The age, growth, reproductive biology and stock assessment of grass emperor, *Lethrinus laticaudis* in Shark Bay, Western Australia

Principal investigator: S. Ayvazian

Co-Investigators: B. Chatfield, D. Gaughan, I. Keay and G. Nowara

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PRINCIPAL INVESTIGATOR: Dr. Suzanne Ayvazian

CONTACT AUTHOR: Dr. Daniel Gaughan

ADDRESS: Fisheries Research Division
Department of Fisheries, WA Marine Research Laboratories
PO Box 20 North Beach, WA, 6920
Telephone: (08) 9246 8442
Fax: (08) 9447 3062
Email: dgaughan@fish.wa.gov.au

OBJECTIVES

- Examine stock delineation using stable isotope analysis.
- Determine the age structure of grass emperor.
- Determine the growth rate of grass emperor.
- Determine the reproductive biology of the grass emperor.
- Develop a stock assessment model for grass emperor for the inner gulfs of Shark Bay.

NON-TECHNICAL SUMMARY

OUTCOMES ACHIEVED

This study has determined the basic biological characteristics of grass emperor from Shark Bay in the Gascoyne Bioregion of Western Australia (WA). Grass emperor has not previously been the subject of focused study in WA. Furthermore, the Shark Bay population is at the southern end of this species distribution along the west coast of Australia and is also a key target species for anglers in the region. The information collected has provided a scientific basis for increasing the legal minimum length (LML) of grass emperor in the Gascoyne Bioregion and for decreasing the bag limit for emperor species (known locally as nor' west snapper). These data were thus used during the development of the Gascoyne Fishing Strategy. In particular, this study has described the reproductive biology and ascertained the length at 50% maturity, and sex ratio at length. This study has confirmed that the WA legal minimum length of 32 cm for grass emperor is appropriate as it is well above the sizes at 50% maturity of 23 cm for females and 18 cm for males. The need for a conservative (i.e. relatively high) LML was supported by the stock assessment model; this model indicated that the optimal age at first capture for grass emperor is 5.1 years old and hence a conservative LML is therefore also expected to improve the yield of the fishery as well as specifically allowing all mature fish to spawn before becoming large enough to legally capture. The stock assessment model has also indicated that the increased pressure on grass emperor, following implementation of severe restrictions on the take of pink snapper, did in fact result in a higher risk to the stock, with fishing mortality likely to have been high enough to over-exploit the stock. Any future changes in the management of pink snapper, the icon angling species in Shark Bay, should not be made without also considering the flow-on effects of transferring effort to other angling species in the region.

Grass emperor *(Lethrinus laticaudis)* comprises a significant finfish resource within Shark Bay, Western Australia (WA). Shark Bay is the southern and western limit of this common inshore species, whose distribution extends across northern Australia to Queensland. Within Shark Bay, *L. laticaudis* has become increasingly targeted by recreational fishers due to tighter management restrictions on the catch of pink snapper in this region. Angler surveys conducted along the Gascoyne region of WA, which encompasses Shark Bay, in 1998-99, 2000-01 and 2001-02 indicated that *L. laticaudis* is the second most frequently caught fish in Shark Bay. Despite the popularity of this species, there has been no validated and published literature on which to formulate management policy. Therefore, the objectives of this research were to collect relevant biological and fisheries data and to use these data to underpin the development of management policy.

Grass emperor were collected monthly between August 1999 and June 2001, in October 2001 and between December 2001 and February 2002, at sites in both the eastern and western gulfs of Shark Bay. The key biological factors of age, growth and reproduction were analysed in detail. Chemical composition of the otoliths (earbones) was undertaken to determine whether there is sufficient spatial demarcation of grass emperor within shark Bay to influence how the species is managed.

Spawning occurs in both the western and eastern gulfs. Grass emperor begin spawning in December, then peak in January and conclude spawning in March. Histological (microscopic) evidence indicated that developing eggs of all stages are present together within reproductively active ovaries; therefore this species is considered to be a batch spawner, i.e. a batch of the most advance eggs are spawned while another batch is developing in the ovary. This next batch then matures sufficiently to be spawned, and are again replaced by another developing batch. Batch spawners thus spawn several times each spawning season. While a small percentage of specimens showed gonads with both male and female reproductive tissue, there was no evidence to indicate that grass emperor in Shark Bay change sex from female to male, as has been reported from the Northern Territory. The size at 50% sexual maturity was 22.6 cm total length (TL) for females and 18.2 cm TL for males. Estimates of numbers of eggs spawned were calculated from 33 pre-spawning females; individual grass emperor in the size range of 200-250 mm TL each produced between 50,000 and 150,000 eggs at each spawning event.

Thin sections of otoliths from grass emperor proved to be a useful tool for age-estimation in the adult fish. A comparison of ring counts between whole and sectioned otoliths showed that sectioned otoliths gave more reliable estimates of age. The clear pattern of internal rings on the otoliths were found to be deposited annually in response to seasonal changes in growth rates, and hence could be used to age this species. The annual deposition of each growth ring concluded between August and February. This timing was coincident with the seasonal onset of reproductive maturation and increases in water temperature following winter.

Grass emperor catches were predominantly comprised of the 3^+ to 7^+ age classes, within a range of 1 to 16 years; grass emperor are likely to live to 20 years. The asymptotic lengths (average length of the oldest fish) for the eastern and western gulfs and the ocean region, calculated from growth equations, were 469 mm TL, 404 mm TL and 592 mm TL respectively. Like other members of the emperor family, grass emperor are thus relatively slow growing and long-lived.

There appears to be minimal movement of adult grass emperor between reef habitats that are separated by non-reef habitat. Thus, there was evidence of negligible mixing of grass emperor between the two closest sites examined in this study (Hamelin Pool and Dubaut Point). It was therefore assumed that grass emperor from other sites would also show a high degree of site fidelity unless "patches" of suitable habitat were sufficiently close to warrant more extensive movement of individual fish. A high degree of site fidelity indicates that isolated reefs are more susceptible to localised depletions of grass emperor. This study did not investigate patterns in the movement of the planktonic (egg and larval) and juvenile stages of grass emperor; however, a detailed study of currents within the gulfs has demonstrated that pink snapper exhibit self-recruitment at quite localised scales within each of the eastern and western gulfs. Therefore, it can be assumed that this may also be the case for grass emperor.

Stock assessment was performed by developing a yield per recruit (YPR) model and assessing the rate of fishing mortality. The YPR model determined that the most appropriate age at first capture was 5.1 years, which corresponds closely to the current LML. Combining the results of the YPR model with the estimates of fishing mortality indicated that exploitation levels were likely to have been too high during the study period and that some degree of over exploitation may have occurred.

Keywords

Grass emperor *(Lethrinus laticaudis)*, black snapper, age, growth, reproductive biology, spawning, stock assessment, stock delineation.

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CHAPTER 1.0

1.1 Background

In the Shark Bay region of Western Australia, grass emperor is under increasing pressure from recreational fishers as stocks of the highly sought after pink snapper are being protected from overfishing. The grass emperor, *Lethrinus laticaudis* is a member of the family Lethrinidae, commonly called the emperors. The grass emperor is found from the Gascoyne region (Shark Bay) of Western Australia across northern Australia to Oueensland. Along this distribution the grass emperor is called the blue lined emperor, black snapper, spangled emperor, grass emperor, and tricky snapper. However, genetic analyses indicate that *L. laticaudis* constitutes on individual species (Johnson et al. 1993). While this increasingly popular species is being exploited in Shark Bay, and is an important inshore target in both the Pilbara and Kimberley regions, there is little biological information on its age, growth and reproductive biology in Western Australian waters. This information is critical to the development of a stock assessment model of the population and implementation of appropriate management policies.

It is difficult to ascertain the level of commercial exploitation for this species as they are regularly grouped under the category 'lethrinid' with several other similar species. This results from the fact that the fishers get paid the same price for all lethrinids Some data from Shark Bay indicates that grass emperor is caught by haul net/beach seine as a part of a suite of species along with sea mullet, tailor, skipjack trevally, whiting and yellow fin bream. Grass emperor catches reported from the catch and effort statistics for the Shark Bay fishing block are 7 tonnes in 1996; 281 tonnes in 1997; and 62 tonnes for the first several months of 1998. These catches represent between 3 and 12.4% of the total statewide catches, most of which come from the Pilbara and Kimberley fisheries.

The grass emperor are a species targeted by recreational fishers in Shark Bay. During a recent Shark Bay creel survey by Sumner and Steckis (1999) this species ranked fifth in importance to boat anglers. Currently a more comprehensive shoreline and boat survey is being conducted throughout the Gascoyne region. The one year survey, which commenced in April 1998, has provided some preliminary results on the status of grass emperor. The anglers' survey indicates that grass emperor is the second most frequently caught fish in the Shark Bay area. The average length of fish caught is 39.9 cm total length and ranged between 25 and 61.5 cm total length.

There is limited biological information on this species, and none which has been collected from WA waters. A small scale survey of this species in Northern Territory indicated that recreational fishers caught fish between 16 and 46 cm fork length with weight correspondingly between 0.1 and 2.0 kg. Fish over 50 cm FL have been reported by fishers. All fish under 32 cm FL were female with progressively more males occurring at larger sizes. This species matures to female at about 30 cm (50% maturity), then to male at 38 cm (50% maturity). This life history strategy is termed sequential protogynous hermaphroditism. Very preliminary results indicate that they may live over 10 years and reach sizes about 50 cm FL (Knuckey et al. 1996). The diet of the grass emperor in northern Queensland waters is composed of teleosts, annelid worms and molluscs (Brewer et al. 1995). Studies from the Great Barrier Reef (Newman and Williams 1996) indicate the preferred habitat of this lethrinid is inshore reefs and seaward of rocky shores and headlands.

These studies provide basic information about the species. However, a comprehensive study is needed to assess the status of the species in the inner gulfs of Shark Bay. This study is well timed as increasing fishing restrictions on pink snapper in Shark Bay are causing a shift in desired catch to grass emperor. This shift has elevated grass emperor to the number 2 priority species for the Gascoyne region by the Recreational Fishing Advisory Committee. Presently the recreational bag limit for this species is 8 per day (in a mixed bag of reef fish) with a minimum size limit of 28 cm total length. If the reproductive information from the Northern Territory applies to Shark Bay, then the population is being fished prior to maturity. Additionally, depending on the size at which the females change to males, and depending on what size fish are vulnerable/accessible to recreational fishing gear, the impact of fishing could differentially influence the sex ratio.

1.2 Need

Grass emperor stocks in the inner gulfs of Shark Bay are under increasing pressure by the recreational fishing sector. This is a result of both the increasing number of recreational anglers fishing in the Shark Bay area and the reduction in the stock size of pink snapper. Current management for the recreational sector consists of a bag limit of 8 fish and size limit of 28 cm. However, there are no validated or reliable biological data on the age, growth and reproductive biology on this species, which are required to develop stock assessment models and evaluate current management regulations.

1.3 Objectives

1. Examine stock delineation using stable isotope analysis.
2. Determine the age structure of grass emperor.
3. Determine the growth rate of grass emperor.
4. Determine the reproductive biology of the grass emperor.
5. Develop a stock assessment model for grass emperor for the inner gulfs of Shark Bay.

This report is structured as a series of stand-alone chapters in which the specific Objectives are addressed:

- Chapter 2 - reproductive biology (Objective 4)
- Chapter 3 - age and growth (Objectives 2 and 3)
- Chapter 4 - stable isotope analyses (Objective 1)
- Chapter 5 - stock assessment (Objective 5)

Each chapter has a detailed *Material and Methods* and a *Discussion*. Following the report, sections on *Benefits and Adoption*, *Further Development* and *Planned Outcomes*, the *Conclusion* will provide an overview of the project.

