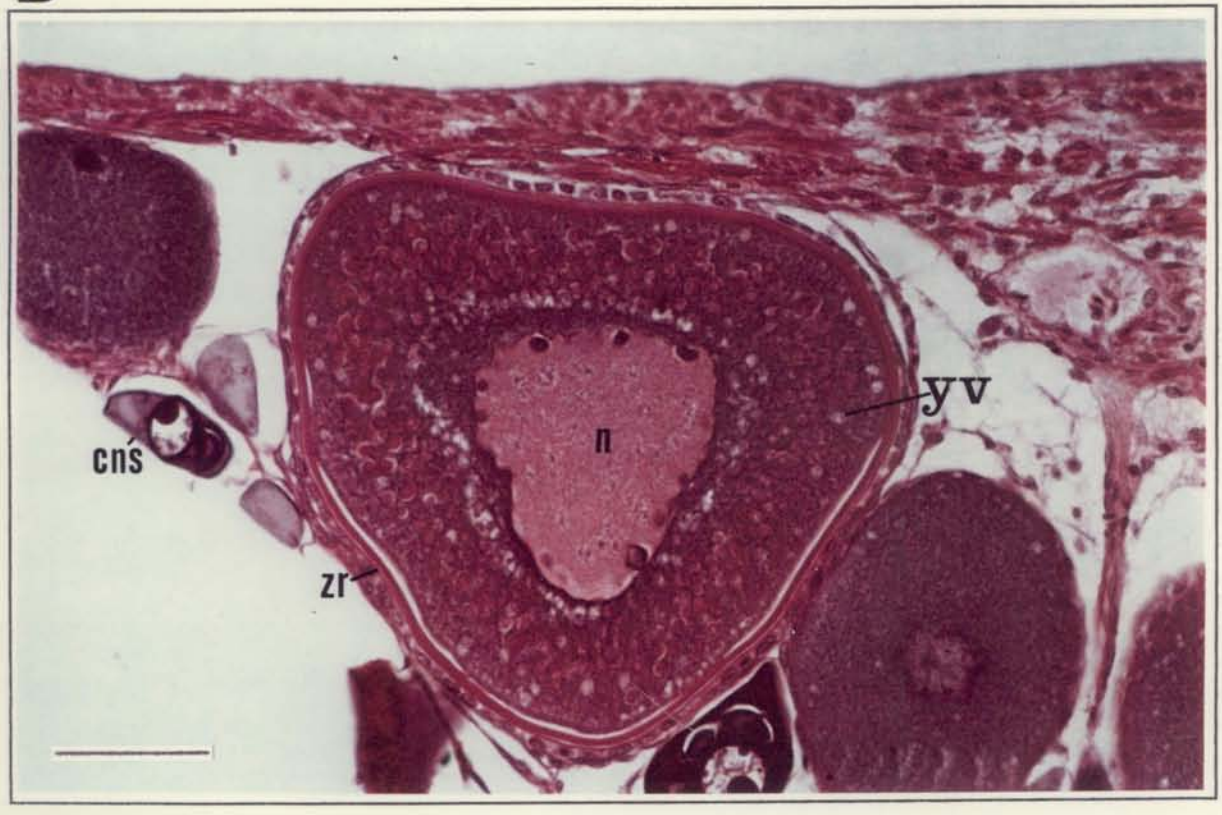

PLATE 3

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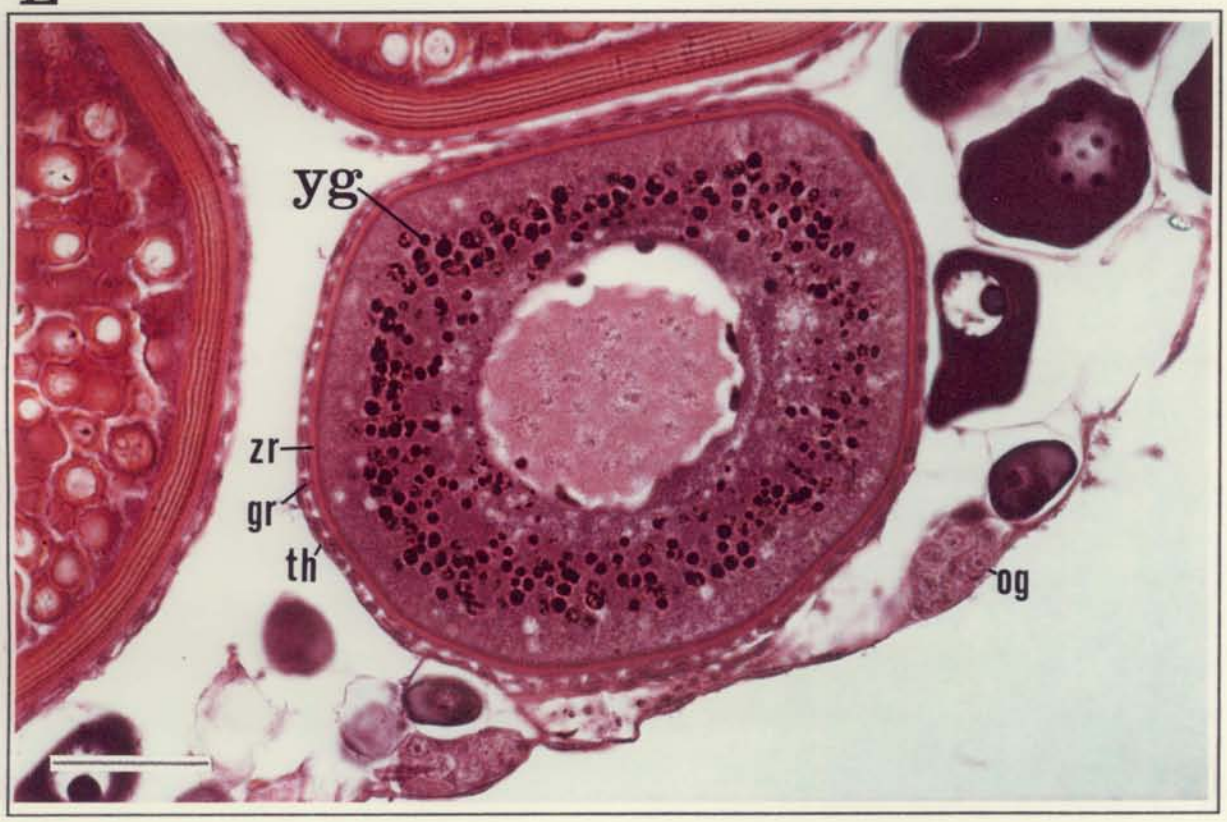

