Research on two serranid species (Serranidae: Epinephelinae) in Western Australian waters. (I) The biology, ecology, and mechanisms of sex-change in the chinaman cod (*Epinephelus rivulatus*) at Ningaloo Reef. (II) The biology of the bar-cheeked coral trout (*Plectropomus maculatus*) within the Pilbara trawl fishery.

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Summary

This study provides detailed biological and ecological information on the chinaman cod, *Epinephelus rivulatus*, and biological information on the bar-cheeked coral trout, *Plectropomus maculatus*, in West Australian waters. Both of these species are members of a large group of predatory fish known as the epinepheline serranids. Serranids are found throughout the tropical and subtropical marine waters of the world, and are valued catches within numerous fisheries. Although some species in the Caribbean have been well studied, there is generally a lack of detailed biological and ecological data on the serranids. In particular, there is little information on the processes controlling sex change in serranid species. This life history pattern, along with the fact that many are long lived and slow growing, may leave serranids particularly vulnerable to fishing pressure. However, without information on the mechanisms controlling sex-change, specific management options for serranid species remains somewhat speculative.

Various serranid species are targeted or caught as by-catch in Australian tropical waters. Nevertheless, apart from coral trout inhabiting the Great Barrier Reef, the biology and ecology of Australian serranids is poorly understood. The present study focussed on the chinaman cod since it is an important species within the Ningaloo Reef recreational fishery. Because it is abundant and accessible, the chinaman cod was also amenable to the logistically difficult task of determining the mechanisms of sex-change in this species. The study of coral trout biology was commenced in response to the capture of undersized fish within the Pilbara trawl fishery. As the current size limit may not be biologically appropriate, samples obtained from the trawl fishery were used to learn more about the biology of this species.

Within the lagoon of Ningaloo Reef, the chinaman cod is typically site attached and may form quite dense populations with sex ratios of about 5.5 females to each male. Individuals of this species undergo female-to-male sex-change, and consequently males are significantly larger and older than females. Males inhabit territories ranging from 94 to 200 m^2 that are defended from other males, whilst the home ranges of females overlapped considerably and measured between 58 and 151 m^2. Males formed alliances with one or two large females whereas smaller females had relatively little intersexual contact and often moved through the habitat of more than one male. Home range size varied widely and appears to be mainly influenced by habitat and resource availability than by body size or sex.

Chinaman cod may spend about 37 days in the plankton prior to settlement. The size at which 50% of females was 194 mm total length. Mature females within the samples ranged from 144 to 350 mm and 1 to 15 years (mean = 4.6 yrs), whilst males ranged from 221 to 381 mm and 2 to 24 years (mean = 9.4 yrs). Growth rate of the chinaman cod is rapid compared to other serranid species.

Size and age at which female chinaman cod change sex varied considerably. The size at sex-change was estimated at 264 to 315 mm total length depending on the location, whilst the age for all locations combined was 5 years. Sex-change in females is controlled by social factors such as the suppressive dominance of the males. Since males have greater reproductive potential, removal of this domination through the loss of a male can result in sex-change by a female. The transition of the gonad from ovary

to testis can be completed within three weeks, although the process may be delayed or halted by the intrusions of other males.

Reproductive activity in female chinaman cod probably begins in June, although most are reproducing from July through to December, and by January only a small number of females are still reproductive. The reproductive season spanned a water temperature range from the annual minimum of 21^0 C to 24.5^0 C (annual maximum was 28.8^0 C). Observations indicate that through the reproductive season *E. rivulatus* spawn during a short six-day period and are quiescent for about three weeks as a new batch of gametes are ripened. The spawning cycle is probably influenced by the lunar cycle and other environmental factors. Spawning takes place in the evening. Over the short spawning period males become much more active and aggressive towards each other whilst females show preference for spawning site and control the timing of spawning. Females may spawn two to three times each month, although competitive interactions probably influence the spawning success of both males and females.

The chinaman cod is a prominent species within the lagoon of Ningaloo Reef and may be more resilient to fishing than other serranid species. It is nevertheless vulnerable to overfishing and should be managed cautiously. This study has provided the first unequivocal evidence that sex-change in a species of serranid is socially controlled. Although caution should be taken when inferring that this is also the case with other serranids, it is likely that such information would be impossible to gain for many species. The chinaman cod is a useful species for further investigations into serranid ecology.

Information about the biology of the bar-cheeked coral trout was limited by the poor quality and the narrow size and age range of many of the fish obtained for sampling purposes. This species also has a life history in which individuals undergo female-to-male sex-change. The sex ratio of mature females to males in the samples was approximately 5.8 : 1. Mature females ranged from 289 mm to 576 mm total length and 1 to 8 years, whilst males ranged from 347 to 710 mm total length and 2 to 10 years. The size and age at sex-change were estimated as about 404 mm total length and 3 to 4 years.

Young coral trout may spend about 30 days in the plankton prior to settlement. The reproductive season of adult fish was July through to February, although there was a decline in reproductive activity during November and December. It was not possible to estimate the size and age at sexual maturity because of the lack of small fish within the samples.

A change in the state-wide size limit for coral trout from 450 mm to 410 mm total length may be recommended. This is nearer the size at sex-change in the bar-cheeked coral trout and will allow more of the undersized fish to be kept by the Pilbara fish trawlers. However, this recommendation is subject to analyses of the catch data for coral trout in other fisheries (such as the trap and line fisheries) showing that a reasonable proportion (e.g. at least 30%) of male fish are smaller than 410 mm total length. Otherwise overfishing of coral trout may occur in these fisheries if the cues for sex-change in this fish are endogenous.

Background

The epinepheline serranids (cods, groupers and coral trout) are major components of recreational and commercial fisheries throughout the world (Roberts & Polunin 1991). Coral trout are particularly important in Australian waters, comprising about 33% of the commercial and recreational catches within the Queensland line fishery. In Western Australia, coral trout are targetted by both commercial and recreational line fishers at the Houtman Abrolhos. In more northern waters of WA they are also caught by trapping, droplining and demersal fish trawling. The Rankin's cod (*Epinephelus multinotatus*) is the dominant catch within the North West Shelf trap and line fishery, and the chinaman cod (*E. rivulatus*) is a major component of the Ningaloo Marine Park recreational fishery. Serranids also comprise about 22% of the total catch of fish taken from the "Timor Box" (an area to the West of Darwin) and are a significant part of the bycatch in various other trawl, line and trap fisheries (Western Fisheries Research Committee Report 1992, Kailola *et al.* 1993).

Little research has been done on the serranids in Australian waters. Thus management decisions are usually made with minimal understanding of the biology or ecology of the exploited species. Of considerable concern is the fact that the majority of serranid species have a protogynous hermaphroditic life history. As a consequence, males are generally fewer in number and larger in size than females within the population. Being larger and often more aggressive, males are also more prone to capture by fishers (Roberts & Polunin, 1991). A major determinant of how loss of males affects population stability is the nature of the mechanisms that control sex change in females. There is, therefore, a real need to learn more about the phenomena of sex-change in the serranids (Ferreira 1993, Williams & Russ 1991, Huntsman & Schaaf 1994). However, whilst much research has been accomplished into protogyny in small species of hermaphroditic reef fish (e.g. wrasses; Fisherson 1970, Robertson 1972), the intensive fieldwork needed for these studies has thus far proven impossible on the larger serranids, because they are usually far more dispersed and inhabit more remote areas.

Need

This study provides comprehensive data on the biology and ecology of the chinaman cod, *Epinephelus rivulatus*, at Ningaloo Reef, Western Australia. This data is essential for the conservation of *E. rivulatus* within the Ningaloo recreational fishery and in other areas where this species is harvested. This study also provides unequivocal evidence of the mechanisms of sex change in an epinepheline serranid. Such research is regarded as vital yet difficult and costly to achieve, and the results of this research will be of interest to fisheries managers throughout Australia and overseas. Finally, this study provides biological information on the bar-cheeked coral trout (*Plectropomus maculatus*) caught within the Pilbara trawl fishery of Western Australia.

Objectives

The aim of this research programme was to provide data needed for the effective management of serranids, including:

- Ecological and biological data on the chinaman cod within the Ningaloo recreational fishery.
- Experimental simulation of line fishing on chinaman cod populations to determine how the social system of this fish is affected by fishing, and its ability to withstand such pressure.
- Experimental manipulation of chinaman cod social units to determine the mechanisms controlling sex change and the effects of sustained fishing pressure on these mechanisms.
- Biological and catch data on the bar-cheeked coral trout caught in the Pilbara trawl fishery. A solution to the present wastage of undersized trout caught in this fishery will also be sought.

Most of these objectives were accomplished. The experimental simulation of line fishing on chinaman cod populations was not completed because of the high rate in which the tags used to identify individual fish were lost. Nevertheless, the effects of fishing on populations of the chinaman cod were examined using the biological and ecological data. Due to the poor quality of the coral trout samples that were received from the commercial trawler operators, the biological data on this species is not as comprehensive as that of the chinaman cod. Because catch data on the coral trout were rarely recorded by the trawler operators, this information is also not presented.

1 Biology And Ecology Of The Chinaman Cod, *Epinephelus rivulatus.*

1.1 Reproductive Biology

1.1.1 Materials And Methods

Study Sites

Ningaloo Reef is the largest fringing reef in Australia, enclosing a shallow lagoon which varies in width from 200 m to over 6 km. Within this lagoon *E. rivulatus* is abundant over sand and limestone substrata with high algal cover, but is rare in coral rich habitats (Ayling & Ayling 1987, *pers. obs.*). Ningaloo Reef was protected for its high conservation value in 1987 as the Ningaloo Marine Park, which stretches for 260 km along North West Cape. The Marine Park is open to recreational fishing apart from certain areas (Sanctuary Zones) in which fishing has been prohibited.

Information on the population structure of *E. rivulatus* was obtained from study sites in Sanctuary Zones at Mangrove and Osprey Bays (Figure 1.1.1). In Mangrove Bay the lagoon was 2 - 3 m deep and the topography uniformly flat with few coral heads or other structures present over a wide area. The substratum was hard limestone covered by a thin veneer of sand. Two study sites were located in this area where algae and holes in exposed limestone provided cover for *E. rivulatus*. Currents through the study areas were either from the south or north, and there was often a surge present at high tide. At Osprey Bay the two sites were located along a limestone reef slope and adjoining reef flat. Most *E. rivulatus* and other fish were located along the reef slope which abutted a sand channel and extended upwards at angles between the vertical and 45º. Tidal currents flowed through the channel in a southerly direction and water depths varied from 6 m within the channel to 2 m on the reef flat. The reef was comprised of an eroded limestone base covered by sand and algae. In many areas the limestone was exposed and provided numerous small ledges and caves for shelter. Coral colonies (mainly *Acropora* and *Pocillopora* spp.) were present but these were usually small and scattered.

Other reproductive data were obtained from *E. rivulatus* captured from areas open to fishing activity at Tantabiddi and between North West Cape and the Murion Islands (*E. rivulatus* captured here are referred to as NWReef fish; refer to Figure 1.1.1). At Tantabiddi the water was 2 - 4 m deep. The habitat was flat and mainly sand, with the occasional large porites massive or smaller coral head, and low mounds of exposed limestone and algae on which *E. rivulatus* were most abundant. This shallow area at Tantabiddi was near a deep oceanic passage through the reef and was probably not subject to high fishing pressure. NWReef fish were captured from a large area outside the reef lagoon where currents were typically strong and depths ranged from approximately 10 - 50 m.

Reproductive Biology

E. rivulatus used for analysis of reproductive biology were collected between August 1994 and December 1995 within the lagoon at Tantabiddi and Osprey Bay. On each sampling occasion approximately 20 - 30 *E. rivulatus* were captured using speargun or

hook and line. The fish were captured between 0800hrs and 1100hrs in 2 - 3 m of water and processed within four hours of capture. *E. rivulatus* captured from NWReef by recreational anglers were also used in reproductive analyses. These fish were stored frozen before being processed. Total and cleaned (viscera and gills removed) weights to the nearest gram, and fork and standard lengths to the nearest millimetre were recorded for each fish (in *E. rivulatus* fork length and total length are the same). All lengths given in this paper are of fork length. Gonads were removed and preserved in 10% formalin or FAACC (Formaldehyde 4%, Acetic Acid 5%, Calcium Chloride 1.3%; Winsor 1984). Preserved gonads were weighed to 0.001 gm, sectioned transversely at 5 - 7 μm and stained using Haematoxylin and Eosin for histological examination.

Sex ratio data were estimated for populations of *E. rivulatus* within the study sites at Mangrove and Osprey Bays. The sex of most *E. rivulatus* within these sites was determined by cannulation as part of a study into the home range and spatial organisation of *E. rivulatus* (see Chapter 1.4). Cannulation involves the insertion of a small diameter polyethylene tube (cannula; outside diameter 0.96 mm) into the genital pore. The cannula is then slowly withdrawn while gently aspirating by mouth to obtain a gamete sample (Shehadeh *et al.*1973). Samples from non-reproductive or immature females were difficult to obtain because previtellogenic oocytes were usually too tightly bound within the stroma. Cannula samples were preserved in an aqueous solution of 1% formalin and 0.6% NaCl, so that they could be verified in the laboratory with a microscope (Shehadeh *et al.*1973). Even during the reproductive season some fish could not be sexed because gamete samples were not obtained (between 4 and 20% of total number cannulated, depending on the site). Nonetheless, the relative size of these fish and subsequent behavioural observations usually gave a reliable indication of gender. Many small individuals (<185 mm fork length) were not cannulated because the cannula could not be easily inserted. These were deemed immature females (IF) because they were smaller than the size (194 mm fork length) at which 50% of female *E. rivulatus* were sexually mature, based on histological samples (see Results). The structure of the male gonad prevented insertion of the cannula tube further than a few millimetres, although samples of sperm were usually easy to obtain from reproductively active males.

Description of the internal structure of the gonad, gonad maturity and evidence for hermaphroditism were determined from the histological samples. Nomenclature for description of the stages of oogenesis and spermatogenesis were taken from Yamamoto (1956). The developmental stage of each gonad was classified according to a modified version of that used by Moe (1969). This scheme was modified so that fish that had recently spawned but were still capable of further spawning during the course of the present reproductive season (F4) were distinguished from those fish which had recently spawned and were not likely to spawn again during the present season (F5). The presence of post-ovulatory follicles was used as evidence of recent spawning, with the description of these in northern anchovy (Hunter & Goldberg 1980) used to estimate the age of post-ovulatory follicles in *E. rivulatus.* The presence of oocytes in the migratory nucleus or hydrated stage of development indicated that spawning was imminent and likely to have occurred on the day of capture (Smith 1965). The definition by Hastings (1981) was used to describe transitional (sex-changing) individuals. The gonads of these fish had proliferating testicular tissue and degenerating ovarian tissue but did not yet have peripheral sperm sinuses containing spermatids. The criteria outlined by Sadovy & Shapiro (1987) were used in the diagnosis of protogynous hermaphroditism, and atretic oocytes were classified into alpha or beta stages according to Hunter & Macewicz (1985).

Spawning Season

Staging of histological samples provided the most accurate means of determining the duration of the spawning season. The external appearance of the gonad was not used because of uncertainty in distinguishing between resting ovaries and testes. Additional information of spawning season was obtained from the ratio of ovary weight to somatic weight (body weight minus viscera, gonads and gills) using the formula:

Gonadosomatic Index (GSI) = (gonad weight / somatic weight) x 100.

Before calculation of GSI the relationship between fork length and gonad weight was examined in ripe females captured from Tantabiddi. Gonad weight was not independent of body size over the full range of mature female lengths (regression $r^2 = 0.341$; $P < 0.001$; n = 44), indicating that GSI values were influenced by fish size. However, females of 248 mm (fork length) and larger showed little change in the relationship of body weight to gonad weight ($r^2 = 0.103$; $P = 0.095$; n = 28), and hence only these fish were used in the analysis of GSI.

Seawater temperature and length of daylight were compared with the annual reproductive cycle of *E. rivulatus* as likely cues used to determine the timing and duration of the spawning season. Temperature data were obtained using a datalogger secured 1 m above the substratum within Osprey Bay. Length of daylight was calculated from sunrise/sunset data obtained from the Perth Bureau of Meteorology.

Statistical Analysis

A two-way, fixed effects analysis of variance (ANOVA) was used to compare the size of *E. rivulatus* between sexes and locations. Analysis for this comparison was made on the untransformed data although the variances of this and the log-transformed data were not homogeneous. Given the large F-ratios of the test and distribution of the means and standard errors, the test was nonetheless useful. A posthoc comparison of mean lengths was completed using a Tukey HSD test for unequal sample sizes. A Kruskal-Wallis nonparametric ANOVA was used to examine differences in gonadosomatic indices between months. Samples gathered in 1994 and 1995 from four locations along Ningaloo Reef for reproductive analysis were pooled. This assumes that there was minimal variation between years and location, as was indicated from examination of the raw data prior to pooling. Chi-square tests were used in the analysis of size at sex-change and sex ratios. Critical *P* values for all analyses were set at $P < 0.05$.

1.1.2 Results

Population Characteristics

Based on the histological samples, the smallest of 38 immature females selectively sampled at the end of the reproductive season (during February and December 1995) was 48 mm in length. The smallest mature female within the histological samples was 144 mm and the largest 350 mm. Depending on location, the mean length of mature females was 240 to 294 mm (Table 1.1.1). Fifty percent of females taken from Tantabiddi within the reproductive season were sexually mature by 194 mm, and all were mature by 240 mm (fork length; Figure 1.1.2). The smallest male was 221 mm and the largest 381 mm (means between 286 and 330 mm).

The mean lengths of males and females were significantly different within all locations except NWReef. Mean lengths of each sex also varied between locations (Table 1.1.2; Figure 1.1.3); the posthoc comparison of means indicating that males from Tantabiddi, Osprey Bay and NWReef had similar mean lengths and were significantly larger than males from Mangrove Bay (Table 1.1.2). Mature females from Tantabiddi had a larger mean length than those at Osprey and Mangrove Bay, but were smaller than mature females taken from NWReef. The NWReef females were statistically similar in size to males from Mangrove Bay although this may be due somewhat to bias in the sampling of fish from the NWReef area (captured by recreational fishers). The latter may also have resulted in the non-significant difference ($P = 0.0685$) between males and females from NWReef.

Analysis of covariance showed that the regressions between standard length and fork length of male and female *E. rivulatus* captured at Tantabiddi were not significantly different ($P_{\text{intercept,n=243}} = 0.3092$, $P_{\text{slope, n=243}} = 0.2476$). Similarly, the regression between cleaned body weight and fork length for these fish were also not significantly different ($P_{\text{intercept, n=216}} = 0.9925$, $P_{\text{slope, n=216}} = 0.9851$) . Therefore, relationships for the pooled data are:

Standard length (mm) = -2.472 + (fork length x 0.815) (n = 243, $r^2 = 0.999$)

Clean weight (gms) = 0.0000104 x fork length$^{3.042}$ (n = 216, $r^2 = 0.997$)

Only two immature (M1) males and one transitional fish (351 mm fork length) were found in the samples, indicating that the transition from female to mature male is a rapid process and/or occurs infrequently. Both immature males and the transitional fish were captured in February or April, soon after the reproductive season had finished. Median values of the size range in which males overlap with females can be used to estimate the size at which sex change occurs within the population (Shapiro 1987). Calculation of these values for the sites along Ningaloo Reef show that size at sex change was significantly different among the populations (Table 1.1.3). Within the sites at Mangrove Bay *E. rivulatus* had the lowest estimated size at sex-change as well as the smallest range over which sex-change occurred. Although sex-change also happened at a relatively small size among the population at Osprey Bay there was a large range of sizes over which it occurred. The largest size and range for sex-change was among *E. rivulatus* taken from the NWReef area, although this is probably influenced by the wide area over which the fish were captured. The range of the male-female overlap divided by the maximum size can be compared between species (Shapiro 1987). In *E. rivulatus* the overlap range was between 8 and 34% of maximum fork length for fish within the sites at Mangrove Bay and NWReef (Table 1.1.3).

Sex ratios for the unfished populations within the study sites at Osprey and Mangrove Bays differed significantly from unity, with between 4.6 and 6.5 mature females present for every male. These ratios did not differ significantly between the four sites (Table 1.1.4). The female biased sex ratio, larger mean lengths of males, and size overlap range is highlighted in the size frequency distribution of *E. rivulatus* captured from Tantabiddi (Figure 1.1.4).

Reproductive Biology

The gonads of male and female *E. rivulatus* were bi-lobed, elongate organs, joined posteriorly to form a short gonoduct which led to the urogenital pore. In both sexes the gonad was suspended from the rear of the body cavity by mesenteries and bound by a

muscular wall and tunica. The testes and ovaries of non-reproductive *E. rivulatus* weighed less than 1% of somatic weight and were similar in size and appearance. However, differences during the reproductive season were much more obvious, with the ovaries of ripe females weighing between 0.7 and 11% of somatic weight (mean of 3.4% ± 0.31SE), compared to an average of 0.197% (± 0.04SE) for the testes of ripe males.

Internally the ovary consisted of numerous lamellae attached to the dorsal and lateral gonadal walls at a slightly oblique angle. Oocytes were produced and ripened within the lamellae. During spawning the ovulated eggs were shed into the lumen, transported through an oviduct and released externally through the urogenital pore. Atretic oocytes (mainly late yolk globule stage) were a common feature within the ovaries. Few ovaries contained post-ovulatory follicles, although when these were present they were quite numerous, suggesting that they are short lived structures and indicative of recent spawning activity. Post-ovulatory follicles within the ovaries of *E. rivulatus* were small and comprised of a convoluted layer of light staining columnar cells with a large nucleus, similar in appearance to those of the northern anchovy (Hunter & Goldberg 1980). These cells were arranged around a narrow, folded lumen (Figure 1.1.5a). Assuming that post-ovulatory follicles in *E. rivulatus* age in a similar way to those of the northern anchovy, they become progressively smaller, denser and less convoluted with increasing age. In order to estimate timing of spawning for *E. rivulatus* the post-ovulatory follicles were classified as either “new” (spawning occurred within the past 24 hrs) or “old” (spawning occurred within the past 24 - 48 hrs) based on their appearance. In new post-ovulatory follicles the columnar cells formed a single unbroken layer around the lumen. In old post-ovulatory follicles the columnar cells were difficult to define. They no longer formed an unbroken layer and they were often separated from the outer connective tissue theca.

Histological examination of *E. rivulatus* showed that the ovaries of 64 (46%) mature females captured at Tantabiddi contained crypts of spermatic tissue in the spermatocyte or spermatid stage of development (Figure 1.1.5b). Six out of 32 (19%) juvenile female ovaries that were examined also contained scattered sperm crypts. These crypts tended to be aggregated together amongst the ovarian tissue and were more common near the dorsal blood vessel. The number of crypts present within the transverse sections of the gonad was usually less than 20. However, ten of the 64 mature ovaries had up to 70 crypts of sperm within the gonad section, and two contained over 160 crypts of sperm. Despite the large number of sperm crypts the ovaries of the latter two females showed no other indication of sex-change, with one being in spawning (F4) condition and the other resting (F2).

Within the testis the spermatogenic tissue tended to be more squat and lobate in shape than the ovarian lamellae. However, these differences were less obvious during the non-reproductive period when gamete reserves were low. Gonadal tissue of male *E. rivulatus* is of the acinus type (Smith 1965), with sperm forming in small crypts, and individual sperm within a particular crypt at the same stage of development. Sperm sinuses form within the walls of the testis to enable transfer of sperm. A lumen was present but no evidence was seen for its use in the transfer of sperm. In ripe males mature sperm were transferred to the peripheral sperm sinuses via sinuses within the testicular lobule. These intralobular sinuses developed within ripe testes through the rupture and joining of crypts of spermatozoa. The peripheral sperm sinuses ran longitudinally in the gonadal wall, uniting and passing down between the junction of the

two lobes to form a duct that led beneath the urinal duct to the common urogenital opening.

Distinctive clusters of cells were usually present within the muscle of the gonad wall (Figure 1.1.5c). These cells tended to be concentrated near the dorsal blood vessels and in some gonads appeared to be organised around a lumen. They were present within ovaries and testes during all reproductive states and are probably the same endocrine tissue described by Smith (1965), Moe (1969) and Sadovy & Colin (1995) for other serranid species. Melano-macrophage centres (Ferguson 1989), or yellow-brown bodies were also a common feature among the gametic tissue in ovaries and testes. These are repositories for the end products of cell breakdown and were most conspicuous soon after the reproductive season. They were also occasionally present within the ovaries of immature females. Parasitic nematodes (*Phylometra* spp.) were commonly found within or attached to the walls of the ovaries and testes of *E. rivulatus*, sometimes taking up a considerable portion of the gonad, causing inflammation of the surrounding tissue and a substantial reduction in gametic tissue. These nematodes are ovoviviparous and in some sections young could be seen within the parent.

Reproductive Stages

Given below are description of the cycles of reproductive maturation for the ovaries and testes of *E. rivulatus*. The ovarian developmental stages include modification of the scheme used by Moe (1969) so that spawned but still reproductive ovaries could be distinguished from spent ovaries.

F1 *Immature Female*. Macroscopically the ovary was small, rounded in cross-section and a translucent, pale rosy-pink. Microscopic examination showed that the lamellae were narrow and in fairly orderly rows. They were packed with previtellogenic oocytes. Blood vessels were small, there was little stroma or connective tissue and the gonad wall was thin and close fitting. Occasionally small yellow-brown bodies and atretic oocytes were also present amongst the oocytes.

F2 *Mature Resting Female* (Figure 1.1.6a). Ovaries were longer and more angular than the F1 stage and an opaque, pale pink colour. Yellow-brown bodies were often visible through the gonad wall. Lamellae within the ovaries of females that had only recently finished spawning were long, narrow and misshapen, and the tunica thin, loose and convoluted. Oocyte stocks were low and relatively few previtellogenic oocytes or oogonia lined the periphery of the lamellae, which were mainly filled with loose stroma and vascular tissue, large muscular blood vessels, and yellow-brown bodies. As time since final spawning increased the gonad wall became progressively thicker and tighter fitting as it contracted back to the resting state. Between February and April there was no sign of reproductive activity and there was a reduction in the amount of vascular tissue within the lamellae as the number of previtellogenic oocytes increased. The muscular blood vessels and yellow-brown bodies became reduced in size such that there were few signs in the ovary of F2 females to distinguish them from immature females. As the reproductive period approached the occasional cortical alveoli stage oocyte appeared amongst the other previtellogenic oocytes.

F3 *Ripe Female* (Figure 1.1.6b). The onset of reproductive activity was marked by the maturation of oocytes into the early yolk globule stage of development. The growth of this batch of oocytes, and of another into the cortical alveoli stage, caused the lamellae to expand and fill the lumen. Previtellogenic oocytes and stroma were still numerous

although the tunica became thinner as the ovary began to enlarge. In fully ripe females the ovary was expanded, round and firm. Colour was a pale apricot with an opaque, granular appearance. The dorsal blood vessel and its numerous branching vessels were usually prominent. Lamellae were large and filled with late yolk globule stage oocytes, with few other structures evident. The lumen was reduced and the gonad wall stretched thin.

F4 *Spawning Female* (Figure 1.1.7a). In running ripe females the ovary reached its maximum length and diameter, and was a semi-translucent, pale apricot colour. If spawning had not occurred but was imminent then the lamellae were dominated by late yolk globule and ripe (migratory nucleus or hydrated) stage oocytes. If spawning had occurred then post-ovulatory follicles were present and the oocytes were less densely packed. The presence of post-ovulatory follicles and ripe oocytes together within the ovary of a particular female (Figure 1.1.7b; n = 19 (17% of ripe females)) shows that females may spawn at least twice over two or three consecutive days. However, the short duration of each spawning period (a few days of each month during the reproductive season; see Chapter 1.2) may preclude more than three spawns before spawning is finished. The variable proportion of F4 females within the samples indicates that *E. rivulatus* do not spawn consistently throughout the reproductive period and only a certain proportion of females spawn at a particular time. This proportion varied from approximately 15% to almost 90% in samples containing at least eight mature females.

F5 *Spent Female* (Figure 1.1.8a). At the end of the reproductive season the ovary was reduced in size, flaccid, and often bloody in appearance. Lamellae were misshapen and kinked. Internally they were disorganised with loose stroma and few previtellogenic oocytes. If many unspawned stage 4 oocytes still remained, then the female was only classed as F5 if 50% or more of these were atretic. Otherwise the fish was classed F4 (Hunter & Macewicz (1985) found that the probability of spawning in anchovy was very low when more than 50% of the advanced oocytes were atretic). Inflows of vascular tissue (leucocytes) were often found within the lamellae as the clean-up of unspawned oocytes and other debris of spawning occurred.

T *Transitional* (Figure 1.1.8b). The sole transitional fish was captured in late April from NWReef and frozen after capture. The gonad was non-reproductive, small and compact in cross-section. It had a thick, muscular tunica and the lamellae were narrow and tightly packed. Muscular blood vessels, yellow-brown bodies and connective tissue filled the central portions of the lamellae and were evidence of change occurring within the gonad. Outer regions of the lamellae contained numerous previtellogenic oocytes and crypts of spermatogenic tissue. Many of the crypts contained spermatocytes although crypts of spermatids were also common and indicative of rapid proliferation of the male tissue. Most of the previtellogenic oocytes appeared intact and viable, although many showed the effects of freezing and had a granular cytoplasm. The remaining oocytes were atretic, with fragmented cytoplasm and ruptured follicular layer. The tunica showed signs of splitting although no peripheral or intralobular sperm sinuses could be distinguished.

M1 *Immature Male* (Figure 1.1.9a). Two immature males were captured. Both were non-reproductive and had testes that were small in cross-section with a thick tunica. Blood vessels were common within the gonad wall and the lamellae were long, narrow and misshapen, resembling the lamellae of F2 females. Peripheral sperm ducts were small and filled with spermatozoa. Central areas of the lamellae contained connective

tissue, vascular tissue and yellow-brown bodies whilst outer regions were stocked with numerous crypts of spermatogenic tissue in all stages of development from spermatocytes to spermatozoa. Previtellogenic oocytes were also common among the sperm crypts, with more than 300 vestigial oocytes in the transverse section of the testes of both males. In comparison, twelve other mature males were also found with vestigial previtellogenic oocytes in their testes, although only two of these had more than 100 oocytes within the transverse section.

M2 *Mature Resting Male* (Figure 1.1.9b). Testes were small, straplike and quite angular in cross-section. The gonad wall was relatively thin and loose after spawning but soon contracted and became thick and tighter fitting. Peripheral sperm sinuses may still contain sperm but were much reduced in size. Testicular lobes were relatively small and well separated from each other. They varied in shape, some lobes appearing elongate and narrow, others short and squat. Gametic tissue in the early stages of spermatogenesis dominated and connective tissue formed thick bands in some inner regions. Yellow-brown bodies were large and numerous, and the intralobular sperm sinuses contained sperm but were reduced in size and disjunct. Muscular blood vessels were conspicuous in the gonad wall.

M3 *Ripening Male*. Testes were whiter and longer than M2 males although still relatively small with a thick tunica. Lobes were dominated by later stages of spermatogenesis with most crypts containing spermatocytes and spermatids. Intralobular and peripheral sperm sinuses were beginning to fill with spermatozoa. Connective tissue was also conspicuous within the lobes. This was a brief stage, with few males classified as such.

M4 *Ripe Male* (Figure 1.1.9c). Testes were large and white and may release milt when squeezed. The tunica was stretched thin by the expanded testicular lobes and the lumen was small. Spermatocytes and spermatogonia were common early, but became confined to peripheral regions of the lobes as spermiogenesis proceeded. During early M4 the intralobular sinuses were narrow and followed a complex pathway through the centre of the lobe. As more crypts of spermatids ruptured and released their contents the intralobular sinuses became wider and dominated the lobe. These drained into the large peripheral sinuses which were full of sperm. Yellow-brown bodies and connective tissue were inconspicuous.

M5 *Spawned Male*. As the reproductive period progressed the tunica became looser and the lobes more flaccid and folded. Empty spaces appeared within the lobes as the reserves of spermatic tissue were used up, and the peripheral sperm sinuses contained variable amounts of sperm. In males which had recently completed spawning for the season the testis was small with a loose, convoluted tunica. The testicular lobes were reduced in size, containing few crypts of spermatocytes, numerous spermatogonia and reduced intralobular sinuses which still contained sperm. Thick bands of connective tissue filled some areas. Large yellow-brown bodies were conspicuous within the centre of the lobes, as were blood vessels within the gonad wall.

Reproductive Season

Early signs of reproductive activity by some females began in May with the appearance of cortical alveoli stage oocytes within their ovaries. Although samples for June were not obtained it is likely that the ovaries of many females ripened noticeably during this

month, and whilst few ovaries sampled during July contained a fully mature (late yolk globule stage) batch of oocytes, nine percent of the females taken during this month showed signs of recent or imminent spawning (Figure 1.1.10a). By August most females in the samples were ripe, and from this month through to December the peak of reproductive activity and spawning occurred. By December the larger females had depleted reserves of vitellogenic oocytes in their ovaries and a few had finished for the season and were inactive. The January samples consisted mainly of resting females as well as a few spent individuals that had recently finished spawning for the season, and the February samples consisted solely of resting females.

The duration of reproductive activity spanned the time of coolest seasonal water temperature (Figure 1.1.10b). At the start of the reproductive season in June the water temperature averaged 23^{o} C and was dropping. Lowest water temperatures were recorded during August (21^{o} C), and by December when the reproductive season was finishing, temperatures had climbed to 24.5^{o} C. The warmest water temperature was 28.8^{o} C, recorded during March when *E. rivulatus* were non-reproductive. Annual changes in length of day preceded the water temperature cycle, and reproductive activity of *E. rivulatus* began when daylight hours were at their lowest (average 10.1 hrs) and starting to increase. The reproductive season continued until the length of daylight peaked in December with an average of 14.2 hrs. No reproduction occurred throughout the downward slope of the daylight cycle.

Annual changes in the gonadosomatic index confirm the spawning pattern described by the histological data (Figure 1.1.10c). The GSI was not homogenous between months (Kruskal-Wallis ANOVA; $H_{(9,n=141)} = 95.668$, $P = 0.0000$), with gonad weight (relative to body weight) increasing in July and peaking in September. Since *E. rivulatus* may mature batches of oocytes on a monthly cycle throughout the reproductive season (Chapter 1.2), the GSI data indicates that the production of oocytes was higher than the loss rate through spawning until September. After this month there may have been no more production of oocytes, resulting in a rapid decrease in ovary weight as spawning continued and the ovaries became depleted of gamete reserves. The high variability in GSI values of females captured between August and December may be due to differences between individuals in the time since last spawning. By January mean ovary weight had declined and was similar to that of resting females. From February to May the GSI remained low with little variation between females as the ovaries remained inactive.

1.1.3 Discussion

Protogynous Hermaphroditism

Although only one transitional fish was captured during the study, the population structure and gonad morphology of *E. rivulatus* provide strong evidence that this species is a protogynous hermaphrodite. For instance, the female biased sex ratio and larger body size of male fish are typical of haremic protogynous species (Sadovy & Shapiro 1987). In such species large males can gain extraordinarily high reproductive success by defending and mating with a group of females. In contrast, smaller individuals are less capable of maintaining a harem and hence should gain optimal reproductive success as a female (Warner *et al.* 1975, Nemtzov 1985, Warner 1988). However, a female biased sex ratio and larger male size can also be a result of other

mechanisms such as differential mortality rates or habitat preferences between the sexes and hence do not provide unequivocal proof of hermaphroditism by themselves alone (Sadovy & Shapiro 1987).

More reliable evidence of protogyny is provided by the structure of the testis. In *E. rivulatus* the presence of a lumen that is not used in the transport of sperm, the lamella-like form of the testicular tissue, and the common occurrence of previtellogenic oocytes in the male gonad are all indicative of prior ovarian function (Sadovy & Shapiro 1987). However, such evidence is also found in *Epinephelus striatus* which may undergo prematurational sex-change and is therefore functionally gonochoristic (Sadovy & Colin 1995). No bisexual *E. rivulatus* were found in the present study though, and all juvenile gonads examined were ovarian, which leaves little doubt that this species is functionally a protogynous hermaphrodite. The common occurrence of non-functional sperm crypts within the female ovaries also indicate that females are primed to change sex if the correct cues are provided, and the low frequency of transitional individuals and immature males (recent sex-changers) suggest that the process of changing sex is rapid.

Sex-change in smaller hermaphroditic species of fish is typically controlled by social cues such as the aggressiveness of more dominant individuals or changes in either the sex or size ratios within a social group (Robertson 1972, Shapiro & Lubbock 1980, Ross 1990, Lutnesky 1994). However the mechanisms controlling sex-change in the epinepheline serranids have not yet been elucidated (Shapiro 1987, Coleman *et al.* 1996), although the influence of exogenous cues are indicated in species such as *Plectropomus maculatus* (Ferreira 1993) and *P. leopardus* (Samoilys & Squire 1994) because of the wide range of lengths and ages in which individuals change sex. Substantial variation in the estimated median size and the range of sizes over which sex-change occurred was evident also in *E. rivulatus*. This is expected in typical sampling schemes where individuals are captured over a relatively large area from populations experiencing different social and environmental conditions (e.g. North West Reef). However, even the data obtained from the population within Osprey Bay exhibited a wide variation in the estimated size at sex-change and range of sizes over which sex-change can occur, particularly when compared with the populations within Mangrove Bay. This disparity is most likely a reflection of the differing social and environmental conditions that were evident between the locations. For instance, at Osprey Bay where the habitat was heterogenous and competition for space along the reef slope was strong, there were considerable size differences between male and female *E. rivulatus* occupying the slope and the adjacent reef flat (Sectio 1.2). As a result there was also considerable variation in the range of sizes over which sex-change occurred. In contrast, there was little variation in the size at sex-change of *E. rivulatus* within Mangrove Bay where the habitat was uniformly flat and featureless, and the fish were less likely to be distributed according to size and competitive advantage. Despite this variability between sites, the range of sizes over which sex-change occurs in *E. rivulatus* is relatively low compared to other protogynous serranid species (size ranges of sex change for ten species varied between 15 and 62% of maximum length; median = 42%, Shapiro 1987). This may be a general rule for smaller species according to the data presented by Shapiro (1987), perhaps because they live in stable, site-attached social groups in which the timing of sex-change is finely tuned to changes in the social environment. In contrast, individuals of the larger species tend to be more solitary throughout much of the year except when they form short-lived annual breeding aggregations, and as a consequence the timing of sex-change may not always be optimal.

Annual Reproductive Cycle

Serranids tend to spawn from early spring to early summer and be reproductively active over 1 - 5 months (Thresher 1984, Shapiro 1987). The late winter start and five month duration of the *E. rivulatus* reproductive period is therefore both early and long for a serranid. In addition, the range of water temperatures in which *E. rivulatus* reproduce (21 - 24.5^{o} C) is relatively cool compared to other Australian serranids such as *Cephalopholis cyanostigma* (28 - 29.5^{o} C) and *Plectropomus leopardus* (24.2 - 28.5^{o} C) from the central Great Barrier Reef (Mackie 1993, Samoilys 1997). Whilst there was no clear correlation between water temperature and spawning in *E. rivulatus*, the start and finish of the reproductive season and the trough and peak of the annual cycle of daylength may explain the reproductive cycle of *E. rivulatus* better. Colin (1992) also recorded that the initial spawning aggregation for *E. striatus* occurred around the shortest day of the year. Little correlation has been found between length of day and spawning season in other epinephelines though (Thresher 1984, Shapiro 1987, Tucker *et al.* 1993), and given that environmental factors such as temperature and photoperiod vary less in tropical than temperate waters, the influence of the environment on reproduction is likely to be more complex in fish species inhabiting tropical waters (Lam & Munro 1987).

Management Concerns

High air temperatures, strong winds and the threat of cyclones during spring and summer (September through to March) limit fishing activity at Ningaloo Reef during much of the *E. rivulatus* reproductive season. Through this time only minimal impact on the reproductive output of spawning populations would thus be expected. Of concern though, is the heavier fishing pressure at other times of year, especially from June to August when the reproductive period of *E. rivulatus* has commenced and the loss of spawning partners and disruption to the social environment by fishing activities may adversely affect reproductive output over the rest of the spawning period. This is particularly so in species such as *E. rivulatus* which reproduce in or near their normal living area (Sectio 1.2) and spawning partners may only be replaced by immigration or recruitment. The relatively high removal of males because of their larger size and dominance in attacking a bait (*pers. obs.*) may have the biggest impact, since their replacement requires the immigration of nearby males or sex-change by females. Under light fishing pressure the replacement of males by either of these methods can happen relatively quickly in *E. rivulatus* (Chapter 2.1) and may cause relatively little disruption to the social environment. However, if fishing pressure is heavy or prolonged the impact on the populations and their reproductive output are likely to be considerably more severe and long term.

Table 1.1.1. Number and mean lengths of male and female *E. rivulatus* captured at various locations along Ningaloo Reef. Tantabiddi and NW Reef samples were captured over a relatively broad area from locations open to fishing activity, and were used in histological analyses. Samples from the sites at Mangrove and Osprey Bays include virtually all individuals within specific populations that are not subject to fishing pressure. The sex of fish within the latter sites was determined by cannulation. M, male. MF, Mature Female. IF, Immature Female. FL, fork length.

	Tantabiddi		NW Reef		Mangrove Site 1			Mangrove Site 2			Osprey Site 1			Osprey Site 2		
Sex	M	MF	M	MF	M	MF	IF	M	MF	IF	M	MF	IF	M	MF	IF
Number	41	142	26	36	11	69	32	31	143	42	5	30	8	32	201	31
Mean FL (mm)	314	258	315	294	286	240	160	287	240	160	330	247	161	323	245	149
SE	3.2	2.7	7.1	6.5	4.7	2.4	4.8	4.7	2.7	2.8	4.5	6.9	12.8	3.9	2.5	6.2
Min FL (mm)	277	144	221	190	264	205	126	252	205	127	319	200	133	256	186	107
Max FL (mm)	358	324	380	350	310	290	188	314	277	189	342	325	197	358	341	188

Table 1.1.2. Results of (a) a 2-way, fixed effects analysis of variance comparing the mean lengths of male and female *E. rivulatus* at Tantabiddi, Osprey Bay, Mangrove Bay, and NWReef. (b) A posthoc comparison of means between location and sex (Tukey HSD for unequal sample sizes). Analysis made on untransformed data. F = female, M = male.

(a)

Source	df	MS	F	*P*
Location (L)	3	20571.0	23.0	0.0000
Sex (S)	1	232299.6	259.3	0.0000
S x L	3	12189.1	13.6	0.0000
error	631	895.8		

(b)

		Tantabiddi		NWReef		Osprey Bay		Mangrove Bay	
		F	M	F	M	F	M	F	M
Tantabiddi	F								
	M	***							
NWReef	F	***	ns						
	M	***	ns	ns					
Osprey Bay	F	*	***	***	***				
	M	***	ns	***	ns	***			
Mangrove	F	***	***	***	***	ns	***		
Bay	M	***	**	ns	*	***	***	***	

Table 1.1.3. Median values of the size range in which male and mature female *E. rivulatus* overlapped. Data taken from fish captured at various locations along Ningaloo Reef and are used to estimate the size at sex change. Also shown is overlap as a percentage of the maximum size of *E. rivulatus* in the population, and the results of a Kruskal-Wallis median test to compare median values among locations. FL, fork length in millimetres.

	Tantabiddi	NWReef	Mangrove Site 1	Mangrove Site 2	Osprey Site 2
sample size	64	57	10	26	89
median FL (mm)	298.0	315.0	278.0	263.5	285.0
overlap range	277-324	221-350	264-290	252-277	256-341
% of max FL (mm)	13	34	8	8	24
median test	Chi-square = 56.41376		df = 4		*P* = 0.0000

Table 1.1.4. Sex ratios of *E. rivulatus* within the study sites at Mangrove and Osprey Bays. Also shown are the results of a Chi-square goodness of fit (GOF) test to analyse deviation of the sex ratios from unity, and a homogeneity test to compare the sex ratios between sites. M; male. MF; mature female.

	Mangrove Site 1	Mangrove Site 2	Osprey Site 1	Osprey Site 2
number (M:MF)	11:69	31:143	5:30	32:201
ratio (M:MF)	1:6.3	1:4.6	1:6.0	1:6.5
GOF	Chi-square = 254.63		*P* < 0.001	
homogeneity	Chi-square = 1.47		0.5 < *P* < 0.75	

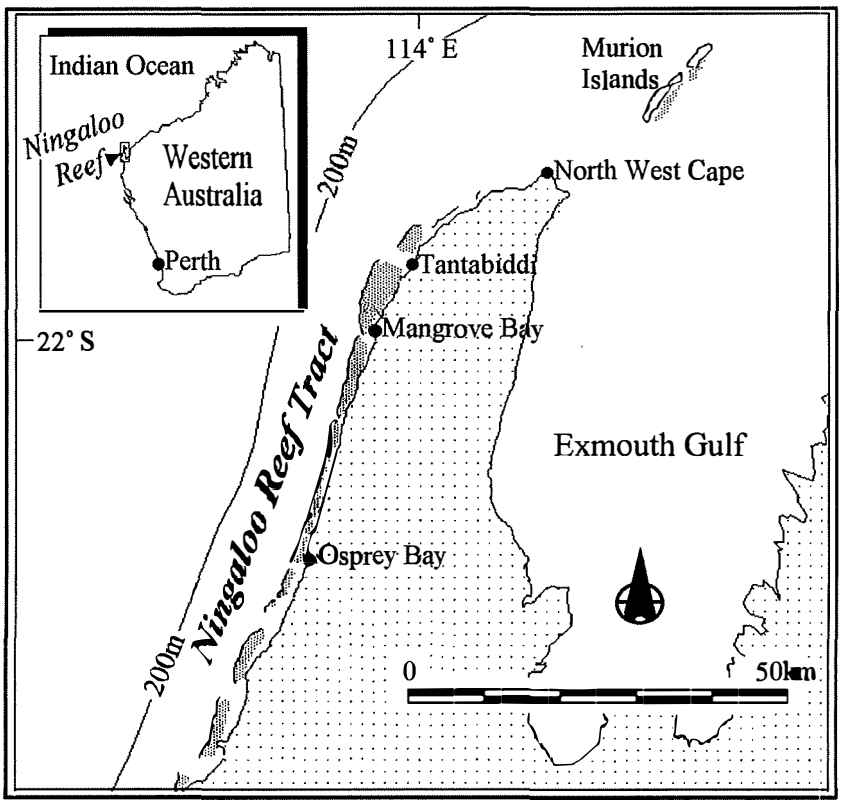


Figure 1.1.1 Location of the sites used in the study of the biology and ecology of *Epinephelus rivulatus* at Ningaloo Reef, Western Australia.