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CHAPTER 2.0 Reproductive biology of the grass emperor, *Lethrinus laticaudis,* in Shark Bay, Western Australia

S. Ayvazian, I. Keay, M. Mackie and D. Gaughan

Objective: Determine the reproductive biology of the grass emperor, *Lethrinus laticaudis*

The reproductive biology of grass emperor, *Lethrinus laticaudis,* in Shark Bay and adjacent oceanic waters was examined between July 1999 and February 2002. Analyses involved determination of reproductive season using the gonadosomatic indices, macroscopic and microscopic staging of gonads, size at 50% maturity, sex ratio and batch fecundity. The current legal minimum length of 28 cm is well above the sizes at 50% maturity of 23 cm for females and 19 cm for males. *L. laticaudis* became reproductively active in December and spawned from January to March, with spawning peaking in January. Females are batch spawners and within the spawning months released eggs every two or three days during the active spawning periods that commenced on the first quarter of the moon, extended over the full moon and finished on the third quarter. *L. laticaudis* showed some evidence for forming spawning aggregations, but it could not be determined if this was a ubiquitous spawning behaviour.

2.1 Introduction

The Lethrinids, or emperors, comprise a significant finfish resource throughout the tropical and sub-tropical Indo-Pacific region (Carpenter and Allen, 1989). They are also major components of multi-species demersal fisheries in Australian waters (e.g. Moran et al. 1993). *Lethrinus laticaudis* is a common inshore species distributed from Shark Bay in Western Australia (WA) across northern Australia to Queensland and northwards into Southern Indonesia, New Guinea and Solomon Islands (Randall et al., 1990). Adults are most commonly found on coral reef or rubble patches while juveniles reside in seagrass habitats (Knuckey et al., 1996). Along the Western Australian coastline the taxonomic status of *L. laticaudis* has been confused with the spangled emperor, *L. nebulosus* (Forsskal), and the lesser spangled emperor, *L. choerorynchus* (Bloch & Schneider) (Sainsbury et al., 1985). The latter two species comprise a substantial portion of the commercial multi-species scalefish fishery of north-western Australia (Moran et al., 1993). An electrophoretic analysis determined that *L. nebulosus*, *L. choerorynchus*, and *L. laticaudis* are genetically distinct species (Johnson et al., 1993). In Shark Bay, the grass emperor, *L. laticaudis*, is known locally as the black snapper or blue-lined emperor.

Within Shark Bay *L. laticaudis* has become increasingly targeted by recreational anglers due to recent restrictions on the catch of pink snapper in this region. Angler surveys of the Gascoyne region of Western Australia, including Shark Bay, conducted in 1998-99 and 2001-02 indicated that *L. laticaudis* is the second most frequently caught fish in the Shark Bay area. The recreational catch of grass emperor landed at public ramps has decreased from 15.9 tonnes in 1998-99 to 11.6 tonnes in 2000/01 to 7 tonnes during 2001-02. (Sumner and Malseed, 2002). The average size grass emperor landed from both the eastern and western gulfs of Shark Bay was 37.9 cm and most fish kept were between 28 and 44 cm. As at October 2003, the recreational catch of *L. laticaudis* is subject to a legal minimum length of 32 cm (total length) and is included in a mixed bag limit of 4 reef fish. However during the course of this research program, the recreational fishing regulations for grass emperor stipulated a legal minimum length of 28 cm total length and a mixed bag limit of 8 fish. The reported average annual commercial catch of this species in Shark Bay between 1998-2002 was 248 kilograms (Department of Fisheries, Western Australia).

Reproductive biology, in particular size at sexual maturity, batch fecundity, spawning frequency and occurrence of hermaphroditism, may have important implications in determining the effectiveness of current and future fisheries management. Young and Martin's (1982) examination of eight species of the economically valuable Lethrinidae from north-western Australia suggested that protogynous hermaphroditism was the prevalent reproductive mode in all eight species, based on sex ratios, length distributions and gonad morphology, although there was considerable overlap in the size distribution of the sexes. One of the species examined by Young and Martin (1982), *L. rubrioperculatus*, also has been reported to exhibit protogynous hermaphrodism in Japanese waters (Ebisawa, 1997). Wassef and Bawazeer (1992) described *L. elongatus* in the Red Sea as a multiple spawner, with spawning occurring over a four-month period. There was a predominance of females among fish aged <6 years and of males at older ages, suggesting protogynous hermaphroditism for this species. Histological examination of the gonads confirmed this sex-reversal (Wassef and Bawazeer, unpubl. data). Despite these widespread reports of protogynous hermaphroditism amongst the lethrinids, Moran et al. (1993) found no evidence of sex-reversal based on the age compostion of males and females of the spangled emperor, *L. nebulosus*, a species of commercial and recreational importance in north western Australian waters. The only report of sex reversal in *L. laticaudis* is by Knuckey et al. (1996) who noted that all fish under 32 cm FL were female, with increasingly greater proportion of males occurring at larger sizes. However, this preliminary study provided few other details on reproduction.

The objectives of this research were to provide information on the reproductive biology, annual reproductive cycle, size at maturity and sexual development of *L. laticaudis* from Shark Bay.

2.2 Material and methods

Shark Bay is a large marine embayment of almost 13,000km^2 between the latitudes 24° 30'S and 26° 45'S, and longitudes 113°E 114° 20'E (Figure 2.1). It is adjacent to a surrounding land area of low relief in an arid to semi arid climate where evaporation exceeds precipitation. Shark Bay is enclosed by Bernier, Dorre and Dirk Hartog Islands and subdivided internally by dune ridges and submerged banks or sills into numerous inlets, gulfs and basins. The only entry for oceanic water is through the wide northern channels (Geographe and Naturaliste) with limited flow through South Passage between Steep Point and Dirk Hartog Island (Marsh, 1990). Shark Bay had an oceanic metahaline salinity regime from 8,000 to 4,500 yrs BP with the hypersaline salinity regime of Hamelin Pool and Lharidon Bight having developed during the past 3,000–4,000 years (Logan, 1974). The low rainfall and runoff, and restricted circulation combined with the high evaporation rates result in an increase in salinity from the normal oceanic in the northern and western parts of the bay to hypersaline in the southern extremities (Logan and Cebulski, 1970). The salinity gradient ranges from oceanic values around $35^0/_{00}$ in the northern and western parts of the bay through metahaline (40-$56^0/_{00}$) to hypersaline in Hamelin Pool and Lharidian Bight (56–$70^0/_{00}$) in the southern regions.

Collection and processing of samples

Research fish sampling was conducted monthly between July 1999 and June 2001, in October 2001, and between December 2001 and February 2002, at sites in both the eastern and western gulfs of Shark Bay (Figure 2.1). Strong southwesterly winds hampered attempts to collect regular samples from the western gulf during summer. Intensive sampling was conducted between December 2000 and February 2001, and December 2001 and February 2002 to enable detailed examination of reproductive activity and batch fecundity because preliminary examination of data indicated that this was the peak spawning period. *L. laticaudis* samples were collected by research staff and occasionally by recreational fishers. Frozen frames of fish caught in oceanic waters adjacent to Shark Bay were also collected opportunistically from recreational fishers.

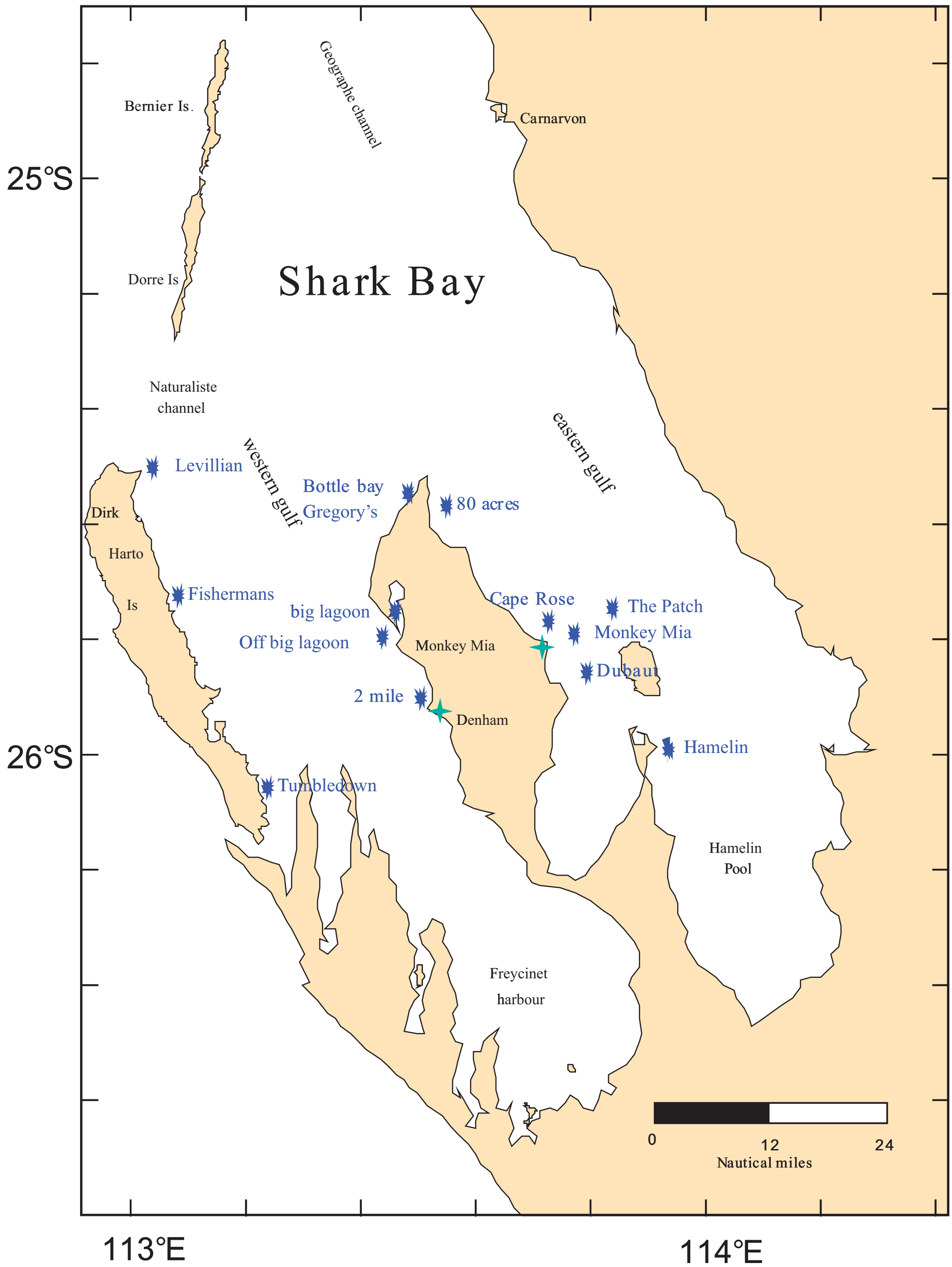


Figure 2.1 Sampling sites for *L. laticaudis* in the eastern and western gulfs of Shark Bay, Western Australia.

Fish were captured by rod and line or baited fish traps. The steel-framed fish traps were circular with a diameter of 110 cm, 48 cm high and two entrance funnels. Two different mesh sizes, 2.4 cm and 5.5 cm, were used on the outside. The smaller mesh was used to capture fish of lengths <100 mm that may have been able to escape from the larger mesh. The traps were baited with chopped fish and deployed from 1 to 8 hours, with occasional overnight sampling. The traps were initially tested in all habitat types, and then selectively placed on more successful substrates.

After capture, fish were placed on ice and returned to the laboratory. Each fish was measured (total length (TL), fork length (FL) and standard length (SL), to the nearest mm, weighed (whole and clean weight = viscera and gonads removed, to the nearest 0.01g). The viscera and gonads were removed and weighed. The gonads were usually removed from the fish within hours of capture, sexed and weighed to 0.01 g. Ten to 20 gonads every month were stored in 10% formalin mixed in seawater solution for histological analyses. All female and a selection of male *L. laticaudis* gonads were processed and kept for histological analysis during the intensive sampling period from December 2000 to February 2001.

A transverse section of ovary approximately 3 mm thick was removed from the central region of the preserved gonad and processed using standard histological techniques to provide 5–7 µm sections that were stained using Harris's haematoxylin and eosin (H&E) for microscopic examination to verify sex and gonad development.

Annual reproductive cycle

Ovaries were classified into reproductive stages according to macroscopic and microscopic criteria, using the staging system of Mackie and Lewis (2001). Ovaries were also classified histologically (Table 2.1). Males were staged macroscopically, however the assignment of some stages of the developing male testes are difficult, until stage 4 when mature and milt can be extracted with pressure (Table 2.2).