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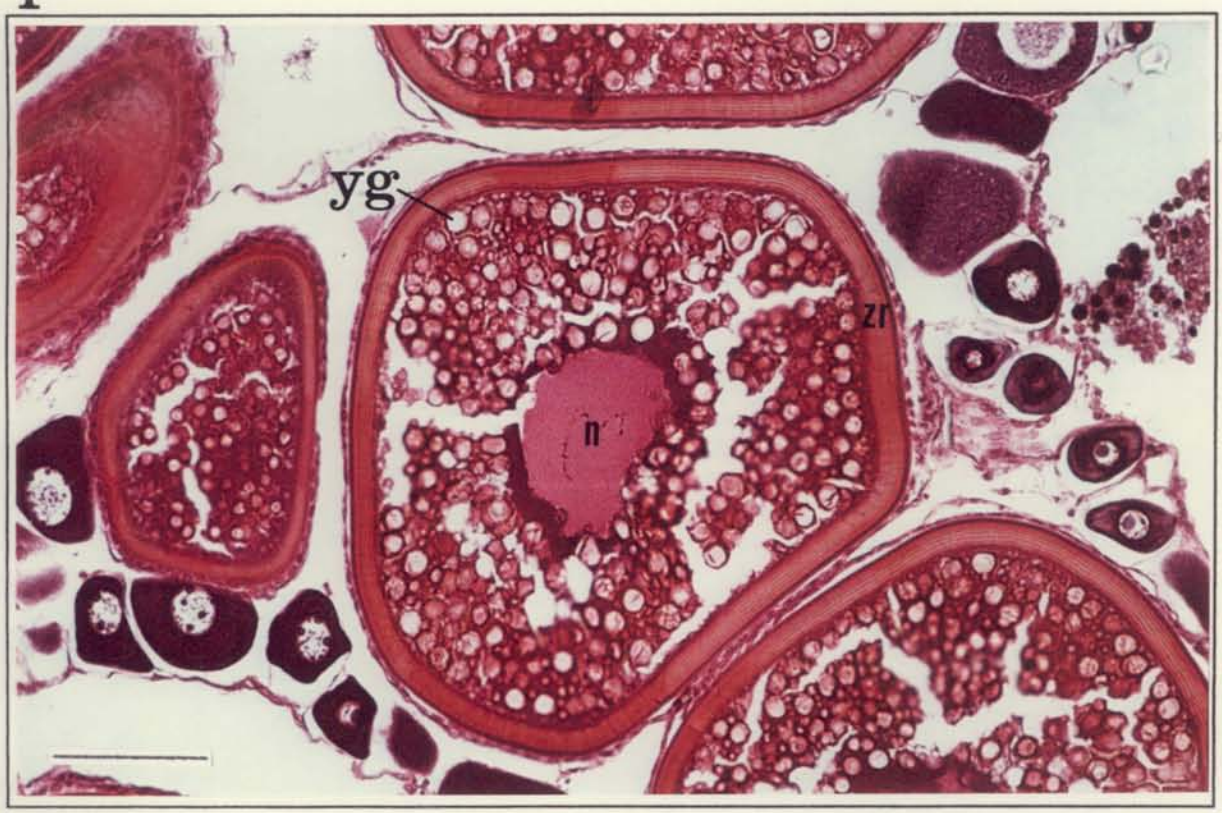