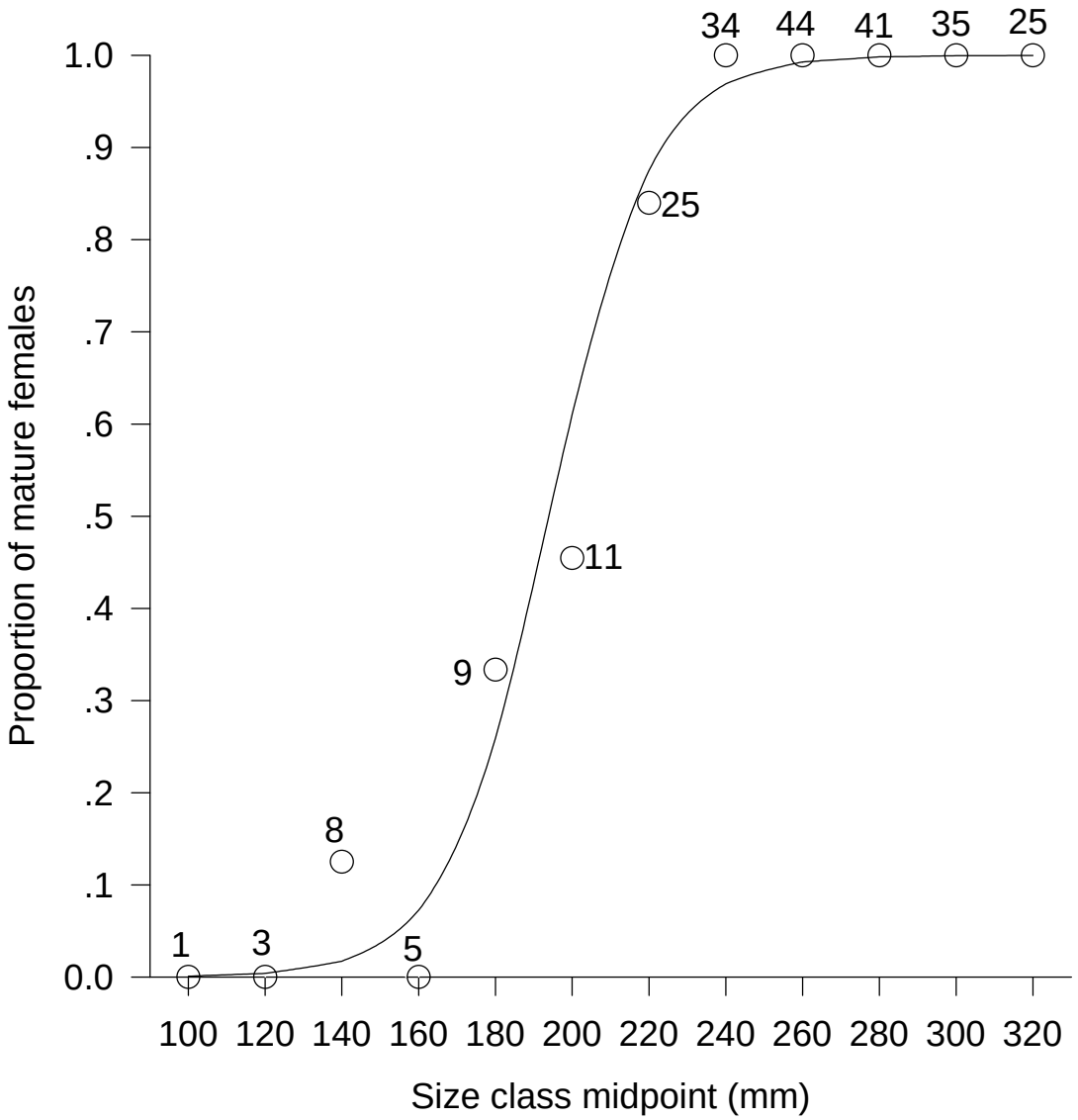


Figure 1.1.2. Maturity curve showing the proportion of mature females within the samples of female *E. rivulatus* from Tantabiddi. Also shown is a logistic curve fitted to the data and sample sizes. Size class intervals are 20 mm.

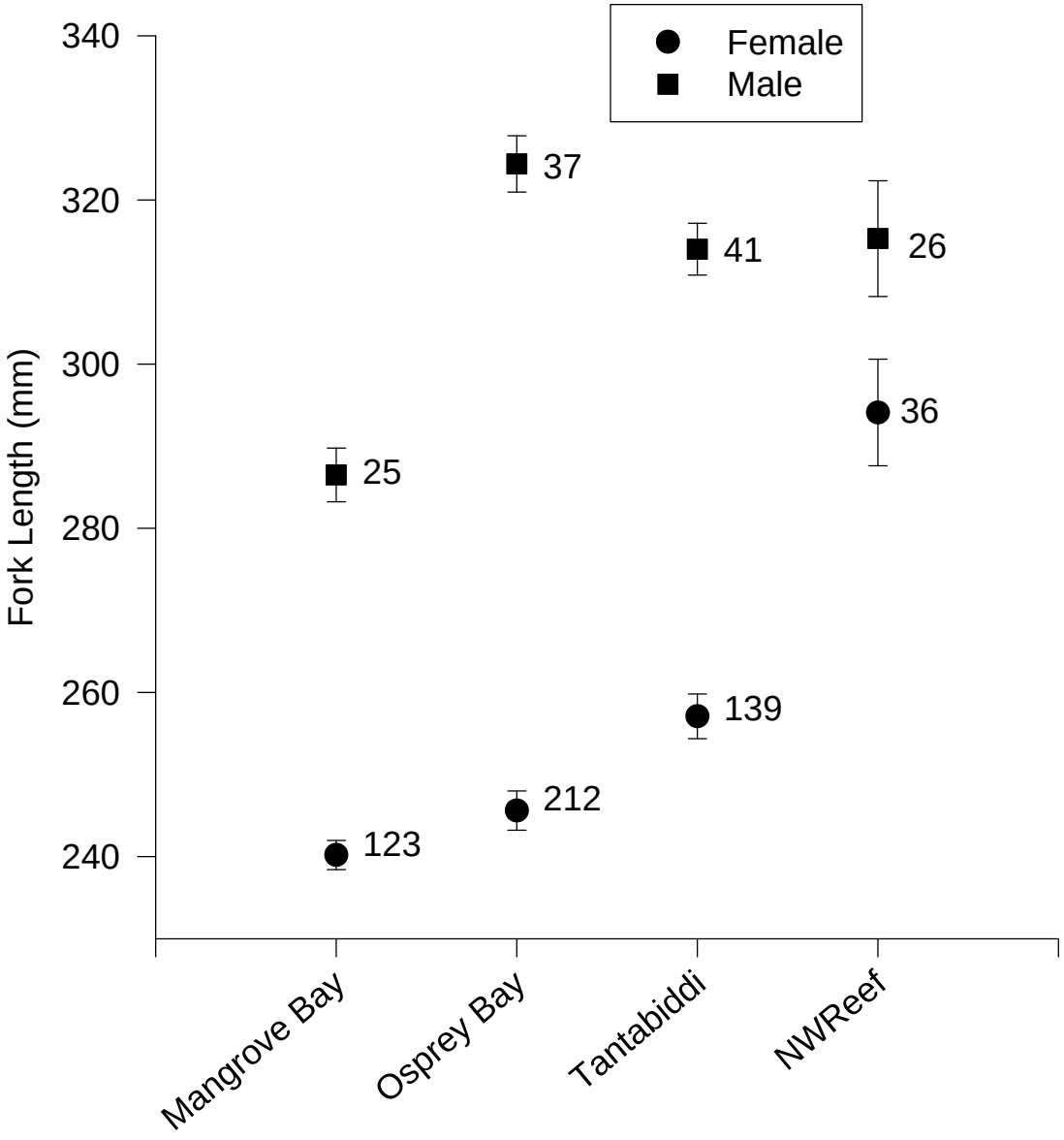


Figure 1.1.3. Mean lengths (± SE) of male and female *E. rivulatus* captured from Ningaloo Reef between 1994 and 1995. Numbers beside error bars indicate sample size.

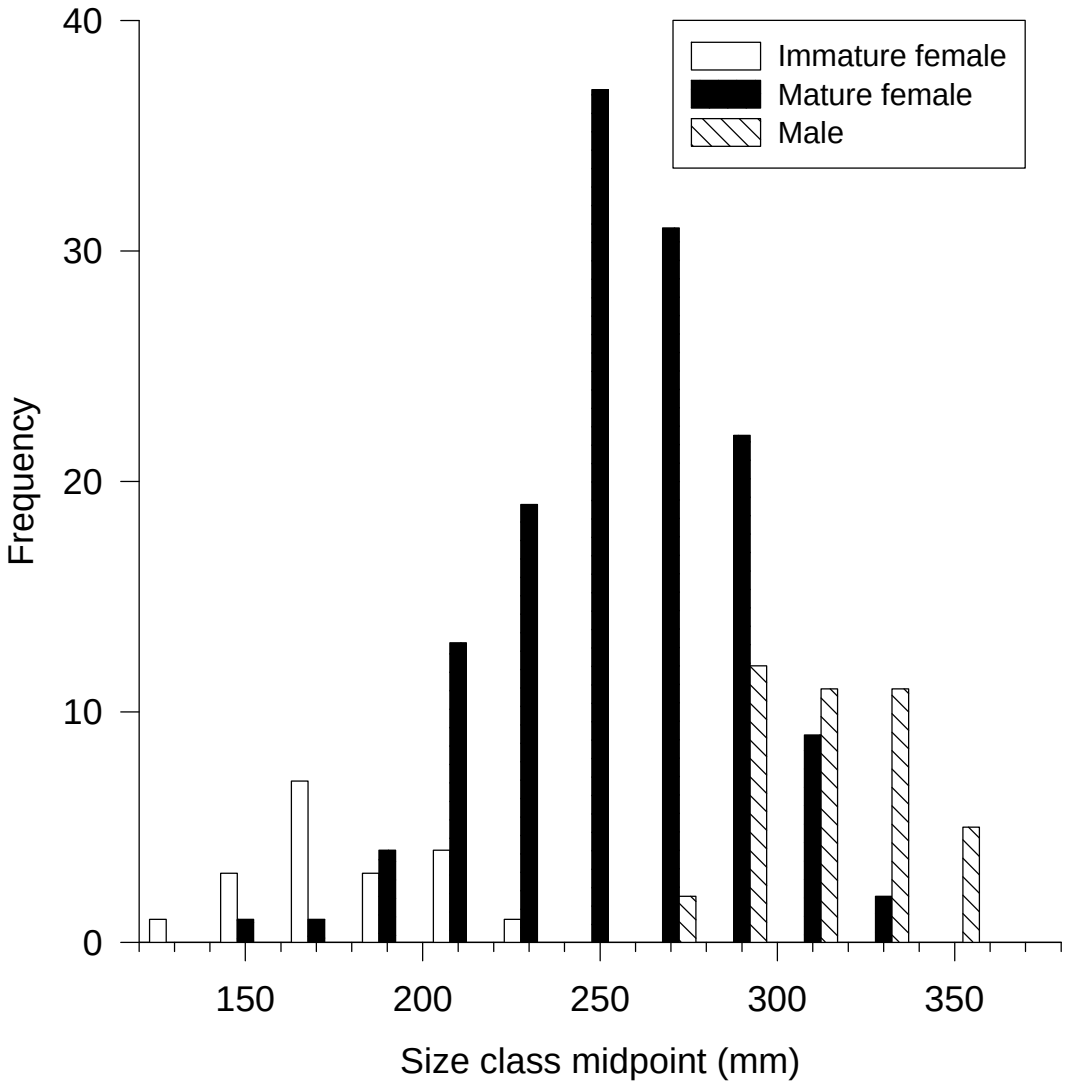


Figure 1.1.4. Size frequency distribution of *E. rivulatus* captured at Tantabiddi. Sexual status of individuals was determined from histological analysis. Measurements are of fork length and size class intervals are 20 mm.

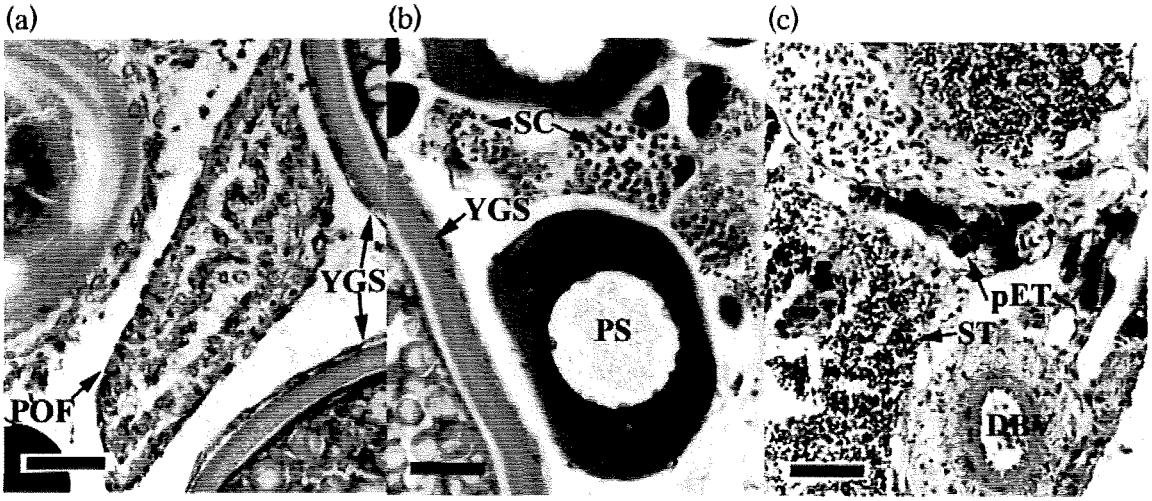


Figure 1.1.5. Transverse sections of *E. rivulatus* gonads showing (a), a post-ovulatory follicle within the ovary of a mature female. Designated age of the follicle was >24 hrs ('old'). (b) Crypts of spermatocytes within the ovary of a mature female. (c) Putative endocrine tissue within the testis of a mature male. Scale bar in each photograph = 30 μm. **DBV,** dorsal blood vessel. **pET,** putative endocrine tissue. **POF,** post-ovulatory follicle. **PS,** perinucleolus stage oocyte **SC,** sperm- at ocytes. **ST,** spermatids. **YGS,** yolk globule stage oocyte.

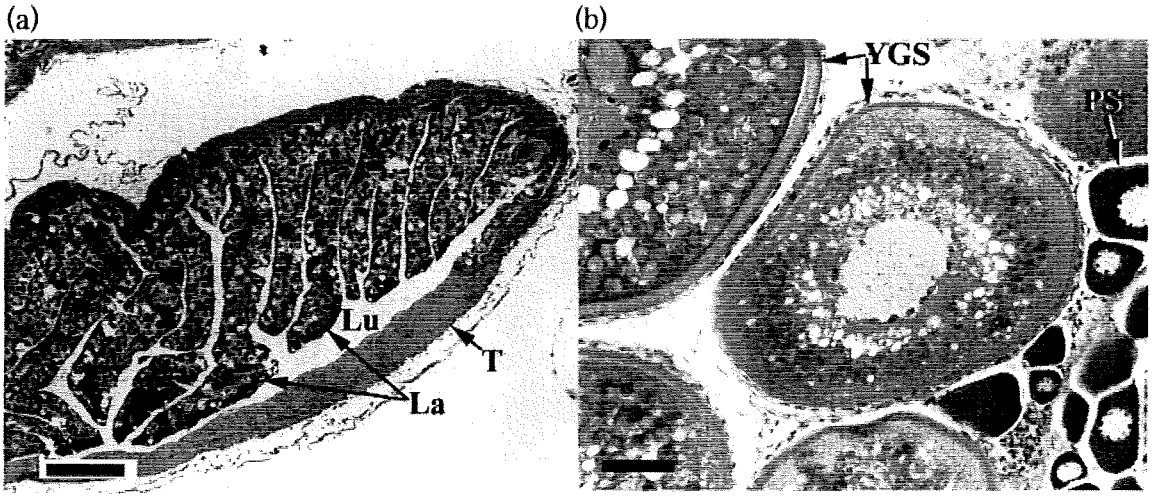


Figure 1.1.6. Transverse sections of *E. rivulatus* gonads showing (a), general structure of the ovary of a mature, non-reproductive female. Scale bar = 500 μm. (b) Detail of the ovary of a ripe female *E. rivulatus* showing early and late yolk globule stage and perinucleolus stage oocytes. Scale bar = 50 μm. **La,** lamellae. **Lu,** lumen. **PS,** perinucleolus stage oocyte. **T,** tunica. **YGS,** yolk globule stage oocyte.

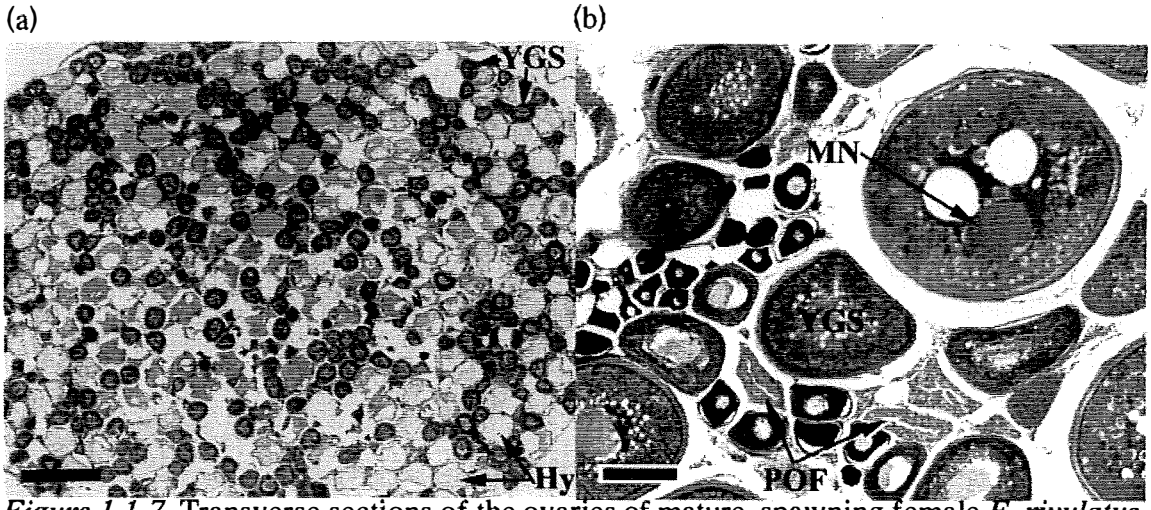


Figure 1.1.7. Transverse sections of the ovaries of mature, spawning female *E. rivulatus* showing (a), general structure just prior to spawning. The ovary is dominated by yolk globule stage and hydrated oocytes. Scale bar = 500 μm. (b) Detail showing post-ovulatory follicles and a migratory stage oocyte in the ovary of a female that had recently spawned and was soon going to do so again when captured. Scale bar = 100 μm. **Hy,** hydrated oocyte. **MN**, oocyte with migratory nucleus. **POF,** post-ovulatory follicle. **YGS**, yolk globule stage oocyte.

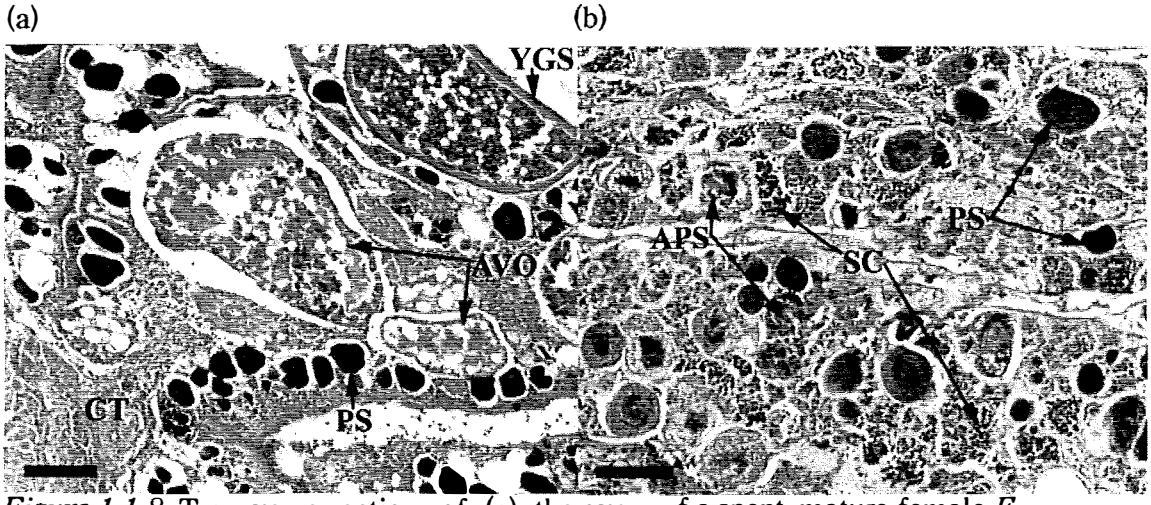


Figure 1.1.8. Transverse sections of (a), the ovary of a spent, mature female *E. rivulatus*, in which oocyte stocks are low and most vitellogenic oocytes are being resorbed. Scale bar = 100 μm. (b) The gonad of the sole transitional *E. rivulatus* captured within the reproductive samples showing numerous crypts of spermatic tissue and some atretic previtellogenic oocytes. Note that the gonad of this fish had been frozen before processing which has affected the appearance of some oocytes. Scale bar = 60 μm. **APS,** atretic perinucleolus stage oocyte. **AVO,** atretic vitellogenic oocyte. **CT,** connective tissue. **PS,** perinucleolus stage oocyte. **SC,** spermatocytes. **YGS**, yolk globule stage oocyte.

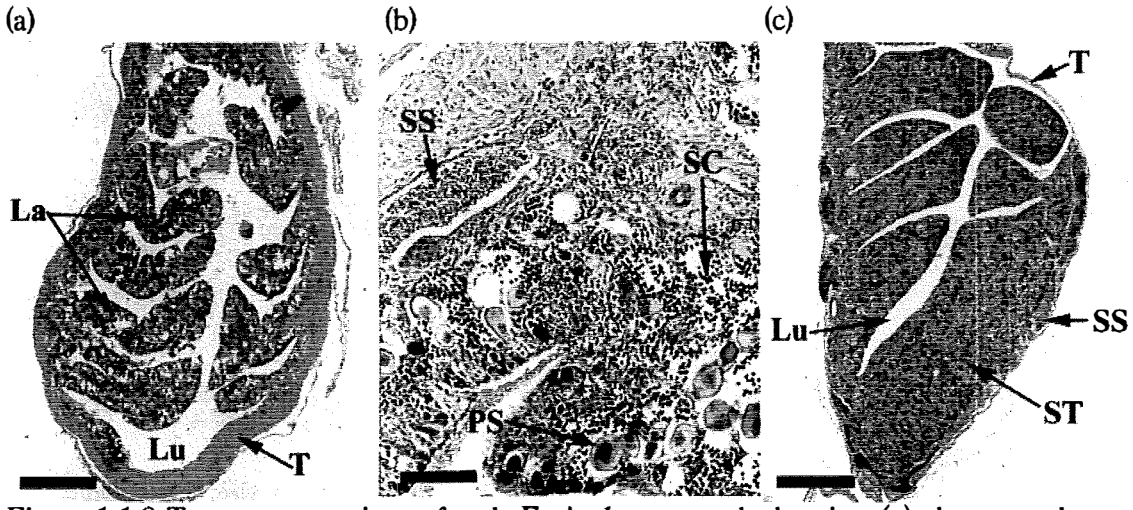


Figure 1.1.9. Transverse sections of male *E. rivulatus* gonads showing (a), the general structure of an immature male testis. Scale bar = 700 μm. (b) Detail of the same immature testis showing vestigial perinucleolus stage oocytes among the developing crypts of spermatic tissue and recently formed peripheral sperm sinus. Scale bar = 30 μm. (c) General structure of the testis of a mature, ripe male showing the lobular structure of the gametic tissue. Scale bar = 800 μm. **La,** lamellae. **Lu,** lumen. **PS,** perinucleolus stage oocyte. **SC,** spermatocytes. **SS,** sperm sinus. **ST,** sperm tissue. **T,** tunica.

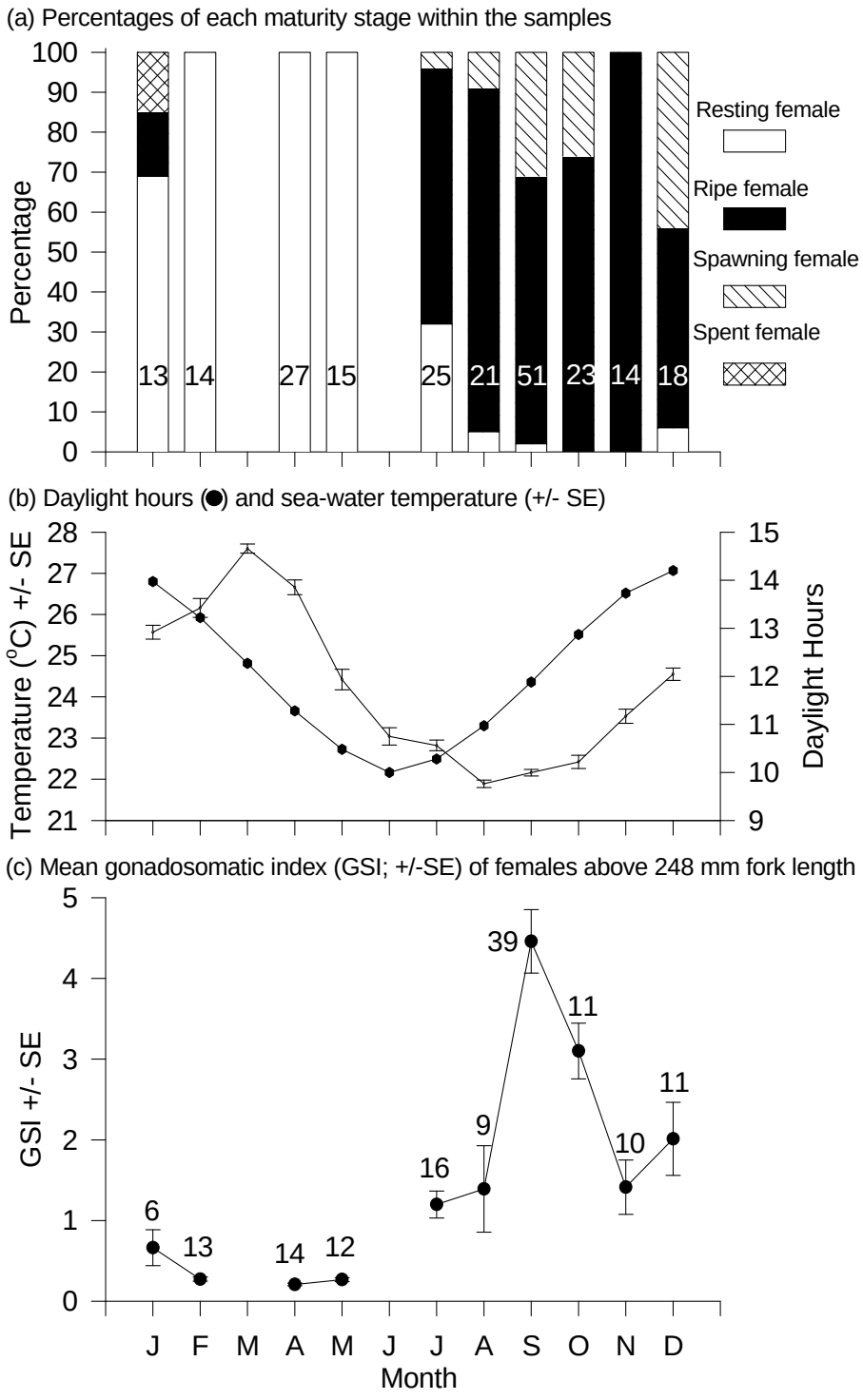


Figure 1.1.10. Seasonal cycles of female *E. rivulatus* reproductive activity and environmental parameters at Ningaloo Reef. Reproductive data obtained during 1994 and 1995 were pooled to increase sample sizes, which are indicated by the numbers within the bars or above the standard error bars.

1.2 Reproductive Behaviour And Mating System

1.2.1 Materials And Methods

Study Site

The present study was conducted within the Sanctuary Zone at Osprey Bay, approximately 200 m from the site used in the study of reproductive biology, and along the same reef slope (Chapter 1.1). Because of the similarities in study sites refer to Chapter 1.1 for description.

Intra-Seasonal Cues And Behaviours Associated With Spawning

Description of the behaviours associated with spawning in *E. rivulatus* were obtained from a series of SCUBA dives made at sunset between 23 August and 18 September, 1996. Prior to these observations, 24 large female and 8 male *E. rivulatus* within the behavioural observation area were captured by snorkel divers using baited hook and line, sexed by cannulation and double tagged with a specific tagging code so that individuals could be identified during the subsequent observations. Refer to Chapter 1.1 for cannulation methodologies. T-bar anchor tags were secured into the muscle below the dorsal fin, in one of three positions on either side of the body. Each fish was then released at the precise location of capture. *E. rivulatus* usually recover quickly after being tagged and cannulated, and will readily take a bait on the following day. Thus the evening observation study was begun on the second evening following the tagging exercise.

Two males were chosen as focal individuals and observed by divers who recorded the time and type of behaviour they exhibited, and the identity, sex and behaviour of conspecifics with which they interacted. The behaviours and interactions were categorised using pre-defined definitions (Table 1.2.1), and each observation period began approximately half an hour before sunset and ran for one hour (the time of sunset progressed from 1754 to 1810 hrs during the course of the study). The two focal males were reasonably large and had neighbouring territories (Figure 1.2.1), and were identified as males 1 (334 mm fork length) and 2 (339 mm fork length). The observed *E. rivulatus* habituated well to the presence of the divers and appeared to return to normal behaviour after the first evening of observation. No behavioural observations were made within the study site at other times of day because no spawning activity had previously been recorded between early morning and late afternoon despite many hours of home range and behavioural observations (Chapter 1.4 and unpublished data).

Video recordings of *E. rivulatus* inhabiting a nearby section of the reef slope were also made each evening by a third diver. There was a relatively high concentration of male and female *E. rivulatus* in this area, and special attention was given to the activities of a particularly large male (male 3; 381 mm fork length). From these recordings a detailed examination and description of the behaviours and interactions of *E. rivulatus* was subsequently made. On completion of the observation study the territory size of the focal males (1 - 3) were estimated by measuring the perimeter of their defended territories and calculating the area these enclosed.

In addition to the evening observations, approximately six medium to large mature females were captured every third day for histological examination of their gonads (n = 53). These fish were taken from various locations between 0.4 and 5 km from the

observation area. The purpose of these samples was to compare maturity cycles of the ovary with behavioural cycles over the observation period. Refer to Chapter 1.1 for methods used in the preparation and analysis of these gonads.

1.2.2 Results

The evening observation study began 23 August 1996, one day after the first quarter of the moon and nine days since the previous new moon (age of the moon was thus 9 d). Full moon was 28 August (age of moon = 14 d), the last quarter 4 September (moon = 21 d), and new moon 12 September (moon = 29 d). The evening observation period ended 18 September, three days short of a full lunar cycle, and two days before the next first quarter.

Behaviour Of Males During The Spawning Period

Swimming activity of the males was generally on the increase when the observation period began (23 August) and peaked for males 1 and 2 on the evening of the full moon. On this evening the two focal males spent over 50% of their time swimming at a rapid pace between vantage points (Figure 1.2.2a), where they would rest briefly, perched on their pectoral fins with strong pale-tail colouration (described below) and an alert appearance. Sometimes during their swims the males would alter course and speed up over a female that lay motionless and often difficult to detect upon the bottom (defined as a swimover; Table 1.2.1).

The number of times that males made these swimovers peaked on the night of the full moon (Figures 1.2.2b). Male 2 was particularly active in this behaviour, swimming over as many as seven females in quick succession. Approximately ten females were grouped near position B at the base of the reef in the territory of this male (Figure 1.2.1), with several more at positions A and C. These females had very distended abdomens and were clearly ready to spawn (Figure 1.2.3), and although male 2 had conflict with other males he showed far more interest in the females. Many of these females were untagged and had not previously been seen within the territory of male 2, which contained between three and six resident females during inactive times of the month. Whilst the origins of these immigrant females was unclear, it is assumed that most came from nearby areas of the reef flat, where *E. rivulatus* (generally smaller and more dispersed) were also found. No spawning activity or build up of fish was noted over the reef flat during the observation study. Male 1 made far fewer swimovers than male 2 during the short reproductive period but was more active in this behaviour at other times of the month (Figure 1.2.2b). The territory of male 1 contained about nine to eleven resident females, with only two or three females moving in during the reproductive period. Swimovers by both focal males were mainly directed at the larger females and rarely at smaller females.

Activity levels of male 3 peaked one day before the full moon and the peak of activity shown by males 1 and 2. Male 3 was bigger than other males in the area and dominated in territorial standoffs with neighbouring males. Compared to neighbouring males, male 3 also had the earliest and largest build up of females within his territory, which measured 61 m^2 and contained six to eight females during non-reproductive times of the month. In contrast, approximately sixteen to eighteen females were present within the territory of male 3 on the evening before the full moon. Most of these lay scattered amongst the algae and limestone lumps that spread out onto the sand channel

(Figure 1.2.1) whilst several others rested on the reef slope. It was also on the evening prior to the full moon that male 3 was observed spawning separately with two females (described below). Both spawning events occurred within two metres of the observation position (in the channel amongst the limestone and algae), in quick succession at 1825 and 1827 hrs (29 and 31 minutes after sunset). No more spawning was observed before the dive was completed at 1837 hrs although *E. rivulatus* were still active in the dim light.

On the following night (full moon) male 3 remained active, swimming around his territory and stopping frequently for short periods of time. There were three other males that male 3 shared territorial borders with, and standoffs (Figure 1.2.4) frequently occurred between them. Male 3 was observed fighting with the largest of these males (ca 350 mm fork length) and chased off a smaller male who intruded into his territory. Torchlight showed that after dark there was ongoing activity among the males, and the females were still appeared alert and watchful when the dive ended at 1840hrs (43 minutes after sunset).

On the evening following the full moon male 3 was less active than during the previous two nights, perhaps because he had finished spawning as there were few females left within his territory. Male 3 was less aggressive towards the neighbouring males and he seemed to tolerate the presence of a small intruding male that appeared within his territory. This small untagged male (ca 310 mm fork length) had scrape marks on his sides and a tattered caudal fin, and during a fight observed between it and one of the resident males the small male was badly bitten near the dorsal fin. Nonetheless, he remained within the territory of male 3, limiting his movements to an area surrounding a small coral colony. The neighbouring males were more active than male 3 on the evening following the full moon, frequently engaging in territorial disputes and female swimovers. Numerous females with distended abdomens were present within the territories of these males although no spawning was observed.

Most of the females that had moved into the territories of males 1 and 2 over the previous three days were also gone on the evening following the full moon. Activity levels shown by these males were still high, but this was associated more with interactions with other males than with females. The number of territorial standoffs that these focal males had with each other and other neighbouring males peaked two days after the full moon (30 August; Figure 1.2.2c). In the case of male 2, many of the standoffs were against a slightly smaller male (ca 320 mm fork length) that moved in on 30 August and took over a substantial portion of the territory of male 2 (Figure 1.2.1). This intruding male had not previously been seen, and despite being smaller it showed more aggression than male 2 and kept control of this area of reef throughout the rest of the observation period. As a result, the territory of male 2 decreased from 41 to 34 m^2. Subsequent to this loss male 2 appeared to be extending his range further across the reef flat where a smaller, less aggressive male (ca 300 mm fork length) resided. Territory gains on the reef flat would not, however, compensate fully for the loss of territory on the reef slope where the female *E. rivulatus* were larger and more numerous in number. The territory of male 1 remained unchanged throughout the study at 41 m^2.

Two days following the full moon there were only a few females visible within the territory of male 3 and this male showed little activity, resting for much of the time and rarely displaying pale-tail colouration. Male 3 occasionally patrolled his territory but showed little aggression towards neighbouring males. In contrast, the other males in the area were still active, swimming low over females and having confrontations with each

other. Over the following couple of evenings there was progressively less activity among the males as *E. rivulatus* in the area entered the non-spawning period.

Behaviour Of Females During The Spawning Period

Females exhibited different behavioural patterns to the males. Over the peak reproductive time when the males were most active, the females spent much of their time resting motionless on the bottom, perched on their pectoral fins with an alert appearance. Occasionally a female would move to a new location, sometimes rubbing her side along the sand as she did so. Although this behaviour was also seen in non-reproductive fish it was observed more frequently during the spawning period. Larger females would also move in and displace a smaller female out of its resting spot. Females usually showed little reaction when a male swimover was directed towards them, but they appeared watchful of the males' territorial disputes and would sometimes swim nearer to where the dispute was occurring. There appeared to be more activity by females towards the end of the peak in spawning activity, possibly by individuals still searching for a male mating partner, although it was not clear whether these fish were gravid. At this time females were occasionally observed making unusual movements away from cover, by swimming up about two metres into the water column before slowly descending, or by swimming rapidly across the sand channel to a small coral lump that lay at least 20 m from the nearest shelter. Females swimming into the territories of neighbouring males would usually have to contend with the aggressive advances of a female already resident in the area, and would sometimes be chased back out of the territory.

Spawning Behaviour

Only two events that were interpreted as spawning were observed during this study, both involving male 3 and occurring approximately half an hour after sunset and one day before the full moon. During both spawns, male 3 and a single female rose quickly and vertically up off the substratum in a tight spiralling motion. At a height of about 1-1.5 m the two fish separated and quickly arched back down towards the substratum, leaving behind a faint pale ball of presumed gametes that measured approximately 7 cm in diameter. During the short period immediately following spawning the gametes were not subject to predation by other fish, and they were quickly lost from view in the dim light. These two spawning events were preceded by intense activity by male 3 as he moved at a fast pace over the motionless, attentive females. Already swimming fast as he approached a female, male 3 would speed up and move in alongside, nudging at the operculum of the female with his head but with little direct contact. This behaviour was observed on numerous occasions over the peak reproductive period, but usually the female would remain motionless or swim away a short distance whilst the male would circle briefly and move on. Although reproduction by males 1 and 2 was not observed, it is likely that they spawned sometime after the observation period on the evening of the full moon (i.e. at least 30 mins after sunset).

Other Behaviours Associated With Reproduction

Fights between the focal males and other males during the course of the observation period were rare, recorded only once for male 1 (on 31 August, the day after the number of standoffs peaked). Male 2 had more fights, all with the intruding male that took over part of his territory. These two males were first observed fighting on the evening after the full moon, and had two to three fights each evening over four consecutive

observation periods. No fighting was observed during the non-reproductive period until the final two days of the observation period, when male 2 was again observed having one fight on each night with the same male that had previously taken over part of his territory. After a fight the males usually rested for some time. Bullying and chasing of females by the focal males were also recorded at a low frequency, generally occurring zero to three times per evening throughout the study. These behaviours peaked over the reproductive period. Bullying occurred more frequently than chases, but never at more than five recordings for a particular night.

Behaviours During The Non-Spawning Period

Activity of the focal males and other *E. rivulatus* during the non-reproductive time was characterised by long periods of rest and relatively few intraspecific interactions. The locations at which the focal males rested were similar to those used over the reproductive time although the route taken between them varied depending on the activities that occurred in the area. Males were not as aggressive towards each other and they showed little interest in the females apart from the largest female and, to a lesser extent, one or two other large females who resided within their territories. Whilst male 2 concentrated his attention on one female in particular, male 1 appeared to divide his time equally between two females (272 mm and 287 mm fork length). The large (dominant) female(s) had special status within the territory of the male and moved freely within it. However, the male was wary of her movements, occasionally bullying her, often swimming nearby her, and quick to move in if the female approached a neighbouring male (or vice versa). The male would usually spend some time lying next to or nearby the dominant female during a tour of his territory.

The dominant female was also alert to the actions of the male, swimming up to him at any of his resting locations, following him or swimming past him. In contrast, the smaller females were usually ignored by the male, even though they would regularly swim close by or rest next to him. Occasionally the male or large female would bully or chase a smaller female, sometimes into the territory of a neighbouring male. If the male bullied the dominant female she would swim away from him. However, if smaller females were bullied they usually remained close by and endured the aggression of the male. Females moved between the territories of neighbouring males, having occasional confrontations with other females and alert to the activities of other *E. rivulatus*, especially the males. The larger females, in particular the most dominant individual, were less inclined to move between male territories.

Colouration

Several colour patterns were exhibited by *E. rivulatus*, often in association with a particular behaviour. Males were usually a barred tan colour whilst resting, swimming or bullying females, although when resting over sand they were sometimes an overall pale colour. When swimming fast or swimming over females the males typically displayed a cream pale-tail colouration (Figure 1.2.5). This colour pattern was commonly observed on fish identified as males but very rarely on a female. It was a temporary paling of the posterior and underside of the fish, with the intensity and extent of the cream colour positively correlated with the dominance and aggressiveness of the individual. The anterior of the body was usually tan and particularly dark along the border of the pale colouration. Over the reproductive period the males nearly always displayed this colour, even during the brief rests. During standoffs the males were typically barred (Figure 1.2.4) although this could change to a mottled, dark or pale

colour, and when fighting or chasing they were often a dark hue. When another male was nearby, a male would take on a mottled colour and raise his dorsal fin, especially if the dominant female approached. Females were typically a pale tan colour overlaid by faint bars during the reproductive period. At other times the smaller females were usually pale or barred whilst the dominant females were dark or barred in colour.

Sex Ratios

The movement of females between the territories of neighbouring males resulted in variation in the sex ratios. Male 1 controlled a loose-knit harem of between three and six mature females during the non-reproductive period, whilst the territory of male 2 contained between nine and ten mature females, and that of male 3 had approximately seven mature females. There were also a few small juvenile and immature females within these areas. These ratios were similar to that determined for populations within other sites at Ningaloo Reef (mature female to male ratio of about 6:1; Chapter 1.1). Over the short reproductive period however, the influx of females resulted in a significant increase in the sex ratios within the territories of the focal males (one-tailed paired t-test to compare increases in number of females of 6 to 13, 10 to 13, and 7 to 16 for males 1 - 3 respectively, $t = 3.5$; $0.025 < P < 0.05$). These ratios do not take into account the presence of the small male that appeared within the territory of male 3 during the spawning period.

Periodicity And Synchronisation Of Spawning

The observation study ended nine days before the next full moon with little apparent change in most behaviours to indicate a build up towards spawning. However this is not surprising given the rapid increase in activity that preceded spawning among the observed fish. The resumption of fighting between male 2 and the untagged male that took over part of his territory suggests that another period of spawning was approaching. Gonadosomatic indices of the ovaries of mature female *E. rivulatus* captured during the behavioural observation period also indicate that these fish were again becoming ripe (Figure 1.2.6). The GSI shows a good correlation with the behavioural observations, having maximum values just prior to spawning and dropping substantially during the time when spawning was observed. By the end of the sampling period on 17 September (three days after the new moon), the GSI values were near that of the females sampled at the start of the sampling period, indicating that the females were again approaching spawning readiness.

Histological analysis of the gonad samples also showed a close correlation between the reproductive status of the ovary and the observations of spawning activity. Ovaries of spawning (F4) females which contained oocytes in the hydrated or migratory nucleus stage of development were only collected on the days following the full moon. The ovaries of two females within the samples taken the day after the full moon contained post-ovulatory follicles which indicate that these fish had spawned the day before the full moon. All females captured four days after the full moon also had post-ovulatory follicles within their ovaries and had probably spawned one or two days earlier. The ovaries of females collected at the end of the sampling period were ripe (F3), being full of late yolk globule stage oocytes and approaching spawning readiness.

The close synchrony between gonad and observation data suggests that *E. rivulatus* throughout the sampling area were reproducing at a similar time during the course of this study. The evidence also indicates that spawning would occur again over the next

full moon in September. However the occurrence of spawning (F4) females in histological samples obtained during 1994 and 1995 from other locations along Ningaloo Reef (Chapter 1.1), shows that reproduction may occur over a wide range of the lunar cycle (Figure 1.2.6). Although the data are limited there is some indication that spawning is concentrated between the full and new moon periods.

1.2.3 Discussion

Spawning Cycle Within The Reproductive Season

This study showed that during the reproductive season *E. rivulatus* spawn during short periods of intense activity (ca 6 days) and then become quiescent for periods of about 20 days whilst gamete stores are replenished. The histological, GSI, and behavioural data provided similar evidence for the timing of spawning and indicated that this cycle of mating and replenishment may occur on a monthly basis. If so, then approximately six to eight spawning events may happen each year and females have the potential to spawn up to 24 times a season, based on the length of the reproductive season and a likely maximum of three spawns per spawning event (Chapter 1.1). In reality though, the spawning frequency of individual females is probably much less than this because spawning success may vary between individuals and many will obtain less than three opportunities to spawn (discussed below). Furthermore, not all females appear to spawn during most months of the spawning season (Chapter 1.1). A more detailed understanding of spawning frequency in *E. rivulatus* will be useful because spawning frequency, body size and fecundity are important determinants of spawning strategies in the serranids (Donaldson 1989).