Table 2.1 Macroscopic and microscopic histological stages of development of *Lethrinus laticaudis* ovaries.

Stage	Histological stage	Macroscopic
Juvenile	Undifferentiated	Gonad very small, sex of the fish cannot be determined
1 Virgin	Chromatin nucleolus stage (CNS) Peri nucleolus stage (PNS)	Very small gonads Colourless transparent
2 Mature resting	(CNS & PNS)	(Stage 2-3) Ovaries small and rounded. Usually translucent and pale pink in colour but can be bloodshot and flaccid soon after spawning season. No opaque oocytes visible.
3 Developing	Cortical alveoli (CA) Early yolk globule stage (YGS)	
4 Developed	Late yolk globule stage (YGS)	Ovaries considerably larger pink to pale orange colour. Opaque oocytes and blood vessels present
5a Pre-spawn	Hydrated and migratory nucleus stage (MNS) oocytes within lamellae	(Stage 5) Ovaries rounded and swollen, occupying most of the ventral cavity. Hydrated oocytes visible and may be extruded with pressure. Large blood vessels visible. Ovaries may become bloodshot and flaccid towards end of the spawning season.
5b Spawning / Running ripe	Hydrated oocytes released into lumen	
5c Post-spawn	(CA and YGS) Post ovulatory follicles (POFs)	
6 Spent	> 50 % atresia of YGS oocytes	-

Table 2.2 Macroscopic stages of development of *Lethrinus laticaudis* testes.

Stage	Macroscopic
Juvenile	Gonad very small, sex of the fish cannot be determined
1 Virgin	Very small gonads, ribbon like. Colourless and transparent
2 Mature resting	Testes flat or wedge shaped, greyish to cream in colour
3 Ripe	Testes large lobular & white, becoming flaccid and bloodshot towards end of the spawning season. Spermatozoa evident in cut cross-section
4 Spawning	Sperm extruded with little pressure Large blood vessels visible

Gonadosomatic indices (GSI) were calculated as:

$$GSI(\%) = [(W_g / W_f)*100]$$

Where: W_g = gonad weight, and W_f = weight of the fish minus the weight of the viscera and gonads. The weight of viscera was subtracted to reduce the variation that can occur as a result of large quantities of bait being ingested by some fish. Prior to the GSI analyses the relationship between gonad and whole weight was investigated so that only data within the range where they were not correlated were used.

Reproductive biology

Criteria outlined by Sadovy & Shapiro (1987) were used in the diagnosis of protogynous hermaphroditism. The definition by Hastings (1981) was used to describe transitional (sex-changing) individuals. Gonads of these fish had proliferating testicular tissue and degenerating ovarian tissue but did not yet have peripheral sperm sinuses containing spermatids. Atretic oocytes were classified into alpha or beta stages according to Hunter & Macewicz (1985).

Size at maturity was estimated from histological and fresh macroscopically staged gonads collected during the peak spawning period (December to February) using a logistic general linear model, with standard errors estimated by bootstrapping.

Batch fecundity (BF) was calculated for preserved spawning stage ovaries as:

$$BF = [E_c/W_s] * W_o$$

Where: E_c =count of hydrated oocytes; W_s = sample weight; and W_o = total weight of both ovaries.

Preserved ovaries used for estimates of batch fecundity were drained, dried and weighed (± 0.1 mg). Tissue samples were taken from the middle region and one-fifth of the distance from each end of one lobe. These samples were cut to include a 1 mm square of outer membrane plus the connected ovarian tissue, and weighed between 0.1–0.3 mg so that 40–120 hydrated oocytes could be counted. Each sample was then covered with several drops of glycerin. After 10-15 minutes the oocytes were loosened by gently tapping the piece of ovary with the blunt tip of the forceps, 3–4 more drops of glycerin were added, and the sample spread over the slide. A glass cover slip was gently placed over the tissue sample and the number of hydrated oocytes counted using a dissecting microscope with x 10 objective. Hydrated oocytes were easily distinguishable from other oocytes by their large size, wrinkled appearance compared to other non-hydrated oocytes when preserved in formalin and by their translucence (non-hydrated oocytes are relatively opaque). In the case of damaged hydrated oocytes, only fragments judged to be a major portion of the oocyte were counted. Empty chorions (follicles) were not counted, although these were noted as indicating that spawning by that fish had already occurred.

The assumption that one ovarian lobe provides a reliable estimate of batch fecundity for the whole ovary was tested by taking three samples as detailed above from the right and left lobes of five ovaries and comparing the fecundity estimates from each pair using a paired t-test. A transverse section of ovary approximately 3 mm thick was also removed from the central region of the lobe in which the tissue samples were obtained. This section was processed using histological techniques to confirm whether the ovary was suitable for fecundity estimates.

Spawning frequency of female *L. laticaudis* was estimated using the hydrated oocyte method (DeMartini and Fountain 1981). Spawning fraction was estimated from the histological samples as the number of ovaries with hydrated or migratory nucleus stage oocytes divided by the total number of mature ovaries in the total catch. Spawning frequency was determined as the inverse of the spawning fraction.

2.3 Results

Reproductive biology

A total of 4895 *L. laticaudis* were used in analyses of reproductive biology (Table 2.3). The gonads of 1121 of these were processed using histological methods. Ovaries varied from 0.01 to 140.12 g and testes from 0.02 to 66.48 g in weight. Body weight of sampled fish ranged from 5.02 to 3500 g, and TL from 68 to 637 mm (Table 2.4). Relationships between length and weight parameters were similar for males and females (Figure 2.2).

Relationships for the combined data set are (lengths in mm, whole weight (WW) in g):

Eastern gulf:

$FL = -2.332 + (TL \times 0.927)$ n = 2540, adj $r^2 = 0.999$
$TL = 2.822 + (FL \times 1.077)$ n = 2540, adj $r^2 = 0.999$
$SL = -3.218 + (FL \times 0.873)$ n = 2464, adj $r^2 = 0.997$
$WW = 0.00002 \times FL^{2.991}$ n = 2342, adj $r^2 = 0.993$

Western gulf:

$FL = -2.539 + (TL \times 0.927)$ n = 2188, adj $r^2 = 0.998$
$TL = 3.168 + (FL \times 1.077)$ n = 2188, adj $r^2 = 0.998$
$SL = -3.632 + (FL \times 0.873)$ n = 2150, adj $r^2 = 0.996$
$WW = 0.00002 \times FL^{3.0319}$ n = 1999, adj $r^2 = 0.991$

Table 2.3 Total numbers of *Lethrinus laticaudis* collected between July 1999 and February 2002 from Shark Bay, Western Australia.

Region	Sex				
	Female	Male	Juvenile	Total	Sex ratio female:male
eastern gulf	1,357	1,136	112	2,605	1.19:1
western gulf	1,347	698	114	2,159	1.93:1
ocean	88	43		131	2.05:1
Total	2,792	1,877	226	4,895	1.48:1

Table 2.4 Length and weight statistics of *L. laticaudis* collected between July 1999 and February 2002 from Shark Bay, Western Australia.

Region	Sex	Min TL (mm)	Max TL (mm)	Min FL (mm)	Max FL (mm)	Min Total wt (g)	Max Total wt (g)
eastern gulf	F	143	637	131	598	42.4	3,358.1
	J	121	206	110	191	24.3	127.9
	M	145	556	134	514	45.1	2,399.1
	T	182	182	168	168	90.1	90.1
ocean	F	175	619	161	579	76.4	3,500.0
	M	251	599	230	546	280.3	2,640.0
western gulf	F	153	561	140	524	51.7	2,180.7
	J	68	270	63	249	5.0	288.4
	M	165	573	150	534	62.9	3,060.0

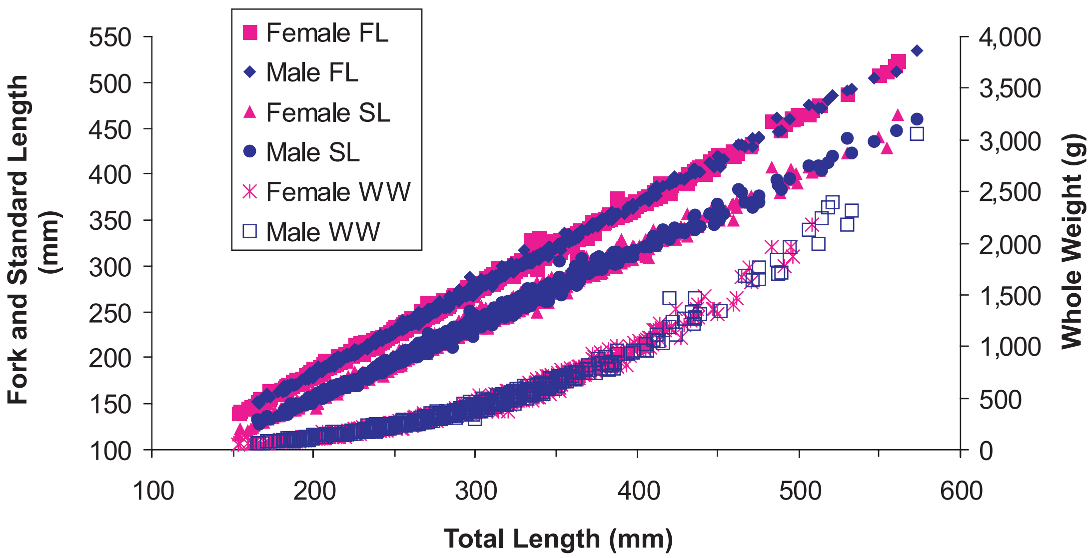


Figure 2.2 Length and weight relationships for *L. laticaudis* in the western gulf of Shark Bay. FL; fork length. SL; standard length. WW; whole weight.

Histological evidence shows that *L. laticaudis* ovaries are the asynchronous type, with oocytes of all stages present together within reproductively active ovaries (Wallace and Selman 1981; Plate 1). This developmental type, along with the presence of ovaries with both post-ovulatory follicles (POFs) and hydrated or migratory-nucleus stage (MNS oocytes show that female *L. laticaudis* are serial or partial spawners (Hunter and Macewicz 1985). Oocytes develop within narrow lamellae, and prior to spawning are ovulated as eggs into the lumen where they are released externally via the gonoduct.

L. laticaudis testes are lobular in cross-section and have a vestigial ovarian lumen characteristic of protogynous species (Plate 2). Sperm are produced and undergo spermiogenesis in crypts. Breakdown of crypts containing spermatozoa produce radial sperm sinuses that transport the mature sperm to a peripheral sperm sinus running longitudinally along the gonad. From here the sperm are released externally via the gonoduct. The testis of one reasonably large male (313 mm TL) caught in February 2001 was ripe but also contained areas of ovarian tissue in peripheral regions that were comprised of CNS, PNS and either late CAS or early YGS oocytes (Plate 3). Many of these oocytes were atretic, however there also appeared to be some developing early stage oocytes.

The gonad of one fish was transitional between male and female. This fish was small (182 mm TL) and captured in November 2000. The gonad was also small with a fairly indistinct lumen. Ovarian tissue filled approximately 30% of the tissue mass and was comprised of CNS and PNS oocytes with many in alpha and beta stages of atresia (Plate 4). Many crypts of primary and secondary spermatocytes were present as well as fewer crypts with spermatids and spermatozoa. These comprised approximately 40% of the tissue mass and appeared to be proliferating. The remaining 30% of tissue mass was somatic tissue including blood vessels and connective tissue.