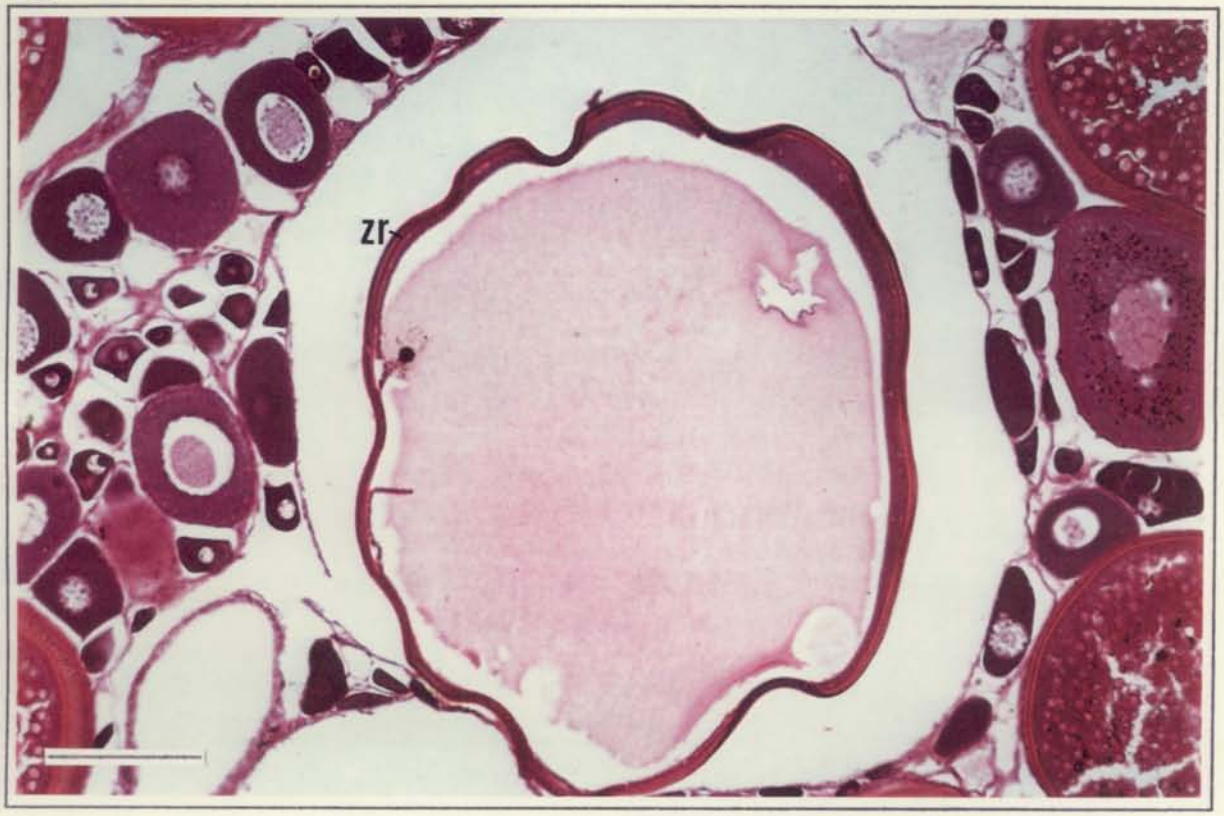


PLATE 4

G



H