Peaks of spawning at intervals throughout the reproductive season have been shown for other serranid species (Shapiro 1987), although the conditions that induce spawning in this group of fish remain an area of conjecture (Domeier & Colin 1997). The behavioural observations indicated that the full moon was an important cue to spawning in *E. rivulatus*, as has been found in other serranids (Johannes 1978, Thresher 1984). However, the histological evidence suggests that this was coincidental to the present study, and therefore *E. rivulatus* may not follow a particular lunar-spawning pattern or there may be other environmental factors that also influence the timing of spawning. The latter could be correlated to the lunar cycle (e.g. tide, current and light intensity cycles), with the precise timing of spawning activity varying with the characteristics of a particular locality, including depth, topography and tidal regime. An ebb tide occurring after sunset may also be favoured for spawning by certain fish species at Ningaloo Reef (J. McIlwain, personal communication), and as a consequence it is likely that peak spawning times relative to the lunar cycle may vary between locations and years depending on the timing, synchronisation and importance of the various cues.

Mating System, Mate Choice, And Competition For Mates

The type of mating system exhibited by a species is dependent on which sex is limiting and how the limited sex controls access to mates (Emlen & Oring 1977). In *E. rivulatus* the female biased sex ratio and ability of the males to monopolise these females result in a polygynous mating system, although the particular form of polygyny is dependent on the means used by males to control access to the females.

In the present study, the ability of male *E. rivulatus* to monopolise female mating partners was largely dependent on the control of territory along the reef slope. This area was a favoured living habitat, and as a result *E. rivulatus* were more numerous and larger in body size on the slope than on the adjacent reef flat. The reef slope was also a preferred spawning site, offering reproductive advantages that attracted non-resident *E. rivulatus* and temporarily increased local densities. Preferences for particular spawning sites are also characteristic of larger serranid species that aggregate to spawn (Thresher 1984, Domeier & Colin 1997). These sites may enhance the survival and dispersion of fertilised eggs (Johannes 1978, Barlow 1981), and this was a likely benefit to *E. rivulatus* in the present study because the spawned eggs would be rapidly transported away from reef predators by the currents that flowed down the sand channel and through nearby breaks in the reef to the open ocean.

Thus the mating system of *E. rivulatus* observed during this study was most likely based on the defence by males of preferred spawning sites ('resource defence polygyny', Emlen & Oring 1977). Ripe female *E. rivulatus* showed further preference for certain locations along the reef slope, probably through choice of a particular area for its environmental features as well as for the qualities of the resident male. This choice of mate by female *E. rivulatus* was indicated by their attentiveness to the activities of the males on the days of spawning, and by the movement by females between the territories of neighbouring males. The larger, most dominant male (male 3) was clearly most successful at monopolising access to the spawning females, whilst smaller males probably had fewer spawning chances and faced high levels of aggression. Although 'sneak-spawning' was not observed by these smaller males, this act of releasing sperm into the gamete cloud of a spawning pair has been observed in another serranid, *Mycteroperca tigris* (Sadovy *et al.* 1994).

The competition between males for access to spawning females was demonstrated by the number of standoffs and fights that occurred between them. Less evident was the selective choice of female spawning partners by the males, although the attention towards the largest females during the non-reproductive period and the number of ripe females that were present when the fish were spawning suggests that the males may exhibit some choice. Furthermore, since *E. rivulatus* are pair spawners there is likely to be no competition between males at the time of spawning. As a consequence the males may release relatively small amounts of sperm compared to males of group spawning species. The testes of male *E. rivulatus* are thus relatively small and contain limited supplies of sperm (Chapter 1.1), and they may therefore allocate sperm carefully between female mating partners in order to maximise the number of eggs they fertilise (Dewsbury 1982, Shapiro *et al.* 1994).

Whilst competition between male *E. rivulatus* was noticeable, competition between the females for mating opportunities was less obvious, even during the spawning period when the number of females per male doubled. Nonetheless, competition is expected in *E. rivulatus* given the female biased sex ratio (Berglund *et al.* 1993), although the rigid size-structured social hierarchy may defuse aggression (Sale 1978) such that only females of similar size or social status were ever observed having aggressive confrontations (present study and *pers. obs*). This is because relative position of these females in the social hierarchy is most uncertain and therefore challenged more frequently.

As a consequence, competition between the females is more subtle and may occur, for instance, through the choice of resting positions which may enhance an individual's

chances of making contact with a male during his territorial patrols. Competition for these preferred resting locations would therefore occur, as was indicated in the present study by the displacement of smaller females from their resting positions by larger individuals. Because of their size disadvantage, smaller females may therefore take any opportunity to spawn, even if conditions are suboptimal (Lutnesky & Kosaki 1995). Under such conditions a small female could gain higher reproductive success because of lowered competition for spawning opportunities with the male. However by spawning early the female may incur a risk of greater interference from unspawned females and increased predation of mating partners and the spawned eggs, although there was no evidence of this in the present study. By spawning late there is also a risk of not spawning at all if the male has exhausted his supply of sperm. In this case an unspawned female may move into the territory of another male, as observed in *E. rivulatus*. But this again incurs a risk of being confronted and chased out by other unspawned females within the new male's territory.

Spawning Strategies And Behaviours

There are few detailed accounts of spawning behaviour in the serranids. In *E. rivulatus* the build up to spawning began about a week prior to the full moon when the males became more active in patrolling and defending their territories from other males. Two or three days prior to spawning the level of activity picked up dramatically as the males began courting the females which were gathering in their territories, by making rapid, powerful swims over them (swimovers). Due to competition and mate choice, the build up in activity and the evening on which spawning occurred varied by a day or two between the males. The largest male in the area (male 3) began spawning the evening before the full moon and again on the night of the full moon. However the activities of *E. rivulatus* within the neighbouring territories suggests that many of his neighbours may have begun spawning a day or two later and spawned on one night only, perhaps quickly exhausting their sperm supplies after mating with several females. Low levels of courtship behaviour were still observed two days after the full moon, indicating that a few (probably less dominant) individuals were still spawning, however many of the females had departed by this time and the males were more interested in the defence of territory that had been gained or lost over the previous few days.

Distinctive courtship behaviour and colouration are characteristic of spawning in most serranids, although the details vary between species. Changes in colour patterns may signal courtship intentions and readiness to mate, and can also be used in dominance interactions (Colin 1992, Donaldson 1995). The pale-tail colouration observed in *E. rivulatus* was mainly used in dominance displays. It was exhibited by males towards females during the non-reproductive period, and on rare occasions by reproductive females engaged in particularly aggressive intra-sexual confrontations (*pers. obs*). This colouration was also a distinctive feature of males over the spawning period, and the intensity with which it was displayed may have conveyed the relative dominance of the bearer to other males and the gathered females. However neither female or male *E. rivulatus* displayed colouration that was interpreted as a signal of readiness to mate. Instead, the rapid swimovers by the males were an obvious display of courtship, whilst the energetic approach and nudging behaviour that males made towards the females were probably intended to show the male's readiness to mate and also induce the female to move with him into a spawning spiral.

Spawning in other serranids also occurs around sunset (Thresher 1984, Domeier & Colin 1997). However in most species it has rarely been observed and may often happen

after dark when unobtrusive observations are impossible. Certain aggregating species that spawn whilst light is available for observations appear to do so within a narrow time period of about 20 to 30 minutes (Colin 1992, Sadovy *et al.* 1994, Samoilys 1997), although non-aggregating species may take longer to complete spawning because of their more elaborate courtship rituals. In the present study, the two observed spawnings by *E. rivulatus* occurred approximately 30 minutes after sunset. Even if these spawnings involved less dominant females and occurred when conditions were suboptimal, it is likely that most spawning activity was commenced soon after this time and completed in the early evening. The precise timing of spawning appears to be under the control of females, as has also been noted in *Plectropomus leopardus* (Samoilys 1997), and may only occur when the females become responsive to the appropriate environmental cues and are induced to spawn by the activities of the males.

There are two main patterns of reproduction in the serranids: the non-migratory, single male/multi-female pair spawning strategy of smaller species such as *Epinephelus fulvus, E. cruentatus, Cephalopholis spiloparaea* and *C. urodeta* (Sadovy *et al.* 1994, Donaldson 1995), and the migratory, aggregating, single male/multi-female pair spawning strategy of larger species such as *E. guttatus, Mycteroperca tigris*, and *Plectropomus leopardus* (Colin *et al.* 1987, Shapiro *et al.* 1993, Sadovy *et al.* 1994, Samoilys & Squire 1994). Two additional size related strategies have also been observed in species at extremes of the serranid size range, that of *C. boenak* which is non-migratory and has a single male/single female pair spawning mode of reproduction (Donaldson 1989), and that of *E. striatus*, which is a migratory, aggregating, and group spawning species (Colin 1992).

The strategy observed in *E. rivulatus* was that of a non-migratory single male/multi-female pair spawning species, but with certain features that suggest this species is near the upper size limit of non-migratory species. Firstly, the movement by some individuals into the spawning sites could be described as short migrations. Further, the mating behaviour of *E. rivulatus* was similar to that of aggregating species such as *Plectropomus leopardus* and *Mycteroperca tigris*, in which males spend much energy in aggressive defence of their breeding territory, whilst the females are relatively inactive (Sadovy *et al.* 1994, Samoilys & Squire 1994). In contrast, courtship in smaller serranid species such as *Cephalopholis spiloparaea* and *C. urodeta* involves elaborate behavioural rituals by the male and active participation by the females (Donaldson 1995). Information on the social and reproductive patterns of species with body lengths between that of *E. rivulatus* (380 mm total length) and the smaller aggregating species such as *E. guttatus* (550 mm total length) may be particularly useful in evaluating the adaptive reasons for the reproductive strategies of small and large serranid species. In these species where the advantages in one spawning mode over the other are likely to be minimal, the importance of environmental parameters such as population density outside of the reproductive period and habitat topography may be examined.

This study shows the usefulness of abundant, site-attached and accessible species such as *E. rivulatus* in the study of serranid mating and social systems. The data obtained in the present study provides a focus for further research on *E. rivulatus* and is useful in determining future sampling regimes, such as when gravid females may be captured for fecundity estimates. Furthermore, because *E. rivulatus* use the same habitat for living and reproduction, the management of this species will require different decisions from those used in the management of the aggregating species, which live as scattered individuals in habitats that are quite distinct from their spawning area.

Table 1.2.1. Description of the behaviours recorded during observations of male *E. rivulatus* at Ningaloo Reef.

Behaviour	Description
Resting	Remaining within a half metre diameter circle on the substratum. Includes small changes in body position. May lie next to a conspecific if female and if no response is made (otherwise is considered an interactive behaviour as described below).
Swimming	Movement through the water. May be fast or slow and towards another fish, but does not include movement that elicits a response from a conspecific.
Bully	Interpreted as an aggressive show of dominance by the male towards a female, this behaviour usually consisted of a short (ca 2 m), medium to fast chase with the male directly behind the female. The swim was often in a half circle as the female evaded the male. No body contact was usually made, and neither fish appeared very stressed or alarmed after the behaviour. Varied from a 'light' bully in which the male showed less aggression and little change to body posture (may display 'pale-tail colouration'), to a 'heavy' bully, in which the male flared his gills out, had erect dorsal fin and outstretched pelvic and pectoral fins, distinct pale-tail colouration, and chased the female in a rapid, stiff and aggressive manner.
Standoff	A territorial ritual between two fish of similar sex (most notably between males) that rarely resulted in physical aggression. Intensity, duration and frequency varied, both between opponents and over time, with reproductive status an important determinant of how aggressive each male was. At peak spawning times standoffs were particularly intense and more frequently resulted in a fight, however, during non-reproductive times the standoffs were half-hearted and less common. One or both males would move towards the opponent in a slow, exaggerated swimming action, body angled so that the dorsal fin was towards the other fish. If the other male responded, they came together, perched on their pectoral fins, operculum to operculum and only millimetres apart. Colouration of both fish was barred. The males would then jostle, moving back a few centimetres and then lunging forward again, with the opposite opercula facing. During less intense confrontations the two fish appeared to be pushing each other, however when the fish were particularly aggressive they appeared to be ramming. One fish would break off and move with an exaggerated action in front of the other, the erect dorsal fin was towards the opponent. This behaviour appeared to be a display of size. The contest would continue for a variable amount of time, both males pushing and displaying, until one or both would move away (or a fight erupted). It was often not clear whether there was a winner or loser in these disputes.
Fight	An extreme and rare act of aggression between two individuals of the same sex (usually male), and often the culmination of a standoff during peak reproductive activity. Fights were usually brief and violent, with

Table 1.2.1 continued…

the two fish swirling around each other, dorsal fins erect, in a blur of

	activity that kicked up a cloud of sand and sometimes resulted in torn fins and other injuries. If one individual managed to bite the other andmaintain a hold the two fish may remain motionless for a time in this position. Colouration was generally barred.
Chase	Fast movement by one fish after another. Usually between individuals of the same sex and associated with defence of territory, with the intruder fleeing the aggressive advances of the territory holder. More often seen between females than between males. Colouration was generally barred.
Swimover	A short burst of speed made by a male over the top or close alongside a resting female. Tail motion was exaggerated and the male often swam with his body at an angle from the vertical.

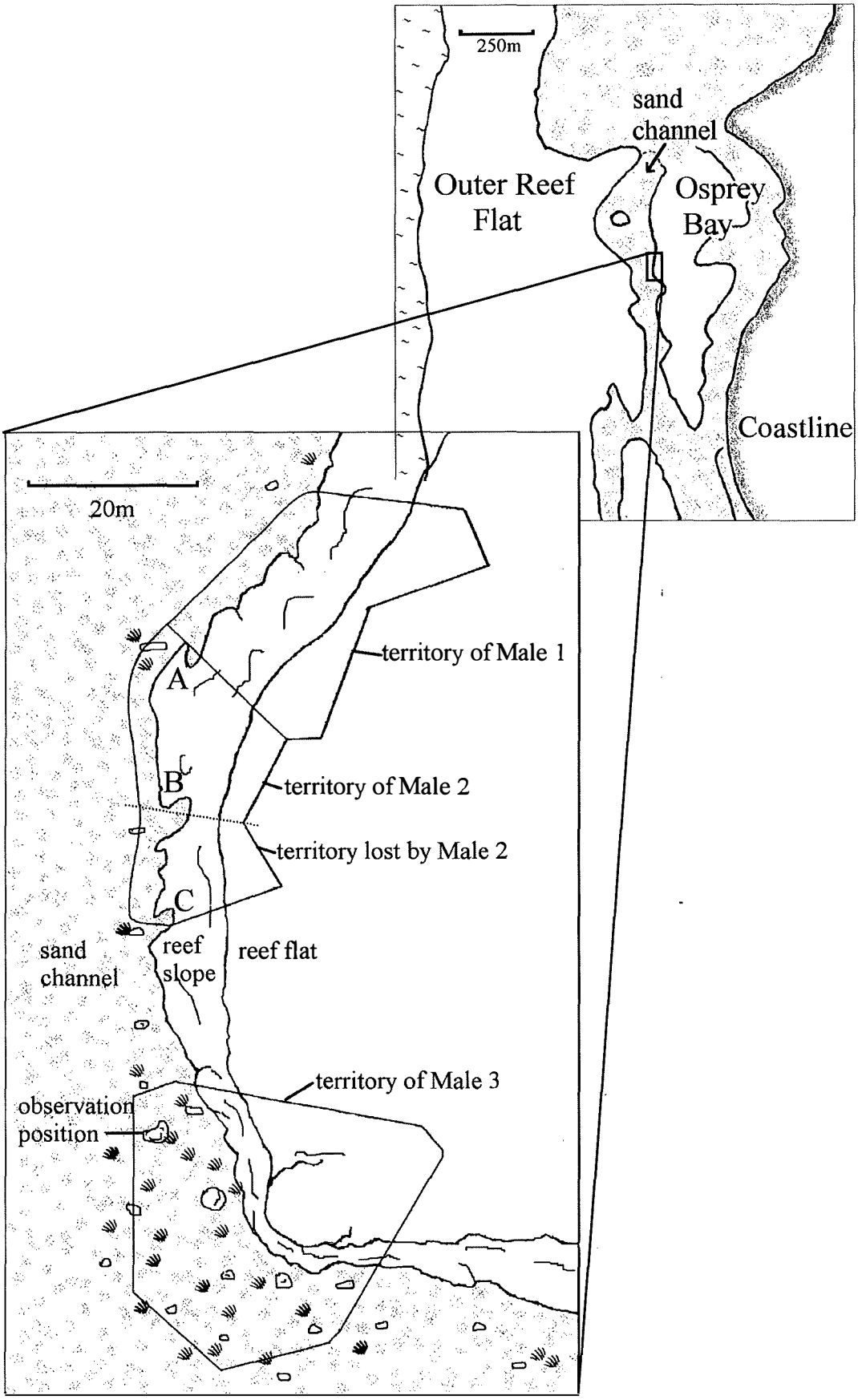


Figure 1.2.1 Map of Osprey Bay, Ningaloo Reef, showing details of the site in which spawning behaviours of *E. rivulatus* were studied. Also shown are the territories of the focal males observed during the study.

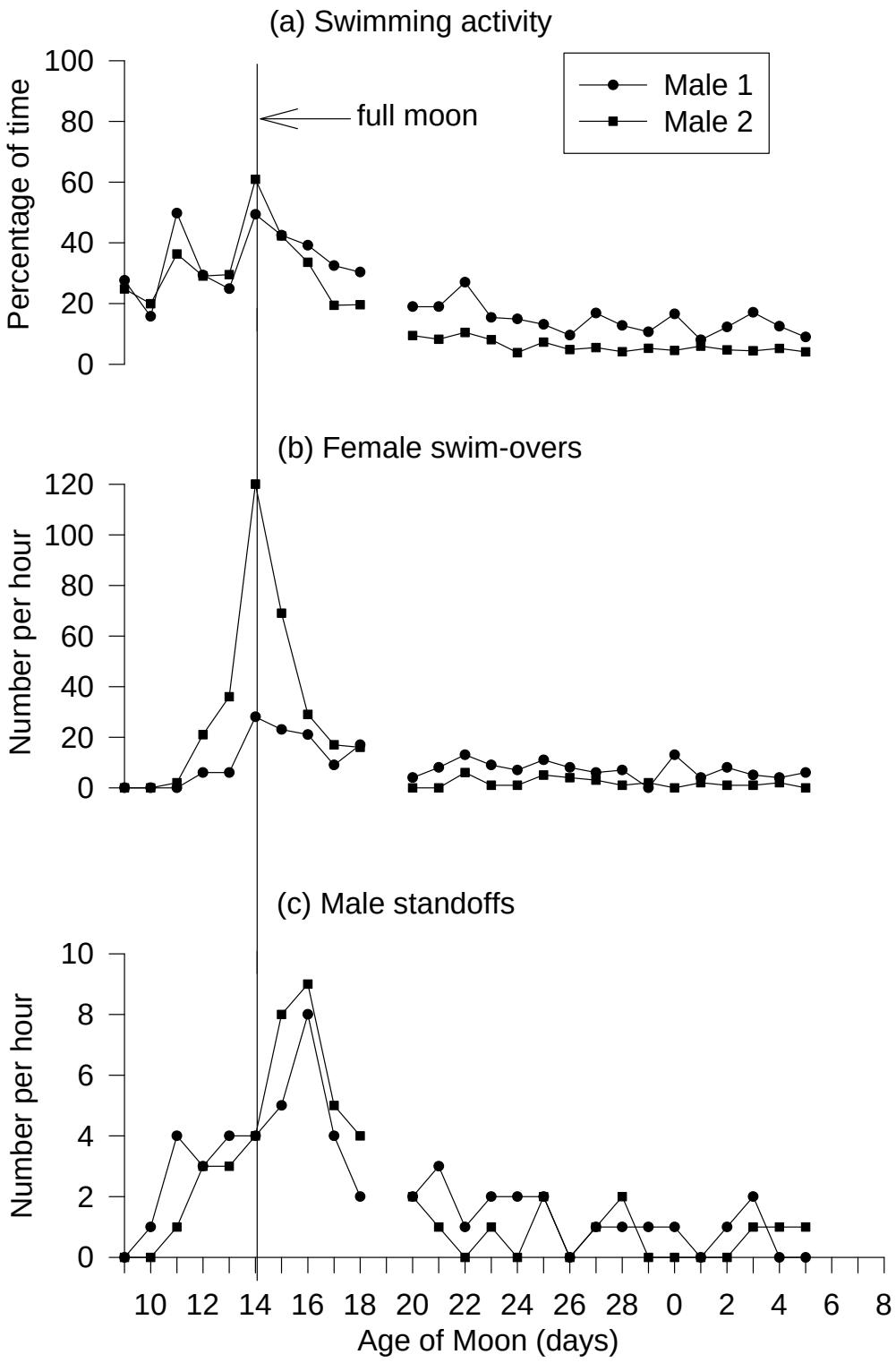


Figure 1.2.2. Behavioural activity of focal males 1 and 2 during the 60 minute observation periods (23rd August to 18th September 1996). Moon at day 0 = New, moon at day 14 = Full.

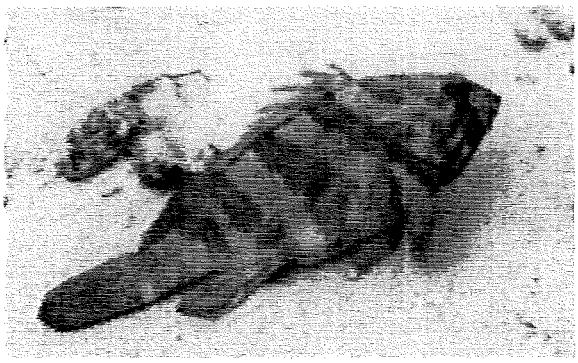


Figure 1.2.3. A ripe female *E. rivulatus* with barred colour pattern and swollen abdomen, resting at the base of the reef slope immediately prior to the peak in spawning activity.

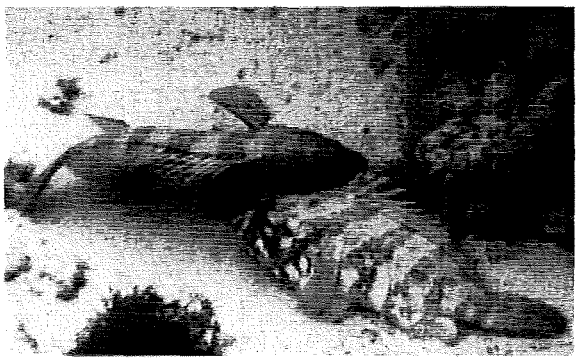


Figure 1.2.4. Neighbouring male *E. rivulatus* engaged in a territorial standoff during the peak in spawning activity.

Figure 1.2.5. A male *E. rivulatus* with the pale-tail colouration, swimming fast between vantage points during the peak in spawning activity. Note the tag beneath the dorsal fin.

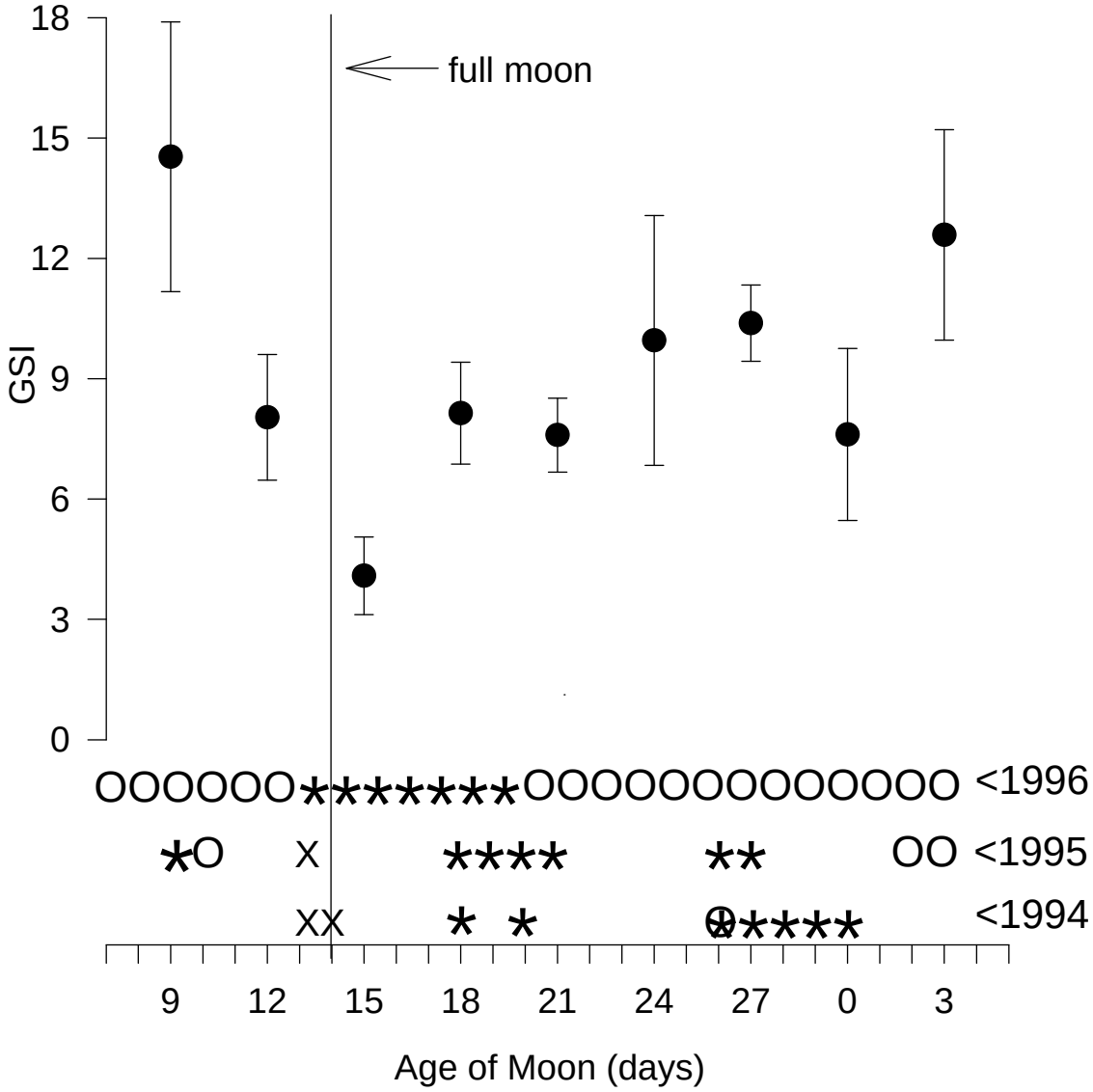


Figure 1.2.6. Mean gonadosomatic index (GSI, ± SE) of mature female *E. rivulatus* captured during the observation study at Osprey Bay (n = 6 for each sample). Also shown are the lunar ages of spawning for females, as estimated from histological evidence (asterisks). Data for 1996 were obtained during the present study, whilst data for 1994 and 1995 were obtained from previous samples. Hollow circles indicate lunar days when no spawning fish were found in the samples. Crosses show days when observations were made of behaviours associated with spawning.

1.3 Age And Growth

1.3.1 Materials and Methods

Study Area And Sampling Procedures

Otoliths (sagittae) were obtained from *E. rivulatus* captured along Ningaloo Reef between August 1994 and September 1996 using speargun and hook and line. Sampling locations included Tantabiddi, Mangrove Bay, North West Reef (NWReef), and Osprey Bay. Refer to Chapter 1.1 for details of these sites. Samples from Osprey Bay included fish captured at random over a wide area as well as from a site in which a study was made on the social structure of *E. rivulatus* (Chapter 1.4), followed by an experiment on sex-change in this species (Chapter 1.5).

Prior to removal of the otoliths, each fish was weighed (gm) and measured (mm), with measurements of fork length (FL) used in the present study (in *E. rivulatus* fork length is the same as total length). Otoliths were cleaned and stored dry in a gelatine capsule within a paper envelope. Unbroken otoliths of 203 *E. rivulatus* captured from all sites were weighed (to 0.1 mg) and measured (to 0.1 mm, maximum length along the longitudinal axis). The slope of the regression between the weights or lengths of the left and right otoliths did not differ significantly from one (Type II regression, $t_{(weights,\ 117df)} = 0.8391$, $0.5 > P > 0.2$, $t_{(lengths,\ 117df)} = 0.4697$, $P > 0.5$), showing that there were no significant differences in these parameters between the two otoliths. Thus the right otolith was used in length and weight analyses due to larger sample size.

Otolith Preparation

Preliminary analysis indicated that growth rings could not be reliably interpreted in whole otoliths. Thus a 400-500 μm thick section was cut transversely through the central region of the otolith to provide a clearer view of the internal structure, using the left otolith where possible. In preparation for sectioning, each otolith was embedded in clear casting resin. The section was then cut using a Struers Accustom-2 low speed saw with a diamond blade, and both sides sanded with 1200 grade ebony paper and 9 micron lapping film to smooth the surfaces. The section was then glued to a glass slide using DPX mounting medium, with the most interpretable surface uppermost. Immersion oil was placed on the exposed face of the section and the otolith analysed, mainly at 60x magnification, with a stereo microscope and transmitted light. The smaller otoliths of immature females which were to be inspected for daily increment pattern were similarly embedded and sectioned before careful sanding using 9 and 3 micron lapping film until between 170 and 300 μm thick. Regular inspection of the section using a compound microscope was required to avoid removing the inner ring structure. The section was glued to a glass slide with DPX mounting medium and covered with immersion oil prior to interpretation at 200-400x magnification using a compound microscope and transmitted light.

Otolith Interpretation

Terminology used in the description of *E. rivulatus* otoliths follows that of Wilson *et al.* (1983), except for the putative daily growth increments which are referred to as microbands in this study. These were defined as a pair of adjacent bands, one light (translucent) and one dark (opaque), with consecutive microbands radiating in a regular

pattern from a central core (focus). These relate to the proteinaceous discontinuous zone and calcium carbonate incremental zone described by Watabe *et al.* (1982). Where possible, counts of these microbands were made from the core to the settlement mark (band formed at the time of transition from the pelagic to demersal habitat), and from the settlement mark to the otolith edge. The first discernible microband after the focus was presumed to have formed on the day of hatching.

The number of microbands in each otolith section were counted on two occasions. Where the discrepancy between two counts differed by less than five percent the average was taken. If the discrepancy was greater than five percent a third count was made and the average of the two counts that differed by less than five percent was used. Otherwise no counts were used from that particular otolith.

Annuli (singular, *annulus*) in the otoliths of *E. rivulatus* were also defined as a pair of adjacent translucent and opaque bands (rings), but in this case refer to putative annual marks which showed large variation in width and clarity. As preliminary observations indicated that the otoliths of *E. rivulatus* were difficult to interpret, all otolith sections were initially examined twice, with knowledge of fish length and date of capture, in order to gain a better understanding of the characteristics of the ring structure. The approximate location of the first annulus was determined from a regression of the distance between the focus and margin of immature female otolith sections versus the average number of daily increments within this distance. The distance to the 365th microband was then calculated, giving a rough but useful guide to the likely position of the first annulus. After I (MM) was familiar with the otolith structure, I made two further counts, at least three weeks apart and without reference to fish size, although the approximate date of capture was sometimes known. During these counts, the readability of each otolith was categorised from 1 (unreadable) to 5 (perfectly readable). Each otolith was read from the centre outwards along the proximal surface. Usually the ring structure was most interpretable in the ventral region of the section, between the sulcus acusticus and ventral apex. However, where possible counts were also made along the dorsal region. If counts along the ventral and dorsal regions differed a decision was made on the reliability of each and hence which to use in analysis.

Otolith Readability

After conversion of the annuli counts to ages (see below), the two age estimates made on each otolith were compared and the level of confidence in the interpretation of the otolith structure assessed. This was done by determining the percent agreement between the two estimates, and by calculating the precision of the age estimates using the Index of Average Percent Error (Beamish & Fournier 1981). Otoliths assessed as unreadable (category 1) were discarded, whilst those assigned conflicting ages were re-read a third time. This final age was used if it was within ten percent of one of the other estimates, otherwise the otolith was discarded. A subsample of 72 otolith sections were also aged by an experienced otolith reader from Fisheries Western Australia.

Validation Of Otolith Rings

Tetracycline: The temporal frequency in which the otolith growth rings were formed was validated using oxytetracycline (Terramycin™ , 100 mg/ml) to label the otolith with a mark visible under ultra-violet light (McFarlane & Beamish 1987). Dosage rate was 50 mg/kg, with the correct amount for each fish calculated from a length-weight

relationship for *E. rivulatus*, and injected into the peritoneal cavity. To validate the microbands, 23 small immature females (84 – 180 mm) were captured in August 1996 from Osprey Bay, using quinaldine and a hand net. Each fish was measured and injected with a tetracycline-saline mixture *in situ.* The dorsal fin rays were then clipped to allow future identification, prior to release of the fish at the location of capture. Between six and 23 days later 14 of these small *E. rivulatus* were recaptured and their otoliths processed as described above. To validate the annuli, 71 mature *E. rivulatus* (201 – 327 mm) were captured using hook and line during May 1994. Each fish was injected with tetracycline and double tagged with t-bar anchor tags prior to release. After periods of 357 to 405 days, 13 of these tetracyclined *E. rivulatus* were recaptured and their otoliths prepared as above. The otolith sections were then examined using a compound microscope fitted with an ultra-violet light source, and the number of annuli between the fluorescent mark and the margin of the otolith counted and compared with the time between captures.

Marginal increment analysis: Analysis of the otolith edge (margin) was also used to examine the frequency and timing of deposition of the annuli. Because of the widely varying structure of these rings and the inability to obtain a consistent measurement along a particular axis within the otolith sections, the analysis of otolith marginal increment was made using a simple categorical system. This system was based on the relative amount of new translucent material at the margin compared to the width of the translucent material between the outer two opaque zones, as follows:

Category	*Amount of translucent material at otolith edge (as a % of distance between the outer two opaque zones)*
0	Opaque zone at edge
1	0 – 50%
2	50 – 100%

Conversion Of Ring Counts To Ages

Absolute age of each fish was estimated from the annuli count, marginal increment category, and date of capture. If the otolith of a fish captured during the time period when an opaque band was most likely to be formed had a wide translucent otolith margin (category 2), then the assigned age was the ring count plus one. The rationale here was that formation of the opaque band was imminent and should be accounted for. However, if the otolith already had an opaque or narrow translucent band (categories 0 and 1) at the margin, then the assigned age equalled the ring count. November 1 to February 28 was chosen as the critical time period during which the opaque band was most likely to form after analysis of the marginal increment data (see Results). For other fish captured outside this period, the absolute age was the same as the ring count.

Microband counts obtained from the otoliths of immature females were converted into proportions of a year so that these data could be used in the analysis of *E. rivulatus* growth. All these fish were younger than one year of age. Fish older than one year were placed in whole year classes prior to further analysis.

Growth Parameters From Length-At-Age Data

The von Bertalanffy growth function (VBGF, Ricker 1975) was used to describe growth in *E. rivulatus* because of the form of the plotted length-at-age data. This equation is typically used to describe the age and growth of serranids, thus permitting comparisons of the growth parameters between species. The VBGF was fitted to the size-at-age estimates using the FishParm software (Prager *et al.*1989), which utilises Marquardt's non-linear least-squares estimation procedures. The VBGF is defined as:

$$L_t = L_\infty \{1 - \exp[\ -K(t\ -\ t_0)\ ]\},$$

where L_t is the mean length at age t, L_∞ is the asymptotic mean length, K is the Brody growth coefficient that defines the growth rate towards L_∞, t is the age of the fish (in years), and t_0 is the theoretical age at which mean length is zero.

Comparison Of Growth Between Sites

An analysis of residual sum of squares (ARSS) was used to compare the VBGFs between the different populations of *E. rivulatus*. This required the calculation of an *F*-statistic from the data for comparison with the critical *F* value, with the degrees of freedom of the numerator and denominator equal to $3(K - 1)$ and $N - 3K$ (respectively), using the following formula (from Chen *et al*. 1992):

$$F = \frac{\dfrac{RSS_p - RSS_s}{DF_{RSSp} - DF_{RSSs}}}{\dfrac{RSS_s}{DF_{RSSs}}} = \frac{\dfrac{RSS_p - RSS_s}{3(K - 1)}}{\dfrac{RSS_s}{N - 3K}}$$

Where RSS_p = the residual sum of squares (RSS) of each VBGF fitted by pooled growth data, RSS_s = sum of the RSS of each VBGF fitted to growth data for each individual sample. N = total sample size, and K = number of samples in the comparison.

Whilst the ARSS will indicate an overall differences between growth curves, separate one-way analysis of variances were used to detect differences between the lengths of fish within each age group. Ages were grouped by two year intervals for this analysis to improve sample sizes. Posthoc comparisons of means were also performed using the Tukey's HSD test for unequal sample sizes, with the assumptions of normality and homoscedascity examined and data log transformed as appropriate. Because of their protogynous nature it was assumed that the growth curves of male and female *E. rivulatus* were similar, although a t-test for independent samples was used to test the overall difference in mean sizes between the sexes.

Estimation Of Natural Mortality

Because the collection of fish samples were biased towards medium to large females for the sake of reproductive analyses, it was not possible to obtain estimates of total mortality from catch curves. However, a preliminary estimate of natural mortality (*M*, per year) was obtained for *E. rivulatus* within Osprey Bay using the equation of Pauly (1984, page 75). This equation is a general relationship used to predict natural mortality

from the von Bertalanffy growth parameters, K (per year) and L_∞ (cm), and mean annual surface temperature, T (°C):

$$\text{Log } M = -0.0066 - 0.279 \log L_\infty + 0.6543 \log K + 0.463 \log T$$

The *E. rivulatus* for which M was determined had been protected from fishing activity since 1987, within the Osprey Bay Sanctuary Zone. In this case natural mortality equates to total mortality (Z), and from this value annual survivorship (%) was estimated (= 100 exp(-Z)). *Mean annual water temperature for* Osprey Bay (24.28 °C) was obtained using temperature data loggers that were secured to the substratum at a depth of approximately 4 m.

Relationship Between Fish Growth And Otolith Growth

Relationships between age and length of *E. rivulatus* and otolith weight and length were examined using linear and nonlinear regression. The FishParm software (Prager *et al.* 1989) was used to fit a number of nonlinear models to the raw and transformed data in order to select the one which best explained the variability of the data. Multiple regression analysis was also used to determine whether age was better predicted by a combination of otolith length, otolith weight and fork length, using either the raw data or the square/log transformations of them. The independent variables were fitted in a stepwise manner in order of best to least fit, with the inclusion level set at $P = 0.10$.

1.3.2 Results

A total of 531 sagittal pairs were obtained from *E. rivulatus* at Ningaloo Reef for the analysis of annuli. Readability of the otolith samples varied considerably between locations (Table 1.3.1). Otoliths of Mangrove Bay fish were particularly difficult to interpret due to the variability in the structure of the opaque bands (detailed below), and no agreement was reached on the ages of 13 *E. rivulatus* from Mangrove Bay that were aged by myself and independently by Fisheries WA personnel. Since the percent agreement between my own two counts on these fish was also zero, none were used in further analyses. The otoliths of *E. rivulatus* from other sites were not as difficult to interpret as those of the Mangrove Bay fish, although reliability of the age estimates was still fairly low (Table 1.3.1). The percent agreement between myself and Fisheries WA personnel on the ages of 47 *E. rivulatus* from Tantabiddi and 11 from NWReef were 47 and 0%, respectively. Forty-eight immature females between 51 and 175 mm fork length were also captured from Mangrove Bay for analysis of the microband structure of their otoliths. It was possible to obtain microband counts from 14 (29%) of these, whilst in the remainder the core was missing or it was covered by a dark crystalline matrix or by fissures that obscured the central region.

Microband Structure

In transverse section the microstructure of *E. rivulatus* otoliths was quite complex. Viewed under compound microscope the central regions were often dark and fissured, with the inner ring structure and core partially or wholly obscured (Figure 1.3.1a). Fan-shaped clusters of finely grained material were often present along the dorsal and ventral axes. These were similar in appearance to the clusters of aragonitic needles in the otoliths of the tropical snapper *Lutjanus vivanus* (Pannella 1980). Although there was often little microband structure within these clusters, a regular pattern of growth

increments was typical in most sections (Figure 1.3.1b). The microband structure was generally clear and easy to interpret, with three main sources of error. (1) Counts of the first few rings were difficult to resolve using light microscopy. (2) The choice of settlement mark, which was assumed to be a band or group of several bands with distinctive optical characteristics that marked a decrease in subsequent microband widths (Figure 1.3.1b). However, the identity or existence of a settlement mark was not validated in this study and it was sometimes difficult to distinguish a mark that could be identified as such. (3) Identification of microbands near the otolith margin were also difficult to distinguish and were sometimes sanded away whilst trying to uncover the central core. It was also difficult to identify outer bands in fish that were older than about 150 days.

Annuli Structure

The annuli within the otoliths of *E. rivulatus* were generally difficult to interpret, and relatively few otoliths could be read with any degree of confidence. Figure 1.3.2a shows one of the few otoliths with a clear, unambiguous ring structure. Reasons for the difficulties in reading the otoliths of *E. rivulatus* include: (1) the variation in width and clarity of the opaque bands, even within the same otolith section. At one extreme these were distinct, dark and narrow, whilst at the other they were faint and diffuse, and in some cases a band was only recognisable as a short smudge of brown located at a position where a ring was expected. When the rings were wide and diffuse it was sometimes difficult to determine whether there was only one ring, or two or more close together. (2) Variable deposition of otolith material along the margin. This often resulted in an irregular ring structure, with some opaque bands widely spaced in one area of the otolith whilst almost touching in another. (3) Split or secondary rings. It was often difficult to identify these from annuli given the variable nature of the ring structure. A comparison between counts on ventral and dorsal portions of the otolith was useful, although these rings were a major source of the imprecision between counts. (4) No one axis in the otolith section provided a consistently clear ring structure, which prohibited standardised measurements from being made. The most consistent area for counting rings was midway between the ventral apex of the section and the ridge of the sulcus acusticus. The dorsal region of the section was often more opaque and unreadable than the ventral region.

In general the first annulus beyond the core was relatively easy to identify. It was often preceded by one or more secondary rings which were also distinct but discontinuous, whilst the first one or two rings that followed the first annulus were typically faint and difficult to interpret. Figures 1.3.2b and 1.3.3a and b illustrate some of the above problems. The otolith section shown in Figure 1.3.2b has narrow, reasonably distinct ring structure in the ventral region of the otolith whilst the dorsal region is more opaque with poor ring structure. There are several possible secondary rings in the ventral region which made aging of this fish difficult. In the section shown in Figures 1.3.3a and b, the annuli in the dorsal region are diffuse and it is difficult to distinguish rings near the margin and sulcus. The early rings are also faint. Annuli in the ventral region are faint, discontinuous and have poor structure such that it is difficult to distinguish between true annuli and secondary rings.

Validation Of Daily And Annual Growth Increments

It was only possible to obtain a partial count of the number of microbands between the tetracycline band and the otolith margin in the otoliths of one juvenile. This fish was

recaptured 23 days after it had been tetracyclined and was 133 mm in length. Although only 7 microbands were visible at the otolith margin of this fish, extrapolation of the width of these bands to the width between the fluorescent mark and the otolith margin provided an estimate of 22.8 microbands (Figure 1.3.4a and b). Thus, whilst the evidence is not conclusive, it is likely that the microbands in the otoliths were laid down daily in these otoliths. In all other fish, left at liberty for 6 to 16 days, the fluorescent band lay close to the otolith margin and the ring structure was too poor for estimates to be made.

Twelve tetracyclined adult *E. rivulatus* were recaptured after periods ranging from 357 to 405 days. All except two of these had a clear fluorescent mark in their otoliths, and in all of these only one opaque band was present between this mark and the otolith margin (Table 1.3.2, Figures 1.3.5a and b). The recaptured *E. rivulatus* were aged 6, 7, 9, 10, 11, 14 and 24 years, providing validation over a range of older ages. Analysis of the marginal increment provided further evidence that only one opaque band forms each year (Figure 1.3.6). Formation of this band mainly occurred during the summer months between November and February. By April the otoliths of most *E. rivulatus* had a translucent edge, which increased in width through the winter months until August and September when the appearance of a new opaque band appeared imminent.

Age And Growth

Assuming daily production of microbands in the otoliths of all juvenile *E. rivulatus*, the time period between hatching and settlement for the 14 immature females from Mangrove Bay varied from 21 to 61 days, with a mean of 37 days (± 3 SE, Table 3). Most of these fish were three to five months old when caught, and were estimated to have hatched from mid August to late November, within the peak spawning time for *E. rivulatus* at Ningaloo (Chapter 1.1). Average fork length of these 0+ females was 85.1 mm (± 4.6 SE, Table 1.3.4).

Mean ages of mature female and male *E. rivulatus* were significantly different (t-test for independent samples on pooled, square-root transformed data = -14.598, P_{317df} = 0.000. Levenes test for homogeneity of variances, F_{1df} = 0.404, P = 0.525, Figure 1.3.7). The ages of sexually mature female *E. rivulatus* ranged between 1 and 15 years (average 4 - 5, Table 1.3.5) , with 30% of the one year old and 90% of the two year old females reproductively active. Average length of females in these two age groups was 177 and 227 mm which agrees with the estimated length at 50% maturity of 193 mm (Chapter 1.1). Males ranged between 2 and 24 years (average 9 - 10). The two year old male in the NWReef sample was abnormally small (221 mm fork length). The average age at which sex-change occurs in *E. rivulatus* at Ningaloo was estimated at five years, being the median value of all ages within the overlap of male and mature female ages (Shapiro 1987). The range of this overlap (2 to 15 years) and presence of transitional fish between the ages of 4 and 10 years shows that sex-change in *E. rivulatus* occurs over a wide range of ages.