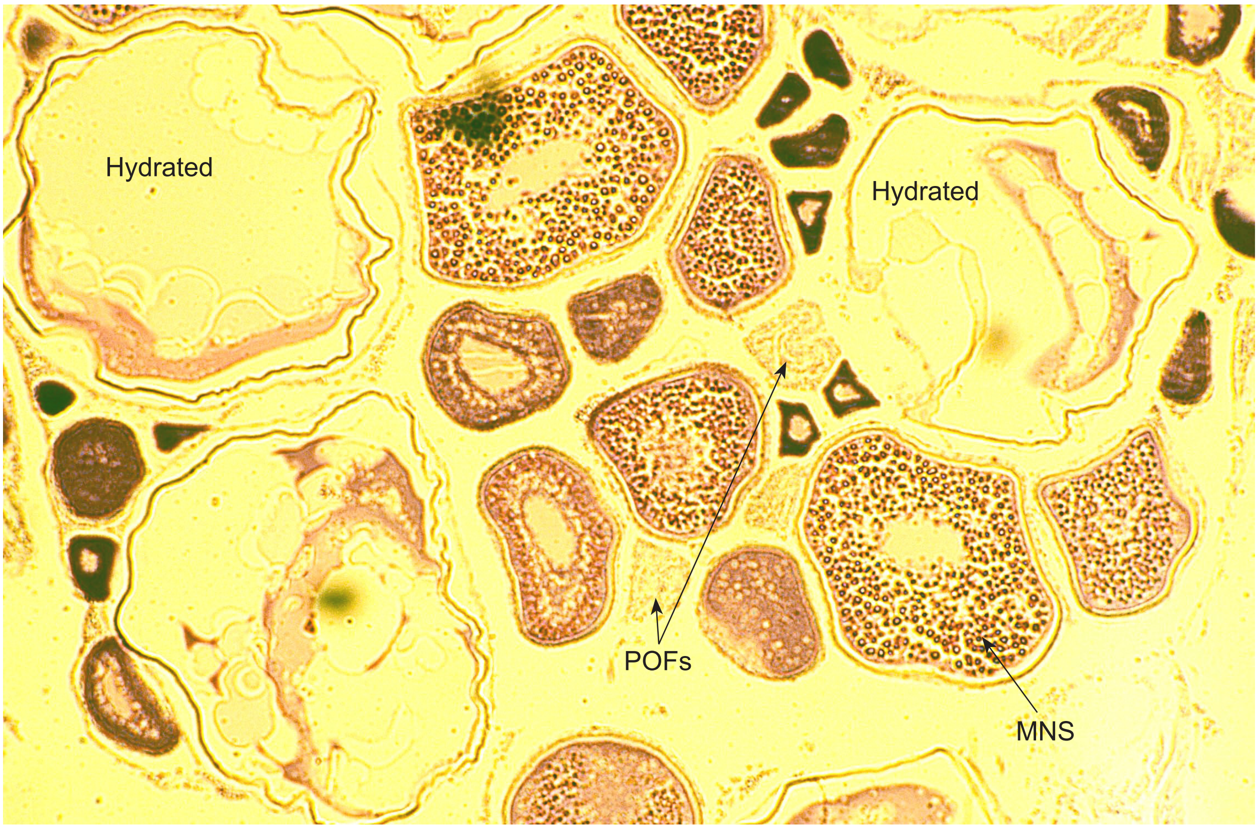


Plate 1. Histological section of *L. laticaudis* ovary.

a)

b)

Plate 2. Histological section of *L. laticaudis* testis.

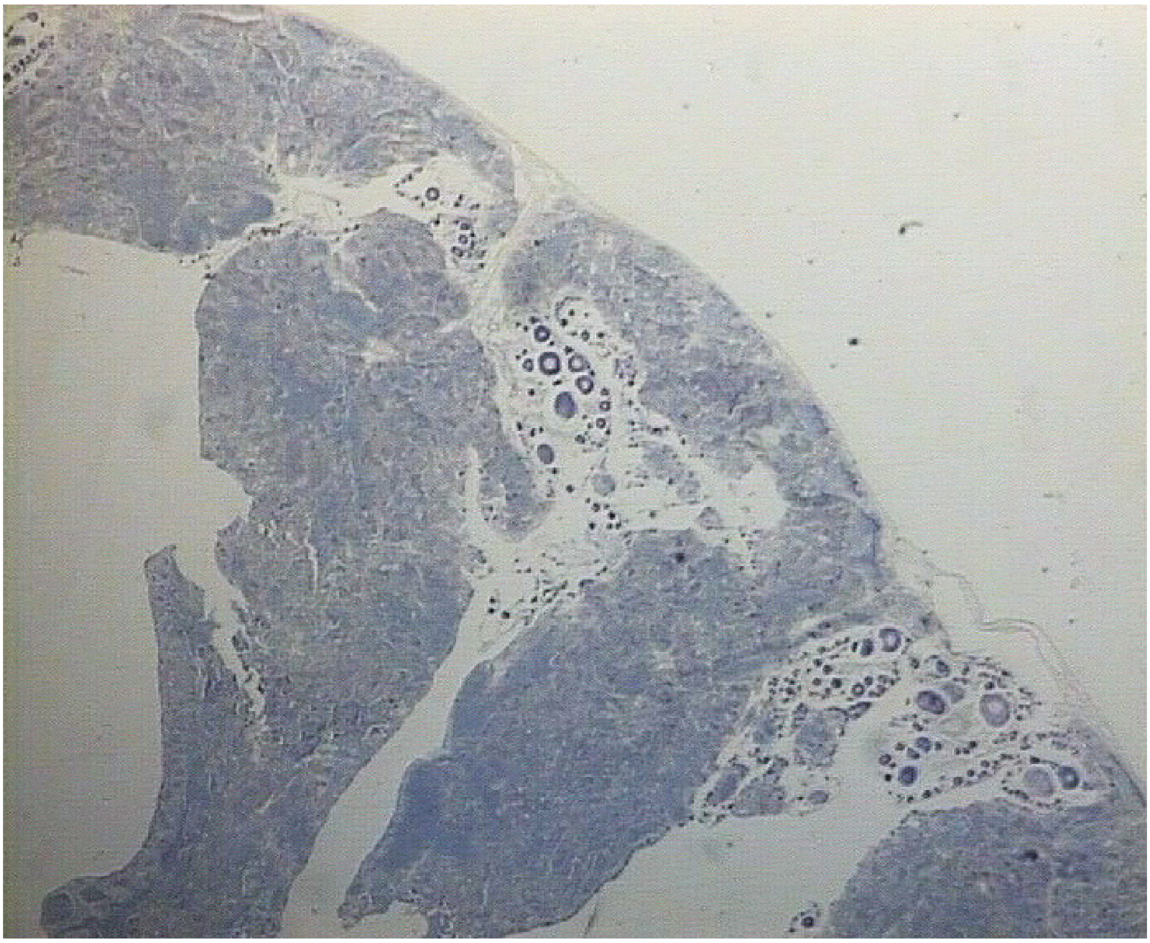


Plate 3. Histological section of *L. laticaudis* testis that also contains ovary with visible oocytes.

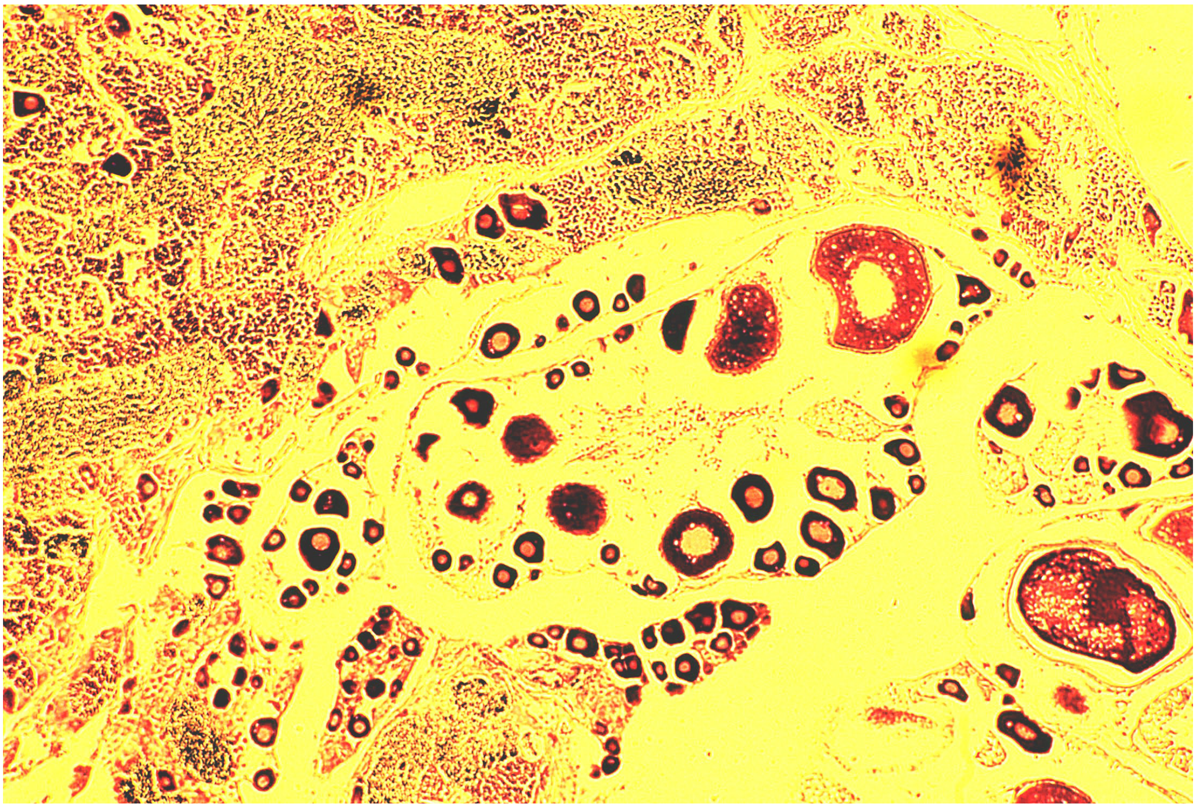


Plate 4. Histological section of *L. laticaudis* gonad in a transitional stage between male and female.

Sex ratios

Overall sex ratios were biased towards females in each region, but particularly in the western gulf and ocean regions where approximately twice as many females to males were obtained (Table 2.3). Only during the summer months were males more abundant than females in the eastern gulf (this pattern occurring in consecutive years), but this was not mirrored in the western gulf where females always dominated the catches (Figure 2.3).

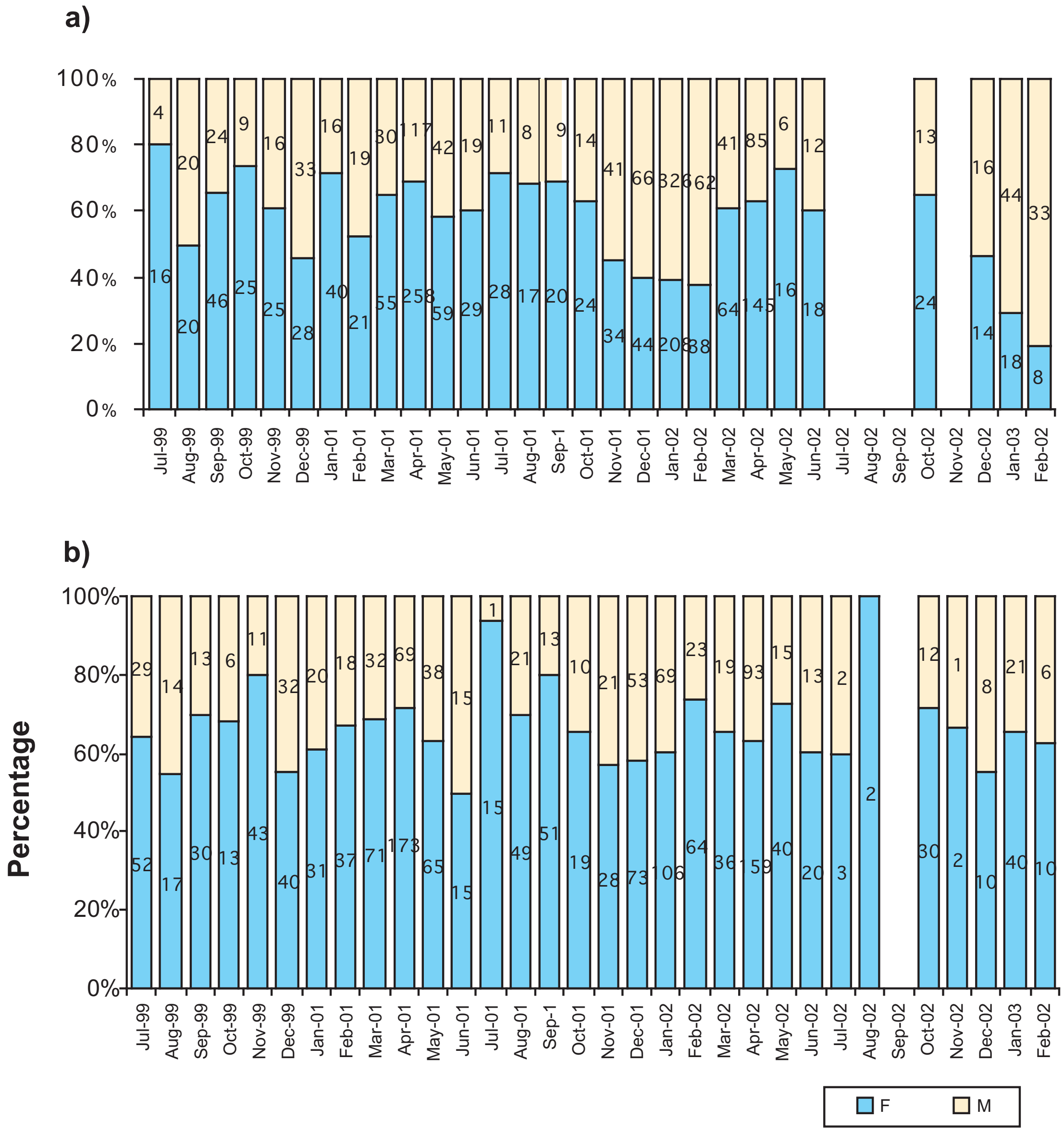
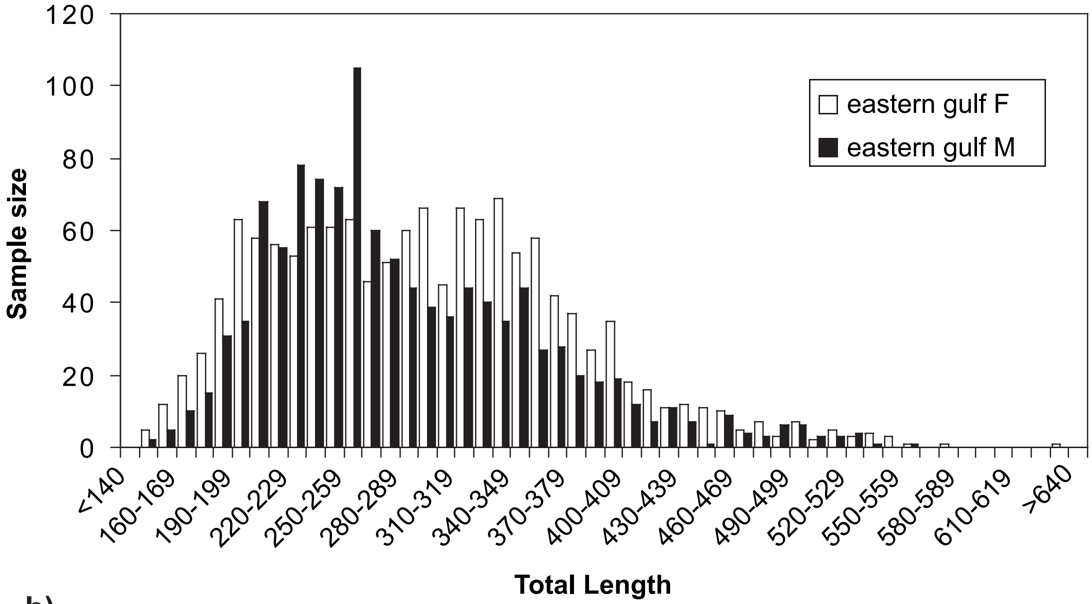


Figure 2.3 Monthly percentages of male and female grass emperor between July 1999 and February 2002 from a) the eastern gulf and b) the western gulf of Shark Bay, Western Australia. Number in bars equals number of fish.

The length distribution of both sexes overlapped completely in both gulfs (Figure 2.4). Females were usually more abundant in each length class, even amongst larger fish. The largest fish captured was a 637 mm TL female. Males were more abundant only between 200 and 270 mm TL in the eastern gulf.

a)



b)

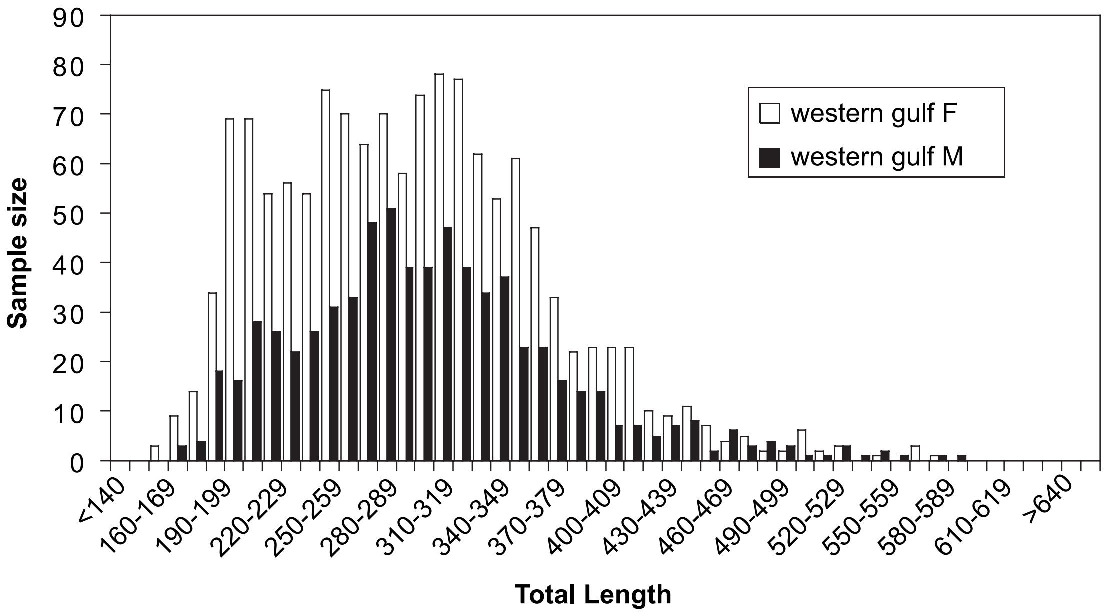


Figure 2.4 Length distribution of male and female *L. laticaudis* in A) the eastern gulf and B) the western gulf of Shark Bay.

Size at sexual maturity

The size at which 50% of female *L. laticaudis* were mature was 226 mm TL (95% CI = 213 -239 mm TL; Figure 2.5a. The size at which 50% of males were mature was 182 mm TL (95% CI = 174 – 189 mm TL; Figure 2.5b).

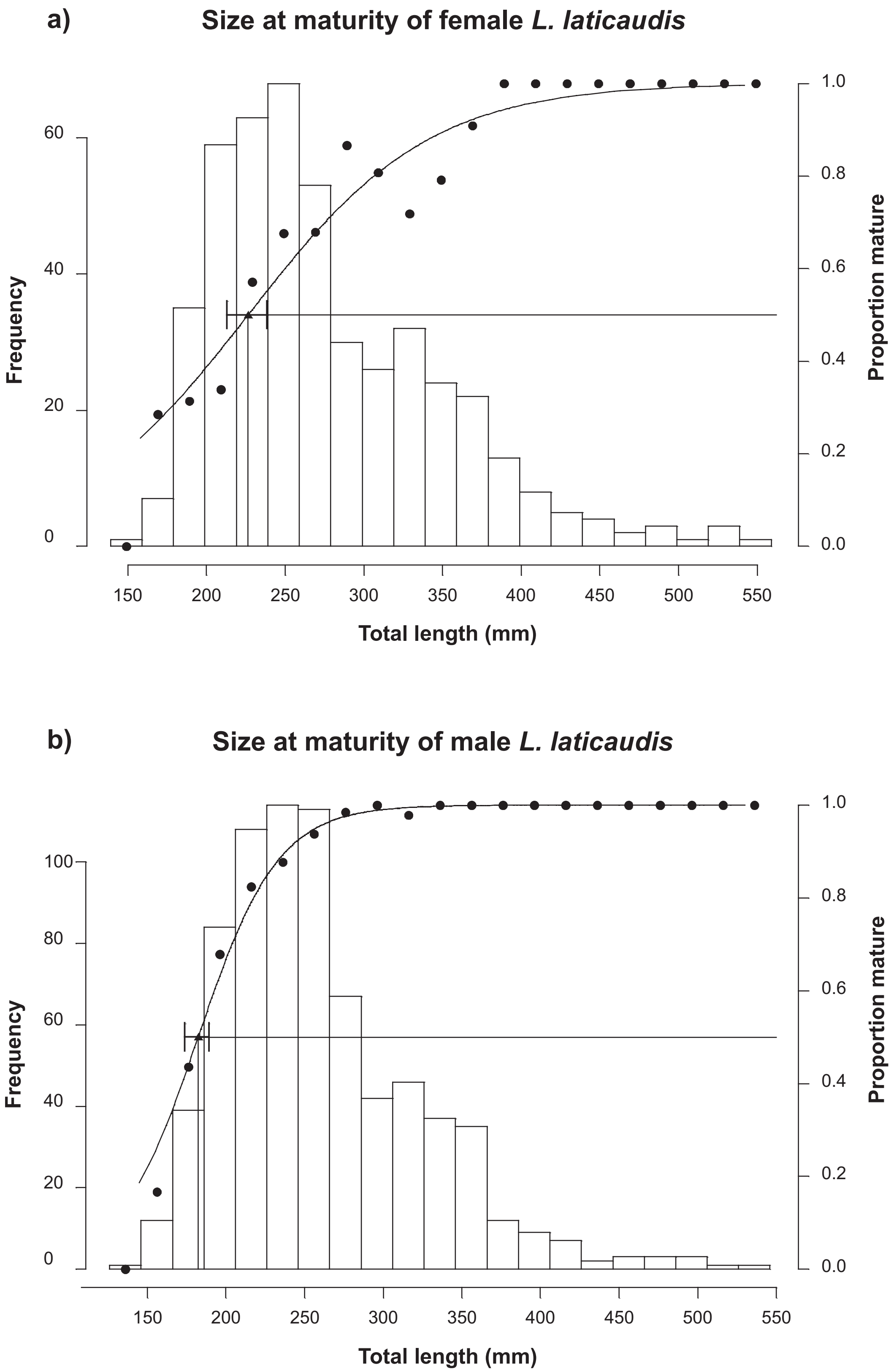


Figure 2.5 Size at maturity of a) female and b) male *L. laticaudis* at Shark Bay.

Annual Reproductive Cycle

Histological stages

Analysis of histologically prepared *L. laticaudis* ovaries shows that the annual cycle of reproductive activity in both gulfs is similar (Figure 2.6). Non-reproductive (stage 2) female occurred throughout the year but dominated samples from May to November. Early indications of reproductive activity occurred in November and December when developing (stage 3) were common in the samples, with developed (stage 4) ovaries appearing in December, most prevalent in January, and decreasing until few were present in the March samples. In both gulfs pre-spawning (stage 5a) ovaries were found in January and February. These were more common in the eastern gulf where they comprised approximately a third of the sample in these two months, compared to 10 – 15% of the samples in the western gulf. Post-spawning (stage 5c) ovaries also occurred in January and February but were most common in March. Only one actively spawning (stage 5b) ovary was sampled, obtained from the eastern gulf in December.