Growth Model From Length-At-Age

The plotted length-at-age data and fitted growth curves show that growth in adult *E. rivulatus* is most rapid during the first four years of life and asymptotes after about seven years (Figures 1.3.8 and 1.3.9). The data for Tantabiddi and Osprey Bay also suggests that growth of females older than 6 years of age may be suppressed whilst males may experience a burst in growth soon after sex-change. Von Bertalanffy growth

curves fitted to the combined male and female data explained between 85.9 and 96.8% of the variability in length-at-age (Table 1.3.6). Values for the growth coefficient *K* indicate that *E. rivulatus* within each site have a rapid growth rate of up to 0.530 at Tantabiddi, and 0.416 for all sites combined. L_∞ estimates of 307, 338 and 318 mm fork length for *E. rivulatus* from Tantabiddi, NWReef and Osprey Bay compare to the maximum observed lengths of 358, 380, and 358 mm for these sites, respectively. The growth curves also show that females dominate the samples up until about the seventh year, however there were occasional old females and young males. For a given age the males were typically larger in size than the females, suggesting that mean growth rate after sex-change is greater than before, or that there is a tendency for only the larger females to change sex.

Comparison of the individual growth functions by analysis of the residual sum of squares was done without the data for immature females from Mangrove Bay. The test indicated significant differences in growth between *E. rivulatus* from the different sites (Table 1.3.7). Further analysis showed that fish in the 6 - 7 year age group from NWReef were significantly larger in size than similarly aged fish from Tantabiddi and Osprey Bay (Table 1.3.8). This is due to the slight depression in the mean lengths of 6 - 7 year old fish compared to 5 year old fish in the latter two sites (Figure 1.3.8, Table 1.3.4). The analysis of variance also showed that 2 - 3 year old fish from NWReef were similar in size to fish of those ages from Osprey Bay and Tantabiddi, whilst 2 - 3 year old fish from Osprey Bay were significantly larger than similarly aged fish from Tantabiddi. All other age groups were similar in size within the three sites.

Preliminary Estimation Of Natural Mortality

Using the general equation of Pauly (1984), an approximation of the rate of natural mortality (*M*) for *E. rivulatus* at Osprey Bay was 0.994. This equated to total mortality (*Z*) in the absence of fishing in this area, and represents an annual survivorship of 37%.

Relationships Between Fish Age And Growth, And Otolith Growth

The usefulness of otolith length and weight in predicting the age and body length of *E. rivulatus* was examined using regression analysis. The relationship between fish age and otolith weight was best described using either a power function (with intercept) or a logistic function, since both explained approximately 88% of the variability in the natural log transformed data (Figure 1.3.10a). Although the relationship between age and otolith weight appears to be linear in older fish, linear regression of the raw and transformed data for fish older than four years did not describe the data as well as the nonlinear functions.

A power function also gave the best fit to the fish age versus otolith length regression, explaining 74% and 84% of the non-transformed and log-transformed data, respectively (Figure 1.3.10b). Because linear growth of the otolith slows in older fish, otolith length is a poorer predictor of fish age than is otolith weight, which increases at a steady rate with age. Examination of the otolith sections showed that the relatively constant gain in weight was due to a thickening of the otolith as the fish ages.

Relationships between fish length and otolith weight or length were high ($r^2 = 0.9600$ and 0.9454, respectively, Figures 1.3.11a and b). The best fit for the relationship between fish length and otolith weight was provided by the Weibull cumulative distribution function (Prager *et al.* 1989), whilst the relationship between fish length and otolith length was linear. The combination of otolith weight, log transformed otolith

length, and fish length provided the best prediction of age in the multiple regression analyses, explaining 84.1% of the variability in age. These data show that otolith length is a good predictor of fish length due to similarities in their growth trajectories, whilst otolith weight is by itself the best predictor of fish age.

1.3.3 Discussion

Interpretation And Validation Of Annuli

The aging of fish from otoliths can be a notoriously difficult and subjective task. This is particularly so for species inhabiting tropical reefs (Pannella 1980, Brothers 1982, Manooch 1987), and was the case for *E. rivulatus*. The otoliths of *E. rivulatus* also showed differences in internal structure and hence readability between locations, as has been noted in tropical damselfish (Fowler 1995), and the congener *E. guttatus*, in which only 33% of otoliths were legible in samples from St Thomas compared with 63% of the otoliths in fish from Puerto Rico (Sadovy *et al.* 1992).

Otolith growth in fish is a complex issue and the subject of numerous yet inconclusive studies (Pannella 1980, Fowler 1990, Beckman & Wilson 1995). It is the result of interactions between endogenous and/or exogenous cycles of somatic growth rate and/or protein and aragonite metabolism, and the ecology, behaviour and physiology of each fish (Brothers 1982, Fowler 1995). Formation of the opaque zone often occurs through spring and summer during periods of fast otolith and somatic growth (Fowler 1995, Beckman & Wilson 1995), when the deposition of calcium carbonate (aragonite) on the otolith occurs as long, thick needles around the organic matrix (Pannella 1980). Deposition of the opaque zone in the otoliths of serranids generally follows this pattern, usually occurring sometime between March and July in species of the genera *Epinephelus* and *Mycteroperca* from the western Atlantic (Moe 1969, Matheson & Huntsman 1984, Matheson *et al.* 1986, Bullock *et al.* 1992, Hood & Schlieder 1992, Potts & Manooch 1995), and November to February for *E. rivulatus* at Ningaloo Reef, as determined in this study. As shown in Chapter 2, formation of the opaque band in the otoliths of *Plectropomus maculatus* from the north west of Western Australia also form during spring and summer. However, notable exceptions to this rule are *Plectropomus maculatus* and *P. leopardus* from the Great Barrier Reef, in which opaque zone formation occurs during winter and spring (McPherson *et al.* 1988 *in* Williams & Russ 1991, Ferreira & Russ 1992).

These seasonal patterns in the formation of annuli in the otoliths of fish are due in part to changes in water temperature (Beckman & Wilson 1995). Pannella (1980) noted that a range of at least 4 – 5^{o} C is needed to sufficiently alter otolith growth such that annuli are produced, which is well below the annual fluctuations in water temperature of 19.7 to 29.7^{o} C at Osprey Bay, and 16.6 to 29.3^{o} C at Mangrove Bay (Chapter 1.1). Differences in the temperature range between these two sites provides one explanation for the differences in otolith readability, with fish at Mangrove Bay possibly producing more opaque material in their otoliths as a consequence of the wider range in temperatures they experience. Similarly, variation in temperature regimes between seasons may be a cause of the considerable intra-otolith differences in the width and character of adjacent annuli.

Formation of one annulus per year was confirmed in *E. rivulatus* above six years of age. However, for an aging technique to be considered accurate it must be validated in

fish of all ages (Beamish & McFarlane, 1983). Thus a more comprehensive, tetracycline-based validation program is needed for *E. rivulatus*, since the otoliths' rings in younger age groups remain unvalidated. Furthermore, repeat injection of adult fish with tetracycline over a number of years would prove useful in interpreting the ring structure within the otoliths of *E. rivulatus* as this would produce a series of fluorescent rings at known time intervals. *E. rivulatus* would be ideal for such a study given its site-attached nature and accessibility. Despite the difficulties in interpreting the otoliths of *E. rivulatus*, they nonetheless fulfil the requirements needed for aging purposes, because they continue to grow throughout the lifetime of the fish, they display visible growth increments, and these increments are formed on a regular, determinable time scale (Fowler 1990, 1995).

Planktonic Duration And Settlement

Knowledge about the duration of the pelagic phase is important in understanding the recruitment dynamics of a species and in determining the extent to which offspring may disperse (Brothers 1982). This has important implications in the stock assessment of a fishery, for instance in assessing whether there is substantial input of new recruits from other areas, and in decisions on the location and size of marine reserves for recruitment of young into exploited populations.

An estimate of the planktonic duration requires identification of a settlement mark, which was identified with varying degrees of confidence in the otoliths of *E. rivulatus*, but remains unvalidated. Nonetheless, given the complex physiological changes that occur as the fish enters the benthic habitat, it is not surprising that a corresponding mark is left in the otolith (Victor 1982), and the optical characteristics and subsequent decrease in increment width are typical of the settlement mark in other tropical fish species (Victor 1982, Fowler 1989, Wellington & Victor 1992), including the serranid *Plectropomus leopardus* (Doherty *et al.* 1994).

Validation of the microbands is also a necessary prerequisite for determining planktonic duration. The present study failed to provide unequivocal evidence that microbands are formed daily within the otoliths of *E. rivulatus*, mainly due to the difficulty in reading microbands at the margin of the otolith. This problem may be solved in the future by injecting juveniles at two different times (up to two or three weeks apart) and then leaving them at liberty for a longer time period (at least a month) so that the tetracycline bands are clear of the margin when the fish are finally recaptured for examination of their otoliths. Nevertheless, if the microbands in the otoliths of *E. rivulatus* were deposited daily as the evidence suggests, then the duration of the planktonic stage in these *E. rivulatus* was 3 - 9 weeks (mean 37 days). This is typical of reef fish species (Brothers *et al.* 1983), and compared to other serranids is close to the mean for *Epinephelus striatus* in the Bahamas of 42 days (Colin *et al.* 1997), but longer than the mean for *Plectropomus leopardus* on the Great Barrier Reef of 25 days (Doherty *et al.* 1994). *E. rivulatus* appear to have a more variable planktonic life than these two serranids, ranging from 21 to 61 days compared to 37 to 45 for *E. striatus.*

Little is understood about the factors affecting the planktonic duration (Wellington & Victor 1992), and virtually nothing is known about the transportation of *E. rivulatus* or other serranid larvae in the vicinity of Ningaloo Reef, apart from the fact that serranid larvae make up a small but distinct proportion of the larval fish fauna that enter the lagoon of Ningaloo Reef across the reef crest (Doherty & McIlwain 1996). Since water inside the Ningaloo Reef lagoon funnels out to the open ocean via numerous reef breaks

and re-enters across the reef crest (Hearn & Parker 1988), it is likely that many of the larvae spawned by *E. rivulatus* in the lagoon are carried out to sea before making their way back to the reef prior to settlement. The length of the planktonic phase in *E. rivulatus* may therefore be influenced by factors such as characteristics of the water flow at the spawning site, retention time in the lagoon, oceanic current speeds, and behaviour of the larvae.

Back calculation of birthdates for newly settled juvenile *P. leopardus* and *E. striatus* indicated that spawning occurred near the new and full moons, respectively (Doherty *et al.* 1994, Colin *et al.* 1997). No clear lunar pattern was evident for *E. rivulatus*, even with the addition of four days to the age of each fish to allow for a possible time lag between birth and production of the first increment (Colin *et al.* 1997). However, reproduction in *E. rivulatus* appears to be cued by other environmental factors besides lunar cycle (Chapter 1.1). Furthermore, because the ages designated to *E. rivulatus* were often an average, the birthdate estimate is thus an approximation and not reliable for exact predictions.

Age, Growth, And Mortality

Serranids are long-lived and slow-growing fishes, with maximum ages usually exceeding 10 years and values for the growth coefficient, *K*, typically between 0.10 and 0.25 per year (Manooch 1987). For some serranid species the maximum age is more than 20 years, which may also be reached by *E. rivulatus* at Ningaloo Reef although few individuals older than 14 years were present in the samples. The high value for *K* (> 0.4) shows that *E. rivulatus* reach their maximum length at a rapid pace compared to many other serranids. Female *E. rivulatus* may also become sexually mature at a relatively early age since most were reproductive by age two. In comparison, only 50% of female *Epinephelus guttatus*, a serranid about 1.5 to 2 times the body length of *E. rivulatus*, had reached sexual maturity by age three (Sadovy *et al.* 1992). Data for *E. rivulatus* captured in the province of KwaZulu-Natal, South Africa, confirm the rapid growth rate ($K = 0.502$) and age at sex-change (4.5 years) (Fennessy 1998). However, these South African fish had a larger asymptotic length ($L_\infty = 351$) and maximum length (435 mm total length), but younger maximum age, since the oldest female and male were only 10 and 14 years, respectively.

The relatively young maximum ages of the South African fish may be due to the heavy fishing pressure they receive (Fennessy 1998), whilst the larger lengths are possibly a result of a deeper habitat, since *E. rivulatus* captured in the relatively deep waters of North West Reef during the present study were generally larger than those captured from the other areas. Recreational fishers also note that *E. rivulatus* captured in deeper waters off Ningaloo Reef are often larger, whilst *E. rivulatus* capture in deeper waters off Geraldton (Western Australia) also reach a larger size than the Ningaloo Reef fish (*pers. obs.*). Combined with a relatively long reproductive season (Chapter 1.1), the fast growth rate and young age at which females become sexually mature help explain the numerical dominance of *E. rivulatus* over other serranid species, especially in areas of sand, algae and eroded limestone that is a common habitat within the lagoon of Ningaloo Reef (Ayling & Ayling 1987, *pers. obs.*).

Although the serranids are notably susceptible to mortality from fishing activities, little is known about their natural causes of mortality apart from the fact that it is typically low (Thompson & Munro 1978). Predation (particularly by sharks), parasitism, cold water shock, and red tides have been cited as the main causes of death

in serranid species, especially of smaller individuals (Olsen & LaPlace 1979, Ralston 1987). Certainly, predation is likely to cause high mortality of smaller *E. rivulatus*. However, parasitism may be a greater source of mortality among larger individuals, since parasitic nematodes (*Phylometra* spp.) were commonly found within or attached to the walls of the gonads (Chapter 1.1). The occasional *E. rivulatus* captured from deeper waters were also host to a large parasitic copepod which caused a cyst approximately 20 mm diameter to form within the flank of the fish. Injury inflicted during particularly aggressive territorial disputes (Chapter 1.2) may also result in death, either directly or by making the individual more susceptible to predation or infection.

Ralston (1987) noted that estimates of natural mortality obtained using the Pauly (1984) equation were about 45% greater than estimates obtained by the catch curve method for several species of serranids. If this were the case for *E. rivulatus* then *M* for this species may be 0.547 (an annual survivorship of almost 58%), which is closer to values determined for other serranid species. However, Ralston (1987) also concluded from a review of the literature that the rate of natural mortality in serranids can be estimated as roughly twice the value of the growth coefficient, *K*. In the case of *E. rivulatus* from Osprey Bay ($K = 0.464$) and other locations at Ningaloo Reef, it is therefore likely that they do have relatively high rates of natural mortality compared to other species of serranids. Given this combination of relatively high growth and mortality rates, it is also likely that *E. rivulatus* have a relatively high productive capacity compared to other serranids.

Otolith Growth

Most body parts follow growth trajectories that are allometric to body length. This is also the case for otolith length in *E. rivulatus*. However, as linear growth of the otolith slows with age, the otolith becomes thicker and heavier at a rate that is consistent with age rather than length. Similar patterns of otolith growth have been observed in other fish, prompting investigation of the potential use of otolith parameters in estimating age for several fish species (Boehlert 1985).

Accurate and efficient determination of fish age is an essential requirement of analyses used in the management of exploited species (Fletcher 1991). In recent times examination of the internal ring structure of otoliths has been the most favoured technique for obtaining these age estimates. However, ageing fish by this method is often a time consuming and expensive task. Furthermore, and as highlighted in the present study, it often produces subjective and imprecise results. This has led to the investigation of otolith parameters as a more rapid and objective ageing methodology. Boehlert (1985), for instance, used a combination of otolith weight, otolith width, and otolith length to age rockfish (*Sebastes* spp.) accurately and objectively. Pawson (1990) showed that sardines (*Sardinops aurita*) could be satisfactorily aged using a combination of otolith weight and fish length, whilst Fletcher (1991) found that otolith weight alone appeared to provide a means of estimating age in pilchards (*Sardinops neopilchardus*), that was as accurate as age estimates gained from scale and otolith readings.

Similarly, the present study has shown that otolith weight may also be a cost effective and objective means of ageing *E. rivulatus*. Certainly, given the difficulties experienced in interpreting the ring structure of this species, further research into the usefulness of otolith weight is warranted. Such research should examine the amount of variation in the otolith weight of similarly aged fish and subsequent overlap between the distributions of otolith weight of different age classes. Comparisons of the age

estimated using the weight of a particular otolith and the internal ring structure should also provide a useful cross-check of each methodology. Ultimately, it may thus be possible to develop an efficient and adequate ageing methodology for *E. rivulatus* that is based on measuring the relevant parameters of a large number of otoliths and examination of the internal ring structure of a sub-sample of these.

Table 1.3.1 The number of *E. rivulatus* otoliths in each readability category. Category 1 = unreadable, categories 2 to 5 = readable with increasing degree of confidence. Percent readable is the percentage of otoliths placed in category 2 or greater and accepted for age analysis. Percent agreement is the percentage of otoliths that were assigned the same age between counts. IAPE is the Index of Average Percent Error. Data from Osprey Bay includes otoliths of fish used in the sex-change experiment.

Location	Readability category					n	Percent readable	Percent agreement	IAPE (%)
	1	2	3	4	5				
Mangrove	31	24	2			57	46	27.5	19.0
Osprey Bay	55	85	54	4		198	73	61	15.8
Tantabiddi	43	106	61	1		211	80	68	17.0
NWReef	22	26	16		1	65	66	54	14.1

Table 1.3.2 Details of *E. rivulatus* captured at Ningaloo Reef and injected with tetracycline in order to determine the temporal significance of opaque growth zones in the otolith. The number of opaque bands refers to the number between the fluorescent tetracycline band and the margin of the otolith.

Fish	Sex	Date of capture recapture	Forklength (mm) capture recapture	Days at liberty	Number of opaque bands	Age	Readability category
OB33	F	1/5/94 23/4/95	230 265	357	1	7	2
OB34	F	1/5/94 23/4/95	230 277	357	1	7	2
OB43	M	2/5/94 10/5/95	314 332	373	1	10	3
OB45	M	1/5/94 10/5/95	? 314	374	1	9	4
OB49	M	2/5/94 10/5/95	306 326	373	1	11	2
OB58	F	2/5/94 10/5/95	236 260	373	1	6	2
RO59	F M	10/8/95 14/9/96	300 325	401	1	10	2
RO69	M	10/8/95 17/9/96	341 350	404	1	24	4
RO73	F	10/8/95 17/9/96	284 295	404	1	7	2
RO75	F	10/8/95 18/9/96	279 285	405	1	14	2

Table 1.3.3 Fork length (FL), estimated age, and backcalculated birth-date of immature female *E. rivulatus* captured at Mangrove Bay.

Fish	Capture date	FL (mm)	Mean microband count		Estimated age (days)	Estimated date of birth
			Core to settlement	Settlement to margin		
MAN1	11Aug 95	121	39.5	218	257.5	26 Nov 94
MAN14	16 Feb 95	88	48.5	86	134.5	4 Oct 94
MAN16	16 Feb 95	98	61	77	138	1 Oct 94
MAN17	16 Feb 95	99	21.5	101	122.5	16 Oct 94
MAN18	16 Feb 95	94	26	156.5	182.5	17 Aug 94
MAN19	13 Feb 95	93	44	111	155	11 Sep 94
MAN22	13 Feb 95	88	41	95	136	30 Sep 94
MAN23	13 Feb 95	100	27.5	107.5	135	1 Oct 94
MAN31	16 Dec 95	81	33	91	124	14 Aug 95
MAN43	24 Dec 95	65	?	?	74	11 Oct 95
MAN44	24 Dec 95	70	41.5	66.5	108	7 Sep 95
MAN46	24 Dec 95	63	40.7	65	105.7	9 Sep 95
MAN47	24 Dec 95	66	25.5	64.5	90	25 Sep 95
MAN48	24 Dec 95	66	31.5	59	90.5	24 Sep 95

Table 1.3.4 Mean fork length (FL, mm) and standard error (SE) of *E. rivulatus* within each age class. Data are shown for all sites combined (Ningaloo), and for each site separately.

Age	Ningaloo		NWReef		Osprey Bay		Tantabiddi		Mangrove Bay	
class	n	Mean FL (SE)	n	Mean FL (SE)	n	Mean FL (SE)	n	Mean FL (SE)	n	Mean FL (SE)
0	16	94.9 (7.8)	-	-	1	162.0	1	165.0	14	85.1 (4.6)
1	19	176.7 (5.8)	1	190.0	4	195.0 (16.8)	13	172.2 (5.9)	1	148.0
2	45	227.3 (2.8)	4	207.0 (8.8)	9	240.0 (4.7)	31	225.0 (3.0)	1	263.0
3	55	254.1 (2.2)	6	264.3 (10.5)	27	253.9 (3.2)	21	252.5 (2.6)	1	236.0
4	38	268.1 (3.3)	2	296.0 (14.0)	16	267.6 (5.2)	18	265.6 (4.6)	2	267.0 (3.0)
5	34	286.1 (3.2)	5	284.0 (11.3)	19	288.0 (4.5)	8	284.1 (4.7)	2	281.0 (13.0)
6	30	290.0 (3.9)	5	314.8 (9.2)	16	284.9 (4.8)	6	280.5 (7.5)	3	295.3 (6.9)
7	28	291.3 (3.8)	1	317.0	16	296.3 (4.6)	10	283.6 (6.3)	1	263.0
8	20	306.1 (4.7)	7	319.4 (3.2)	5	310.2 (7.5)	6	293.3 (9.6)	2	287.5 (24.5)
9	13	318.0 (3.7)	2	328.0 (1.0)	4	320.5 (4.6)	6	315.0 (7.0)	1	306.0
10-12	37	329.0 (3.3)	3	344.3 (3.5)	19	334.6 (4.7)	13	319.7 (4.7)	2	313.5 (12.5)
13+	18	339.7 (4.4)	7	346.3 (7.0)	4	331.8 (15.7)	7	337.7 (2.9)	-	-

Table 1.3.5 Summary of age data for *E. rivulatus* captured within the sampling sites at Ningaloo Reef. Ages are in years.

	Immature females		Mature females			Transitional		Males		
Location	n	Oldest	n	Range	Mean (SE)	n	Age	n	Range	Mean (SE)
Tantabiddi	15	2	99	1-11	3.8 (0.23)	-	-	31	4-19	10.0 (0.58)
Osprey Bay	6	2	93	1-15	4.8 (0.24)	2	7,4	40	3-24	8.9 (0.58)
Mangrove	15*	1	10	2-8	5.0 (0.58)	-	-	5	6-12	9.0 (1.00)
NWReef	1	2	22	1-8	4.9 (0.51)	1	10	19	2-17	9.7 (0.99)

* refer to Table 3 for data on these fish.

Table 1.3.6 Parameters of the von Bertalanffy growth function for *E. rivulatus* captured at sites along Ningaloo Reef, and for all sites combined (Ningaloo). Also shown are the coefficient of determination for the fitted curves (r^2) and sample size (n). The ages of 14 immature females from Mangrove Bay (determined from daily ring counts and converted to a proportion of the year) were included in each analysis to provide data on the 0+ age group. ASE; Asymptotic Standard Error.

Location	L_∞ (ASE)	K (ASE)	t_0 (ASE)	r^2	n
Ningaloo	321.7 (2.7)	0.416 (0.020)	-0.697 (0.089)	0.859	340
Tantabiddi	307.4 (3.4)	0.530 (0.031)	-0.382 (0.073)	0.903	158
NWReef	338.3 (4.9)	0.418 (0.031)	-0.353 (0.073)	0.968	57
Osprey Bay	317.8 (3.9)	0.464 (0.030)	-0.430 (0.086)	0.886	153

Table 1.3.7 Results of an analysis of the residual sum of squares to compare the von Bertalanffy growth functions of *E. rivulatus* populations at Ningaloo Reef. Although data for the immature females from Mangrove Bay were used to obtain von Bertalanffy growth parameters for each site, such data were not included in the analysis shown here. RSS, residual sum of squares. df, degrees of freedom.

Location	RSS_{pooled}	df	RSS_{summed}	df	*F*	*P*
Ningaloo	130845.89	323				
Tantabiddi			51615.95	141		
NWReef			14998.64	40		
Osprey Bay			53072.25	136		
Summed			119686.84	317	4.926	<0.001

Table 1.3.8 Results of 1-way analysis of variances comparing the size-at-age (mm and years) of *E. rivulatus* from three locations at Ningaloo Reef. Data are pooled into two year age classes to provide adequate replication. Also shown are the results of the posthoc comparison of means using the Tukey's HSD test for unequal sample sizes. All analyses completed on untransformed data. Note that the data (either raw or transformed) for age groups 2-3 and 8-9 did not pass Cochran's test for homogeneity of variances.

Age (years)	df	MS effect	MS error	*F*	*P*	Tukey's HSD test		
						Osprey	Tantabiddi	NWReef
2-3	2,95	2168.36	448.47	4.835	0.010	250.4	236.1	241.4
4-5	2,65	853.26	460.44	1.853	0.165	278.7	271.3	287.4
6-7	2,51	2341.34	366.21	6.393	0.003	290.6	282.4	315.2
8-9	2,27	791.14	288.64	2.741	0.082	314.8	304.2	321.3
10-11	2,24	650.27	371.42	1.751	0.195	331.5	320.6	345.0
12+	2,23	469.38	354.23	1.325	0.285	339.0	331.4	345.9

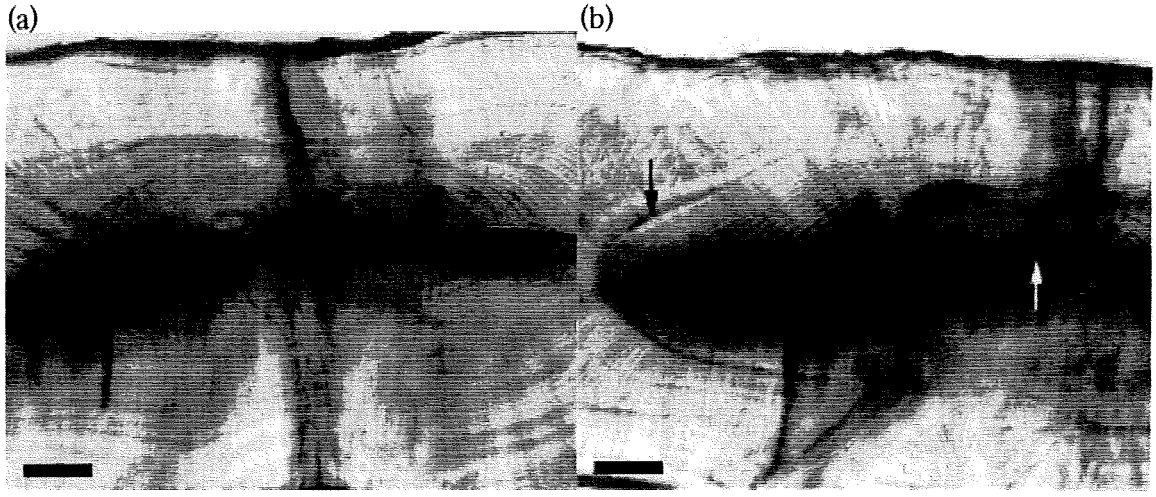


Figure 1.3.1 Transverse sections of the otoliths of immature female *E. rivulatus,* viewed with transmitted light. (a) Cracks running through the core (scale bar = 60 μm). (b) Microbands, settlement mark (black arrow) and focus (white arrow; scale bar = 50 μm).

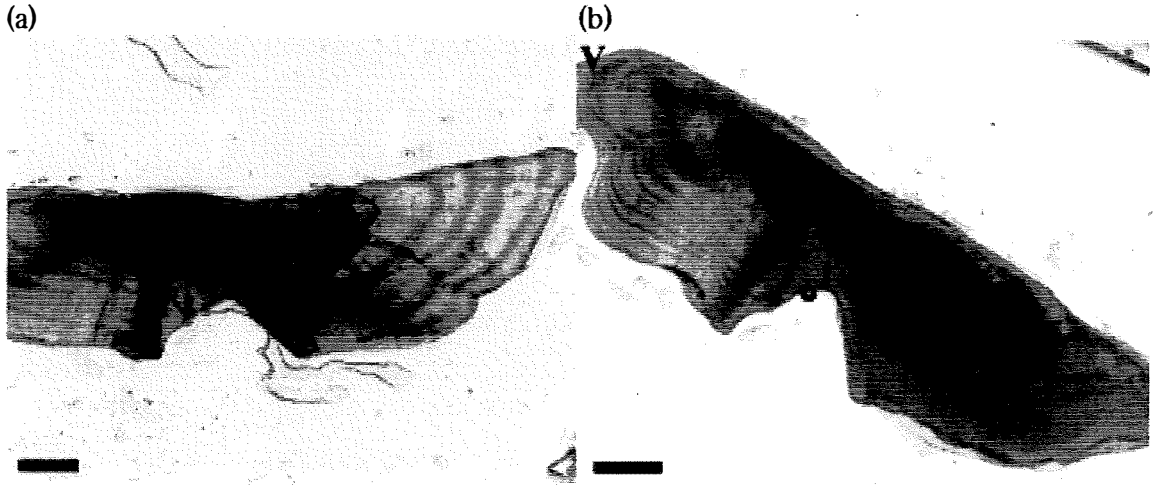


Figure 1.3.2 Transverse sections of the otoliths of *E. rivulatus,* viewed with transmitted light. (a) A five year old male with clear annuli (scale bar = 350 μm). (b) A male with reasonably clear annuli within the ventral (V) half of section, whilst the dorsal half is opaque and unreadable (scale bar = 400 μm).

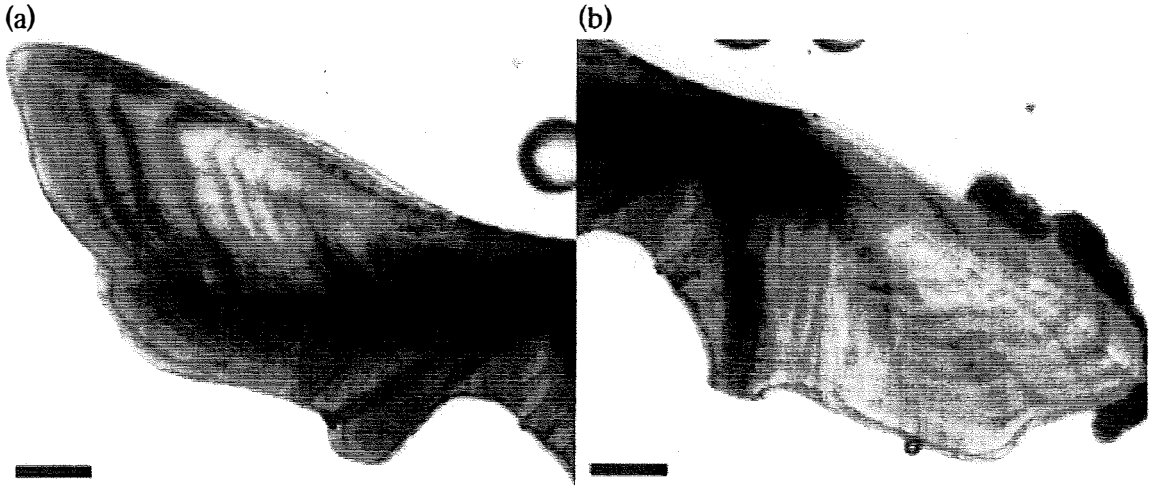


Figure 1.3.3 (a and b) Dorsal and ventral regions of the same otolith section of a male *E. rivulatus,* viewed with transmitted light. Estimated age of this individual varied from 9 to 15 years, depending on the region in which counts of annuli were made and the criteria used to distinguish them. Scale bar in both photographs = 300 μm.

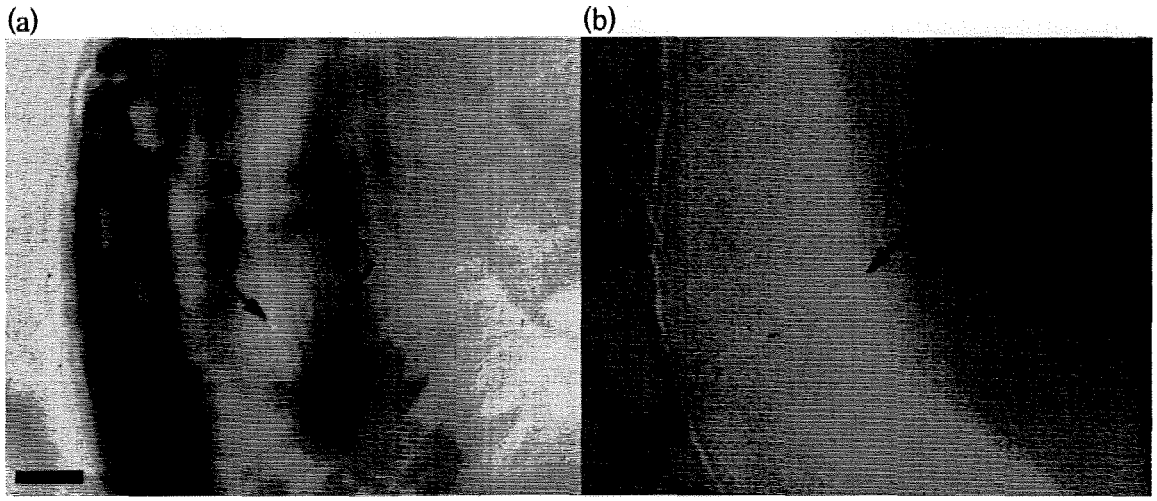


Figure 1.3.4 Transverse section of the otolith of an immature female *E. rivulatus* injected with tetracycline for validation of the microbands. Photographs show detail of the otolith margin, with (a), microbands between black arrows (made clearer by altering microscope focus), and (b), fluorescent tetracycline band. Scale bar in both photographs = 37 μm.

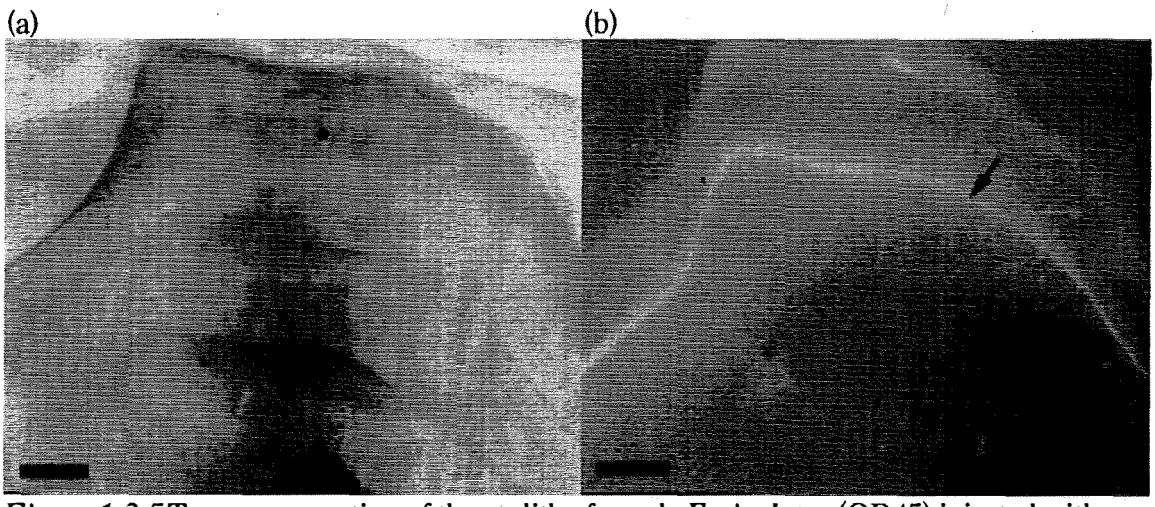


Figure 1.3.5 Transverse section of the otolith of a male *E. rivulatus* (OB45) injected with tetracycline for validation of annuli. (a) Detail of otolith near the sulcus showing annuli (black circles). (b) Same image, with fluorescent tetracycline band. Scale bar in both photographs = 400 μm.

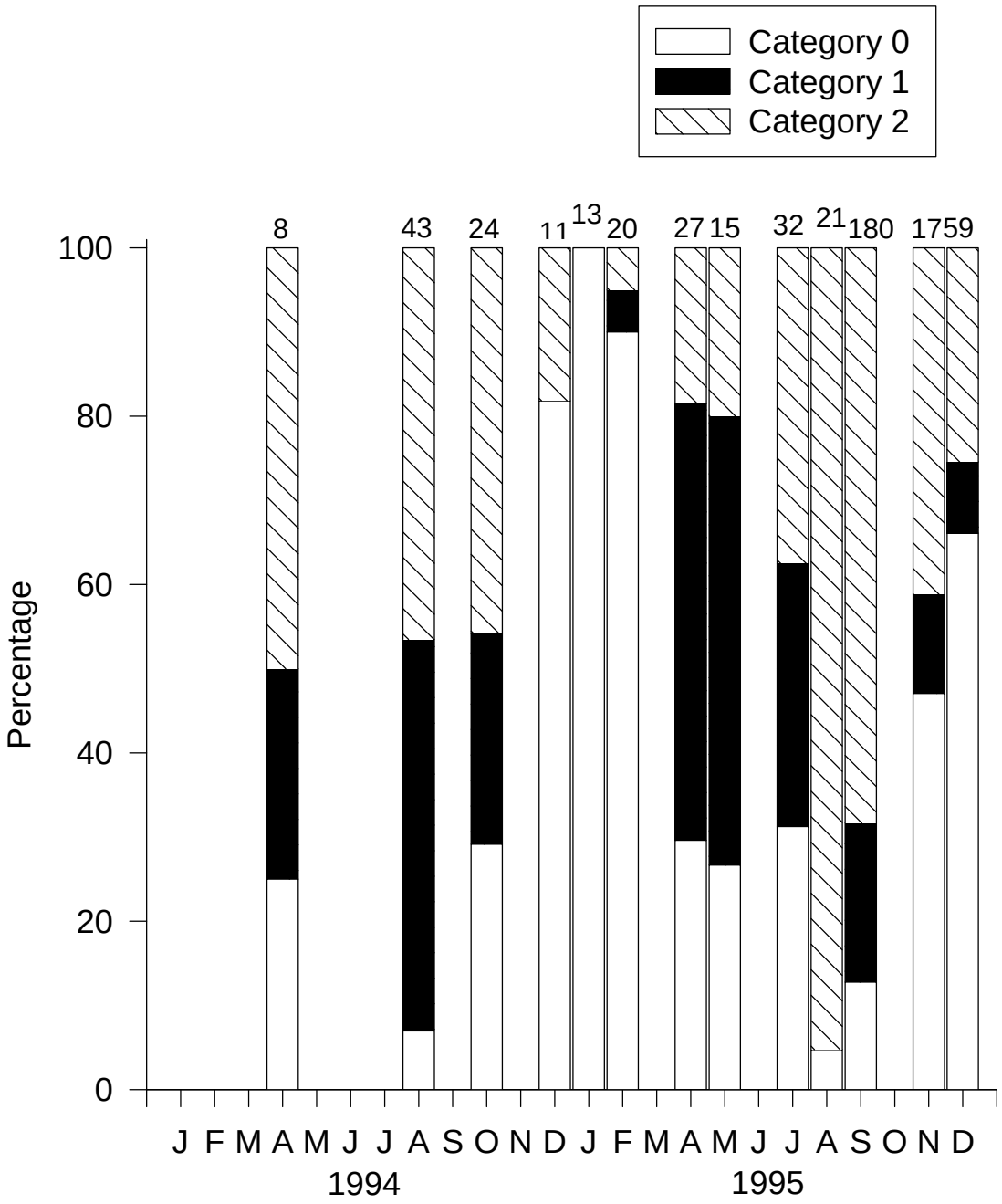


Figure 1.3.6 Percentage of *E. rivulatus* otolith samples with either an opaque (category 0), narrow translucent (category 1), or wide translucent (category 2) margin. Numbers above bars indicate sample size, which include pooled data from different locations.

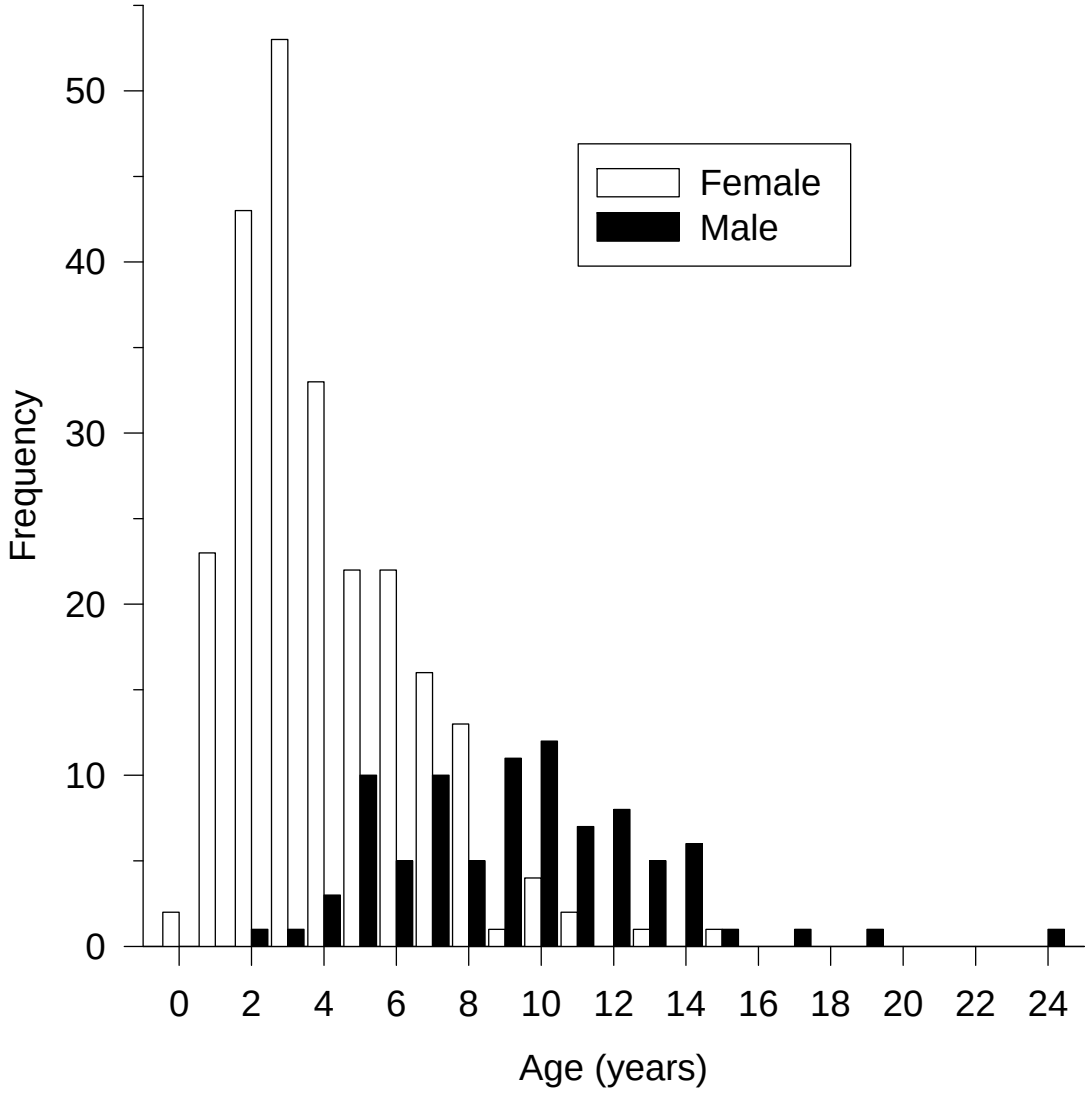


Figure 1.3.7 Age-frequency distribution of sampled male and female *E. rivulatus* at Ningaloo Reef. Data are pooled from various sites.

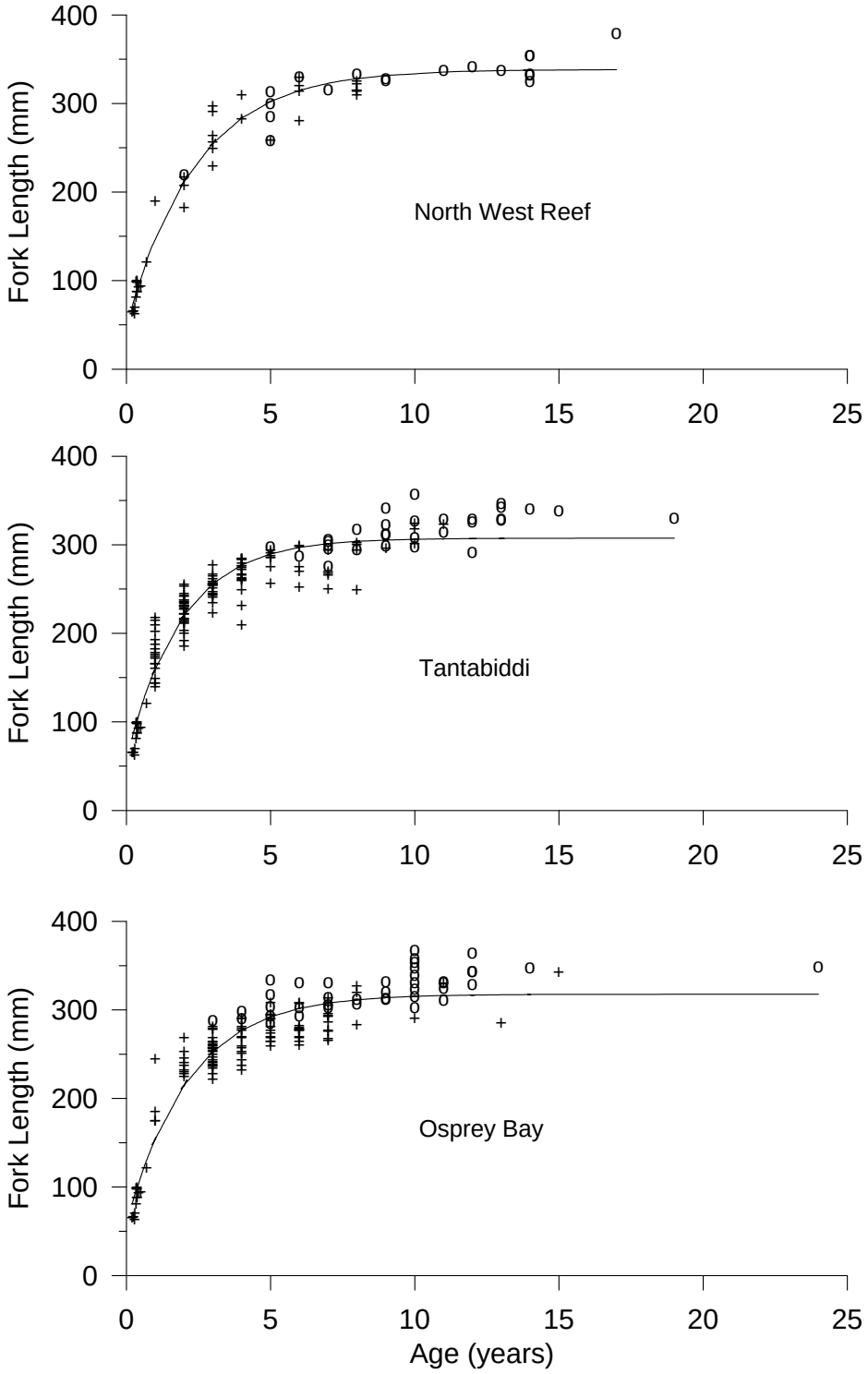


Figure 1.3.8 Von Bertalanffy growth curves fitted to length-at-age data for *E. rivulatus* captured at three locations along Ningaloo Reef. Data for the 0+ age group are from Mangrove Bay. Crosses = females, circles = males.