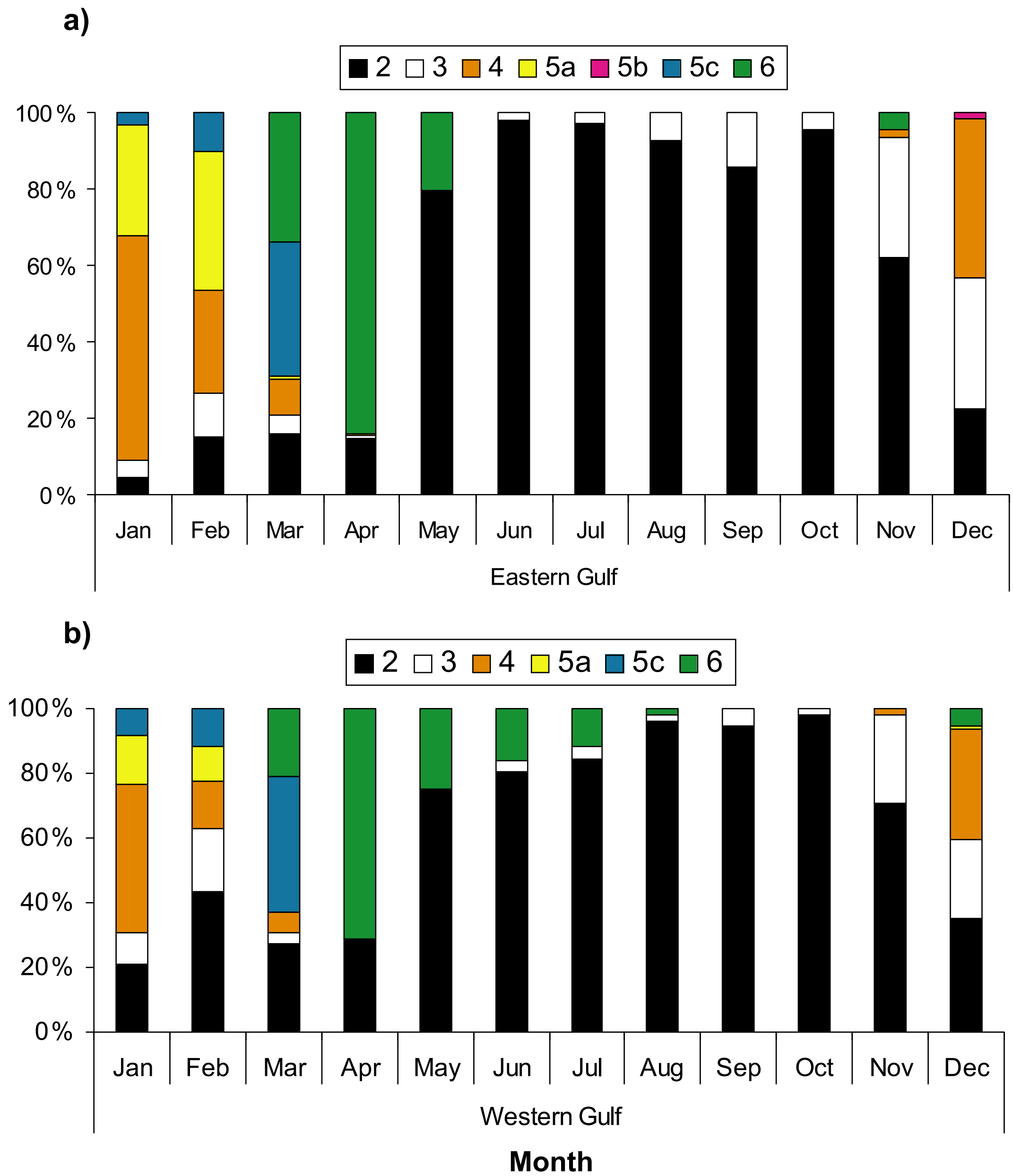


Figure 2.6 Annual reproductive cycle of female L. laticaudis in a) the eastern gulf and b) the western gulf of Shark Bay.

Gonadosomatic Index

Monthly trends in mean gonadosomatic indices (GSIs) confirm the short summer peak in spawning activity followed by a long resting period of female *L. laticaudis* in both gulfs (Figure 2.7). This pattern was consistent between the two sampling years, with the ovaries starting to gain weight in December and reaching maximum weight in January, followed by an abrupt decrease in weight in February as eggs are spawned. By March when most ovaries in the samples were post-spawning or spent, the mean GSIs had dropped back to the level of resting gonads, where they remained until the following December. The maximum mean GSI for females was greater in the eastern gulf where the highest individual GSI was 18.4 compared to 11.7 in the western gulf. The overlay of sea surface temperature (SST) data shows that *L. laticaudis* spawned prior to or at the maximum annual water temperature (Figure 2.7). During January and February 2000 the SST was 29.2 – 29.3°C, compared to 25.5 – 26 °C the following year (when SST peaked in March at 27.6 °C). Trends in mean GSIs for males shows that reproductive activity in the testes commences in November and generally peaks in January, but with a more within-month variability in relative gonad weight compared to females (Figure 2.8). As with females, the maximum mean GSI for testes was greatest in the eastern gulf where the highest individual GSI reached 12.9 compared to 8.0 in the western gulf.

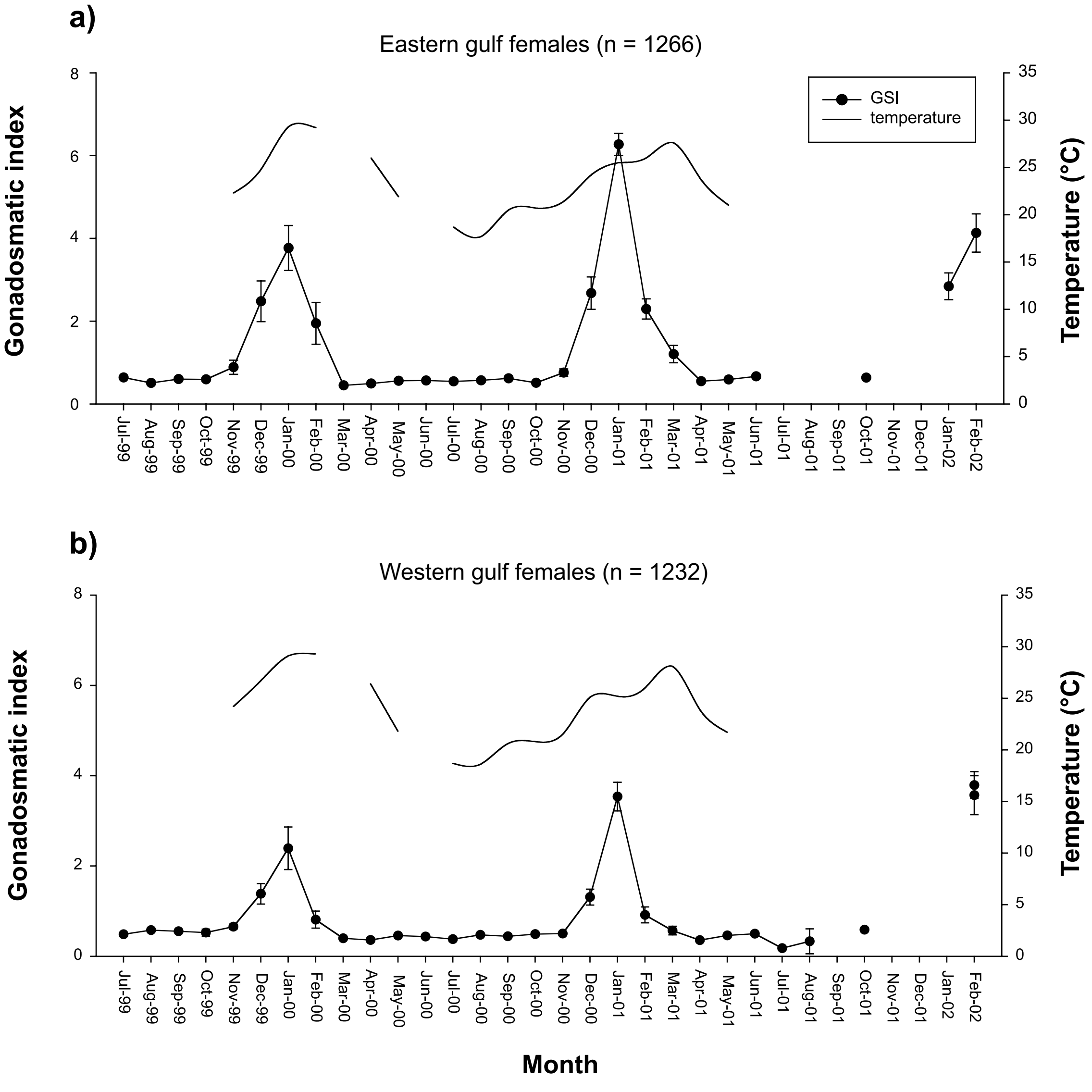


Figure 2.7 Annual cycle of gonadosomatic indices for female *L. laticaudis* in a) the eastern gulf and b) the western gulf of Shark Bay.

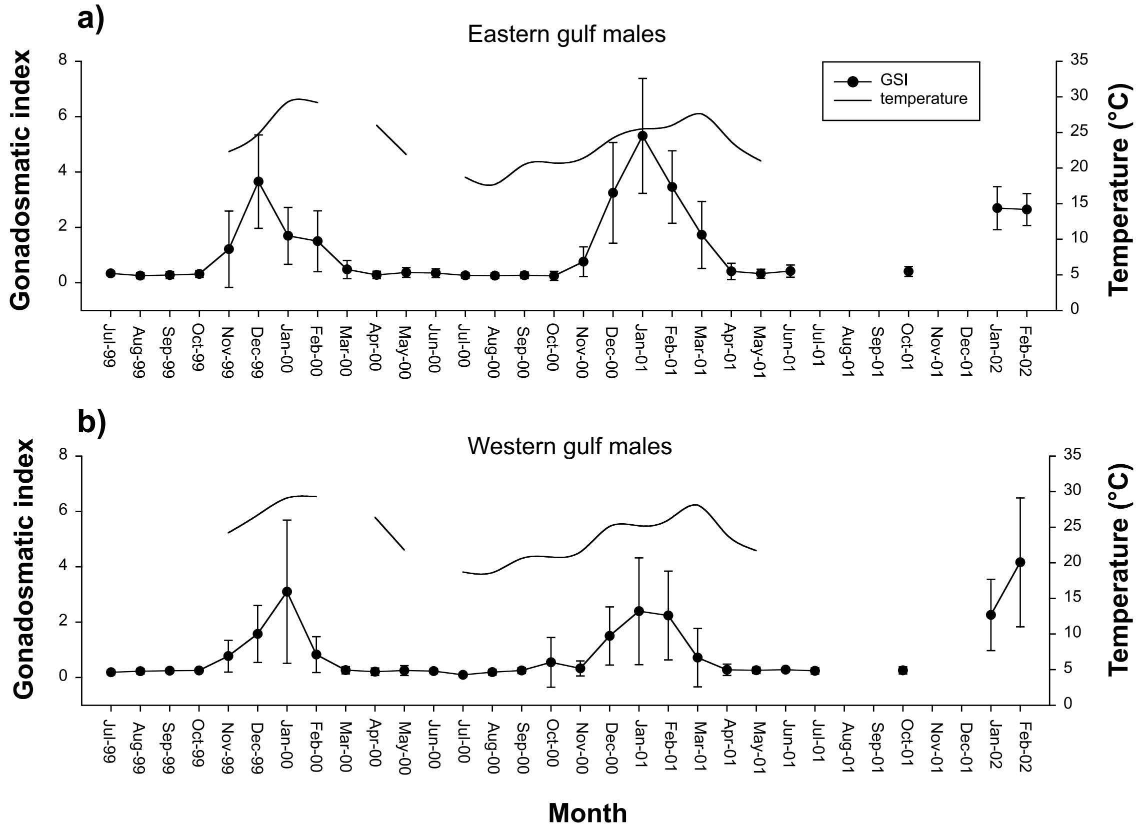


Figure 2.8 Annual cycle of gonadosomatic indices for male *L. laticaudis* in a) the eastern gulf and b) the western gulf of Shark Bay.

Daily Spawning Cycle

Spawning by female *L. laticaudis* exhibited a strong, consistent pattern that coincided with rapid decreases in the GSI each month of the spawning season (Figure 2.9). The monthly spawning cycle largely occurred within an 18-day period, commencing when the moon was 5–6 days old (first quarter) and extending through the full moon to end during the third quarter when the moon was 21–22 days old (Table 2.5). The presence of ovaries with both POFs and hydrated or MNS stage oocytes within each spawning cycle also shows that females are capable of releasing two or more batches of oocytes during the monthly spawning period (Plate 1).

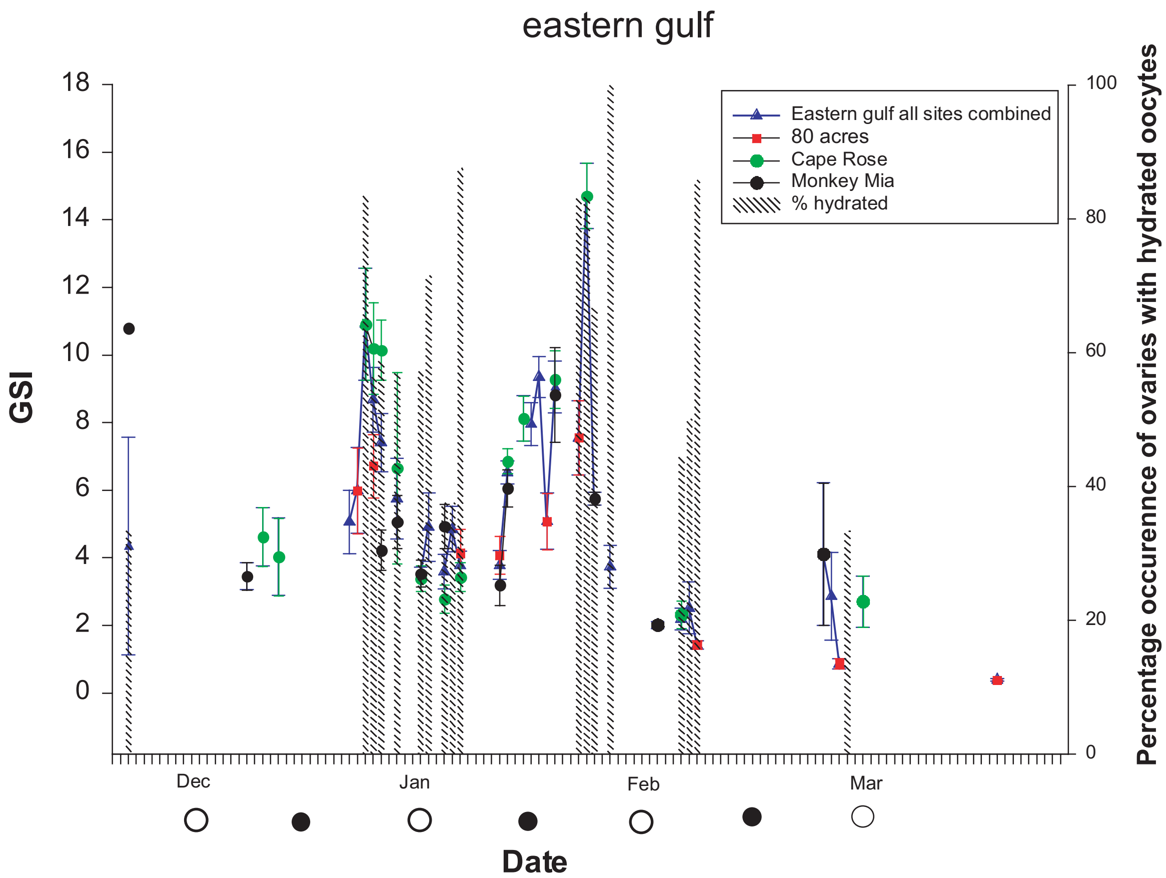


Figure 2.9 Monthly cycle of ovarian gonadosomatic indices (± SE) and percentage of spawning ovaries in samples collected during 2000/2001 from the eastern gulf of Shark Bay. White circles; full moon. Black circles; new moon.

Table 2.5 Percentage of ovaries containing hydrated oocytes within samples obtained from both gulfs of Shark Bay (data for December to March pooled)

Lunar day	% of ovaries with hydrated oocytes	Total n
1-2 (No moon)	0	33
3-4	8	24
5-6	43	44
7-8	49	39
9-10	44	64
11-12	13	40
13-14	33	6
15-16 (Full moon)	50	26
17-18	52	33
19-20	55	29
21-22	62	13
23-24	5	22
25-26	0	34
27-28	0	32
29+ (No moon)	0	4

Potential Batch Fecundity

Estimates of batch fecundity for *L. laticaudis* were taken from the left and right lobes and the anterior, middle and posterior regions of each lobe. A t-test comparing the batch fecundity estimates of these different regions within and between lobes revealed no statistically significant difference and indicated the final maturation of oocytes was similar throughout the gonad (Table 2.6).

Because *L. laticaudis* are serial spawners these estimates are of batch (not annual) fecundity. A total of 33 pre-spawning (stage 5a) ovaries were appropriate for determining batch fecundity. However, the length and weight range of females from which most of these ovaries were obtained was small (197 – 270 mm TL, 120 – 350 g; Figure 2.10a and b). There was also considerable variation in estimates, with the fecundity of females around 200 - 250 mm TL (~ 100 – 350 g) varying tenfold from about 20,000 – 200,000 eggs (most between 50,000 – 150,000 eggs).

Table 2.6 Results of paired t-tests to compare estimates of batch fecundity for *L. laticaudis* ovaries. Samples used in comparisons were taken from three regions along the ovarian lobe (anterior, middle and posterior), and from right and left lobes.

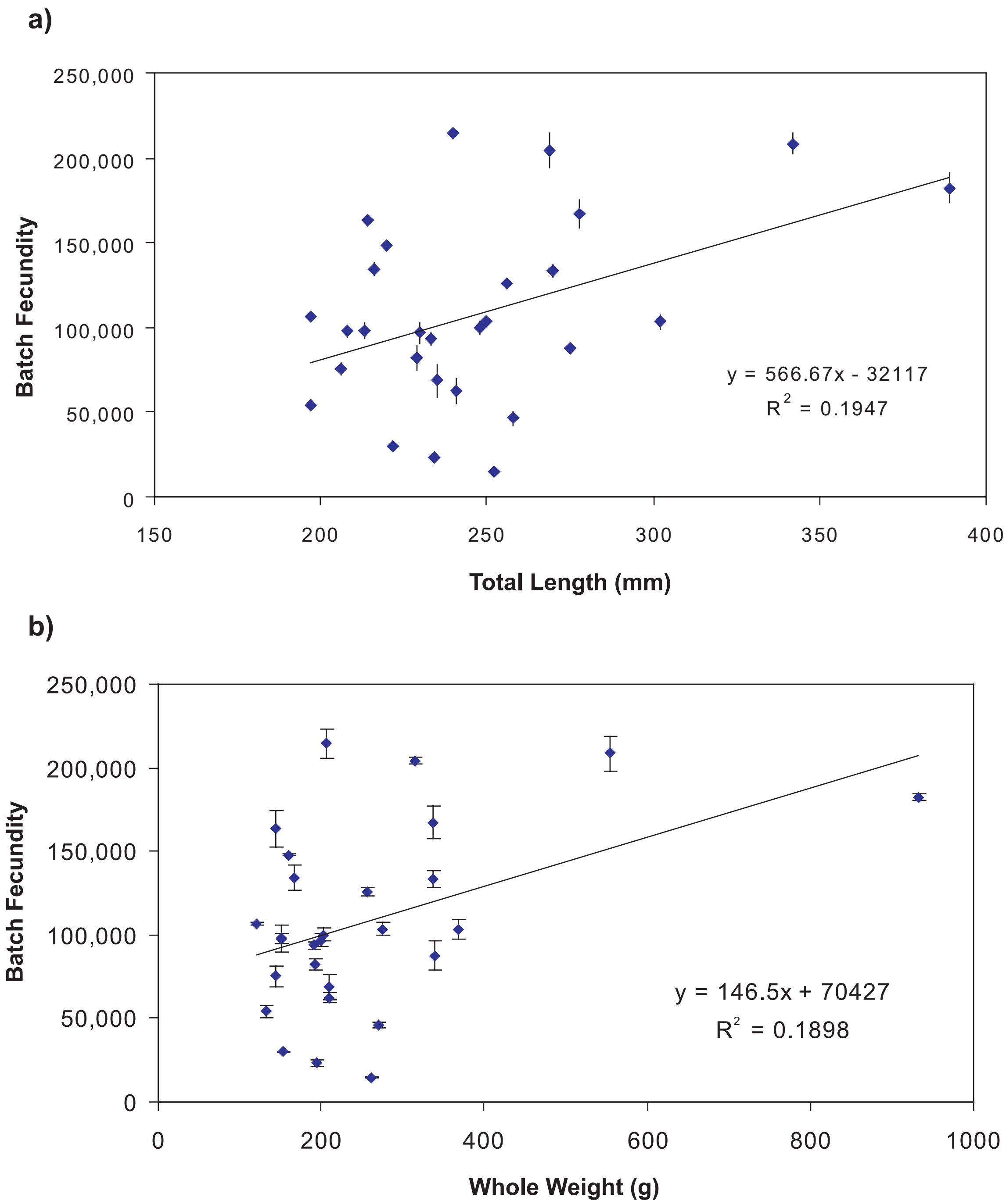
Sample Source	df	t	P
Anterior v Middle	9	-0.1977	0.8477
Anterior v Posterior	9	-0.2178	0.8324
Middle v Posterior	9	-0.0026	0.998
Middle Left v Middle Right	4	-1.632	0.178

Spawning fraction and frequency

Spawning fraction and frequency of females in which ovaries were reproductively developed or spawning (stages 4 and 5a-c) are shown in Table 2.7. About half of the females collected in January and February were spawning, indicating a spawning frequency of every two days. Females could therefore potentially spawn 9 times during each 18 day spawning cycle. Spawning frequency in December was relatively low, with females generally only spawning twice during the month. During March, a third of the reproductively active females were spawning, indicating that individuals may spawn about six times.

Table 2.7 Spawning fraction and spawning frequency for *L. laticaudis* collected from Shark Bay.

Month	Spawning fraction (%)	Spawning frequency (days)
December (n = 49)	8.2	12.25
January (n = 158)	48.10	2.08
February (n = 46)	54.35	1.84
March (n = 23)	30.43	3.29



Figures 2.10 Relationships between batch fecundity and a) total length, and b) whole weight, for *L. laticaudis* females in Shark Bay.

2.4 Discussion

Protogynous hermaphroditism

Species with a protogynous life history present particular concerns to fisheries management because males are generally larger, older, and fewer in number than females. Thus, if too many males are removed by fishing, the reproductive output of the population could be seriously diminished due to a shortage of sperm (Shapiro 1987; Huntsman and Schaaf 1994). Studies on protogynous species of groupers has similarly highlighted concern over the uncertainty of their response to fishing pressure and the inability to produce predictive yield models based on a proper understanding of grouper sex-change (Huntsman and Schaaf 1994; Coleman et al. 1996).

Determination of sexual strategy in lethrinid species is therefore important for their management. However, whilst there is evidence to suggest that protogyny is common amongst lethrinid species (e.g. Young and Martin, 1982; Knuckey et al. 1996), at least some species may not exhibit this sexual strategy. For instance, neither Kuo (1988), and Lee (1990), or Moran et al. (1993) were able to confirm protogyny in *L. nebulosus* sampled in the north est of Western Australia, and Toor (1964) did not describe protogynous hermaphroditism in the pig-face bream, *L. lentjan*.

Prior to the current research, the only information available for *L. laticaudis* was a preliminary study conducted in the Northern Territory by Knuckey et al. (1996). The strongly bimodal length distribution noted during this NT study is suggestive of protogyny, but this by itself is not conclusive since it can also be the result of differential growth and mortality in males and females (Sadovy and Shapiro, 1987). In contrast, the overlapping length distribution of male and female *L. laticaudis* in Shark Bay is indicative of a gonochoristic rather than protogynous lifestyle, particularly given the presence of very small males and large females. Nevertheless, size frequency distributions are not good evidence of sexual strategy *per se*, since they can be affected by such things as sampling biases and differential mortality rates (Sadovy and Shapiro 1987). The slight female bias in sex ratios is also poor evidence of protogyny in this species at Shark Bay.

- For the purposes of management, *L. laticaudis* in Shark Bay should be considered to be gonochoristic.