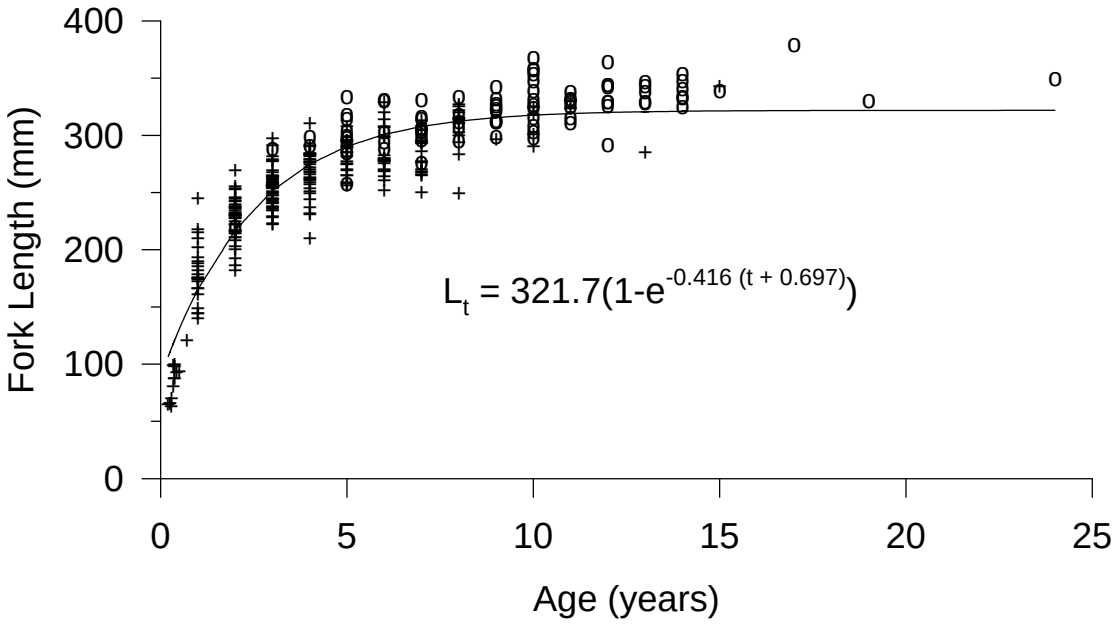


Figure 1.3.9 Von Bertalanffy growth curve fitted to length-at-age data for *E. rivulatus* captured along Ningaloo Reef. Sampling sites for data include North West Reef, Tantabiddi, Osprey Bay, and Mangrove Bay. Crosses = females, circles = males.

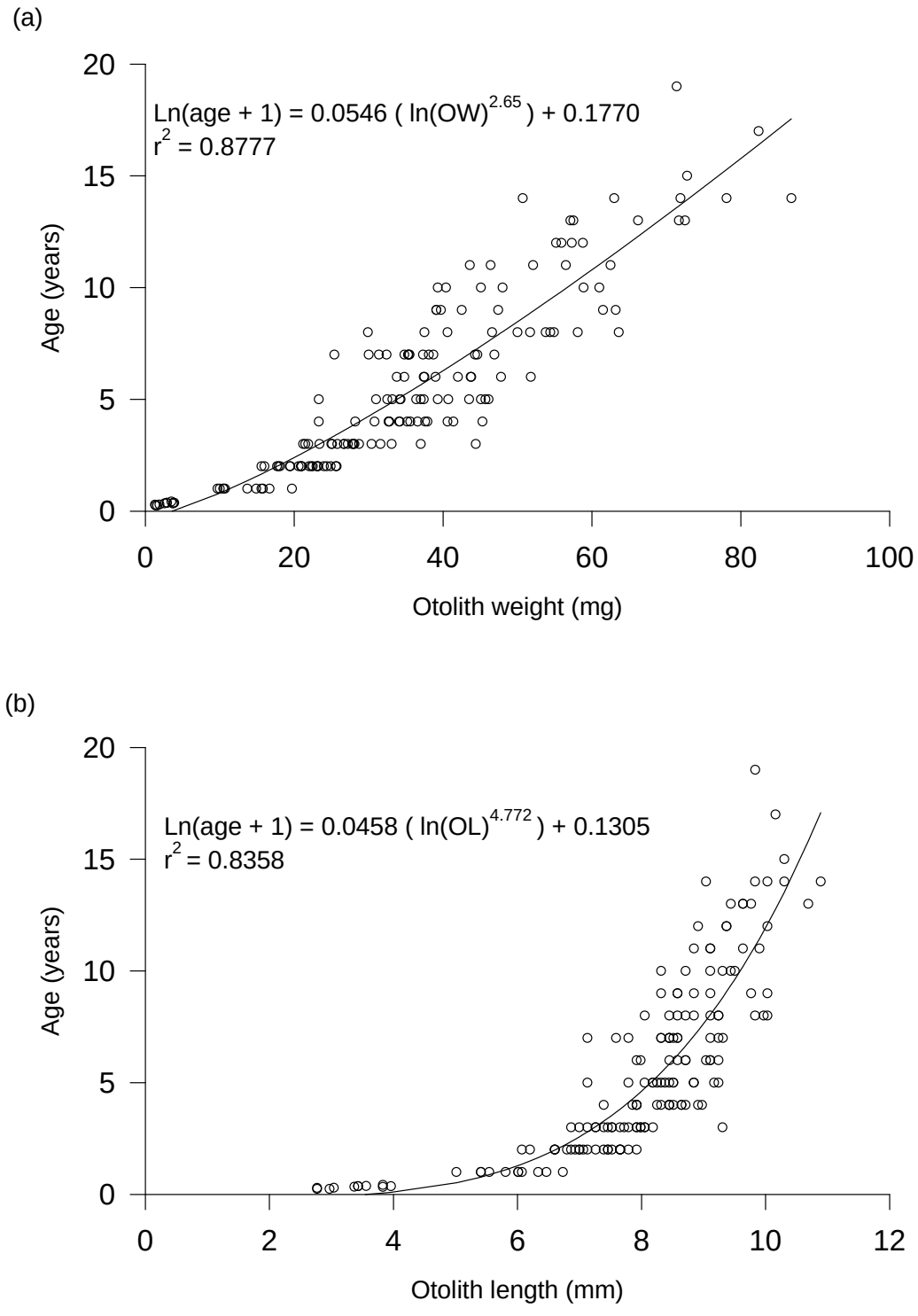


Figure 1.3.10 Relationships between (a) fish age and otolith weight, and (b) fish age and otolith length, for *E. rivulatus* at Ningaloo Reef. The otolith used in each comparison was the right sagitta, *n* = 165. Also shown are the allometric equations fitted to the log transformed data, and the associated coefficients of determination (note that the plotted data is untransformed).

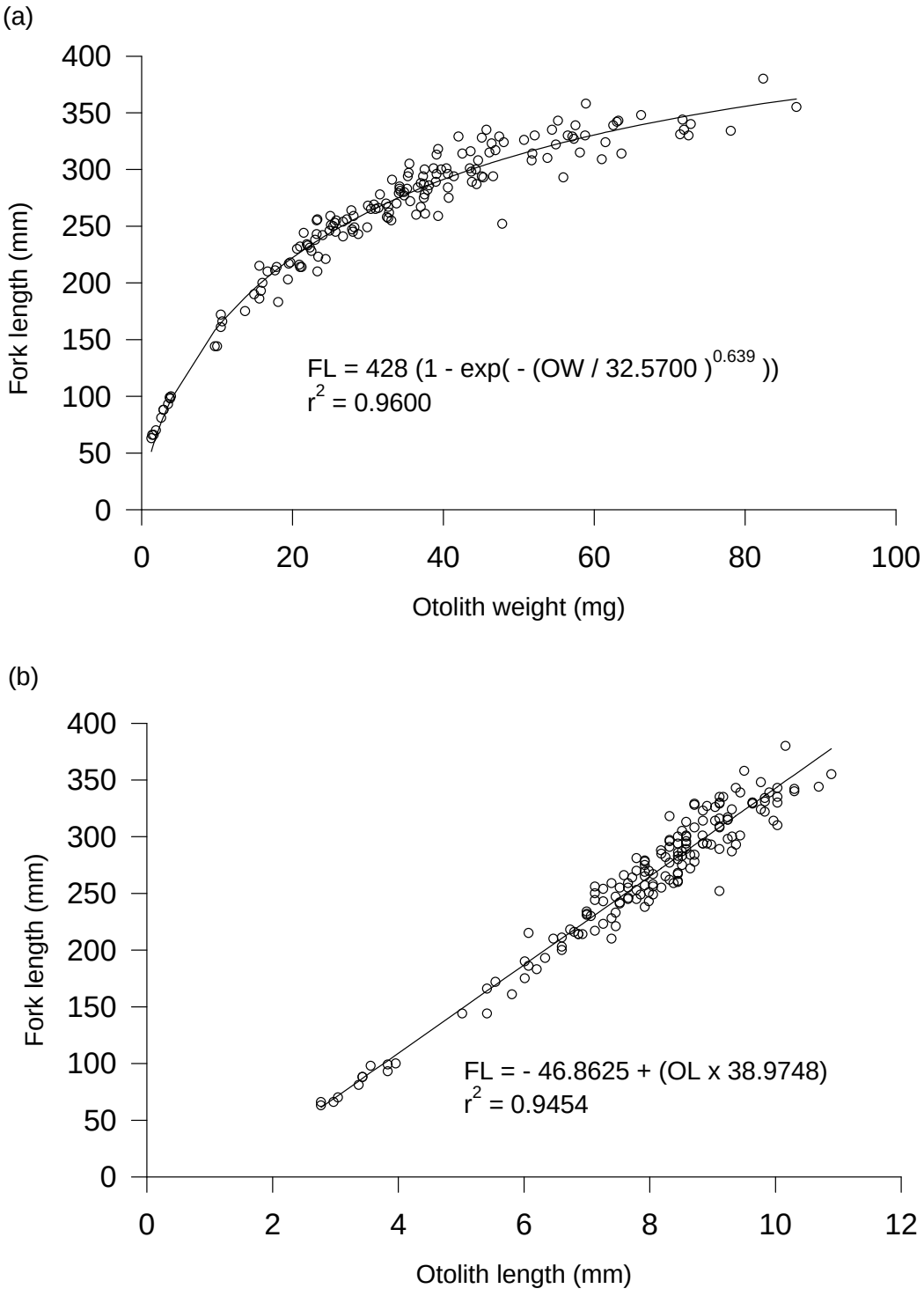


Figure 1.3.11 Relationships between (a), fork length and otolith weight, modelled using a Weibull cumulative distribution function and (b), fork length and otolith length, modelled using a linear function, for *E. rivulatus* at Ningaloo Reef. The otolith used in each comparison was the right sagitta, *n* = 165.

1.4 Home Range And Spatial Distribution

1.4.1. Materials and Methods

The three populations of *E. rivulatus* used in this study were located in Mangrove and Osprey Bays, Ningaloo Reef. Refer to Figure 1.1.1 and the Materials and Methods in Section 1.1 for description of these study sites. Two of the populations were located in Mangrove Bay and one in Osprey Bay, and all three were located in areas protected from fishing (Sanctuary Zones). Data used to describe patterns of space use by *E. rivulatus* within these populations were obtained by marking out the habitat in which each population was found in a grid pattern, and pinpointing the location of tagged individuals during each of a series of dives through the sites. Details are given below.

Gridding And Mapping Of The Study Sites.

The sites used in this study were chosen because of the relatively high concentration of resident *E. rivulatus* compared to surrounding areas. Each site was marked out in a grid pattern using metal rods secured into the limestone substratum at 6 m intervals, with the size of each grid dependent on the number and distribution of *E. rivulatus*. In Mangrove Bay the two sites were within 300 m of each other and are referred to as sites 1 (78 m x 78 m) and site 2 (108 m x 90 m). The site within Osprey Bay was also used in a study on sex-change in *E. rivulatus* (Section 2.1) and measured 214 m x 60 m. After marking out each grid, the topographical features of each site were mapped onto A3 waterproof paper for use during subsequent dives to locate tagged fish.

Capture And Tagging Of E. rivulatus.

Because *E. rivulatus* of all sizes and both sexes are generally similar in colour and morphology, it was necessary to tag individuals using a coded tagging system so that they could be recognised underwater (although some untagged individuals were identifiable from their distinctive body markings). Captured individuals were also measured and cannulated prior to release at the location of capture. Refer to the Materials and Methods in Section 1.2 for details of these methodologies. Although cannulation is a useful sexing technique, it is not always successful, so to minimise injury of the fish only two attempts were made at cannulating them before release. The sex and reproductive status (mature or immature) of fish that could not be captured or cannulated was estimated from their body size, using the size at which 50% of female *E. rivulatus* are sexually mature along Ningaloo Reef (194 mm fork length; Section 1.1). Only samples from reproductive fish could be obtained, and so the first tagging session occurred in October and November 1994 within the reproductive season (Section 1.1). During this time 93 and 101 *E. rivulatus* were caught and processed in sites 1 and 2 at Mangrove Bay (respectively), and 232 within the Osprey Bay site. In January 1995, 84 fish were also captured for tagging within the Osprey Bay site, prior to the home range study in this site between 20th April and 9th May 1995. Of these 84 fish, at least 34 (40%) had previously been tagged but had lost one of these or only had stubs of the tags remaining. The other 50 fish may have lost both tags, moved into the site since the initial tagging session, or had not been captured at that time. There were also evidence of tag loss by fish within the Mangrove Bay sites, and 83 and 137 fish were captured for tagging within sites 1 and 2, respectively, during July 1995. The home range studies within these sites commenced soon after (7-16 September 1995 in site 1, and 1 - 18 August. 1995 in Site 2). Based on the known number of fish that could not be captured,

it was estimated that at least 90% of the fish within each population were tagged prior to the home range studies. Most of these were small-sized individuals.

Space Use Patterns

The location and size of home ranges for *E. rivulatus* within the three populations were estimated from a series of SCUBA dives made over six consecutive days through each site. During each dive the position and identity of *E. rivulatus* were recorded onto a map of the site as I swam slowly in zigzag fashion between the metal rods marking each row of the grid. All areas of the grid and within 6 m of the outer perimeter were thoroughly searched for *E. rivulatus* with special attention given to ledges, holes and other likely shelters. In many cases a particular individual was observed and recorded on more than one occasion as I searched along adjacent rows of the grid. If an individual was swimming in an unhurried manner when observed, the locations at which it was first and last seen were recorded. However, if the individual was swimming fast (and hence possibly spooked by my presence), only the location at which it was first observed was recorded. Two dives (each approximately 2.5 hours duration) per day were required to survey each of the Mangrove Bay sites, with total dive times of 30.3 hours completed over six days in site 1, and 62.9 hours over 13 days in site 2. Due to the size and complexity of the site in Osprey Bay it was split into four, with each quarter taking one day to survey (two dives per day). Total dive time within this site was 108.0 hours completed over 24 days. Dives were swum in an East-West direction across the sites as it was easier to keep track of position, however the starting point of each dive was varied to minimise the possibility of herding the fish.

Only the home ranges of individuals observed at least once on each day of the dive series were used in analyses of home ranges (i.e. those fish with at least six location fixes). The outer perimeter of each home range was estimated from a compilation of the location fixes using the minimum convex polygon method (straight line connection of the outer location fixes; Mohr & Stumpf 1966). In a few cases a location fix was deleted because it was probably due to an unusual movement by the individual, based on the distribution pattern of other location fixes or observations made during the dives. The area of each home range was subsequently determined using a graphics digitiser. Prior to analysis of the home range data, *E. rivulatus* within the site at Osprey Bay were categorised by living area into either reef slope or reef flat inhabitants, depending on where 50% or more of their home range was situated.

Further information on the movement patterns of immature *E. rivulatus* (70 - 180 mm fork length) was obtained from half hour observations made on individuals within site 1 at Mangrove Bay (n = 15). These observations were made by divers who remained on the surface and as far from the fish as possible whilst plotting the distance and direction of movement by the fish onto underwater paper (using the grid markers as a guide).

The amount of overlap between the home ranges of individuals was examined as an indicator of territoriality and social hierarchy, the premise being that greater territoriality amongst individuals will lead to less overlap in their living space. For this analysis each pair of females that had overlapping home ranges were categorised by their differences in body length (15 mm size classes), and by the amount of overlap in their home ranges (less than 10% overlap, 10 - 50% overlap, and more than 50% overlap). Similar analysis was not required in assessement of territoriality between the males since the patterns were more distinct.

1.4.2 Results

The number of tagged *E. rivulatus* that were observed during the dives was low compared to the number that had originally been tagged within each site. One reason for this is tag loss, as indicated by the observed loss of all or part of a tag by twelve fish whilst conducting the series of dives through site 2. Although probably a minor factor, some fish may also have died as a result of the tagging process. For instance, eight fish captured within the Osprey Bay site were foul hooked through the eye or in the gut (hooks left in the latter fish). Five fish were also released outside of their normal living area, and the combined stress of capture and aggression by territorial *E. rivulatus* is known to have killed at least one of these. Mortality is likely to have been higher among smaller fish which were probably more sensitive to the capture and handling procedures. The loss of one or both tags, or overgrowth of the tags by algae also caused confusion in the correct identification of individuals. For example, 32 home ranges within the Osprey Bay site could be attributed to two or three individuals because their sole remaining tag was similar in size, colour and location in the body. For some of these home ranges, the correct identity of the inhabitant was possible based on body length and/or body marks. However, it is likely in a few cases that a home range attributed to one individual may have actually been that of two fish.

Emigration by tagged *E. rivulatus* out of the sites is also likely to have reduced the numbers observed during the dives. This was evidenced by the recapture of a 314 mm male almost two years and twelve kilometres from the location of original capture and tagging in site 1 at Mangrove Bay. However many other individuals exhibited greater site fidelity, and after two years were still located within the site in which they had originally been captured for tagging. At least some of these fish, identifiable because of their large size or particular body marks, were still within the same small area of reef.

Population Densities

Despite lacking data on individuals due to reasons outlined above, the three study sites were obviously well populated with *E. rivulatus* of all size classes which occupied overlapping, restricted home ranges (Figures 1.4.1 and 1.4.2). The population within site 1 included 112 males and females (67 tagged and 45 untagged) and the home ranges of 65 (58%) of these fish could be identified for further analysis (Table 1.4.1). In site 2 there were 216 males and females (137 tagged and 79 untagged), and the home ranges of 105 (49%) of these were identifiable. At Osprey Bay the population was estimated at 264 individuals (153 tagged and 111 untagged) and the home ranges of 117 (44%) of these could be identified.

Densities of fish within the Mangrove Bay populations were similar, with approximately two *E. rivulatus* per 100 m^2. Overall densities of fish within the Osprey Bay site were higher than at Mangrove Bay, although this was due to the large number of fish inhabiting the reef slope whilst densities on the reef flat were relatively low (Figure 1.4.1, Table 1.4.1). The distribution of home ranges shows that most of the fish on the reef slope were males and large females (≥ 275 mm fork length), whilst small to medium sized females (particularly those smaller than 245 mm) were mainly found on the reef flat. A chi-square test for independence of the frequencies of females smaller than 275 mm versus that of large females (≥ 275 mm) and males (pooled data) inhabiting the reef slope and the reef flat confirmed that the distributions were different ($\chi^2_{(0.05,1)} = 29.6$, $P < 0.001$).

Although the distribution of home ranges suggested that females within the smaller age groups (particularly fish below 215 mm, 216 – 230 mm, and 231 – 245 mm) were not evenly distributed within the Osprey Bay site (Figure 1.4.1c), analyses by chi-square were not significant (chi-square goodness of fit comparing the observed frequency distribution of females within five equal sections of the site with frequencies that would be expected if the fish were evenly distributed through the site. Analyses done on each size group separately, and in each case $P > 0.05$). *E. rivulatus* within the two Mangrove Bay populations were more evenly dispersed through the sites compared to the Osprey Bay population. Nonetheless, 65 percent of the fish within site 2 occupied home ranges that included more limestone than sand (Figure 1.4.2), and when diving through these sites it was evident that these areas of exposed limestone provided more shelter and were preferred by *E. rivulatus*.

Home Range Size

Small (immature) females had relatively small home ranges and during the half hour observation periods they usually remained motionless in or near shelter, unless moving a short distance between shelters. These movements were made at a fairly rapid pace and were often less than 2 m, although swims up to 10 m were recorded. Immature females inhabiting the reef flat at Osprey Bay were the most mobile, occupying home ranges that averaged 66 m^2 (Table 1.4.2). In site 2 at Mangrove Bay and the site in Osprey Bay the largest home range was held by a female (Table 1.4.2). However, in all three populations the mean home range size of males was larger than that of the females. Maximum home range size for females was 389 m^2 and for males 434m^2. Mean home range size of mature *E. rivulatus* varied from 58 m^2 for females within site 1 to 200 m^2 for males inhabiting the reef flat at Osprey Bay.

An analysis of variance also indicated that the overall mean home range of male *E. rivulatus* was larger than that of mature females, although within each particular site there were no intersexual differences in home range size (Table 1.4.3, Figure 1.4.3). The analysis of variance also showed differences in the mean home range size of fish inhabiting sites 1 and 2 in Mangrove Bay, and the reef slope and the reef flat in Osprey Bay (Table 1.4.3). The posthoc comparison of means indicates that *E. rivulatus* within the three populations form two distinct groups with respect to mean home range size; one group including fish with relatively small home ranges that inhabited the Mangrove Bay sites and the reef slope in Osprey Bay, and the second group including fish on the reef flat in Osprey Bay (Table 1.4.3). The home ranges of males within site 2 bridged the two groups, being similar to the smaller home ranges of *E. rivulatus* from most other locations except for males occupying the reef flat at Osprey Bay, and females within site 1 at Mangrove Bay. The home ranges of males inhabiting the reef slope were also similar to those of females on the reef flat.

The relationship between body length and home range size was significant for *E. rivulatus* within all habitats except the reef slope at Osprey Bay (Table 1.4.4). However this relationship was due to the relatively small home ranges of small fish, since all regressions with fish less than 215 mm excluded were non-significant (also refer to Figure 1.4.3). Natural log transformations did not appreciably reduce the variability of the data, with the best fit (for transformed data from site 1) explaining 45% of the variability in the data. Therefore, home range size is not influenced by body size in medium and large *E. rivulatus*. The plot of home range size on fish density suggests an inverse, curvelinear relationship because of the relatively low density and large home ranges of *E. rivulatus* inhabiting the reef flat at Osprey Bay (Figure 1.4.4). There was no

pattern in this relationship for the fish inhabiting the reef slope at Osprey Bay and the two Mangrove Bay populations, however.

Home Range Overlap

The home ranges (territories) of male *E. rivulatus* generally did not overlap, whilst there was much overlap between the home ranges of females (Figures 1.4.1 and 1.4.2). Grouping of females into 15 mm size classes show that the home ranges of similarly sized individuals often do not overlap either (Figures 1.4.1 and 1.4.2). Regression analysis further showed that when the home ranges of these similarly sized female do overlap, the frequency of substantial (>50%) overlap is less than for females with larger differences in body size (Figure 1.4.5, regression for Osprey Bay data: $r^2 = 0.4310$, $P_{1,13\ df} = 0.0079$, and for site 2, Mangrove Bay data: $r^2 = 0.3639$, $P_{1,13\ df} = 0.0173$). Intersexual overlap in living area was common, and the home range of many females overlapped those of more than one male (Table 1.4.5). However some small females (< 275 mm) inhabiting the reef flat within the Osprey Bay site appeared to have little or no male contact as there was no overlap in their home ranges.

1.4.3 Discussion

Spatial Distribution And Social Organisation

The data presented here show that *E. rivulatus* is a social species, capable of forming crowded populations in which there is much overlap in living space and the likelihood of frequent intraspecific interactions. However *E. rivulatus* does not form spatially distinct social groups like those of the serranid species *Cephalopholis miniata* and *C. argus*, in which males defend large territories containing up to twelve females (Shpigel & Fishelson 1991). In contrast, *E. rivulatus* of both sexes occupied relatively small, similarly sized home ranges that become smaller as densities increased. Overlap in living areas was high among females but not among males, and females often shared the habitat of more than one male. These spatial characteristics closely resemble those of *Cephalopholis cyanostigma*, a slightly smaller serranid that is abundant within shallow, coral rich habitats on the Great Barrier Reef (Mackie 1993).

The spatial distributions indicate that male *E. rivulatus* are territorial towards each other, as is confirmed by behavioural observations (Section 1.2). Females are far more abundant, share considerable overlap in living area, and are therefore likely to have higher levels of intrasexual contact. Similarly sized females appear to exhibit some territoriality towards each other however, since their home ranges overlapped less frequently. This adds support to the presence of a social hierarchy among the females, in which an individual's status is based on relative size and similarly sized females are social competitors. Nevertheless, it is apparent that the defence of discrete territories by similarly sized individuals is often not possible, perhaps because the social and environmental factors that attract individuals together overcome territorial aggression. As a result, similarly sized females are obliged to share more habitat as densities increase, and under these circumstances other factors such as the relative aggressiveness of individuals may ultimately determine the social relationships between individuals (Myrberg 1972, Carpenter 1987).

The spatial data support the view that *E. rivulatus* have a flexible social system in which individuals may improve their status and reproductive success through growth and opportunistic movement into habitats with better resources and more chance of intersexual contact. Therefore, whilst *E. rivulatus* is essentially sedentary by nature with many individuals occupying the same area for periods spanning at least two years, they may nevertheless shift in habitat as needs and opportunities arise. Small scale shifts are indicated by the distribution pattern of home ranges within the Osprey Bay site, which suggest that individuals are moving from the reef flat to the slope, and possibly along the reef flat as their body size increases. The presence of untagged fish also suggest an influx into the study sites, and the recapture of the male that had originally been tagged in Mangrove Bay proves that they can move considerable distances. Similar movements by individuals searching for better habitat or social opportunities were also noted for *C. cyanostigma*, with one individual recaptured approximately a kilometre from the point of tagging, and small but distinct changes in the location and size of home ranges occurred when males were experimentally removed from the study sites (Mackie 1993). Ontogenetic shifts in habitat are also typical of larger serranid species in which individuals change habitat and move offshore as they grow (Sheaves 1995, Ross & Moser 1995, Sluka & Sullivan 1996). However, in these larger species there generally appears to be a much greater spatial distinction between the habitats of juveniles (nursery grounds) and adults compared to smaller species such as *E. rivulatus*.

Home Range Size

Although the minimum area polygon method of estimating home range size is sensitive to sample size (White & Garrott 1990), the estimates provided here for *E. rivulatus* are probably reasonable given the low mobility and relatively small area of movement of many individuals. The data show that habitat may affect home range size, sex generally does not, and neither does body size once a certain length (about 215 mm) is reached. Habitat is usually regarded as the major determinant of living area in reef fish, affecting the availability of resources such as shelter, food and spawning sites (Ebersole 1977, Hixon 1980, Jones 1984, Shpigel & Fishelson 1986, Hourigan 1989, Shapiro 1991). Habitat quality and competitor densities were also the primary factors affecting territory size of the serranids *Cephalopholis miniata, C. argus* and *C. hemistiktos* in the Red Sea (Shpigel & Fishelson 1991).

The influence of habitat may also vary with ontogeny in *E. rivulatus*. For small females the primary concern is likely to be shelter from predation and aggression. However, as an individual grows, so may the need to move into areas that not only provide more appropriate shelter and greater abundance of prey, but also more chances of interacting with males and better dispersion of gametes during spawning (Shapiro 1991). Thus females (and males) crowd into areas where these resources are in greater supply, their success at doing so mainly determined by body length. The reef slope at Osprey Bay was a habitat that was particularly desirable, resulting in high densities and reduced room for movement, which was offset by a decreased need to search for prey or potential mates.

In contrast, the reef flat was a less attractive habitat, apparently providing less shelter and food, inferior spawning sites, and being more prone to oceanic swell at high tide (personal observations). Smaller fish inhabited this area and roamed over relatively large areas, perhaps because they were less restricted by competitive interactions and were searching for more resources and social interaction. At Mangrove Bay the habitat and potential resources were more uniformly distributed and no area appeared to offer

any particular spawning advantages. Possible reasons for the relatively small home ranges of *E. rivulatus* in this area (compared to those on the reef flat in Osprey Bay) were an apparently high density of invertebrate prey and the need to shelter from large marine animals such as black stingrays (*Dasyatis thetidis*) and a school of large golden trevally (*Gnathanodon speciosus*) that occasionally move through the two sites (personal observations). Whilst these animals may not actually prey upon larger *E. rivulatus*, both were animals to shelter from, particularly the trevally whose arrival out of the often murky waters of Mangrove Bay was always heralded by a wave of small fish fleeing these aggressive benthic hunters.

Management Issues

Observations made during this study are applicable to the management of *E. rivulatus* and more generally to other serranids. The high densities within the three populations are notable for several reasons. Firstly, there were far more fish within the study sites than were estimated from surveys made prior to the study, highlighting the difficulty in obtaining accurate counts from visual censuses of cryptic species such as *E. rivulatus*. Secondly, the high abundances combined with a wide variety and frequent consumption of prey species (*unpublished data*) suggests that *E. rivulatus* may influence the distribution and abundance of a number of other species within the lagoon. Finally, because females grow fast, mature early (Section 1.3), and are reproductively active over six months (Section 1.1), the reproductive output of crowded populations like the ones studied here is likely to be high.

The Sanctuary Zones (marine reserves) where the populations used in this study were found are viable management tools, as they provide refuges for exploited species from which the restocking of depleted areas may take place via emigration of adult fish and dispersal of larvae (Russ 1985, Watson *et al.* 1996, Roberts 1997). However, this study provides only half the information necessary to judge the effectiveness of Sanctuary Zones in maintaining healthy populations of *E. rivulatus* for restocking purposes, because detailed information about exploited populations of this species is still lacking. This study has established that the information needed for such a judgement could be obtained with a larger investigation that included populations of *E. rivulatus* in both Sanctuary Zones and non- Sanctuary Zone areas. Future studies need to investigate patterns in the movement of fish and the reasons behind them, and provide direct measurements of the export of larvae, females and males from Sanctuary Zones to non-Sanctuary Zone areas in order to establish that restocking actually occurs.

Table 1.4.1 Estimated number and densities of *E. rivulatus* within the study sites at Ningaloo Reef. The number of fish inhabiting either the reef slope or reef flat within the Osprey Bay site are not provided since the home ranges of many fish could not be identified. Densities of *E. rivulatus* on the reef slope and reef flat are therefore conservative, as they are based on those fish for which home ranges could be identified.

	Mangrove Bay						Osprey Bay		
	Site 1			Site 2					
	immature females	mature females	males	immature females	mature females	males	immature females	mature females	males
n	32	69	11	42	143	31	31	201	32
densities	1.8 fish per 100 m^2			2.2 fish per 100 m^2			overall = 2.6	reef slope = 3.1	
							reef flat = 1.4 per 100 m^2		

Table 1.4.2 Home range size of *E. rivulatus* within the study sites at Ningaloo Reef. The data are for fish with identifiable home ranges and do not indicate total number of fish.

	Mangrove Bay						Osprey Bay					
	Site 1			Site 2			Reef slope			Reef flat		
	immature female	mature female	male	immature female	mature female	male	immature female	mature female	male	immature female	mature female	male
n	17	38	10	3	78	24	0	31	18	7	49	12
min (m^2)	2	5	9	4	20	21	-	20	15	11	22	108
max (m^2)	51	186	434	38	221	173	-	305	218	138	389	357
mean (m^2)	14	58	104	21	76	96	-	70	94	66	151	200
SE	2.8	6.4	39.5	10.0	4.9	9.7	-	9.1	11.6	19.2	12.5	19.5

Table 1.4.3 (a) Analysis of variance and (b) treatment means for the natural log of home range area (m^2), of male and mature female *E. rivulatus* inhabiting sites 1 and 2 at Mangrove Bay and the reef slope and reef flat at Osprey Bay. Note that sample sizes were unequal and a Cochran's test indicated heterogenous variances $C_{260,7} = 0.23$, $P = 0.029$. (c) A posthoc comparison of means between population and sex (Tukey HSD for unequal sample sizes). MF = mature female. M = male. Site 3 = reef slope. Site 4 = reef flat.

(a)

Source	df	MS	F	*P*
Site	3	8.77	21.79	0.0000
Sex	1	2.93	7.29	0.0074
Site x Sex	3	0.14	0.35	0.7921
error	252	0.40		

(b)

Treatment means								
Site x sex	n	Mean (SE)	Site	n	Mean (SE)	Sex	n	Mean (SE)
1:M	38	3.80(0.13)	1	47	3.82(0.12)	M	63	4.49(0.09)
1:MF	9	3.92(0.88)	2	102	4.23(0.06)	MF	197	4.25(0.05)
2:M	78	4.18(0.58)	3	50	4.20(0.83)			
2:MF	24	4.40(0.13)	4	61	4.92(0.79)			
3:M	32	4.08(0.54)						
3:MF	18	4.39(0.63)						
4:M	49	4.84(0.64)						
4:MF	12	5.25(0.32)						

(c)

		Mangrove Bay site 1		Mangrove Bay site 2		Osprey Bay reef slope		Osprey Bay reef flat	
		MF	M	MF	M	MF	M	MF	M
Mangrove Bay site 1	MF								
	M	ns							
Mangrove Bay site 2	MF	ns	ns						
	M	*	ns	ns					
Osprey Bay reef slope	MF	ns	ns	ns	ns				
	M	ns	ns	ns	ns	ns			
Osprey Bay reef flat	MF	***	*	***	ns	***	ns		
	M	***	***	***	*	***	*	ns	

Table 1.4.4 Analysis of the relationship between home range size (m^2) and fork length (mm) of *E. **rivulatus*** at Ningaloo Reef. Regressions were made on pooled male and female data before and after natural log transformation of both home range and length data. *P* is the test statistic of the regression analysis of variance; *a* and *b* are parameters of the regression: home range size (m^2) = *a* + (*b* x fork length (mm)). An outlier in the site 1 data was removed before analysis.

Site	data	n	r^2	*P*	*a*	*b*
Mangrove Bay site 1	raw	64	0.2327	0.0000	-65.25	0.50
	transformed		0.4500	0.0000	ln(-10.29)	ln(2.55)
Mangrove Bay site 2	raw	105	0.1105	0.0005	-22.74	0.39
	transformed		0.1623	0.0000	ln(5.16)	ln(0.09)
Osprey Bay reef flat	raw	68	0.1634	0.0007	-56.25	0.82
	transformed		0.2637	0.0000	ln(-6.62)	ln(2.07)
Osprey Bay reef slope	raw	49	0.0694	0.0674	-2.76	0.26
	transformed		0.0443	0.1465	ln(-0.33)	ln(0.79)
Osprey Bay reef flat & slope	raw	117	0.0034	0.5308	92.66	0.10
	transformed		0.0167	0.1663	ln(1.66)	ln(0.52)

Table 1.4.5 The frequency of female home ranges that overlapped with zero, one, or more male home ranges within the Osprey Bay site. The data are divided into large (≥ 275 mm) and small (< 275 mm) females occupying either the reef flat or slope. Note that this analysis only those females for whom home range size could be determined.

		Number of male home ranges overlapped by a female (and percentage)				
		0	1	2	3	4+
Reef slope	≥ 275 mm	0	6	3	3	0
	< 275 mm	0	5	6	8	1
Reef flat	≥ 275 mm	0	8	2	1	0
	< 275 mm	9	15	13	3	4

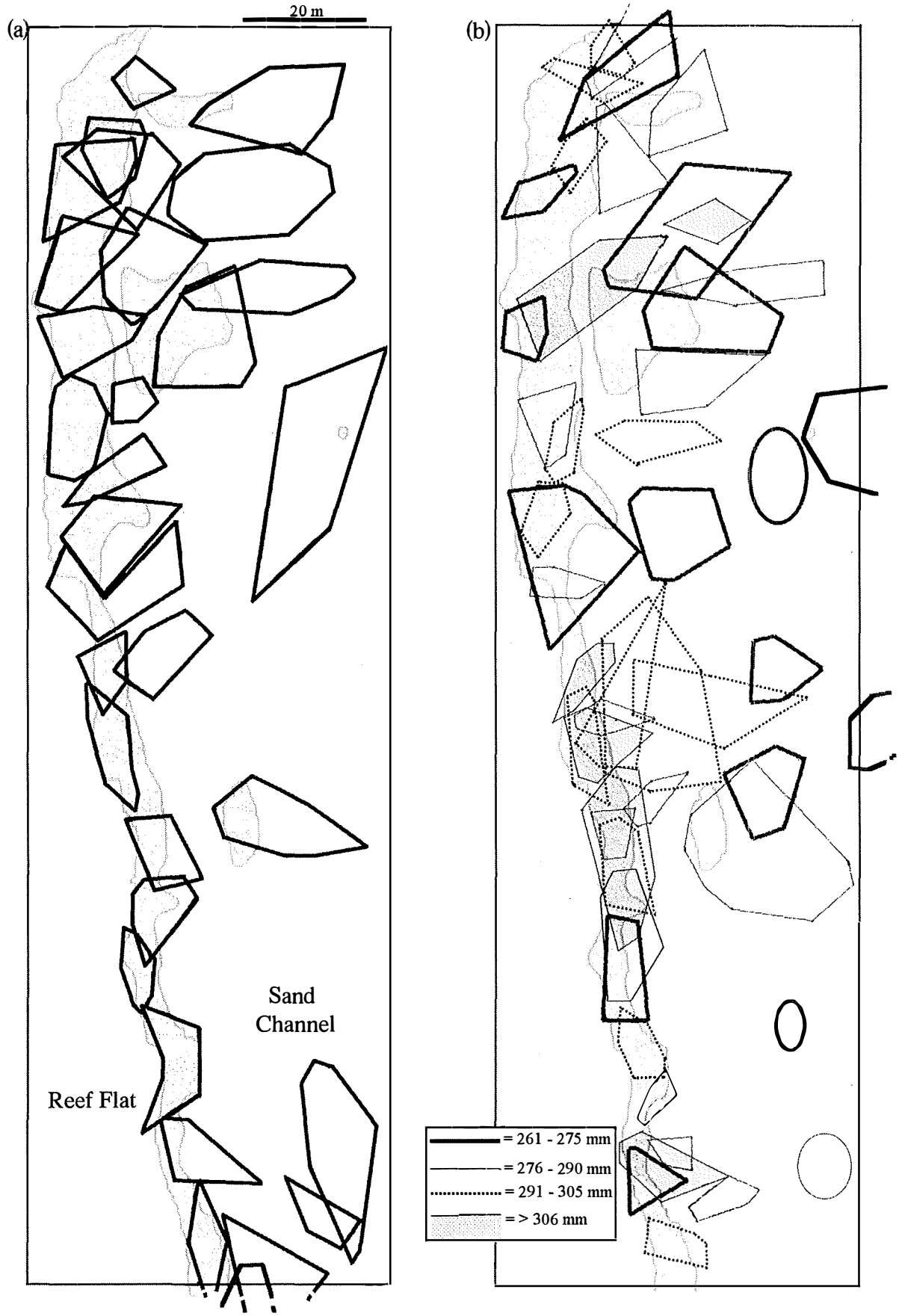
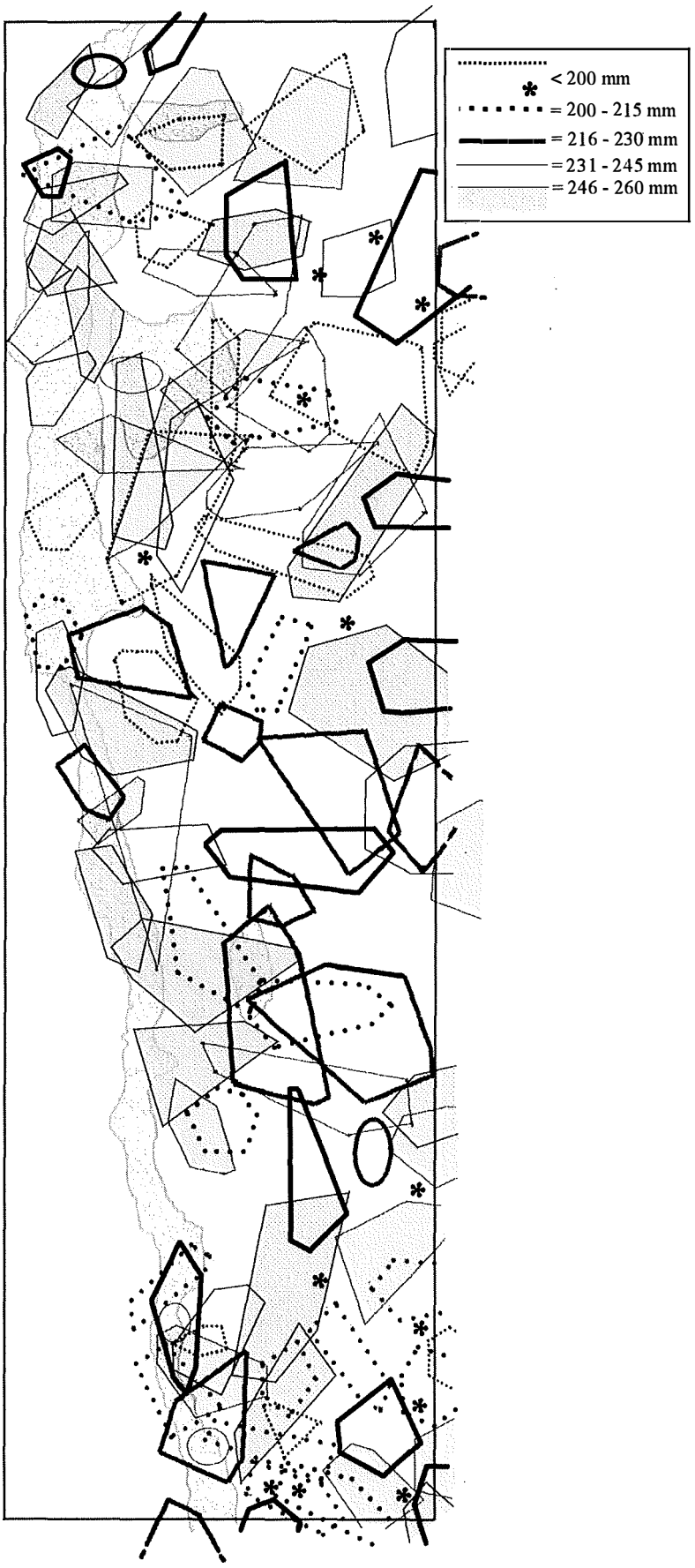


Figure 1.4.1 Detail of the site within Osprey Bay showing the home ranges of (a), all male *E. rivulatus,* (b), females above 261 mm forklength, and (c) (overleaf), females below 260 mm forklength. The home ranges of individuals seen on three or less occasions are shown as ovals. Shaded areas indicate the reef slope and limestone ledges on the reef flat. North is toward the top of the page, the shoreline is towards the right.

1.4.1 (c)



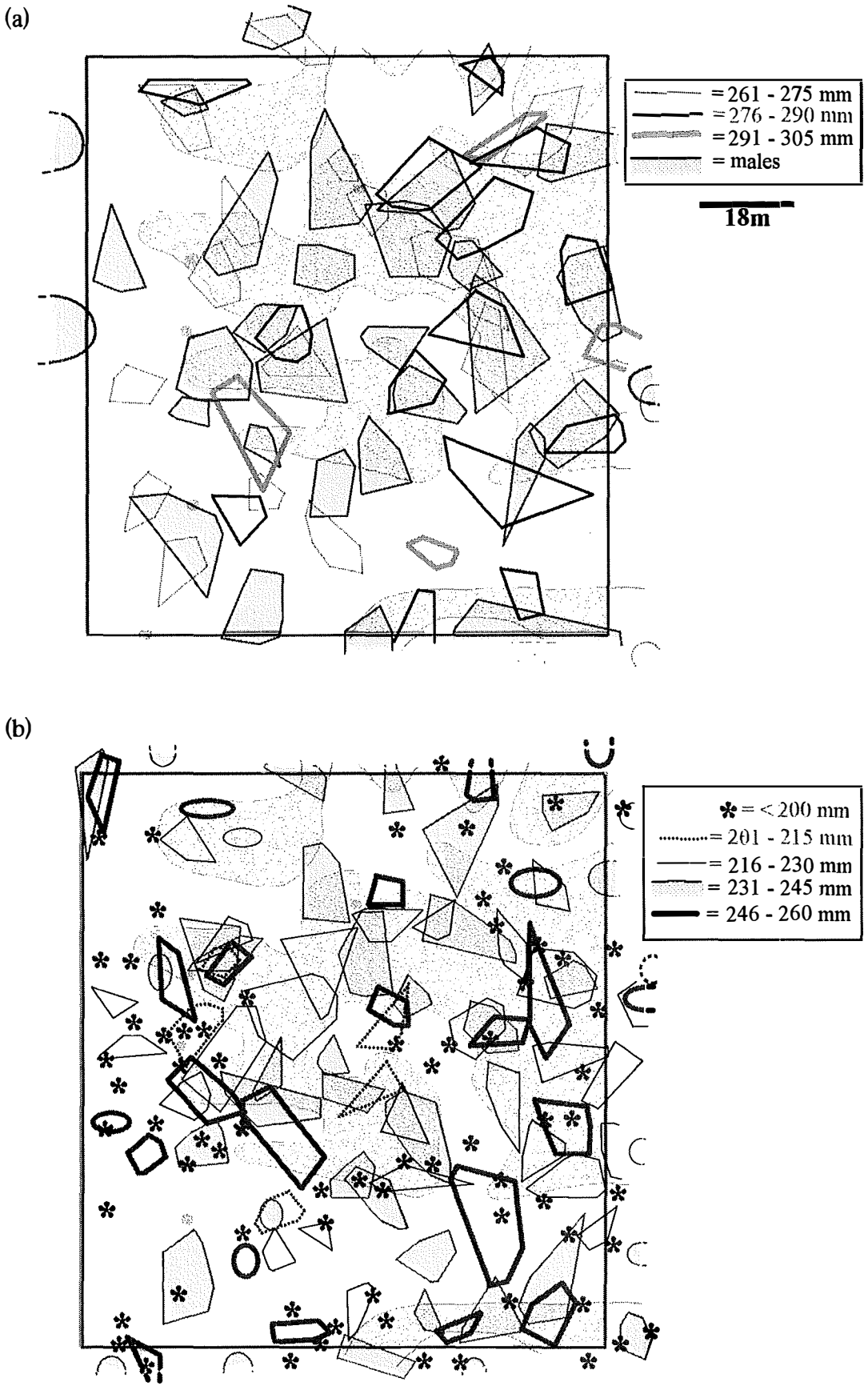


Figure 1.4.2 Detail of site 2 within Mangrove Bay showing the home ranges of (a), male and female *E. rivulatus* above 246 mm forklength, and (b), females below 245 mm forklength. The home ranges of individuals seen on three or less occasions are shown as ovals. Stippled areas indicate substratum of exposed limestone and sand, all other areas are mainly sand. North is towards the top of the page, the shoreline is towards the right.

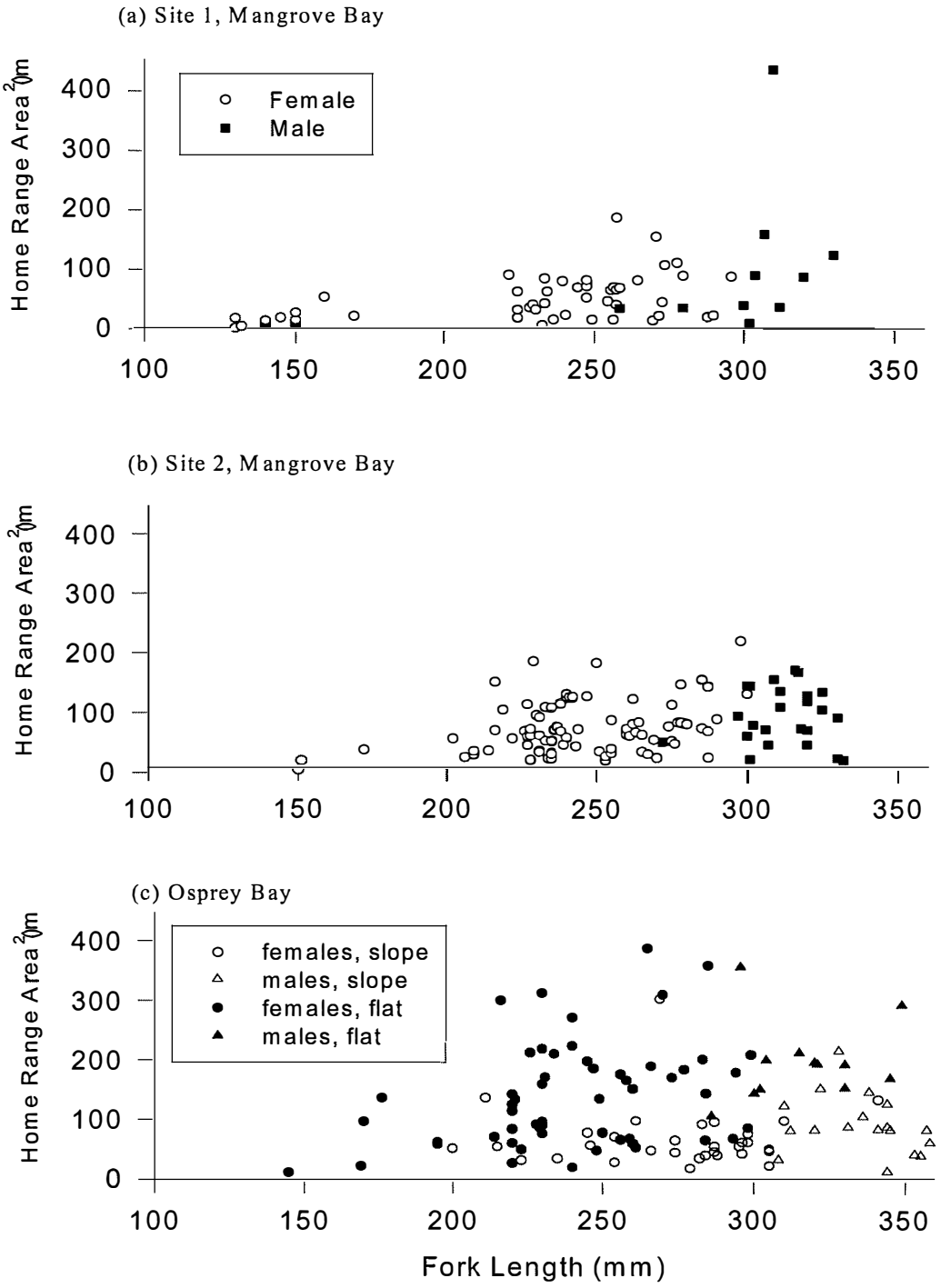


Figure 1.4.3. Relationship between home range size and the body length of male and female *E. rivulatus* within the study sites at Ningaloo Reef.

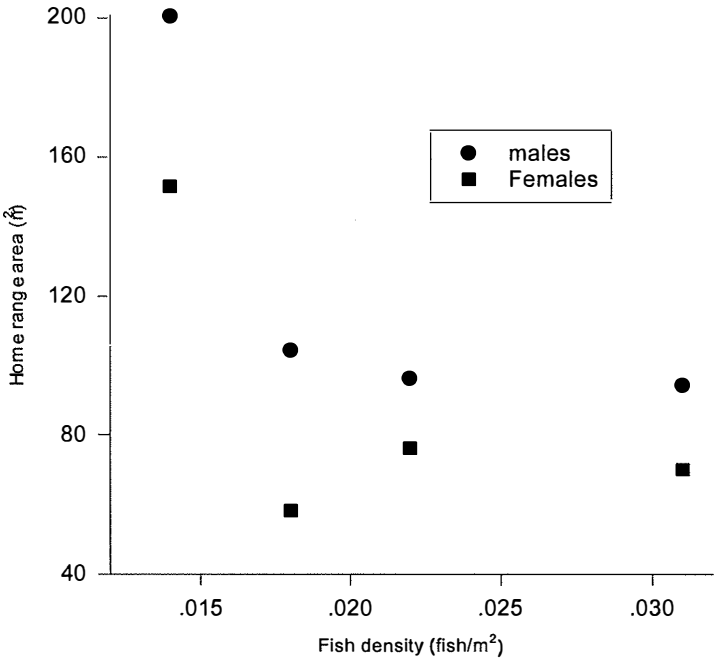


Figure 1.4.4. Relationship between home range size and fish density for *E. rivulatus* at Ningaloo Reef. Populations used in this analysis are (in order from lowest to highest densities): reef flat at Osprey Bay, site 1 at Mangrove Bay, site 2 at Mangrove Bay, and reef slope at Osprey Bay.

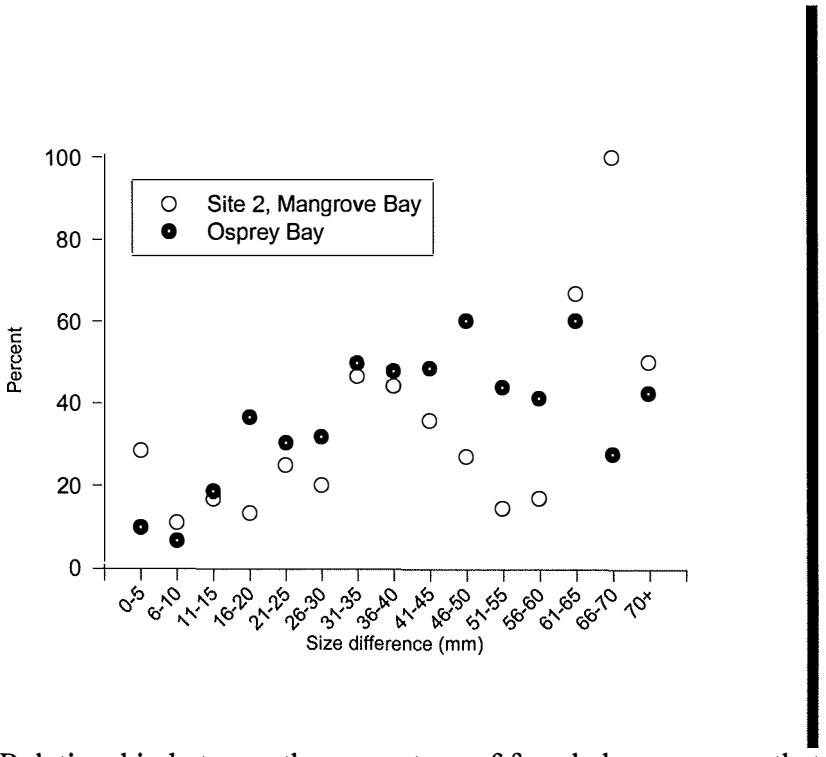


Figure 1.4.5. Relationship between the percentage of female home ranges that overlapped by more than 50% and the size difference of each female pair.

1.5 Experimental Investigation Of Sex-Change In *E. rivulatus*

1.5.1 Materials and Methods

The present study was based on an experiment to determine if protogynous sex-change in a similar sized epinepheline, *Cephalopholis cyanostigma*, could also be induced by removal of males from social groups (Mackie, 1993). However, whilst the latter project did indicate that females changed sex to replace the males, the results were equivocal because of the small number of males removed (four), and because the sex of cod within the sites prior to the experimental removal of males could only be determined by behavioural evidence.

The experiment conducted on *E. rivulatus* was therefore done on a larger scale so that more males could be removed, with cannula used to obtain physical evidence of the gender of fish prior to the experimental removal of the males. Cannulation techniques (insertion of a small diameter polyethylene tube, or cannula, into the gonopore of the fish to obtain gamete samples) are explained in Chapter 1.1. The technique had been used to determine gender of several hundred *E. rivulatus* prior to the cannulation of *E. rivulatus* during the sex-change experiment. As it is difficult to obtain cannula samples from non-reproductive *E. rivulatus*, the sex-change experiment was conducted at the start of the reproductive season in August and September 1995.

The population of *E. rivulatus* used in the sex-change experiment were located along an area of limestone and coral reef bordering a sand channel within Osprey Bay (Figure 1.5.1). Depth of water ranged between 2 and 6 m in the study site, which measured 214 m by 60 m and was located in an area closed to fishing activity. A study to determine the social organisation of *E. rivulatus* in this population had been completed three months prior to the present experiment (Chapter 1.4), providing information on the sex and home range of each *E. rivulatus* in the site. Most of these fish had also been tagged to allow identification of individuals underwater. This information was needed in the sex-change experiment to determine which males to remove, where to find them, and to examine the movement patterns of individuals subsequent to the experimental removal of males. The study site contained at least 264 *E. rivulatus*, including about 32 males (Chapter 1.4). The sex ratio of *E. rivulatus* within the site was 1 male to 6.3 mature females, with mean fork lengths of males and mature females during the home range study being 323 mm and 245 mm, respectively. All body measurements used in this study are of fork length (FL), which is the same as total length in *E. rivulatus*. The estimated length at sex change for *E. rivulatus* within Osprey Bay was 278 mm (Chapter 1.1).

Phase 1

General procedures for the sex-change experiment were to re-cannulate all *E. rivulatus* within the study site that were 250 mm or longer. Selected males were then removed from the population. Finally, after a period of three to four weeks, all fish longer than 250 mm were captured and their gonads examined using histological techniques to determine whether any fish had undergone sex reversal. Re-cannulation of *E. rivulatus* within the study site was necessary because at least seven months had passed since the fish had been cannulated for the home range study and some may have changed sex. Three weeks was considered long enough for sex-change to have commenced among *E.*

rivulatus during the experiment, based on the study made on *C. cyanostigma* (Mackie 1993).

The re-cannulation exercise was completed between the 19th and 27th of August 1995. Of 167 *E. rivulatus* captured during this time, the gender of 88% was confidently determined. *E. rivulatus* were captured for cannulation by snorkellers (myself and two assistants) using a baited hook and line tied to a short stick. Location of capture was noted and each fish was taken to a dinghy anchored nearby where it was put into a bucket of seawater, processed, and released at its site of capture. Processing was done by myself and involved measurement, identification by tags or body marking, cannulation, and clipping of the dorsal fin. The latter was done to identify cannulated from non-cannulated fish. Sixty-three (38%) of the *E. rivulatus* captured for cannulation did not have tags for identification and had either not been tagged during the home range study or had lost their tags. These fish were left untagged in case the process of tagging affected susceptibility to sex-change, but were identified by their length, body markings and location within the site.

On completion of the re-cannulation exercise 20 males were removed from within the central region of the study site on the 28th and 29th of August, 1995 (Figure 1.5.2A). A large female (343 mm) was also inadvertently removed at this time because it was not reliably cannulated and was assumed to be male. This central region from which the males were removed was termed the “manipulated” area. At least 16 males occupying territories on the northern and southern extremities of the site were left untouched so that histological changes to the ovaries of females within these “control” areas could be compared to those of females within the manipulated area where males were removed. This experimental design was considered appropriate because it minimised the potential interference by neighbouring males in the ability of a susceptible female to change sex, as highlighted in the previous work on *C. cyanostigma* (Mackie 1993). Further, because female *E. rivulatus* may move between the territories of more than one male (Chapter 1.2), it was thought necessary to remove all males from an area in order to ensure that a susceptible female’s normal interactions with males were sufficiently altered for sex-change to occur.

Approximately three weeks after the removal of males, all *E. rivulatus* that were 250 mm or larger (apart from most of the control males) were captured from both control and manipulated areas of the site for sampling of gonads. This occurred between the 18th and 25th of September 1995, with completion of the experiment to this stage referred to as Phase 1. Sex-change was deemed to have occurred if the gonad of a particular fish that had been cannulated as female before the removal of males was either transitional or male when captured at the completion of the experiment. Because of the movement by females between male territories and the large number of fish used in the study, it was not possible to match sex-changing individuals with a particular social unit.

Phase 2

A subsequent survey of the manipulated area of the study site was made on the 15th of December to assess numbers and movement of *E. rivulatus* since Phase 1. This survey was limited to the reef slope and about 15 m of the adjacent reef flat where *E. rivulatus* were most abundant. Using SCUBA, I carefully searched the area in a zigzag pattern, with the location, length and identity (if tagged) of *E. rivulatus* marked onto a map of the site printed onto underwater paper. Following the survey most of the larger *E.*

rivulatus observed within the manipulated area were captured for sampling of gonads. The aim of this second phase was to determine if further sex-change had occurred and whether new males had moved into the site. As some of the fish within the site were particularly flighty and wary of divers, not all could be caught for sampling purposes.

Processing Of Samples And Histology

All *E. rivulatus* removed from the study site for sampling purposes were taken by myself using a speargun. Within four hours of capture each *E. rivulatus* was measured and weighed. Gonads were removed and placed in FAACC (Formaldehyde 4%, Acetic Acid 5%, Calcium Chloride 1.3%; Winsor 1984), whilst the otoliths (sagittae) were cleaned and stored dry. The otoliths were subsequently sectioned and an estimate of the age of each fish determined as per the methods outlined in Chapter 1.3. Preserved gonad samples were weighed and several 3 mm thick portions removed from along the length of one lobe for preparation and analysis using standard histological techniques. Sections were cut at 5-7μm and stained using Haematoxylin and Eosin. These sections were examined under light microscope in order to determine the gender and reproductive maturity of each fish based on the following gonad developmental stages (refer to Chapter 1.1 for complete details of these)

Immature Female (F1): Ovary showed no evidence of prior spawning.

Mature Resting Female (F2): Ovary small and relatively light-weight. The ovarian lumen was large and the tunica was thick and contracted, or becoming so. Lamellae were narrow and contained previtellogenic oocytes.

Ripe Female (F3): Ovary large, firm and expanded with a granular appearance. The tunica was narrow and the dorsal blood vessel large. The lumen was filled by the expanded lamellae which were dominated by vitellogenic oocytes.

Spawning Female (F4): Ovary large and expanded, with a thin tunica. If captured just prior to spawning the lamellae were dominated by hydrated and yolk globule stage oocytes. If spawning had recently occurred then post-ovulatory follicles were present and the vitellogenic oocytes were less tightly packed

Spent Female (F5): Ovary reduced in size, flaccid, and often bloody in appearance. The lamellae were misshapen with a loose stroma and disorganised appearance. Few previtellogenic oocytes and sometimes vitellogenic oocytes (mostly atretic) were present.

Transitional (T): Only one transitional *E. rivulatus* had previously been captured (Chapter 1.1). In the present study an individual was considered to be changing sex if the gonad showed an abundance of developing spermatic tissue in the presence of degenerating ovarian tissue, and no peripheral sperm sinuses (Hastings 1981).

Immature Male (M1): As with the transitional stage, immature male *E. rivulatus* were rare, with only two previously captured in samples (Chapter 1.1). Both fish were also taken during the non-reproductive season and had small testes with a thick tunica. Blood vessels were abundant and the lamellae were long and narrow. Central regions of these were filled with connective and vascular tissue, and yellow-brown bodies whilst peripheral regions contained numerous crypts of developing spermatic tissue and previtellogenic oocytes. Peripheral sperm crypts were small and filled with spermatozoa.

Mature Resting Male (M2): Testis small and straplike. The tunica was thick and compact, or becoming so. Peripheral sperm sinuses were small and the testicular lobes of variable shape and well separated from each other. Yellow-brown bodies and vascular and connective tissue were numerous whilst spermatic tissue in the early stages of spermiogenesis dominated.

Ripening Male (M3): Testis still relatively small with a thick tunica. The lobes were dominated by later stages of spermatogenesis and the intralobular and peripheral sperm sinuses were filling with spermatozoa.

Ripe Male (M4): Testis large and white with a thin tunica and expanded testicular lobes. The latter were dominated by spermiogenesis whilst connective tissue was reduced. Peripheral and intralobular sperm sinuses were well developed and filled with spermatozoa.

Spawned Male (M5): Testis small with a loose tunica. The lobes contained little spermatic tissue and thick cords of connective tissue. Large yellow-brown bodies and blood vessels were also common.

Atretic oocytes were classified into two stages according to Hunter & Macewicz (1985). During the initial (alpha) stage of atresia, the entire oocyte is resorbed (all yolk and cytoplasm gone), whilst the second (beta) stage involves degeneration of the follicle (granulosa and thecal cells).

1.5.2 Results.

Phase 1

Following the removal of 20 male *E. rivulatus* from the manipulated area, between 20 and 27 days elapsed before capture and sampling of all possible sex-changing fish. Of the 117 *E. rivulatus* taken during this time, 40 came from the control areas and 77 from the manipulated area. The sex of these fish and the changes that occurred within the control and manipulated areas are explained below.

Manipulated Area.

Two *E. rivulatus* captured within the manipulated area were classified as transitional and four as recent sex-changers (new males) at the end of Phase 1. Nine *E. rivulatus* captured in this area were mature males that had immigrated into the study site. Figure 2b shows the location where these immigrant males, new males and transitional fish were captured. Two control males had moved or extended their home ranges into the manipulated area, two resident males had been inadvertently left in the area when the others were removed to begin the experiment, and 58 were mature females (Table 1.5.1).

One of the four new males was immature (M1) whilst the other three were maturing (M3). Three of these four males were cannulated as female at the start of the experiment. The fourth had not been cannulated and was not tagged. However the testis of this male was similar to that of the other recently sex-reversed males (described below) and it was likely that this fish had only recently changed sex. Figure 1.5.3a shows the cannula sample of the immature male taken just prior to the start of the

experiment, whilst Figure 1.5.3b shows the histological section of the gonad of this same individual when captured at the end of Phase 1.

The nine immigrant males captured within the manipulated area were mature (M4) and had apparently moved in to the area to replace the males removed at the start of the experiment. Most of these immigrant males were taken along the slope at the northern section of the manipulated area where the highest concentration of *E. rivulatus* was found during the home range study (Figure 1.5.2b, Chapter 1.4). Three of these males had previously been tagged but were only seen once or twice during the home range study. These males apparently inhabited territories outside the study site but occasionally roamed into it. They had moved at least 30-40 m from the border of the study site to their location of capture. The largest immigrant male (335 mm) had also been tagged previously but was only observed outside the study site. This male had moved at least 115 m to the location at which it was captured at the end of Phase 1. The remaining five immigrant mature males taken within the manipulated site had not previously been tagged or cannulated. The testes of five of the nine immigrant males contained vestigial oocytes, with one in particular having a high number (approximately 350 within the transverse section of the gonad). However none of the testes of these males showed any other histological evidence of sex-change within the time period of the study.

Two males had inadvertently been left in the manipulated area of the site at the start of the experiment. These were both small in size (286 and 292 mm) and were not captured for cannulation prior to the experiment. Both were tagged and had changed sex since October 1994 when they were cannulated prior to the home range study. The testis of one contained relatively few vestigial oocytes whilst the testis of the other contained none, and both were reproductive (M4) males and therefore unlikely to have changed sex within the time period of the experiment.

All 58 female *E. rivulatus* captured from within the manipulated area of the site were reproductively ripe (F3). The ovaries of 29 of these females (49%) contained small crypts of spermatic tissue (usually less than 20 crypts in the transverse section of gonad). However, these fish were not considered to be changing sex because the ovaries of mature female *E. rivulatus* often contain spermatocrypts (Chapter 1.1), and there were no other histological indications of sex-change, as described previously for the transitional stage.

Lengths of the original males removed from the manipulated area at the start of the experiment were relatively large, being within the upper half of the size range of all males that were captured from Ningaloo Reef (Figure 1.5.4). In contrast, the lengths of the immigrant males were smaller (except for one individual), and barely overlapped with the range of lengths of the original males. Lengths of the two transitional fish were similar to that of the larger immigrant males, whilst the newly sex-changed males were generally larger than both the transitional fish and immigrant males, but still relatively small compared to the original males. Few females were of similar size to any of the original males. Some females were larger or equal in size to the immigrant males, two of the newly sex-changed males, and both transitionals (Table 1.5.1, Figure 1.5.4). A comparison of the lengths of immigrant males and the resident males that had been experimentally removed at the start of the experiment showed that the former were significantly smaller (Table 1.5.2). However the influence of the large immigrant male resulted in a non-significant comparison in mean lengths between the immigrant males and the females that had or were changing sex. The two large control males (330 and

333 mm) that appeared to have extended their home range into the manipulated area were not included as immigrants in these analyses because the interest was on males with completely translocated territories.

The ages of male and transitional *E. rivulatus* within the site at Osprey Bay are shown in Figure 1.5.5, excluding data for one transitional, two immigrant males, and four resident males which could not be reliably aged. The age distributions mirror that of the length data for these fish, showing that the original males were relatively old with a mean age of 9.5 (0.58 SE) and a range of 6-14 years. Most of the females that successfully changed sex were also older (mean of 7.5 years (1.5 SE), range 5-11 years), whilst the immigrant males were relatively young (mean = 4.9 (0.44 SE), range 3-7 years). The fish that were still in the process of changing sex were also relatively young (4 and 7 years).

Control Area.

Thirty-three of the 40 *E. rivulatus* taken from the control areas were ripe or spawned females (F3 and F4) and seven were ripe mature males (M4). The testes of none of these males contained vestigial oocytes and all had been previously cannulated as male. A female from the control area with a home range abutting the manipulated area had over 380 crypts of sperm within the transverse section of its ovary (Figure 1.5.6). This is a high number, for whilst the ovaries of female *E. rivulatus* often contain sperm crypts, it is rare to find individuals with more than 160 crypts in the transverse section of the ovary (Chapter 1.1). At 327 mm this female was also large compared to the average size of females and sex-changers within the study site, although it was smaller than the control male (345 mm) with whom it had an overlapping home range. It is possible, therefore, that the large female was in a state of physiological conflict due to the opposing influences of a dominant resident male and the sudden loss of adjacent males.

The ovaries of 17 (52%) of the 33 females taken from within the control area also contained crypts of spermatic tissue but were not considered to be in the process of changing sex. Since 46% of the females sampled at other locations along Ningaloo Reef for reproductive analysis also contained sperm crypts within their ovaries (Chapter 1.1), the occurrence of them within the control females is normal. No *E. rivulatus* taken from within the control areas had therefore changed sex or were in the process of doing so since the removal of the males to commence the experiment.

Description Of The Gonads Of Transitional Or Sex-Changed E. rivulatus

Transitional Fish: The two fish classified as transitional had both been cannulated as female prior to the experimental removal of males, with 21 and 22 days elapsing between removal of the latter and capture of the two transitional fish (Table 1.5.1). The histological sections showed that the gonads of both fish had been ripe ovaries with a full complement of mature oocytes, but at some time prior to capture major physiological and morphological changes had commenced (Figures 1.5.7a,b and 1.5.8a,b). A comparison of sections taken from different sections of the gonadal lobe showed that these changes were occurring simultaneously throughout the length of the gonad. The gonads of both fish were large and relatively heavy (9.70 and 12.47 g), and comparable in weight to the ovaries of reproductively mature female *E. rivulatus* (Chapter 1.1), indicating that the transitional process had only recently begun.

The dorsal blood vessel in the transitional gonads was large, and other blood vessels also ran longitudinally through the tunica. The latter was stretched thin by the expanded

lamellae. These lamellae filled the ovarian lumen and were dominated by yolky oocytes in alpha and beta stages of atresia. Numerous previtellogenic oocytes and crypts of spermatic tissue were also present within the somatic tissue that occurred in pockets amongst the degenerating oocytes. Yellow-brown bodies (melano-macrophage centres) were also a common feature within the gonads of the transitional fish. The somatic tissue was comprised of connective and fibrous tissue, and various types of free cells (mainly leukocytes). It was not possible to identify reliably the nature or purpose of these cells using haematoxylin and eosin stained sections and light microscopy, and they were often mixed together with some showing the effects of post-mortem lysis and fixation. However, it was obvious that they were involved in the dual process of removing ovarian tissue from the gonad and development of the spermatic tissue.

The previtellogenic oocytes were concentrated within this somatic tissue and along the periphery of the lamellae. At least half of these oocytes were atretic. In some the outer cytoplasm was less basophilic and/or had split from the central mass of cytoplasm, and the nucleus appeared necrotic. These oocytes were similar in appearance to those in the red grouper, *E. morio*, which were undergoing "rejuvenilization" and regressing (Moe 1969). In other previtellogenic oocytes the cytoplasm was fragmenting and breaking apart and the oocyte had been invaded by macrophages. The nucleus soon broke down and a layer of hypertrophied cells containing basophilic cytoplasm surrounded the oocyte.

The first signs of atresia in the ripe, yolk globule stage oocytes that dominated the gonad was deterioration of the zona radiata. This lost its striations and rapidly became eroded. Macrophages apparently moved through the zona radiata into the oocyte, but were usually difficult to distinguish within the oocyte until later stages of atresia. The nucleus of the atretic oocyte disappeared early in the resorption process. The granulosa cells became hypertrophied and filled with yolk material. The yolk globules ruptured and oil droplets fused. As atresia continued the oocyte became reduced in size and filled with vacuoles, remnants of yolk and yolk vesicles, which progressively became more grainy in appearance.

Sex-Reversed Fish: The sole immature (M1) male was captured 19 days after experimental removal of the original males. The gonad of this male was small and compact in cross-section, with a thick muscular tunica and long, narrow lamellae. The dorsal blood vessel was large and smaller vessels ran longitudinally through the tunica and down the middle of the lamellae. Numerous capillaries carried blood to the peripheral regions of the lamellae.

As in the transitional gonads, the testis of this immature male was characterised by high levels of cellular activity. The vitellogenic oocytes were gone, although within a conglomerate of cells in one lamella were macrophages that contained an acidophilic substance that may have been yolk (Figure 1.5.9a). Generally the lamellae were dominated by crypts of spermatocytes. Spermatogonia were common in peripheral regions of the lamellae, with scattered crypts of spermatids also present. Numerous previtellogenic oocytes (200+ in the transverse section) in the chromatin nucleolus stage of development still remained. These were mainly located towards the periphery of the lamellae. Central regions of the lamellae contained connective tissue and numerous small yellow-brown bodies. No sperm sinuses were yet present within the lamellae although a sinus had formed within the peripheral regions of the gonad, just below the muscle layers of the tunica and separated from the underlying germ tissue by one to several layers of connective tissue. This sinus was almost empty apart from a few cells,

including a group of sperm that had entered via a ruptured cyst (Figure 1.5.9b). Several other cysts of maturing spermatids also lay next to the sinus, separated only by a thin membrane.

A distinct cell type, similar in size to blood vessels and characterised by an ovoid nucleus and a pink staining cytoplasm was also present among the germ connective tissue within the lamellae. These cells were usually grouped together and were particularly abundant within distal regions of some lamellae (Figure 1.5.9c). These cells were thought to be Leydig cells because they were similar in gross appearance and the timing of their proliferation during the sex change process to Leydig cells in the protogynous rice eel, *Monopterus albus* (Chan & Phillips 1967, Yeung *et al.* 1985). However, the identity and function of these cells in the immature male *E. rivulatus* could not be reliably determined using haematoxylin and eosin stained sections and light microscopy. These cells were not obvious within the gonads of *E. rivulatus* at other stages of development. Similar cells were found though, either singly or in small groups, within peripheral regions of the gonadal lamellae of the transitional fish and maturing males.

Of further note were the cords of putative endocrine cells within the muscle layers of the tunica (refer to Chapter 1.1 for details of these). These cells were also present within *E. rivulatus* of both sexes and all reproductive status, but were more abundant within the gonads of these new males. There were also many cells within the lumen of the gonad, including masses of Leydig cells and other tissue which appeared to have sloughed off from the sides of the lamellae and tunica.

The testes of the two maturing (M3) males were larger and less compact than that of the immature male. In transverse section both gonads were ovarian in structure, with relatively long lamellae and a large lumen. Previtellogenic oocytes at the chromatin nucleus stage were numerous in one testis (almost 200 in the transverse section of the gonad), but relatively few in the other (about 48 in the transverse section). In both testes the connective tissue within the central regions of the lamellae was looser than in the testis of the immature male. The dorsal blood vessel was large and there were numerous other blood vessels within the thick muscular tunica. The lamellae were dominated by crypts of spermatocytes and spermatids in approximately equal proportions. Spermiogenesis was also underway and some crypts of spermatozoa had ruptured and joined together (Figure 1.5.10). Other matured crypts of spermatozoa had joined with the peripheral sperm sinus which was still small but filled with sperm. Numerous putative Leydig cells were also present within the testes of the maturing males. These were reduced in number compared to the immature male and mainly found in the distal regions of the lamellae. In one of the maturing males many of these cells appeared degenerate and the tissue around them loose and disorganised.

The fourth male classified as a recent sex changer (M3, 322 mm) had not been previously cannulated. However the testis of this fish was histologically similar to that of the two other M3 males, with a thick muscular tunica and an abundant supply of blood, numerous endocrine cells, and narrow lamellae that were dominated by spermatocytes, spermatids and connective tissue. There were also about 180 vestigial oocytes in chromatin nucleolus stage of development within the testis of this male. Of particular interest were the presence of several incompletely resorbed vitellogenic oocytes which were surrounded by fibrous tissue (Figure 1.5.11). Some were still within the lamellae whilst others were embedded in a mass of somatic and germinal tissue in the lumen. Only one of these vitellogenic oocytes still contained recognisable yolk.

Immigrant Males: The testes of all eight immigrant males were classed as early to mid M4 (ripe). Spermatic tissue in all stages of development filled the testicular lobes, the tunica was stretched thin and the dorsal blood vessel was large. Spermiogenesis was underway and intralobular sinuses were developing. Peripheral sperm sinuses were relatively large and filled with sperm. Few yellow-bodies were present and there was little connective tissue within the lobes, which varied widely in shape. A transverse section of a particularly ripe immigrant male is shown in Figure 1.5.12.

Phase 2

Survey And Sampling Of Fish Within The Manipulated Area

The survey of the study site to determine whether there had been further immigration by males or sex-change by females, was completed 81 days after sampling of *E. rivulatus* during Phase 1, and 108 days after the initial experimental removal of males. Thirty-three *E. rivulatus* above 230 mm fork length were observed along the slope in the manipulated area of the site. Most of these were untagged and approximately 240-250 mm in length, although three were between 290 and 320 mm fork length. Two of the latter were untagged, whilst one had been tagged but could not be identified because the ends of both tags were gone and only stubs remained. This individual and one of the two untagged fish could not be captured for subsequent sampling because they were too wary and could not be approached. However, when followed both fish limited their movements to a small area of reef (approximately 30 m in length), rapidly doubling back instead of continuing along the slope. This indicated that these fish were not just passing through, but had established territories in the area. Although it was not possible to identify either of these fish, the tagged individual was probably male as it displayed the pale colouration at the rear of the body that is usually only observed on reproductive males (Chapter 1.2). Assuming that these three fish were males, then the ratio of males (including transitionals; see below) to mature females was at least 4:29 (1:7.25) along the reef slope at the end of Phase 2.

Sixteen *E. rivulatus* were captured from within the manipulated area following the completion of the survey. These were selected for capture based on their relatively large size or because they were new to the area. Fourteen of these 16 *E. rivulatus* were females. Seven of these were ripe (F3), six were mature but inactive (F2), and one was spent (F5) and had apparently finished spawning for the season. The sole male taken in these samples was 318 mm in length. The testis of this fish was ripe (M4) with no oocytes present. It was untagged and had moved in from an unknown location.

The gonads of one of the 16 fish were transitional. This individual was 254 mm in length and four years old, thus making it one of the smallest males captured at Ningaloo Reef and one of the youngest within the Osprey Bay samples once the sex-change process was completed (Figures 1.5.3 and 1.5.5). The gonads of this transitional fish were small (0.734 g) and compact in cross-section (Figure 1.5.13). The tunica was thick with a large dorsal blood vessel and conspicuous blood vessels running down the narrow lamellae. Central regions of the lamellae were filled with somatic tissue, whilst peripheral regions contained previtellogenic oocytes and numerous crypts of spermatic tissue. The latter were mainly spermatocytes and gonia (presumably spermatogonia), although small crypts of spermatids were also present. Distinctive clumps of putative Leydig cells were distributed amongst the somatic and germ tissue. These were more dispersed than in the gonads of the immature males sampled during Phase 1. Loosely structured yellow-brown bodies were also common. Only one large, incompletely

resorbed vitellogenic oocyte remained within the transverse section of this transitional gonad, and many of the previtellogenic oocytes were atretic. In the latter the nucleus was no longer present and the cytoplasm was fragmented. These oocytes were surrounded by hypertrophied thecal cells containing oocyte cytoplasm. In addition to these there were numerous smaller oocytes approximately 8-15 µm in diameter. These had a large nucleus with a single nucleolus sometimes evident, and dark staining cytoplasm. They were usually found singly along the periphery of the lamellae and did not show signs of atresia. Peripheral or intralobular sperm sinuses were not evident.

1.5.3 Discussion

This study resulted in the replacement of twenty males by six sex-changing females, nine immigrant males, and two control males. Another sex-changing female and three more immigrant males were also found at the end of Phase 2. At both times the number of sex-changing individuals were not the near one-for-one replacement of removed males by sex-changing females that has been observed in similar experiments (Fishelson 1970, Shapiro 1980, 1981, Warner & Swearer 1991). Nevertheless, in both cases the population was returning to the 1:6 sex ratio of the undisturbed Osprey Bay population (Chapter 1.1).

Proof of sex-change by female *E. rivulatus* was provided by clear cannulation and histological evidence. Furthermore, the number of immature males, maturing males, and transitional *E. rivulatus* taken during the experiment were high, relative to the numbers obtained from the control areas and in samples taken for reproductive and growth analysis (Chapters 1.1 and 1.3). In these samples only one transitional and two immature males were found out of 412 fish. Thus social cues are important to sex-change in this species of serranid, although there is possibly a minimum size and age at which females are not physiologically or psychologically capable of changing sex. This minimum size may be around the size of the transitional fish captured during Phase 2 (254 mm), since it was at the low end of the male size range and there were was no evidence of smaller females changing sex despite the relatively high female bias in the sex ratio at the time.

However, whether a female succeeds in changing sex to replace a male may also depend on the interactions she has with other *E. rivulatus*, in particular with immigrant males. The latter, being generally quite small, were probably seeking to claim vacant male territory along the reef slope as this area was particularly advantageous to reproduction (Chapter 1.2). The interactions between the potential sex-changing females and immigrant males may have been competitive (both trying to gain control of the same area of reef), and/or suppressive (the immigrant males trying to dominate the females and keep them as highly fecund mating partners), and is likely to have lowered the incidence of sex-change. Such interference from outside males has also been noted in similar experiments on other protogynous group living species (Robertson 1972, Nemtzov 1985, Mackie 1993). The ability of a female to change sex may also be dependent on the amount of competition received from other females, particularly when two or more equal sized, dominant females are living in close proximity. In this situation the aggressiveness of each individual may be important in deciding which female changes sex to replace a male (Mackie 1993).

Thus relative size was an important factor in determining whether a female was able to change sex to replace a removed male. In some cases the female was large enough to commence the transition process soon after the males were experimentally removed. For smaller females though, the process was probably delayed or slowed. Judging from the way that the males and transitional *E. rivulatus* were concentrated in the manipulated area, it seems that considerable interactions were still occurring between these fish for control of the more favoured territories within this area at the end of Phase 1.

Phase 2 of the experiment showed that few male or female *E. rivulatus* had moved into the manipulated area to replace those removed during Phase 1. This may be due in part to the small size and low abundance of the remaining *E. rivulatus*, making it socially and reproductively a less attractive habitat. Although given the heavy depletion of the population during Phase 1 and the high quality of the resources available on the reef slope (e.g. food, shelter and spawning sites; Chapter 1.2), it is more likely that there were few fish available to move into the area. Furthermore, the wariness of the *E. rivulatus* during Phase 2 suggests that the depletion process had spooked the remaining fish and made them more cautious of divers and/or the area. Future monitoring of the site will determine whether this population is able to recover to former levels, a likely scenario given the abundance of *E. rivulatus* in many areas of the Ningaloo Reef lagoon. However, when groupers of the genus *Cephalopholis* were experimentally removed from reefs in which they were dominant predators, they showed little sign of recovery after three years (Shpigel & Fishelson 1991), thus indicating that *E. rivulatus* may take some time to re-establish itself in the site. Monitoring the progress it makes will provide useful ecological data on the dynamics of the community and competitive interactions with other predators, and will also show the resilience of this species to fishing pressure.

Mechanisms Of Sex-Change In E. rivulatus

The interpretation of sex-change experiments should be made with care because there is a chance that the process of removing the males was in itself a cue for sex-change to occur (Shapiro 1981). Nonetheless several theories have been proposed to explain the proximate control of sex-change in other hermaphroditic species, and one or more of these might explain such in *E. rivulatus*. The following possibilities are based on the reproductive and social characteristics of *E. rivulatus*, as discussed in Chapters 1.1 and 1.4.

Inhibition Of Sex-Change: In early studies it was hypothesised that presence of the dominant male in a social group suppressed or inhibited the natural tendency of females to change sex (Robertson 1972, Fricke & Fricke 1977). Certainly, in *E. rivulatus* the aggressive dominance of the male is evident, particularly towards the largest one or two females within the social group (Chapter 1.2). These are the only females with which the male maintains a close relationship, possibly because the largest females produce the most eggs and are more likely than smaller females to change sex if the opportunity arises. Thus, the male, at risk of losing a favoured mating partner and at the same time facing increased competition from a new male should benefit by suppressing the tendency to change sex in these females. In *E. rivulatus* this suppression is achieved by maintaining close contact with the female, especially during the brief reproductive bouts that occur throughout the spawning season. Much of this contact consists of lying nearby the females; however, the male will also chase, bully, and make rapid swims over the top of the larger females, thus reinforcing his dominance (see Chapter 1.2 for

details). The performance of the male during territorial disputes with neighbouring males may also influence his domination over the females that observe these disputes.

Inducement Of Sex-Change: Sex-change in a susceptible female *E. rivulatus* may also require the presence of smaller females. According to this theory a minimum number of other females can stimulate sex-change regardless of whether the dominant male is lost or removed. The stimulation may occur when the number of smaller individuals in a social unit relative to the number of larger individuals surpasses a critical ratio for a particular female (size ratio induction; Ross *et al.* 1983) , or when the sex-ratio increases beyond a critical ratio (sex ratio induction; Shapiro 1980, Shapiro & Lubbock 1980). In *E. rivulatus* the latter appears more appropriate given the stable sex ratios that occur between different populations inhabiting different areas of Ningaloo Reef (Chapter 1.1). This mechanism would enable sex ratios to keep relatively constant even under conditions of continuing female recruitment and low male mortality (Coleman *et al.* 1996), although possibly not in the short term due to disruptions such as caused by the present experiment.

The sex ratio induction hypothesis might also explain the presence of relatively small males that appear in populations of *E. rivulatus* (Chapter 1.1). These may have previously been subdominant females living on the periphery of a social unit, and not subject to high levels of intersexual interactions like more dominant females. Thus unlike the latter, the subdominant female may be more capable of changing sex under the stimulation of rising sex ratios. Sex-change by subdominant females that experienced low levels of contact with the male was also observed in the experiment conducted by Robertson (1972) on the cleaner wrasse, *Labroides dimidiatus*. Robertson further noted that the larger females may play a role in suppressing the tendency of subdominant females to change sex.

These small males appearing amongst a population of larger, established males would experience high levels of aggression and relatively low reproductive success. Nonetheless, within the Osprey Bay site where many females move onto the reef slope to spawn these small males may have at least the same reproductive success as a large female. This is achieved by following the females onto the reef slope during the brief spawning events and enduring high aggression from the resident males, but eventually getting to spawn with females once the resident males have exhausted their sperm supplies (Chapter 1.1). Furthermore, the present study also showed that if a larger high status male is lost from the population a smaller male may improve his status and hence reproductive success by taking over the social group and stopping the dominant female from changing sex. No histological evidence was found in the present study or in previous studies on *E. rivulatus* at Ningaloo to show that small males may develop from individuals that undergo prematurational sex-change.

Speed And Timing Of Sex-Change.

The rate in which individuals of a particular species are able to change sex may depend not only on the morphological and physiological complexities associated with the transition process, but also on the mating system and resulting pressure on an individual to make the change (Godwin 1994). Thus, the fast rate in which sex-change is accomplished in protogynous, haremic species may be due to the high risk of males moving in to take over the harem (Ross 1990). Certainly male-takeover was a significant factor in the present experiment and a plausible reason for the speed in which sex-change can be completed in *E. rivulatus*.

Female *E. rivulatus* also appear to be primed for a rapid transition given the appropriate social environment. Their ovaries often contain small amounts of spermatic tissue, and once the required sensory information that instigates sex-change is received, the ovary is rapidly cleared of female tissue. Male hormone-producing (Leydig) cells proliferate during or soon after the resorption of female tissue is completed and the gonad is swiftly transformed into a testis. Thus, a ripe ovary with full complement of mature oocytes can become a ripening testis with few signs of ovarian tissue inside of three weeks.

Whether the speed of sex-change is as rapid during the non-reproductive season when delays are not so reproductively costly is not known. However, this would appear to be so given the rarity of sex-changing *E. rivulatus* in samples taken for reproductive and growth analysis. The sole transitional and two new male *E. rivulatus* in these reproductive samples were all captured three to four months after the reproductive season, indicating that this is the main period during which sex-change will occur under natural conditions. This has also been found to be the case in other species of epinepheline serranids (Moe 1969, Shapiro *et al.* 1993). As noted by Shapiro (1984), this seasonal pattern of sex-change may have no direct selective importance, but is possibly a result of changes in population sex ratios due to peaks in juvenile recruitment after the breeding season and post-spawning increases in adult mortality.

Changes To The Gonad During Sex-Change.

The transition process in *E. rivulatus* began with atresia of vitellogenic oocytes throughout the ovary and the influx of macrophages and other somatic tissue. The latter were similar in gross appearance to the stromal cells of uncertain origin that were also present within the gonads of sex-changing wrasse, *Thalassoma duperrey* (Nakamura *et al.* 1989). The first signs of atresia in the vitellogenic oocytes was the loss of striations and erosion of the zona radiata, disappearance of the nucleus, and the fusion of yolk granules and lipid droplets. The cytoplasm was phagocytosed by the invading macrophages and the hypertrophied granulosa cells. Blood supply throughout the gonad was high and indicative of the large transport of materials and cells to and from the lamellae, and yellow-brown bodies formed as deposits for the end products of the resorption process. Such was the intensity of the resorption process that most of the previtellogenic oocytes were also removed from the gonad. These are usually more resistant to atresia and the number left within the gonads of the new males and transitional *E. rivulatus* after the experiment were much fewer than in some of the males captured for reproductive analysis (Chapter 1.1). This suggests that the transition process in these fish at the start of the reproductive season was more intense than in transitional fish during non-reproductive times of the year, no doubt because delays will be detrimental to immediate spawning success and increase the risk of interference by other males and dominant females.

Slow proliferation of spermatic tissue commenced as the oocytes were still being resorbed. As the gonad was cleared of ovarian tissue the rate of spermatogenesis increased and the lamellae soon become dominated by spermatic and connective tissue. The putative Leydig cells did not appear in abundance until development of the spermatic tissue was well underway, being most prolific within the gonads of the immature males. Although the microscopy and staining techniques used in this study did not allow proper identification of these Leydig cells, such cells do appear to be characteristic of most, if not all teleost species (Grier *et al.* 1980). Furthermore Leydig cells were shown to increase markedly in numbers during mid to late stages of sex-

change in the protogynous rice field eel, *Monopterus albus* (Chan & Phillips 1967). Nakamura *et al.* (1989) also found that proliferation of Leydig cells occurred during later stages of sex-change along with an increase in plasma 11-ketotestosterone levels and proliferation of spermatic tissue in *Thalassoma duperrey*. Ultrastructural details of these Leydig cells within the gonads of *T. duperrey* (numerous mitochondria with tubular christae and extensive smooth endoplasmic reticulum) showed they were the likely site of ketotestosterone production. Use of electronmicroscopy, endocrinology and additional histochemical techniques in further studies of sex-change on *E. rivulatus* would provide answers to the identity and function of the numerous cells and processes occurring within the gonad during sex-change in this species.

As sex-change progressed and the gonad developed into a testis the peripheral sperm sinus formed within basal tissue layers of the tunica. Initially this sinus was small and contained few spermatids and somatic cells, but as the spermatic tissue developed and spermiogenesis occurred the sinuses filled with more spermatids and expanded. Intralobular sinuses were less evident until later when the testis was ripening. At this stage the lamellae were still relatively long and narrow with mid regions filled with connective and other tissue, and had not obtained the thick lobular form found in the testis of ripe males. It is unlikely that *E. rivulatus* changing sex outside of the reproductive season would form mature sperm and sperm sinuses as rapidly as those in the experiment described here.

Significance Of Socially Induced Sex-Change In E. rivulatus

Concern over the exploitation of protogynous epinepheline serranids arises from the uncertainty of their response to fishing pressure and the inability to produce predictive yield models based on a proper understanding of their protogynous nature (Shapiro 1987, Huntsman & Schaaf 1994, Coleman *et al.* 1996). Thus the need to learn more about the sex-change process in this group of fishes has been noted by several authors (Shapiro 1987, Bannerot *et al.* 1987, Williams & Russ 1991, Gilmore & Jones 1992, Ferreira 1995). Information on structural changes that occur during the transition process in the epinepheline serranids is also lacking and remains an area where little has been achieved in the study of hermaphroditism among fishes.

Thus the present study is useful because it addresses both fishery management and fish hermaphroditism issues. It indicates that a population of *E. rivulatus* is able to withstand some degree of fishing pressure and still maintain a fairly constant sex ratio. Such is the advantage of socially rather than genetically induced sex-change. However, the study also showed that the short-term supply of potential males is finite and that sustained fishing pressure may deplete the population to a level where reproductive output is seriously diminished. Huntsman & Schaaf (1994) used life history parameters for the graysby (*Epinephelus cruentatus*), a protogynous grouper of similar size to *E. rivulatus*, to model the effects of fishing under various compensatory mechanisms. The results of these simulations indicated that compensation for loss of males (as shown in *E. rivulatus*) allowed greater reproductive capacity to be maintained with increasing fishing mortality than was displayed for uncompensated stocks. However, it was further shown that the life history parameters of the epinephelines make them inherently fragile to fishing mortality. Bannerot *et al.* (1987) also noted that as increasing fishing mortality disrupts the ability of mates to come into contact with each other, the reproductive success of protogynous hermaphrodites relative to that of gonochoristic (non sex-changing) species declines.

The latter was apparent in the study of sex-change in the blue-spotted rockcod, *Cephalopholis cyanostigma* (Mackie 1993). Observations of the changes in home ranges and behaviours following the removal of males showed that light fishing pressure may produce short term disruptions within social units of *C. cyanostigma* as the social hierarchy is restructured and the movement patterns of the remaining fish are altered to fill the void caused by the removal of fish. However, it was also noted that slow turn-over rates may make populations of *C. cyanostigma* susceptible to heavier levels of fishing if recruitment of juveniles and immigration do not match loss rates.

C. cyanostigma and *E. rivulatus* share social and life history characteristics despite inhabiting quite different habitats. Both species are similar in body size and live in site attached social groups. Males defended territories from other males and associated predominantly with the largest females. Sex-change also appears to be socially induced in both species. This indicates that the social control of sex-change may be common among the epinephelines, at least among the species that maintain fairly stable social units during much of the year. Whether it will be possible to determine the cues controlling sex-change in some of the larger epinepheline species that migrate to specific locations and spawn in large numbers during short periods of the year remains doubtful. Thus, management of these species may have to be based in part on the studies of non-migrating, more easily manipulated epinepheline species such as *E. rivulatus*.

Table 1.5.1 Details of *E. rivulatus* captured within (a), the manipulated area, and (b), the control areas, of the Osprey Bay study site at the end of Phase 1. New males refers to individuals that had changed sex since the start of the experiment. Uncaught residents refer to males that were inadvertently left in the manipulated area at the start of the experiment. Control males refers to males that had extended their home ranges into the manipulated area.

(a) Manipulated area

Fish classed as:	n	Fork length (mm)	Sexual status	Days since removal of males
Transitional	2	303, 306	T	22, 21
New Males (M1)	1	319	M1	20
New Males (M3)	3	305,312,322	M3	22, 27,21
Immigrant Males	9	289,289,292,295,295, 300,302,307,335	M4	na
Uncaught Residents	2	286, 292	M4	na
Control Males	2	330, 333	M4	na
Females	58	232 - 313 (mean=269)	F3	20 - 27

(b) Control areas

Fish classed as:	n	Fork length (mm)	Sexual status	Days since removal of males
Males	7	286 - 349 (mean=320)	M4	20 - 27
Females	33	230 - 327 (mean=271)	F3, F4	20 - 27

Table 1.5.2 Results of (a) an analysis of variance comparing the mean lengths of original resident males taken from the manipulated area at the start of the experiment, the mean lengths of males that had immigrated into the manipulated area, and the mean lengths of males that had recently changed sex, or were in the process of doing so at the end of Phase 1. (b) Results of a Cochrans C test to determine whether the variances of these lengths were homogenous. (c) Results of a Tukey HSD test for unequal sample sizes, used in the pairwise comparison of the mean lengths of the three males groups. New = newly sex-changed males, immigrant = immigrant males, old = original resident males.

(a)

Source of variation	df	MS	F	*P*
Length	2	3555.7	9.6378	0.0005
Residual	34	368.9		

(b)

	Cochrans C	df	*P*
Length	0.654734	2	0.0355

(c)

Fish	n	Mean length	Compared with:	*P*
new	6	311	immigrant	0.6025
immigrant	9	300	old	0.0038
old	22	332	new	0.1569

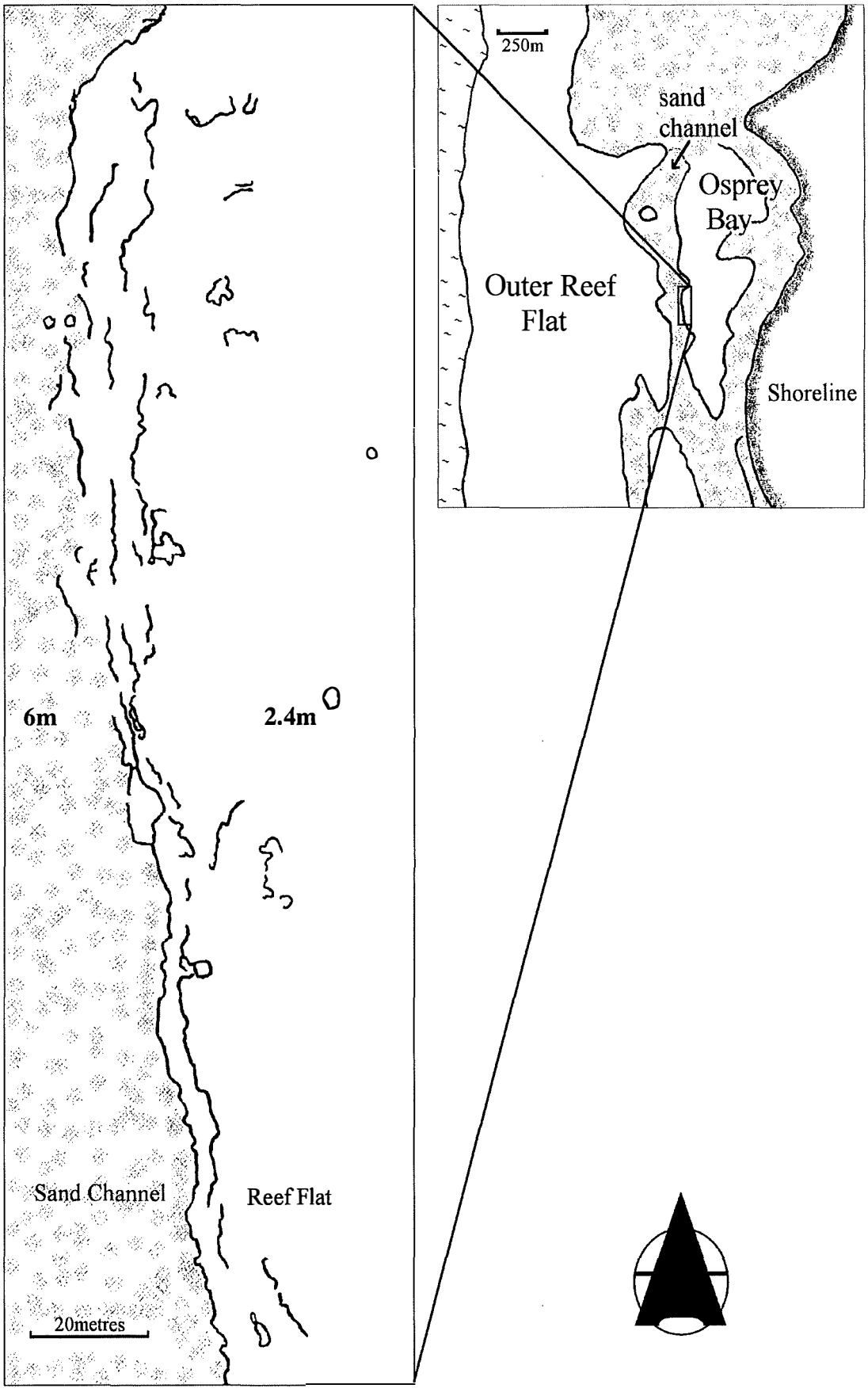


Figure 1.5.1. Detail of Osprey Bay and the study site used in the sex-change experiment.

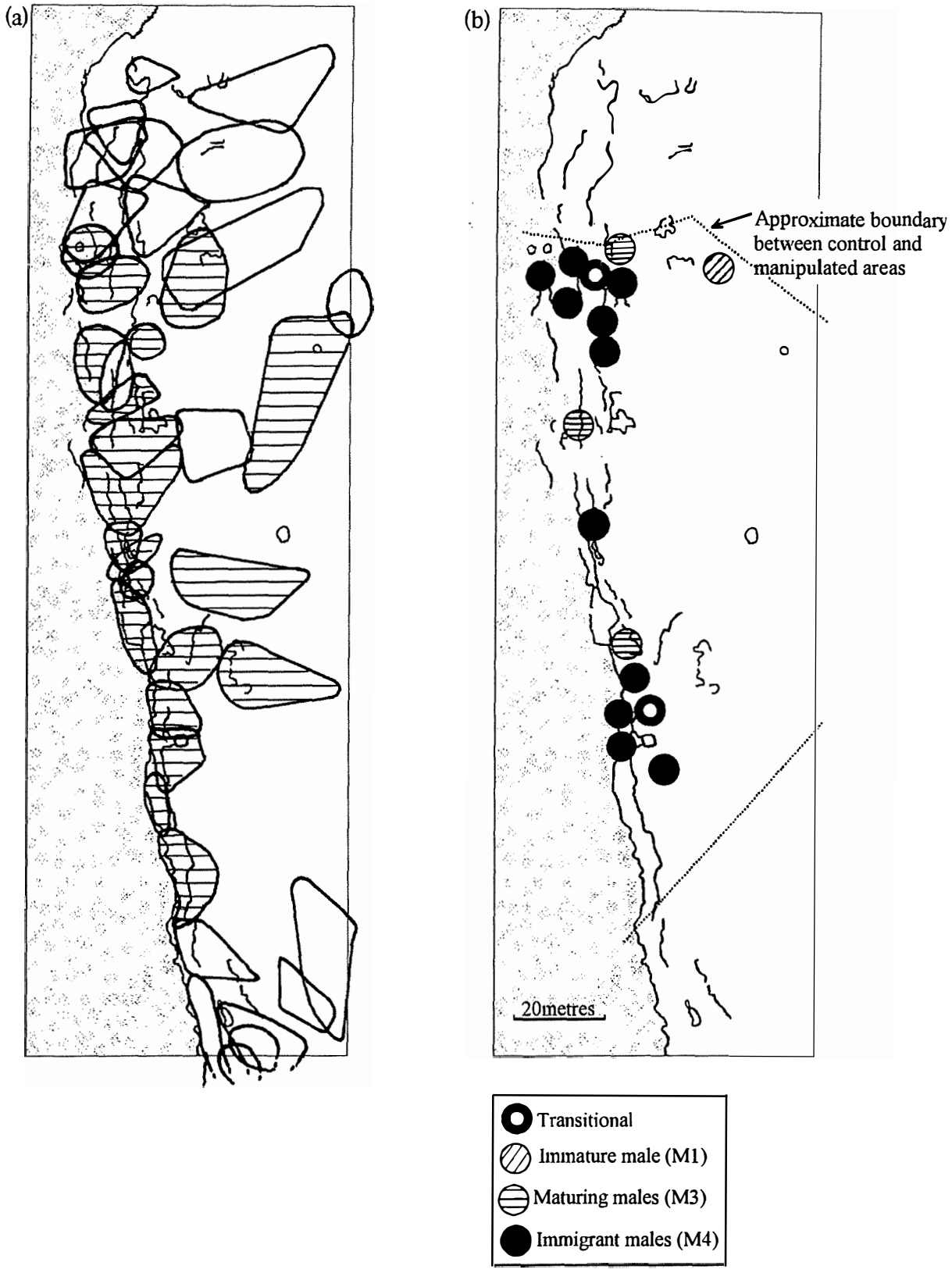


Figure 1.5.2 Map of the Osprey Bay study site showing (a), the home ranges of male *E. rivulatus* prior to the sex-change experiment. Cross-hatched home ranges are those of the males removed at the start of the experiment. (b) Capture location of male and transitional *E. rivulatus* within the manipulated area of the study site at the end of Phase 1.

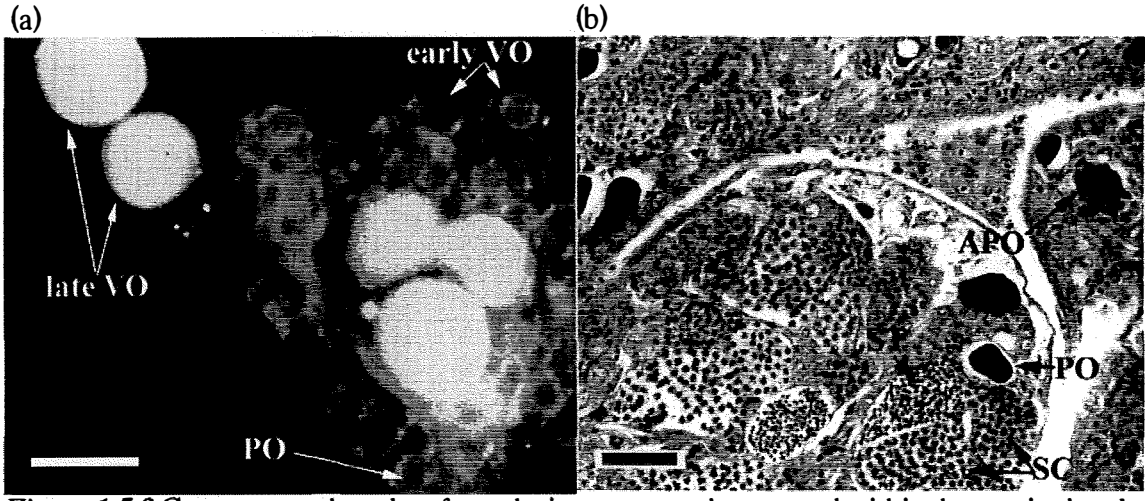


Figure 1.5.3 Gamete samples taken from the immature male captured within the manipulated area of the study site. (a) Sample of whole oocytes obtained by cannula prior to the experimental removal of males at the start of Phase 1. Scale bar = 50 μm. (b) Histological preparation of a transverse section of the gonad taken at the end of Phase 1, showing few vestigial oocytes remaining among the developing sperm tissue. Scale bar = 350 μm. **APO,** atretic previtellogenic oocyte. **PO,** previtellogenic oocyte. **SC,** spermatocytes. **ST,** spermatids. **VO,** vitellogenic oocyte.

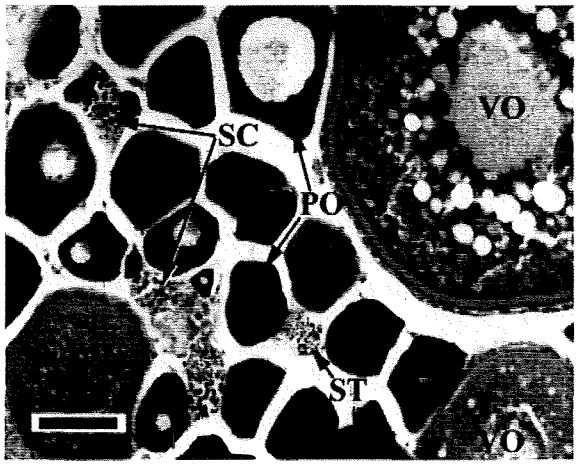


Figure 1.5.6 Transverse section of the ovary of a large female *E. rivulatus* captured within the control area of the study site at the end of Phase 1. Note the crypts of spermatic tissue amongst the previtellogenic and vitellogenic oocytes. Scale bar = 50 μm. **PO,** previtellogenic oocyte. **SC,** spermatocytes. **ST,** spermatids. **VO,** vitellogenic oocytes.

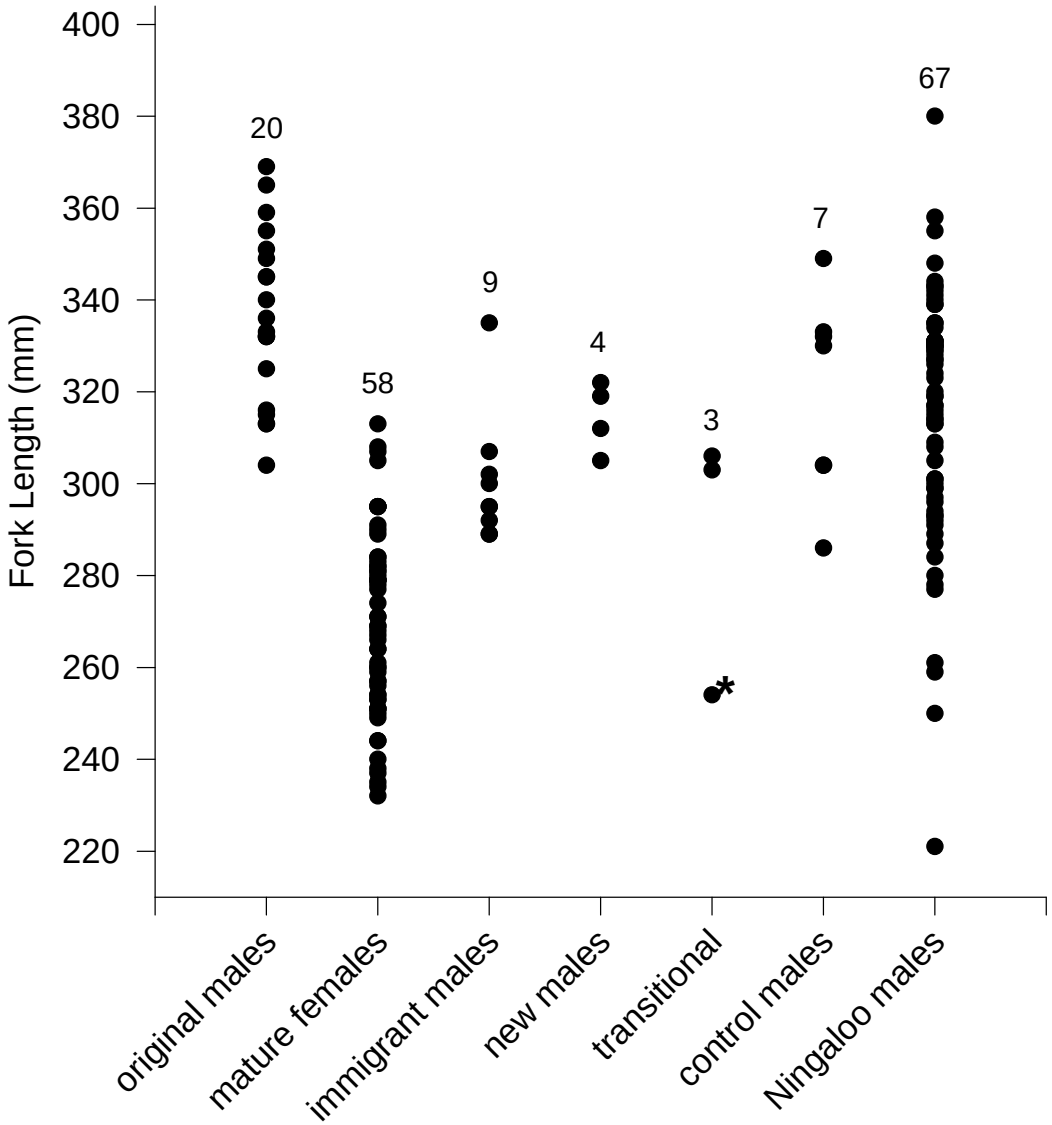


Figure 1.5.4 Lengths of *E. rivulatus* captured within the Osprey Bay study site at the completion of Phase 1 of the sex-change experiment. The asterisk indicates the length of the transitional fish captured during Phase 2. The lengths of male *E. rivulatus* captured for reproductive analysis from other locations along Ningaloo Reef are also shown. Numbers indicate sample size.

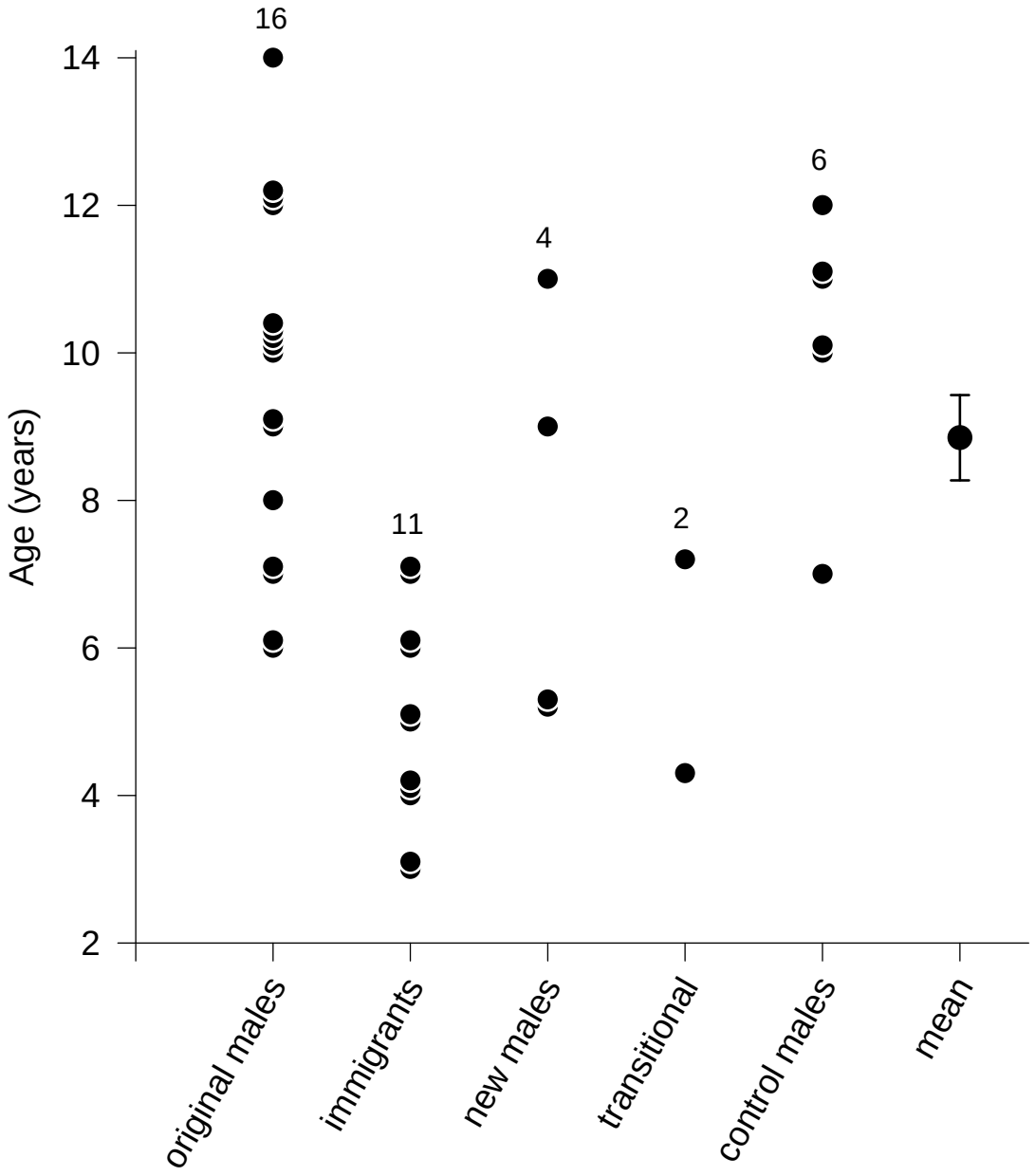


Figure 1.5.5 Estimated age of *E. rivulatus* captured within the Osprey Bay study site at the completion of Phase 1 of the sex-change experiment. Data points of similarly aged fish are shown stacked on top of each other. Also shown is the mean age (± SE) of fish within these samples. Numbers indicate sample size (note that not all individuals within these samples could be reliably aged).

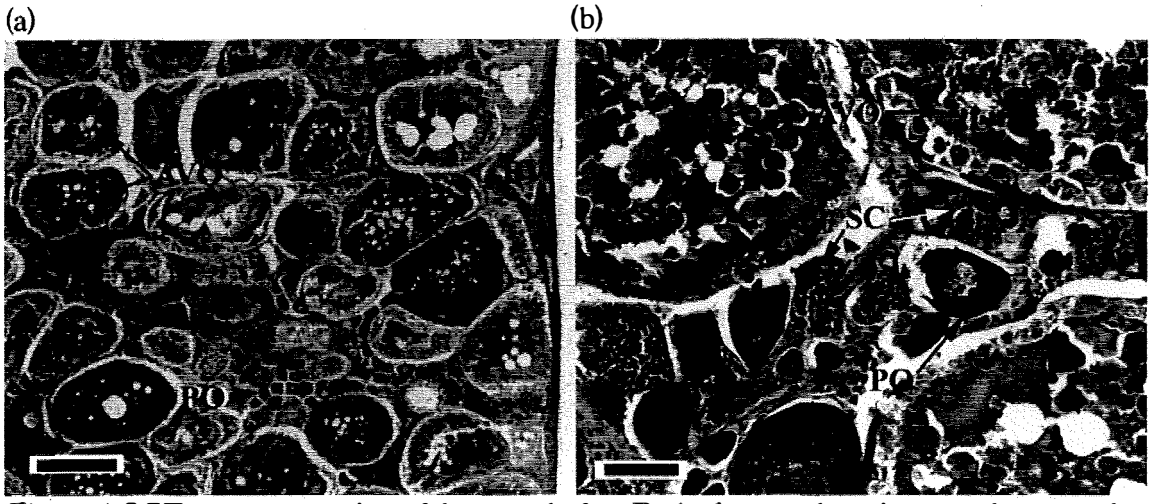


Figure 1.5.7 Transverse section of the gonad of an *E. rivulatus* undergoing sex-change at the end of Phase 1. (a) General structure of the gonad showing lamellae full of atretic vitellogenic oocytes. Scale bar = 270 μm. (b) Detail showing atretic vitellogenic oocytes, previtellogenic oocytes and spermatocrypts. Scale bar = 50 μm. **AVO,** atretic vitellogenic oocyte. **BV,** blood vessel. **PO**, previtellogenic oocyte. **SC,** spermatocytes. **T,** tunica.

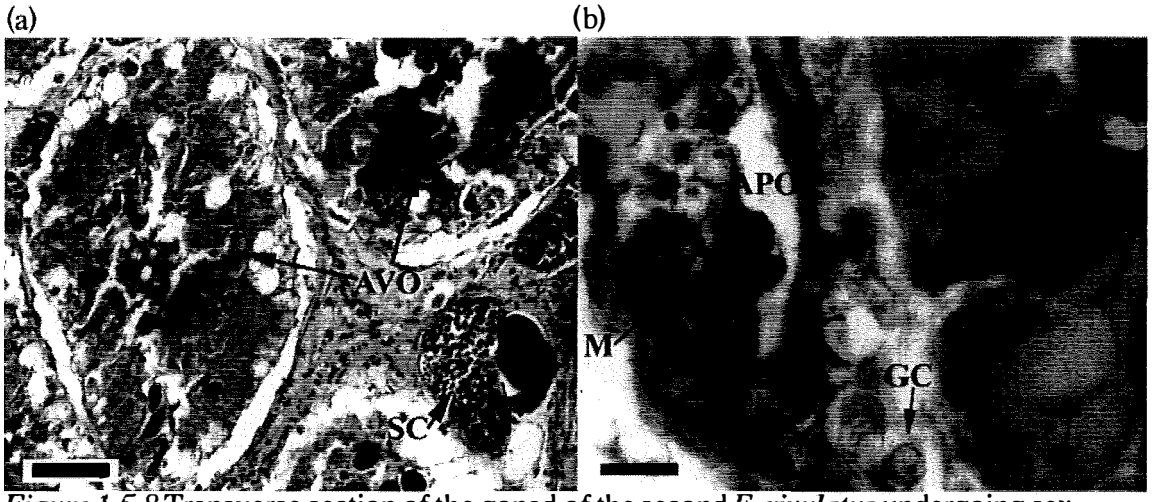


Figure 1.5.8 Transverse section of the gonad of the second *E. rivulatus* undergoing sex-change at the end of Phase 1. (a) Detail showing atretic oocytes and spermatocrypts. Scale bar = 50 μm. (b) Detail of an atretic previtellogenic oocyte lying next to an atretic vitellogenic oocyte. Note the eroded zona radiata and hypertrophied granulosa cells of the latter. Macrophages have invaded the previtellogenic oocyte and phagocytized ooplasm. Scale bar = 14 μm. **APO,** atretic previtellogenic oocyte. **AVO,** atretic vitellogenic oocyte. **GC,** granulosa cell. **M**, macrophage. **SC,** spermatocytes. **ZR,** zona radiata.

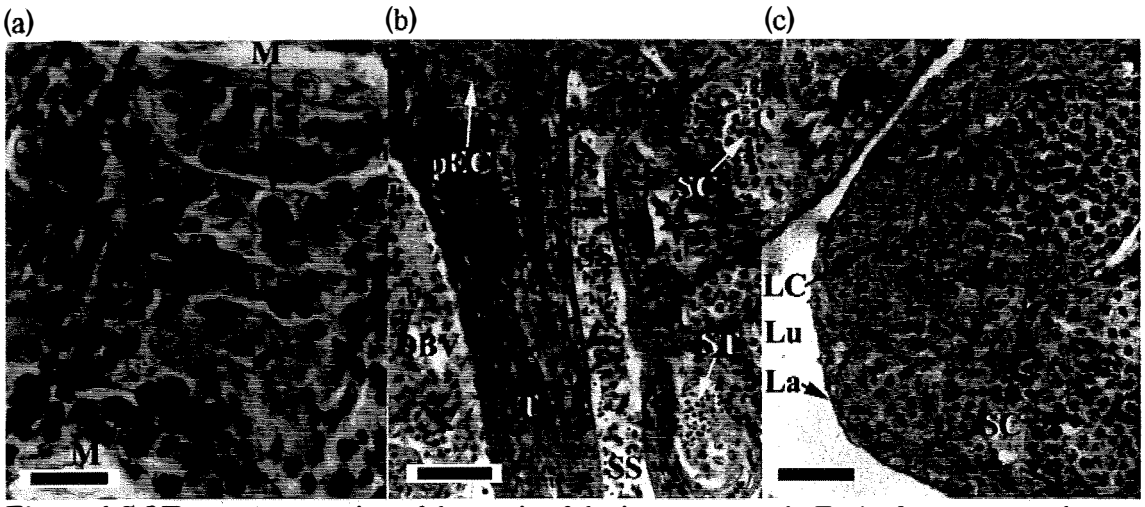


Figure 1.5.9 Transverse section of the testis of the immature male *E. rivulatus* captured within the manipulated area at the end of Phase 1. (a) Detail of somatic tissue showing macrophages containing remnant yolk. Scale bar = 14 μm. (b) Detail of newly developed peripheral sperm sinus. Scale bar = 36 μm. (c) Detail of putative Leydig cells within the distal region of a lamella. Scale bar = 30 μm. **DBV,** dorsal blood vessel. **La,** lamella. **LC,** Leydig cells. **Lu,** lumen. **M,** macrophage. **pEC,** putative endocrine cells. **SC,** spermatocytes. **SS,** sperm sinus. **ST,** spermatids. **T,** tunica.

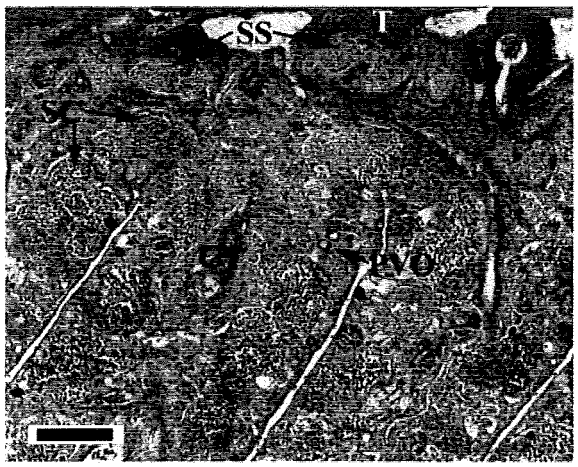


Figure 1.5.10 Transverse section of the testis of a maturing male *E. rivulatus* captured within the manipulated area at the end of Phase 1. Note narrow lamellae filled with connective tissue and developing spermatic tissue. Intralobular and peripheral sperm sinuses are also present and contain spermatids. Scale bar = 100 μm. **CT,** connective tissue. **PVO,** previtellogenic oocyte. **SC,** spermatocytes. **SS,** sperm sinus. **T,** tunica.

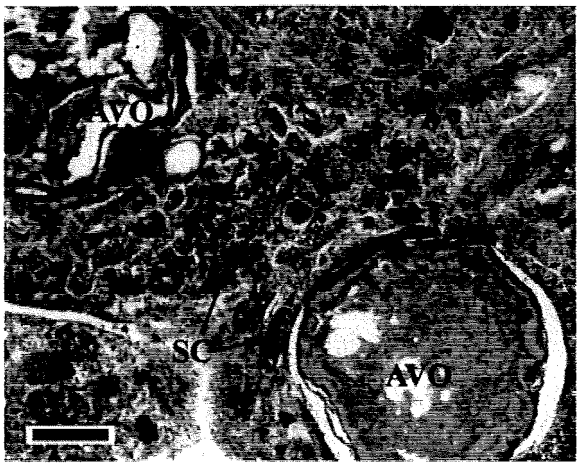


Figure 1.5.11 Transverse section of the testis of a maturing male *E. rivulatus* that had not been cannulated to determine gender prior to the experiment. Shown is detail of incompletely resorbed vitellogenic oocytes amongst the developing spermatic tissue. Scale bar = 100 μm. **AVO,** atretic vitellogenic oocyte. **SC,** spermatocytes. **ST,** spermatids.

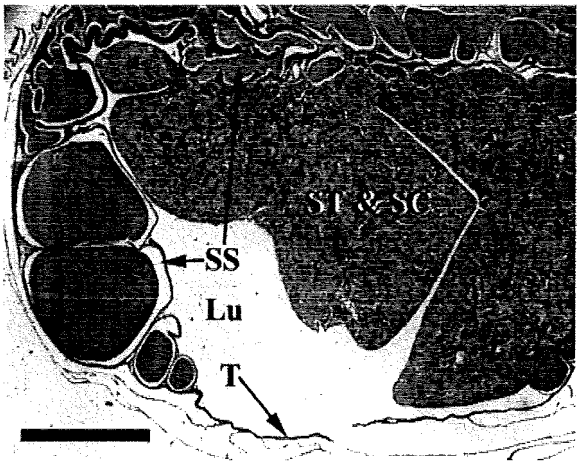


Figure 1.5.12 Transverse section of the testis of a ripe immigrant male *E. rivulatus* removed from the manipulated area at the end of Phase 1. Section taken at the junction of the two testicular lobes and shows the vestigial ovarian lumen, well developed peripheral sperm sinuses and thin tunica. Scale bar = 700 μm. **Lu,** lumen. **SC,** spermatocytes. **SS,** sperm sinus. **ST,** spermatids. **T,** tunica.

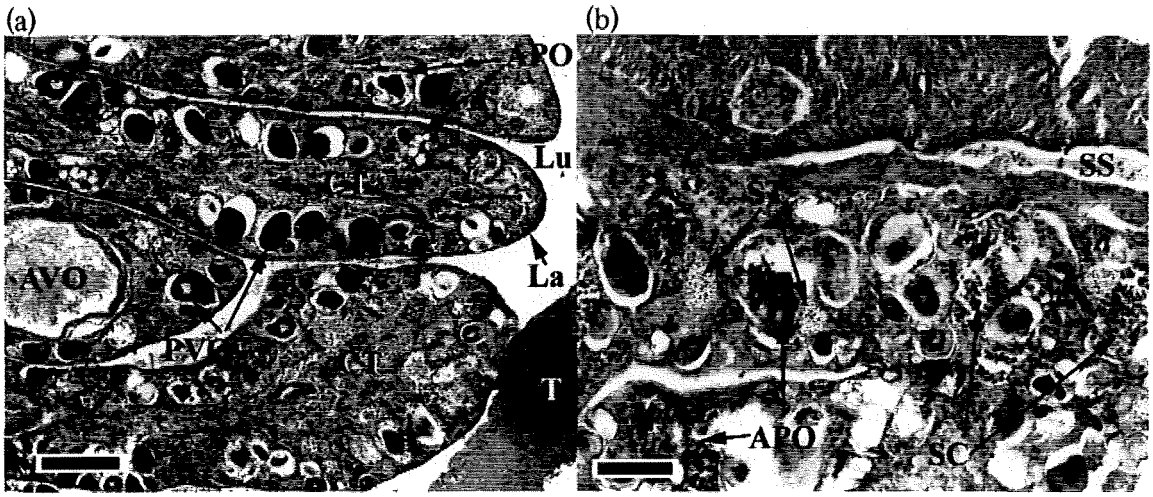


Figure 1.5.13 Transverse section of the gonad of an *E. rivulatus* undergoing sex-change at the end of Phase 2. (a) General structure of the gonad showing the long narrow lamellae and thick tunica. The lamellae are filled with connective tissue, spermatocrypts and previtellogenic oocytes. An incompletely resorbed vitellogenic oocyte also remains. Scale bar = 100 μm. (b) Detail showing developing crypts of spermatic tissue, atretic previtellogenic oocytes and the newly formed peripheral sperm sinus. Scale bar = 50 μm. **APO,** atretic previtellogenic oocytes. **AVO,** atretic vitellogenic oocytes. **CT,** connective tissue. **La,** lamella. **Lu,** lumen. **PVO,** previtellogenic oocytes. **SC,** spermatocytes. **SS,** sperm sinus. **ST**, spermatids. **T,** tunica.

2 Biology Of The Bar-Cheeked Coral Trout, *Plectropomus maculatus* Within The Pilbara Trawl Fishery

2.1 Materials And Methods

Study Sites

Most of the samples used in the analysis of *P. maculatus* biology were obtained between April 1995 and September 1996 from Kraus Fishing Company, that operates fish trawlers within the Pilbara trawl fishery. This fishery extends from north of latitude 21.6^0 S out to the 200 m depth contour, and between longitudes 114. 2^0 and 120.0^0 E, with most of the catch taken from depths between 50 and 100 m. The trawlers operating within this fishery are usually based at Point Samson, to which they return after each 4 - 6 day fishing trip. Whilst at sea the catch of fish is sorted after each trawl, placed in a cold brine solution to cool, and then stored whole in tubs within a refrigerated hold. At the end of each trip the catch is sent to Perth for sale. However, *P. maculatus* that were to be used for biological analysis were frozen and stored at Point Samson until sufficient quantity had been captured. These coral trout were then transported to Perth where they remained frozen in storage until collection. Approximately a quarter of these fish were haphazardly chosen so that a representative sample of about 30 fish per month were obtained for analysis, using the methodologies described below.

Many of the *P. maculatus* obtained from Kraus Fishing Company measured between 300 mm and the legal minimum size of 450 mm total length (TL). In order to obtain samples of larger fish, the filleted frames of 51 legal-sized *P. maculatus* were purchased from a fish processing company (A.J. Langfords Pty. Ltd.). These fish were also captured by trawlers operating within the Pilbara trawl fishery, and therefore had been kept chilled and frozen for about a week prior to collection for sampling. Twenty-three smaller *P. maculatus* between 104 and 331 mm TL, and seven larger individuals between 357 and 539 mm TL were also captured by speargun from coastal sites within the Dampier Archipelago (Boiler's Rock, SW Angel Island, and Conzinc Island), which is in the vicinity of the Pilbara trawl fishery. These fish were captured at depths between 4 and 13 m and processed within hours of capture, as detailed below.

Processing Of Samples

Total, standard and fork lengths of all coral trout were obtained (mm; following Fisheries WA practice, both lobes of the tail were squeezed together to provide an extended measurement of total length). The head length (tip of snout to the most posterior tip of the gill operculum) was also obtained from some fish, so that a regression allowing estimation of total length from head length could be determined for management purposes. Whole and cleaned body weights (gms) were obtained from all fish except the filleted frames. The gonads of each fish were removed, weighed to 0.001 gm and fixed in 10% formalin solution. The otoliths were also removed, cleaned, and stored dry in empty gelatin capsules within paper envelopes.

Processing And Analysis Of Gonads

Analyses of histologically prepared sections of *P. maculatus* gonads were completed by a contracted technician, using the methodologies described in the study of *E. rivulatus* reproduction (Chapter 1.1). Because of the poor quality of the *P. maculatus* gonad sections (due to freezing and decomposure), many of them were also re-analysed by the main research scientist (MM). Length measurements used in description of *P. maculatus* biology are of total length (TL).

Processing And Analysis Of Otoliths

Methodologies used in the processing and analyses of the *P. maculatus* otolith samples were similar to those used for *E. rivulatus* (Chapter 1.3), with the following exceptions:

(1) The two separate counts of annuli in the otolith sections were both made by an experienced otolith reader from Fisheries WA.
(2) The periodicity of opaque zone formation within the otolith was determined by analysis of the marginal increment only. Measurements for marginal increment analysis were made along an axis perpendicular to the outer annuli and as close as possible to the sulcus. The marginal increment ratio was calculated as the distance between the last opaque zone and the otolith edge divided by the distance between the outer two opaque zones. The mean marginal increment ratio was calculated for each month to determine how often this ratio peaked over the year (and therefore how many annuli were formed). Only fish two years and older with an otolith readability of 3 or greater (on a scale of 1 (unreadable) to 5 (perfectly readable); Chapter 1.3) were used in analysis of the marginal increment. The frequency of otoliths with either an opaque or a translucent margin were determined for each month to provide evidence of the periodicity of opaque zone formation.
(3) Counts of annuli within each otolith were made on two occasions without reference to fish size or date of capture. The second count was taken as correct for otoliths with a readability category of 3 to 5, since by then the reader was familiar with the internal structure of *P. maculatus* otoliths. Counts of annuli within otoliths with a readability of 2 were only used in analyses if the two counts did not differ.The amount of precision between the two counts was determined using the Index of Average Percent Error (Beamish & Fournier 1981).
(4) The absolute age of each fish was estimated from the count of annuli, marginal increment ratio, and date of capture.The time period during which the opaque zone was most likely to form in *P. maculatus* was October to April (see Results). Thus, if the otolith of a fish captured between these months had a marginal increment ratio of 50% or more and formation of the opaque zone appeared imminent, then the absolute age was equal to the number of annuli plus one. However, if a fish captured between these months had otoliths with an opaque margin or a marginal increment ratio of less than 50%, then the absolute age was the same as the number of annuli. Similarly, the age of fish captured outside these months was the same as the number of annuli.

The sagittal otoliths were used in all analyses. Linear regressions of left and right otolith weights (slope = 1.0037, r^2 = 0.9952, n = 81) and left and right otolith lengths (slope = 1.0173, r^2 = 0.9846, n = 81) indicated that there was little difference in each otolith pair. The right otolith was therefore sectioned where possible.

2.2 Results

2.2.1 Reproductive Biology

Detailed analysis of the histological samples was not possible, because the onboard storage and freezing of the fish resulted in considerable deterioration and damage to the somatic and germ tissue within the gonads. It was not possible to identify reliably atretic oocytes, migratory nucleus stage oocytes or post-ovulatory follicles. It was also difficult to confim reliably the presence of sperm tissue within the female ovary. Consequently, the identification of individuals in the process of changing sex was difficult, as was distinguishing between and immature and resting females, and resting and recently spawned females.

Lengths And Sex Ratios

Females dominated the samples, comprising 86% (n = 526) of the overall tally of fish. Most of these females were within the size classes 325 - 374 mm TL (32% of all females), 375 – 424 mm TL (39%), and 425 – 474 mm TL (16%). Seven transitional fish were also identified, comprising 1.1% of the overall sample (Table 2.1). Six of these transitional fish were under the legal size limit of 450 mm TL (410 to 440 mm TL) and one was a legal-sized fish obtained from the processing company. The sex ratio of fish within the undersized samples obtained from Kraus was 6.9 females to every male (with transitional fish included as male). The samples obtained from the Dampier Archipelago consisted entirely of females (n = 30), whilst the market sample of legal sized fish included 19 males (37%; sex ratio of 1.7 females to every male). Overall sex ratios for fish captured by the trawlers was 5.8 females per male (Kraus and processing company samples combined).

Mature females within the samples ranged from 289 to 576 mm TL (Figure 2.1). The only immature females that could be reliably identified were in the samples obtained from the Dampier Archipelago (n = 9), ranging in length from 101 to 282 mm TL. Only three females captured by the trawlers during the peak of the reproductive season (August to October) were not reproductive and might therefore have been immature. These fish measured 329, 401 and 421 mm in total length. An estimate of the size at 50% sexual maturity for female *P. maculatus* was therefore not possible. Male *P. maculatus* ranged in size from 347 to 710 mm TL. Comparison of the lengths of mature females and males indicated that males were significantly larger than females (Mann-Whitney U test, $U_{0.05(2)511,79} = 8294.5$, $P = 0.000$). The range of overlap between the total lengths of male and female *P. maculatus* was 31% of the maximum total length (Table 2.2).

There were no appreciable differences in the regressions of total and standard lengths, total and fork lengths, and total and head lengths of male and female *P. maculatus* (Figure 2.2). The relationships between clean weight and total length of males and females were also not significantly different ($P_{\text{intercept,n=554}} = 0.3566$, $P_{\text{intercept,n=554}} = 0.3440$). Length and weight relationships for *P. maculatus* are, therefore:

Pooled:
Standard length (mm) = -9.435 + (total length x 0.831), n = 605, $r^2 = 0.992$
Fork length (mm) = 1.034 + (total length x 0.946), n = 605, $r^2 = 0.998$
Total length (mm) = 21.516 + (head length x 3.417), n = 190, $r^2 = 0.9882$ (for fish larger than 290 mm TL)
Clean weight (gms) = 0.000015 x total length$^{2.943}$, n = 554, r^2 (for the ln transformed data) = 0.981.

Reproductive Biology

The gonads of male and female *P. maculatus* are bi-lobed, elongate organs, joined posteriorly to form a short gonoduct that led to the urogenital pore. In both sexes the gonad was suspended from the rear of the body cavity by mesenteries and bound by a muscular wall and tunica. The testes of males captured during the non-reproductive period weighed a maximum of 0.86% of clean body weight (mean = 0.36% ± 0.02 SE, n = 38), compared to a maximum of 1.4% of clean body weight and a mean of 0.47% (± 0.05 SE, n = 21) during the period of peak reproduction (August to October). The ovaries of resting (F2) females were similar in weight to male testis, with a mean weight that was 0.48% (± 0.12 SE, n = 191) of clean body weight (maximum of 1.1%). In comparison, the ovaries of ripe (F3) females weighed up to 7.4% of clean body weight (mean 1.5% ± 0.6 SE, n = 221). Internally, the ovary consisted of numerous lamellae attached to the dorsal and lateral gonadal walls. Oocytes were produced and ripened within the lamellae and shed into the lumen when ovulated.

Within the testis of male *P. maculatus* the sperm tissue is of the acinus type (Smith 1965), with sperm forming in small crypts, and individual sperm within a particular crypt at the same stage of development. Sperm sinuses form within the walls of the testis to enable the transfer of sperm. A vestigial lumen was also present.

Parasitic nematodes were found within the tunica or gametic tissue of all but a few of the gonads of the trawl caught *P. maculatus.* These sometimes occupied a considerable portion of the gonad, and males appeared particularly prone to infestation. The gonad of only one *P. maculatus* captured from the Dampier Archipelago contained these parasites.

Protogynous Hermaphroditism

Seven transitional fish were identified within the samples, ranging from 410 to 641 mm TL (mean 457 mm TL ± 31.02 SE; Table 2.1). The median value of the overlap between male and female body lengths can be used to estimate the size at which sex-change occurs in the population (Shapiro 1987). For the trawl caught coral trout, the median size at sex-change estimated in this way was 404 mm TL (Table 2.2).

Reproductive Season

Analysis of the histological samples indicated that *P. maculatus* have a long reproductive season, spanning eight months from July through to February (Figure 2.3). During this time spawning peaked in September and October, followed by a decline in reproductive activity through November and December as the proportion of females with spent and inactive ovaries increased. In January and February there was a smaller peak in spawning activity among females, after which the proportion of reproductive females decreased. By April through to June the majority of females were non-reproductive.

Annual changes in the gonadosomatic index (GSI) reflect the spawning pattern described by the histological data (Figure 2.4). The GSI was not homogeneous between months (Kruskal-Wallis ANOVA; $H_{9,df=279}$, $P = 0.0000$), beginning to increase during July and peaking in September and October. A dip in the GSI occurred during November and December, followed by a second peak in reproductive activity during January. After January the GSI dropped steadily, reaching its lowest levels between

April and June, although samples taken in 1995 indicate that base GSI levels were reached in March, a month earlier than in 1996. Note that only females above 360 mm TL were used in the analysis of the GSI, since there was little relationship between body size and gonad weight in fish of this size and greater (r^2 of the regression = 0.1907), thereby minimising the influence of body size on the GSI.

2.2.2 Age And Growth

January 1 was chosen as the nominal birthdate of *P. maculatus*, since this month is near the end of the reproductive season and midway through the period when formation of the opaque band occurs in the otolith (see below). Thus all ages used in the description of *P. maculatus* age and growth refer to one-year age-classes that begin on January 1. Age classes begin at 0, such that fish referred to as age 1 are in their second year of life.

Microband And Annuli Structure

Microstructure of the transverse sections of *P. maculatus* otoliths was similar to that of *E. rivulatus* (Chapter 1.3). Viewed under compound microscope, a regular pattern of growth increments (microbands) was typical in most sections. These varied in clarity and it was usually not possible to get a continuous count of microbands from the core to the margin of the otolith. In those sections where a core was present, the first few microbands were difficult to resolve using the compound microscope. It was also difficult to count microbands at the margin of the otolith. A possible settlement mark was identifiable in some sections as a band or group of several bands with distinctive optical characteristics.

The annuli were variable in structure. Variation within a particular otolith occurred through inconstant deposition of otolith material along the margin. The area of most uniform deposition occurred between the sulcus and about 2/3 the distance towards the ventral tip. Variation in the structure of the annuli also occurred between the otoliths of different fish, with some otoliths tending to have relatively thin opaque and translucent bands whilst in other otoliths these bands were much wider.

Readability Of Annuli

Annuli within *P. maculatus* otoliths were generally not difficult to interpret in sections that were cut through the core and perpendicular to the longitudinal axis. The first band was usually easy to identify. Almost 84 % of the otoliths were read with reasonable to high confidence (categories 3 to 5, Table 2.3). The Index of Average Percent Error for these otoliths was 7.3%, indicating reasonable precision between the two counts. Eleven otoliths (2.7%) were considered impossible to interpret (category 1), mainly because little translucent material had been deposited in the otolith. Thus, whilst a banding pattern was evident in these sections it was impossible to identify reliably the individual annuli.

Validation Of Growth Increments

The periodicity of microband deposition was not determined during this study, and for analysis purpose it was assumed that they were produced on a daily basis.

Evaluation of annuli formation by marginal increment analysis showed that the outer translucent band reached a maximum mean width in June (Figure 2.5). Wide error bars around this mean indicate relatively large variation in the width of the translucent

margin of individual otoliths. In contrast to the June peak in the marginal increment ratio, the period in which most otoliths had an opaque margin extended over several months from October to April (Figure 2.5). These data confirm that only one annulus (one translucent and one opaque band) forms each year. In most otoliths the translucent band forms during a short period in late autumn and early winter, and the opaque band forms over a prolonged period through spring, summer and early autumn. The data also show that there are a small but significant number of otoliths in which formation of the annuli does not follow this general pattern. For instance, approximately 20% of otoliths had an opaque margin when the majority were forming translucent bands (Figure 2.5).

Age And Growth

Assuming daily production of microbands in *P. maculatus* otoliths and their initiation on the day of hatching, the time periods between hatching and settlement of three immature females from the Dampier Archipelago (110, 135, and 185 mm TL) were 28, 37, and 25 days, respectively (mean = 30). Total ages of these fish when captured were 168, 188, and 163 days (respectively), indicating they hatched between the 25th March and 20th April, at the end of the 1996 spawning season. It was not possible to obtain reliable age estimates of the other juvenile fish from microband counts.

The ages of mature females ranged from 1 to 8 years whilst males ranged from 2 to 10 years (Table 2.4). The most abundant female age groups were 2, 3, 4, and 5 year olds, which comprised 32, 27, 22, and 8.7% of the total female sample, respectively. The mean lengths of these dominant age groups (2 to 4 years) fall within the two most dominant length classes described previously (Table 2.5). Mean ages of mature female and male *P. maculatus* were significantly different (t-test for independent samples on natural log transformed data = -6.463, P = 0.0000, n (females) = 289, n (males) = 50; Figure 2.6). Three transitional fish that could be reliably aged were all 4 years old. Total lengths of these fish were 410, 420, and 439 mm.The only legal sized transitional fish (obtained from the processors) was probably 7 years of age, although readability of this otolith was poor. This individual was 641 mm in total length.

Eighteen *P. maculatus* captured from the Dampier Archipelago could be aged. Nine of these were immature females that were 0 to 1 years old (including the three with microband counts; Table 2.4). These fish ranged in total length from 104 to 282 mm (mean 207.3 mm ± 25.0 SE). The other nine *P. maculatus* were mature females between 1 and 2 years old, and 301 to 385 mm total length (mean 340.0 mm ± 10.1 SE). The ages of *P. maculatus* within the undersized samples obtained from Kraus ranged from 1 to 8 years (females), and 2 to 7 years (males). Legal sized coral trout obtained from the fish processors were 3 to 10 years old. There was little overlap in the age range of male and female fish in this sample compared to the undersized samples, with females ranging from 3 to 6 years and males from 5 to 10 years. Mean age of all trawler caught females (Kraus and processor samples combined) was 3.2 years (± 0.07 SE) and of all trawler caught males was 4.7 years (± 0.26 SE). The median value of ages within the overlap of male and female fish was 3 years (mean = 3.4 years ± 0.07 SE), and the range of this overlap was 60 % of the maximum age. The lack of immature females within the samples prohibited a detailed analysis of the average age at which sexual maturity is reached. Nevertheless, the data indicate that all ageable 2 year old females were mature and that most fish attain sexual maturity during their second year (age 1). Two females captured within the peak reproductive period were possibly immature because their ovaries were not reproductively active (see above). These fish were both 3 years old.

Growth Model From Length-At-Age

Growth of *P. maculatus* was most rapid during the first year (age 0), but slowed markedly during the second year (Figure 2.7). Between the ages of 1 and 4 the lengths at ages of fish within the samples varied little despite the large number of fish within these age groups. This is probably due in part to the lack of fish above the legal minimum length. A linear regression of males and females within these ages (1 to 4) explained 69% of the data (total length (mm) = 300.3 + (age (years) x 30.5); n = 272). After age 4 the variance in the lengths of fish within each age group increased (Figure 2.7, Table 2.5). The data suggests that males may have a more variable and higher growth rate than females in the older age groups, although there are too few data for fish older than 6 years of age to varify this.

The asymptotic mean length (L_∞) estimated by the von Bertalanffy growth function was 767.3 mm TL. The growth coefficient (K) was 0.113, and the theoretical age of fish at zero length (t_0) was –3.4 (Table 2.6). The von Bertalanffy growth curve provided good description of the lengths at age of fish within age groups 1 to 5 (Figure 2.7). However, the overall fit of the data is fairly poor, with only 59% of the data explained by the model. In particular, the model fails to explain the growth trajectories of the few fish that were less than 1 years old. By using the inverse of the growth equation, the age that corresponds to the current minimum legal length for *P. maculatus* (450 mm TL) was estimated as 4.4 years. Similarly, the age that corresponds to the median value of the overlap in male and female total lengths (404 mm TL) is 3.2 years. This agrees well with the median value of the overlap in male and female ages (3 years; see above).

2.3 Discussion

Many of the broader issues pertaining to reproduction and growth in the epinepheline serranids are discussed in Chapters 1.1-3 and 1.5. These are relevant to the following discussion of *P. maculatus* biology.

2.3.1 Reproductive Biology Of *P. maculatus*

Protogynous Hermaphroditism

The identification of transitional *P. maculatus* was made with some reservations due to the poor quality of the samples. Nevertheless, the larger mean size and age of males, the female biased sex ratio, and the presence of a vestigial lumen, peripheral sperm ducts, and previtellogenic oocytes within the male testis confirm that *P. maculatus* is a protogynous species (Sadovy & Shapiro 1987). Ferreira (1993, 1995) similarly concluded that *P. maculatus* and the congener *P. leopardus* also had a protogynous life history on the Great Barrier Reef. The wide range of lengths and ages at which sex-change occurred in the Pilbara *P. maculatus* is typical of the serranids (Shapiro 1987), and suggests that females may change sex in response to environmental cues that vary in time and space. This is indicated by differences in the estimated median size at sex-change of the trawl caught *P. maculatus* (404 mm TL), and those inhabiting shallow, inshore waters of the Great Barrier Reef, which had an estimated size at sex-change of 360 mm standard length (Ferreira 1993; this is approximately 445 mm TL according to the length relationships for Pilbara fish).

Annual Reproductive Cycle

P. maculatus inhabiting the Pilbara trawl grounds have a long reproductive season, with at least 50% of females ripe for eight months of the year (July through to February). In comparison, most other serranid species are reproductively active over 1 to 5 months (Thresher 1984, Shapiro 1987), and the congener, *P. leopardus*, may spawn within a three month period (September to October) on the Great Barrier Reef (Ferriera 1995). Data on the reproductive pattern of *P. maculatus* on the Great Barrier Reef are incomplete. Nevertheless, it appears that reproduction by these *P. maculatus* occurs at a similar time of year to Pilbara *P. maculatus*, as spawning fish were obtained in September and November, but not in March (Ferreira 1993).

The relatively long reproductive season of Pilbara *P. maculatus* is reduced somewhat by the apparent drop in reproductive activity during November and December. This dip may be due to a physiological slow down in gamete production over the period of peak water temperatures (which peaked further south at Ningaloo Reef during December and January (Chapter 1.1)), since tropical serranids may spawn at water temperatures below the annual maximum (Thresher 1984). It may also be due to differences in the reproductive season of *P. maculatus* between the two years in which samples were obtained (as suggested by the gonadosomatic index data). Further, it may be a result of spatial differences in the timing of reproductive activity, since *P. maculatus* within the samples are likely to have been captured over a wide area by the trawlers.

Population Characteristics

Sex ratios of serranid species typically differ from unity, with more females than males present in the samples (Shapiro 1987). This is characteristic of a polygynous mating system in which large males gain extraordinarily high reproductive success by monopolising mating access to a number of females (Emlin & Oring 1977), as shown for *E. rivulatus* (Chapter 1.2). The spawning sex ratio may therefore indicate average male spawning success (Shapiro 1987), which may not vary significantly between different populations of a particular species if the mating system remains consistent.

Nevertheless Ferreira (1993) observed spawning sex ratios of 2.6 females to each male for *P. maculatus* on the Great Barrier Reef, compared to the ratio of 5.8 females to each male in the present study. Because the majority of the samples obtained during the present study were undersized it is likely that males were not fully represented. Further, the inclusion of fish from reproductive and non-reproductive times of the year may also create a bias, since non-spawning populations of serranid species which aggregate to spawn can have biased sex ratios. This was the case for a population of *Epinephelus guttatus*, which proved to be all female when sampled (Shapiro *et al.* 1993), and may also be true for populations of *P. maculatus* that inhabit shallow, near-shore areas of the Dampier Archipelago. It is also possible that the samples analysed by Ferreira (1993) were not representative because the males were more easily captured or targeted, or some other reason affected catchability of the females. Biases in sex ratio data due to different sampling tools, different sampling locations, and different sampling times are usually unpredictable, and the estimates are likely to be inaccurate to some indeterminate degree. Thus, when used for management purposes such as the determination of spawning stock biomass via the daily egg production method, the resulting estimates may also be biased. The most reliable estimates of spawning sex ratios in many serranids may, therefore, be obtained from direct observations of

spawning fish of known sex. Unfortunately, this is also likely to be the most expensive and logistically difficult method to use.

The samples were dominated by females between 325 and 425 mm total length, and 2 to 4 years of age. This reflects the sampling bias towards fish below the minimum legal size limit of 450 mm total length. The paucity of smaller fish within the trawl caught samples means that they are either not on the trawl ground or are generally uncatchable. Because the trawl nets have 4-inch mesh, the latter may be true to some extent, particularly given the sleek body shape of *P. maculatus*. Being more prone to predation, these smaller fish are also likely to keep near habitat like the exposed limestone crevices that provide shelter for fish on the trawl grounds (J. Jenke, Fisheries WA, *pers. comm.*). As the trawl approaches these fish may dash into the crevice and evade the net. It is also possible that the smaller individuals are rare within habitats on which the trawlers operate, since ontogenetic shifts in habitat are typical of larger serranid species (Sheaves 1995, Ross & Moser 1995, Sluka & Sullivan 1996).

2.3.2. Age And Growth Of *P. maculatus*

The dominance of certain length and age classes is also likely to affect the results obtained from the data. The von Bertalanffy growth curve provides a good fit of the data for these 2 to 4 year old fish which made up 77% of the samples. However, this is not the case for fish of other ages that were poorly represented in the samples. In particular, the growth of small individuals in their first year of life is faster than that predicted by the growth curve. There are also not enough data for the older age groups, and the results of the length at age analysis may be somewhat misleading. For instance, the growth curve is influenced by the two ten year old fish. However, if as suspected, the population mean length of this age group is lower, then the growth curve would probably reach an asymptote sooner than has been predicted (see Figure 2.7). As a result the t_{zero} and L_∞, may be smaller, and K larger, than has been estimated by the von Bertalanffy growth function. Since this is supported by the data for *P. maculatus* and *P. leopardus* on the Great Barrier Reef, which have a smaller t_{zero} and L_∞, and a faster K than the Pilbara fish (Ferreira 1992, 1993), the results of the present study should be used with caution until more data are available on the older age groups.

Nevertheless, the predicted L_∞ is only slightly higher than the maximum recorded size of *P. maculatus* (76 cm total length; Kailola *et al.* 1993). Under the assumption that L_∞ is approximately 5% greater than the maximum length of fish in the samples (Pauly 1984), then the value for L_∞ estimated for Pilbara *P. maculatus* is reasonable. Furthermore, the growth coefficient (K), is similar to that of other epinepheline serranids, and indicates that Pilbara *P. maculatus* are similarly slow growing. They also reach a reasonable age (10 years), which is similar to that of a sample of Great Barrier Reef *P. maculatus* (12 years; Ferreira & Russ 1992). Further sampling of *P. maculatus* in waters off the West Australian coast is likely to push the maximum age of these fish higher also.

Otolith Readability And Band Formation

The structure of the annuli within *P. maculatus* otoliths was generally easier to interpret than they were in the otoliths of *E. rivulatus* (Chapter 1.3). Otoliths of *P. maculatus* captured on the Great Barrier Reef were also most readable between the sulcus and the

ventral tip, and had a high readability similar to that of *P. maculatus* from the Pilbara (Ferreira & Russ 1992).

A small number of otoliths were impossible to read, mainly because they contained little translucent material and it was not possible to distinguish reliably between their annuli. A decrease in the deposition of translucent material onto the otolith may be a result of unusual environmental or physiological conditions. The fact that the deposition of material onto the otoliths of some fish differs from the norm, at least some of the time, is indicated by the presence of otoliths with an opaque margin when most were forming the translucent band.

2.3.3 Management Of *P. maculatus* In Western Australia

Management of exploited protogynous species such as *P. maculatus* is ambiguous, since traditional management techniques do not consider hermaphroditism and the unique social and ecological characteristics that are usually associated with it. Refer to Chapters 1.1 and 1.5 for discussion of the biological, ecological, and management issues relevant to protogynous hermaphroditism and the epinepheline serranids.

The size at which 50% of females attain sexual maturity is often used as the minimum legal length for exploited species, since this should allow half the females to reproduce before entering the fishery. This criteria was also used to set the minimum legal length of coral trout (including *P. maculatus*) on the Great Barrier Reef at 380 mm TL (I. Brown, QLD Dept. Primary Industries, *pers. comm.*). In comparison, the minimum legal length of coral trout in Western Australia is substantially higher (450 mm TL), which in the absence of biological data, was set at approximately half the known maximum length (M. Moran, Fisheries WA, *pers. comm.*). Because of this large difference in minimum lengths, it was thought the present study would show that a reduction in the limit of west coast *P. maculatus* was biologically acceptable, since the discarded fish are essentially a dead, wasted income to the trawler operators.

A size at 50% maturity of female *P. maculatus* could not be determined in the present study, however, as it appears to occur below the minimum length of females within the samples (approximately 300 mm TL). Nevertheless, this is substantially lower than that obtained for *P. maculatus* on the Great Barrier Reef of 300 mm standard length (Ferriera 1993; or 372 mm TL using the length relationships for Pilbara caught fish). This indicates that *P. maculatus* inhabiting the trawl grounds mature at a much smaller size than their east coast counterparts. Because of such, a reduction in the size limit of *P. maculatus* might therefore be justified in Western Australia.

Reductions in size limits should be made with caution though, and there are several points to consider in the case of *P. maculatus* and the data obtained from this study. Firstly, given the substantial differences in biological parameters of trawl caught *P. maculatus* and those inhabiting shallow waters of the Great Barrier Reef, the relevance of the data presented here to other stocks of *P. maculatus* in Western Australia is unclear. More biological information on shallow, nearshore populations of *P. maculatus* that are targetted by line fishers is particularly essential, since these stocks may be more valuable to the wider community than are those on the trawl grounds..

The criteria of 50% female maturity is also not likely to provide an acceptable minimum length in protogynous species such as the coral trout, since it leaves virtually all the males susceptible to capture. Thus, if sex-change in a protogynous species is *not*

cued by the loss of males through fishing activities, the reproductive output of the population may be seriously diminished due to a lack of males. In this situation, setting the minimum length at the average size at which sex-change occurs will theoretically allow some females to reach the size at which they can change sex and replenish the male population.

On the other hand, if sex-change in females *is* cued by removal of males, then the operational sex ratio may be maintained no matter what the size limit (although small females may be physiologically unable to change sex; Chapter 1.5). However, in this case the size at sex-change may theoretically equilibrate with the size limit anyway, since the loss of fish above this size will cue those below it to change sex. Preferably, therefore, the size limit should be set at the size at which sex-change occurs in unfished populations in order to preserve the biological characteristics of the exploited stock. A final point to make is that females may not be physiologically capable of changing sex at the size at which they are just reaching sexual maturity. Thus, even in those species with socially controlled sex-change, there may not be enough females changing sex to replace captured males with a size limit set at the mean size of maturity.

Based on this argument, the legal size limit for *P. maculatus* within the trawl fishery could be decreased to 410 mm TL, which is the approximate size at sex-change for *P. maculatus*. This may be a reasonable compromise between the uncertainties listed above and keeping more of the fish that are currently discarded. Consideration could also be given to granting the trawlers special licence to keep the undersized *P. maculatus*. However, this opens a channel for operators to sell undersized fish captured by other fishing methods. This creates enforcement problems. Further, fishers utilising other methods may consider the situation unfair, and extra pressure may be placed on stocks if the trawlers can target populations of undersized trout. A decrease in size limit in accordance with the estimated size at sex-change is the only plausible recommendation that can therefore be made, although emphasis is still placed on getting more data for the inshore stocks, and for the young and older age classes.

Table 2.1 Summary of the *P. maculatus* samples collected for reproductive analysis. The samples obtained from Kraus Fishing Company (Kraus) and from the fish processors were all captured within the Pilbara trawl fishery off Pt. Samson. Dampier fish are those captured from the Dampier Archipelago. F, female. T, transitional. M, Male. TL, total length.

	n			minimum total length			maximum total length			Mean (± SE)		
	F	T	M	F	T	M	F	T	M	F	T	M
Kraus	465	6	60	289	410	347	479	440	477	385 (1.8)	426 (5.1)	424 (3.6)
Processors	31	1	19	415	641	501	576	641	742	505 (8.1)	641	631(16.0)
Dampier	30	0	0	104	-	-	539	-	-	289(18.0)	-	-

Table 2.2 Details of the overlap in the lengths of male and female *P. maculatus* caught within the Pilbara trawl fishery. All measurements are of total length.

Minimum male – maximum female lengths (mm)	347 – 576
Range of the overlap (mm)	229
Extent of the overlap*	30.86%
Number of fish with lengths within the overlap range	474
Median value of the overlap (mm)	404
Mean value of the overlap (mm)	409.4
SE	1.944

* = (Range of the overlap/maximum length) x 100

Table 2.3 The number of *P. maculatus* otoliths in each readability category. Category 1 = unreadable, categories 2 to 5 = readable with increasing degree of confidence. Percent readable is the percentage of otoliths used for age analysis, and includes all otoliths with readability of 3 to 5, plus otoliths with readability of 2 if the two annuli counts were the same. Percent agreement is the percentage of otoliths with readability 3 to 5 in which both counts were the same. Percent measurable is the percentage of otoliths with readability of 3 to 5 that were used for marginal increment analysis. IAPE is the Index of Average Percent Error.

Readability category					n	Percent readable	Percent agreement	Percent measurable	IAPE (%)
1	2	3	4	5					
11	55	225	114	2	407	97.2	67	83.8	7.3

Table 2.4. Summary of *P. maculatus* age data. Samples obtained from Kraus Fishing Company (Kraus) and from the fish processors were all captured within the Pilbara trawl fishery off Pt. Samson. Dampier fish are those captured from the Dampier Archipelago. Ages are in years. All otoliths with readabilities of 3 to 5, and those with a readability of 2 in which both counts were the same, were used in this analysis.

	Immature Female		Mature Female			Transitional		Male		
Location	n	Age	n	Range	Mean (SE)	n	Age	n	Range	Mean (SE)
Kraus	2*	3, 3	256	1-8	3.1 (0.07)	3	all 4	35	2-7	3.8 (0.20)
Processors			24	3-6	4.8 (0.19)	1	7**	15	5-10	6.7 (0.41)
Dampier	9	0-1	9	1-2	1.8 (0.14)					

* Maturity status of these females was not certain (see Section on Length And Sex Ratios).
** Confidence in this reading was poor (readability category 1).

Table 2.5. Mean lengths (and standard errors) of *P. maculatus* within each age class. Measurements are of total length.

	Females			Males		
age (years)	n	mean length (mm)	SE	n	mean length (mm)	SE
1	8	330.0	6.557			
2	93	358.7	2.305	4	369.8	8.509
3	83	397.3	3.164	11	403.1	3.308
4	66	416.7	3.691	14	427.5	6.148
5	27	456.0	11.701	9	471.1	25.980
6	12	473.7	22.129	8	592.3	36.432
7				4	596.8	62.037

Table 2.6. Parameters of the von Bertalanffy growth function for *P. maculatus* captured within the Pilbara trawl fishery and from the Dampier Archipelago. Also shown are the coefficient of determination for the fitted growth curve (r^2), and sample size (n). ASE; asymptotic standard error. Data used in analysis were age (years) and total length (mm).

L_∞ (ASE)	K (ASE)	t_0 (ASE)	r^2	n
767.3 (126.6)	0.113 (0.041)	-3.371 (0.800)	0.5872	352

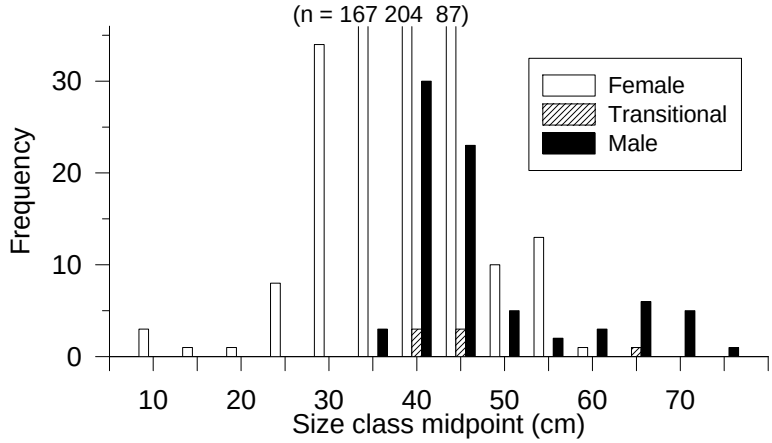


Figure 2.1. Size frequency distribution of *P. maculatus* captured by trawlers within the Pilbara Trawl Fishery, and from the Dampier Archipelago. Size class intervals are 5 cm; measurements are of total length.

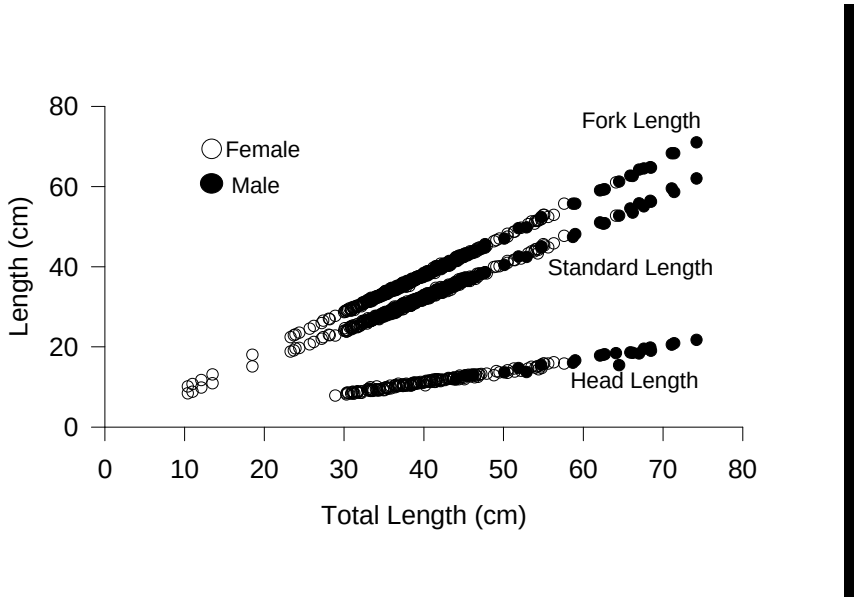


Figure 2.2. Relationships between total length and other body measurements of *P. maculatus*.

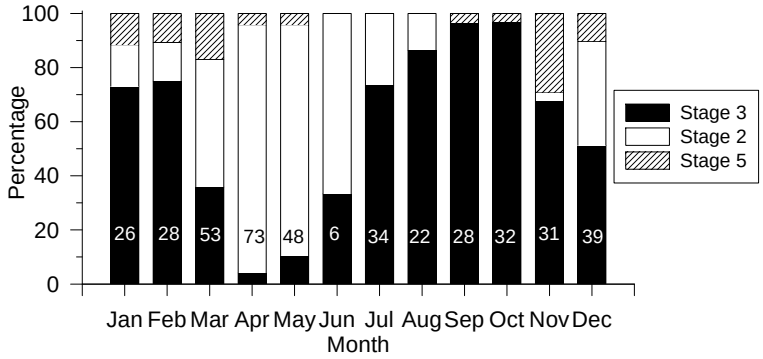


Figure 2.3 Reproductive stages of female *P. maculatus* captured by trawlers within the Pilbara Trawl Fishery. Sample sizes for each month are shown within the bars, and include pooled data obtained during 1995 and 1996. Stage 2 = resting, Stage 3 = ripe, Stage 5 = spent.

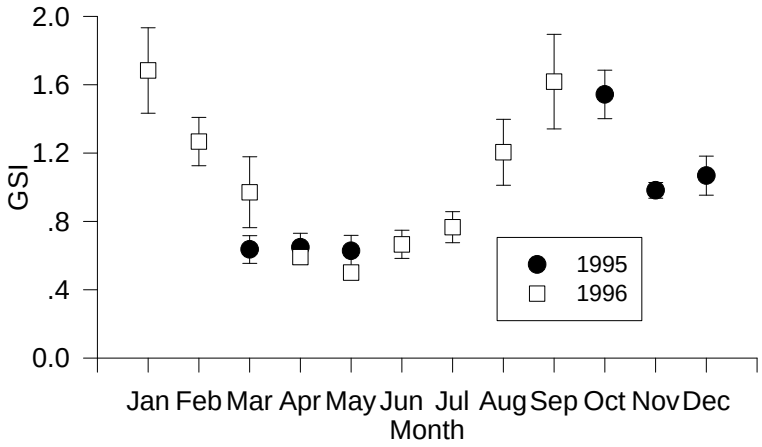


Figure 2.4 Mean gonadosomatic indices (GSI; ± SE) of female *P. maculatus* captured by trawlers within the Pilbara Trawl Fishery. Fish used in the analysis were ≥ 360 mm in total length.

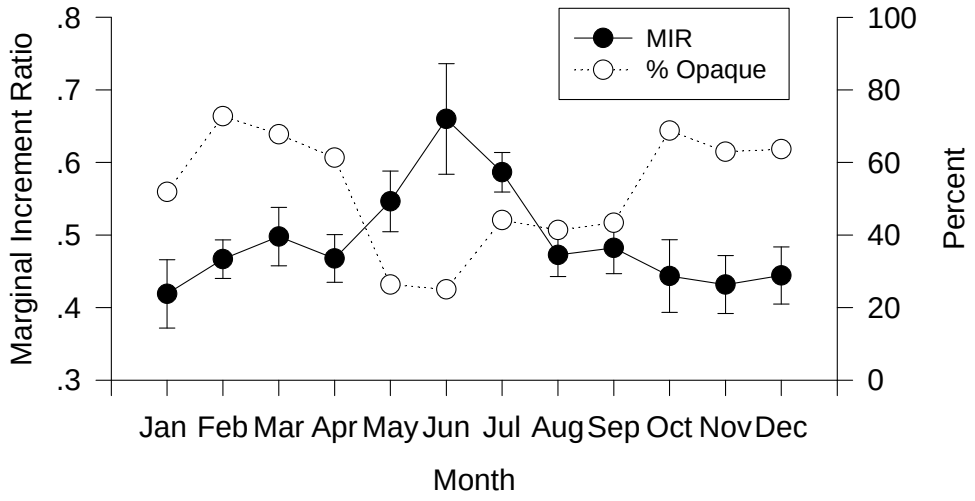


Figure 2.5 Seasonal changes in mean marginal increment ratios (MIR; ± SE) and the percentage of otolith Sections with an opaque margin (% Opaque), for *P. maculatus* captured within the Pilbara trawl fishery.

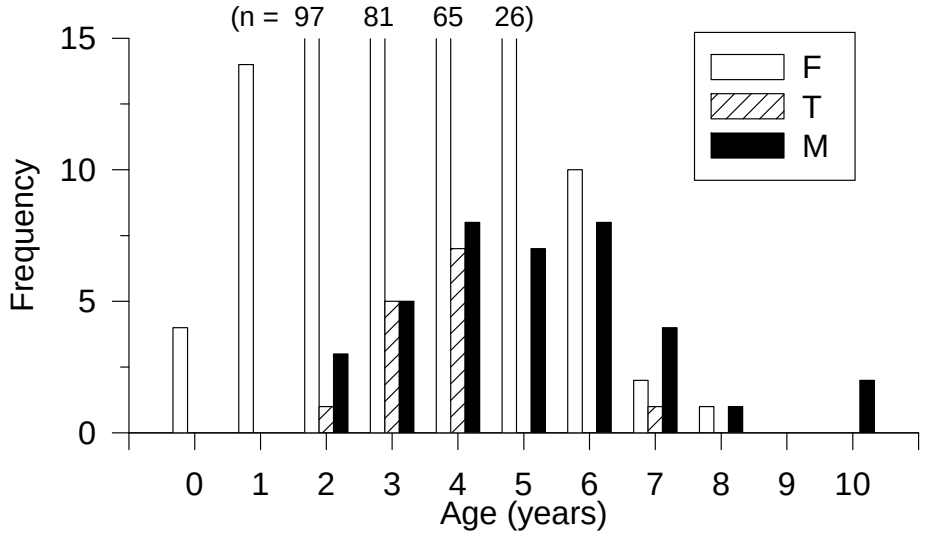


Figure 2.6 Age frequency distribution of *P. maculatus* captured by trawlers within the Pilbara Trawl Fishery, and from the Dampier Archipelago. F; female. T; transitional. M; male.0

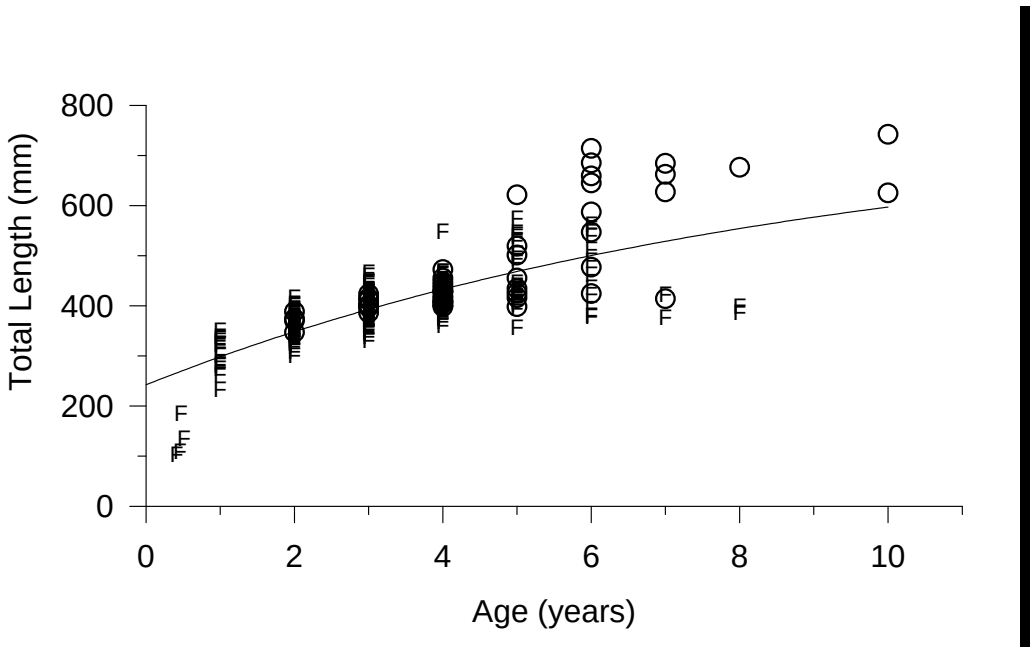


Figure 2.7 Von Bertalanffy growth curve fitted to length-at-age data for *P. maculatus* captured by trawlers within the Pilbara Trawl Fishery and from the Dampier Archipelago. F; female. O; male.

Conclusions

Chinaman cod (*Epinephelus rivulatus*) on Ningaloo Reef, and bar-cheeked coral trout (*Plectropomus maculatus*) in waters adjacent to Point Samson are protogynous hermaphrodites. Sex-change by female *E. rivulatus* is controlled by social cues such as the aggressive dominance of males, and a ripe female can become a male within three weeks.

Sex-ratios of *E. rivulatus* and *P. maculatus* were 5.5 and 5.8 mature females to each male, respectively. Mean ages and lengths of males were significantly greater than those of females in both species. Von Bertalanffy growth parameters (data for separate sampling sites pooled) for *E. rivulatus* and *P. maculatus* were, respectively, K = 0.416 and 0.113, t_0 = -0.697 and -3.371, L_∞ = 321.7 and 767.3. The von Bertalanffy growth curves explained 86% (*E. rivulatus*) and 59% (*P. maculatus*) of the age at length data.

The size at 50% maturity in female *E. rivulatus* was 194 mm TL. This statistic could not be determined for female *P. maculatus* due to the low number of small fish within the samples. In protogynous species, the size at sex-change is more appropriate than the size at 50% maturity of females in setting minimum legal size limits for management purposes. The median size at sex-change for *E. rivulatus* was 300 mm TL, and for *P. maculatus* 410 mm TL.

Current regulations to control fishing pressure on *P. maculatus* and other *Plectropomus* species such as *P. leopardus* (which together are managed as 'coral trout'), include a bag limit of four fish per person per day, and a size limit of 450 mm TL. These regulations appear to provide good protection for coral trout (M. Moran, Fisheries WA *pers. comm.*), except in the Pilbara trawl fishery where mortality of discarded undersized fish is high.

Within the Pilbara trawl fishery, *P. maculatus* is a small but valuable component of the catch, although many of the *P. maculatus* that are caught in the nets cannot be legally kept because they are undersized. This is a waste since the fish are usually dead when thrown back. A reduction in the present size limit to the approximate size at sex-change (410 mm TL) would make better use of this resource, and should therefore be given consideration. However, *P. maculatus* and *P. leopardus* are a valued target species elsewhere in Western Australia, and uncertainty about the relevance of the present study to other locations dictates that a cautious approach is taken. The following options are proposed regarding the minimum legal size limit for coral trout in Western Australia (specifically for *P. maculatus*, although the same management policies will apply to other *Plectropomus* spp.):

1) Keep the present size limit. This is a cautious approach which may be appropriate given the uncertainties in the data and its relevance to *P. maculatus* inhabiting areas outside the Pilbara trawl grounds. However, this approach does not allow better useage of a wasted resource.

2) Change the size limit of *P. maculatus* captured by trawlers within the Pilbara trawl fishery to the size at sex-change (410 mm TL), whilst maintaining the present size limit in other areas. This would reduce the wastage within the trawl fishery whilst populations in other areas are managed conservatively until more relevant data is available. However, this approach may be deemed unfair by

fishers who target coral trout outside the Pilbara trawl fishery. Further, undersized coral trout captured within other fisheries could be sold through the trawl fishery.

3) Change the state-wide size limit for coral trout to 410 mm TL. This is above the current size limit for coral trout within Queensland waters (380 mm TL) and will allow more of the fish to be kept by the Pilbara fish trawlers. This approach is recommended if analyses of the catch data for coral trout in other fisheries (such as the trap and line fisheries) indicate that a reasonable proportion (e.g. at least 30%) of male fish are smaller than 410 mm TL. Otherwise reproductive overfishing may occur if the cues for sex-change in this species are endogenous.

Unlike *P. maculatus* there is not a minimum legal size limit for *E. rivulatus*, although a similar bag limit of four fish per day per person is applied. *E. rivulatus* appears to have a relatively high productive capacity compared to other serranids, and because sex-change is socially controlled the sex ratio of this species should remain fairly constant if males are removed by fishing activities. This reduces the risk of recruitment overfishing, at least under conditions of low fishing mortality. Nevertheless, *E. rivulatus* is susceptible to localised overfishing because of its sedentary nature and ease of capture. The practices of high-grading and use as bait may also add significantly to the mortality of *E. rivulatus* on Ningaloo Reef. Monitoring of catch rates and fish abundance are needed to assess the effectiveness of current management policies. If required, an appropriate size limit for *E. rivulatus* would be 300 mm TL (the size at sex-change). However, changes to bag and possession limits or increased protection of populations within Marine Reserves may be more effective than size limits in curtailing fishing mortality, since these policies should minimise the unecessary capture and injury of unwanted fish.

Benefits

The research on *E. rivulatus* will benefit the local Exmouth community by providing information that is of interest to tourists and which is needed in the preservation of chinaman cod stocks. The information about protogynous hermaphroditism in *E. rivulatus* is of general ecological importance and may benefit the conservation of all epinepheline serranids. Biological data on *P. maculatus* should allow better management of this species, especially within Western Australian and Northern Territory fisheries where current information is minimal.

Flow Of Benefits

State	%	Fishery(ies)/Other Beneficiaries	%
WA	80	Ningaloo Recreational Fishery	25
QLD	5	Point Samson Trawl Fishery	20
NT	15	WA Coral Trout Fisheries	20
		Serranid Fisheries in General	20
		Exmouth Community	15
TOTAL	**100%**	**TOTAL**	**100%**

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Staff

The research on *E. rivulatus* was submitted as a PhD thesis by Michael Mackie. This thesis was co-supervised by Drs Bob Black (University of Western Australia) and Mike Moran (Fisheries WA). No staff were directly employed for the *E. rivulatus* study, although a number of volunteers assisted in the field work.

The research on *P. maculatus* was completed by Michael Mackie under the supervision of Dr Bob Black. Ms Stephanie Chin was contracted to analyse the histologically prepared gonad sections. Mr Iain Dunk was contracted to assist in the sectioning of *P. maculatus* otoliths and to conduct the analysis of these.

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