Size at sexual maturity

Knowledge of the size at sexual maturity is important for input to population dynamics models and as a basis for setting legal minimum lengths. In Shark Bay, there was considerable variability in the lengths of immature and mature females. For females the size at 50% sexual maturity (L_{50}) was 226 mm TL. This was greater than the L_{50} for males of 182 mm TL. The data indicate about 75% of females and 100% of males will be reproductively active before reaching the legal minimum length (at the time of this study) of 280 mm TL.

In Western Australia, during the 1990s the whole of the Lethrinidae were regulated by one minimum length of 280 mm total length. In the past several years the popularity of *L. laticaudis* among recreational fishers has developed and increased, particularly along the Gascoyne region of Western Australia. The legal minimum length was changed to 32 cm from October 2003.

- For the purposes of management, the size at maturity of *L. laticaudis* in Shark Bay is 9–12 cm below the legal minimum length, so this management tool has been appropriately implemented.

Seasonality of reproduction and lunar influences

Environmental cues, such as increasing photoperiod and water temperature during the October to March period, summer months in the southern hemisphere, may be responsible for the transition to active reproductive state. *L. laticaudis* exhibited a very defined summer spawning season that was closely aligned with the lunar phase and varied little between the two gulfs of Shark Bay. Water temperature is known to influence gametogenesis, spawning and gonad atresia in fish (Lam 1983), and may therefore influence reproductive development of *L. laticaudis* gonads. However, other factors associated with lunar phase, such as changes in light intensity and water movement appear to influence the specific timing of spawning in this species. Water movement is particularly of interest since it can affect the dispersal of eggs away from the spawning area (Thresher 1984).

- The peak spawning months for *L. laticaudis* in Shark Bay are January and February.

Male *L. laticaudis* GSI values approached those of the female fish for each year and for GSI values of both sexes are similar between the eastern and western gulfs of Shark Bay. Ebisawa (1990) suggested that similar GSI values are indicative of large spawning aggregations such as those reported for *L. nebulosus* and *L. atkinsonii*. During the present study, *L. laticaudis* spawned in areas where they occurred all year round. However, two spawning aggregations, containing abnormally high numbers of fish, were found at locations where fish were not normally present in the eastern gulf (Cape Rose and Monkey Mia). Both aggregations were near the tips of sand bars (2-3 m depth) along side deeper channels (8–10 m), and were well positioned to maximise the dispersal of eggs with tidal movement. Of interest was the fact that the majority of fish captured at these two aggregation sites were under the legal minimum length during this study of 280 mm TL.

- For the purposes of management, it should be noted that *L. laticaudis* in Shark Bay appear to form spawning aggregations. This may have sustainability implications if location(s) of aggregations are sufficiently consistent, both temporally and spatially, so as to significantly increase vulnerability to exploitation. If targeting of aggregations of Shark Bay *L. laticaudis* became common and this more efficient exploitation necessitated implementation of stricter management measures, this study has provided the basis from which controls on fishing around the appropriate lunar phases during January and February could be considered.

Potential batch fecundity

The paucity of fecundity data obtained for larger individual of *L. laticaudis* in this study preclude any definitive conclusions. Given that fecundity is a function of body size, data for *L. laticaudis* studied elsewhere or those from similar species of *Lethrinus* should be used to infer egg production for *L. laticaudis* larger than 300 mm FL. There is a risk in doing this because *L. laticaudis* in Shark Bay are at the southern end of their range so results obtained from elsewhere may not be appropriate. This uncertainty would therefore need to be considered in any modeling exercise that requires fecundity data.

Spawning frequency and fraction

The spawning frequency for *L. laticaudis* has not been determined previously. In our study the spawning frequency of every 2–3 days during January and February supported the GSI information describing the peak spawning period. Our study also confirms that *L. laticaudis* in Shark Bay exhibit a spawning frequency typical of many substropical and tropcial fish species.

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CHAPTER 3.0 Age and growth studies of *Lethrinus laticaudis* in Shark Bay, Western Australia

S. Ayvazian, I. Keay, M. Craine, B. Chatfield and D.Gaughan

Objective: Determine the age structure and growth of *Lethrinus laticaudis*.

Age structure and growth rate parameters of grass emperor, *Lethrinus laticaudis*, were examined throughout the eastern and western gulfs of Shark Bay and adjacent oceanic regions. Shark Bay represents the southern limit of the grass emperor's distribution along the Western Australia coast.

The sagittal otoliths proved to be a valuable and reliable tool for age estimation in the adult fish. A comparison of ring counts from a sample of whole versus sectioned adult otoliths indicated that there was a bias between the two ageing methods. Plots of the ring count from sectioned otoliths versus the mean difference between ring counts from sectioned minus whole otoliths demonstrated a minimal difference in ring counts between 0 and 8. There was an increasing trend for whole otolith ring counts to be less than the sectioned otoliths for ring counts above eight. A subset of 131 sectioned otoliths were used to demonstrate the precision of ring counts between readers. For this subset, the index of average percent error (IAPE) was 1.79%.

The sectioned sagittae showed very clear opaque and translucent zones. Marginal increment analysis demonstrated that the opaque zones were formed annually between August and February for each of the years sampled. Opaque zone formation coincided with gonadal maturation and spawning activity and the onset of warmer (summer) conditions.

Growth rates for the grass emperor were estimated using the von Bertalanffy growth curve model. The L_∞ values were consistently higher for females than males, however this was only statistically significant for the eastern gulf. There was no significant relationship between K values by sex of the fish. Spatially, the ocean caught fish had a significantly greater L_∞ than the gulfs, while the eastern gulf had a greater value than the western gulf. The K values were also statistically significant between the regions. These parameter estimates were within the range observed for other lethrinids. Despite much fishing effort we were not able to collect a reasonable sample of young of the year grass emperor. The growth rate estimates would be greatly improved with this additional information.

Examination of the relationship between otolith weight and each of age and body morphometrics demonstrates a significant and positive relationship in each case. It may be possible to develop a proxy for the actual determination of age from ring counts, which can be both time consuming and costly.

3.1 Introduction

Fishes of the family Lethrinidae (commonly called the emperors) generally inhabit shallow and deep water coral reefs and the adjacent sandy areas throughout the Indo-Pacific region. Several lethrinid species form a major component of the commercial, artisanal and recreational fisheries in these tropical and sub-tropical waters (Carpenter and Allen 1989). It has been suggested that some lethrinid species will be vulnerable to overfishing due to their sedentary nature and dependence on spatially limited coral reef habitat as well as the more global phenomena of fisheries expansion (Moran et al. 1993). Because of their vulnerability to overfishing it is critical that fisheries researchers provide managers with the biological information necessary to regulate exploitation in a sustainable manner.

Within the Gascoyne region of Western Australia, the grass emperor, *Lethrinus laticaudis,* is an increasingly popular recreationally caught species (Sumner and Malseed 2002). Recent research in north-western Australia has shown this species to be a dominant member of the inshore fish assemblage (Newman et al. 2003). Despite this, there have been no previous age and growth studies conducted on this species in Western Australia. Preliminary information from the Northern Territory indicates that grass emperor may live more than 10 years and grow to 50 cm fork length. However, due to the limited number of fish collected and examined the authors suggest the information be extrapolated with caution (Knuckey et al. 1996).

For some species of lethrinid, age and growth studies have been reported, in order to provide information for stock assessment leading to improved fisheries management. These include, most notably, *Lethrinus nebulosus* in the tropical waters of New Caledonia (Morales-Nin,1988), Fiji (Sharma 1990, Dalzell et al. 1987), north-west Australia (Moran et al. 1993), Arabian Gulf waters (Ezzat et al. 1992), Gulf of Aden, (Aldonov and Druzhinin 1978), Kuwait (Baddar 1987) and Egyptian Red Sea Coast (Sanders et al. 1984), as well as *L. miniatus* from eastern Australian waters (Brown and Sumpton 1998, Church 1995), *L. lentjan* from Sudan (Kedidi 1984), the Red Sea (Wassef 1991) and Indian waters (Toor 1968), *L. mahsena* in the Red Sea (Wassef 1991) and *Gymnocranius audleyi* from the Great Barrier Reef, Australia (Laursen et al. 1999).

An important component of ageing studies is the validation of the method(s) used for ageing the fish from hard parts (Beamish and McFarlane 1983). Historically, scales were used to age fish, however this has been largely replaced by the use of otoliths. Otoliths are believed to produce reliable estimates of age as their configuration is not altered or diminished by age (Secor et al. 1995). The rings on the otolith must be reliably enumerated and proven to be deposited annually in order to be a useful tool for ageing. This validation process is critical for establishing the usefulness of age estimation for understanding the biology of the species and parameterising population models (Beamish and McFarlane 1983).

Teleost growth rates can be summarised using one of many growth curves (Ricker 1975). Among the most widely used to describe the growth of lethrinids is the von Bertalanffy growth function. Williams and Russ (1994) and Dalzell et al. (1987) provide summaries of the growth values for many species of lethrinids. The two parameters estimated by the growth function are K, the growth rate and L_∞ the average maximum size. Among the species reported the K values ranged from 0.056 to 0.87 and L_∞ ranged from 14.0 cm (standard length) to 106.5 cm (fork length). Dalzell et al. (1987) generalised across studies and suggested that lethrinids were slow growing and long lived species.

The objectives of this portion of the project were;

1. assess the suitability of whole versus sectioned otoliths for quantifying annuli,
2. investigate the periodicity of ring formation using marginal increment analysis,
3. estimate growth parameters of grass emperor stocks for the eastern and western gulfs and ocean region of Shark Bay, Western Australia.

3.2 Materials and methods

Collection and processing of samples

Research fish sampling was conducted monthly between July 1999 and June 2001, during October 2001 and between December 2001and February 2002 at sites in the eastern and western gulfs and ocean region of Shark Bay (Figure 3.1). There were also a small number of fish collected from recreational fishers during April, May and June 1999. Grass emperor samples were collected by Department of Fisheries staff and from a few dedicated recreational fishers. No regular monthly samples were available from commercial fishers. In order to produce statistically robust analyses, the aim was to collect 60 grass emperor from a wide range of sizes from each region per month. The local weather conditions determined our ability to access most sites. Strong south-westerly winds during summer months often made it difficult to collect samples from the western gulf. Western gulf sites were sampled whenever possible including, Denham, 2 mile and Tumbledown; and in the eastern gulf including, 80 acres, Dubaut, Monkey Mia and Hamelin Pool.

Grass emperor were captured by rod and line or baited fish traps. Standard and long shank hooks were used in sizes 1/0 to 4/0. The bait used varied from commercially available scaly mackerel *Sardinella lemuru* to small nearshore species, yellow eye mullet, *Aldrichetta forsteri,* sea mullet, *Mugil cephalus* and whiting, *Sillago spp.*

The baited fish traps were circular with a diameter of 110 cm by 48 cm high. The traps were constructed of steel rod with two entrance funnels. Two different mesh sizes, 2.4 cm and 5.5 cm, were used on the outside. The smaller mesh was used to capture fish less than 100 mm that may have been able to escape from the larger mesh. The traps were baited with chopped fish, usually pilchard, *Sardinops neopilchardus, s*caly mackerel, *Sardinella lemuru* or mullet species, *Aldrichetta forsteri* or *Mugil cephalus.* Soak time for the traps varied from 1 to 8 hours with occasional opportunistic overnight sampling. The traps were tested in all habitat types initially and then selectively placed on the more successful substrates.

Young of the year (0^+ cohort) were sampled using traps, seine nets, trawls and snorkelling from a variety of habitats in both the eastern and western gulfs. Researchers from Murdoch University and the Department of Fisheries pink snapper and grass emperor research programs attempted collection of these small fish.

After capture, fish were placed on ice and returned to the laboratory. Each fish was measured (total length (TL), fork length (FL) and standard length (SL), to the nearest mm), weighed (whole and clean weight=viscera and gonads removed, to the nearest 0.01 g). The sagittal otoliths were extracted, cleaned, dried, weighed (nearest 0.001 g) and stored in envelopes until processed.

Length-weight relationship

The power curve is used to model the relationship between the total length L and total weight W. The model is,

$$W_t = aL_t^b + \varepsilon,$$

where $\varepsilon \sim N(0,\sigma^2)$, W_t is the total weight at time t, and a and b are constants to be estimated.

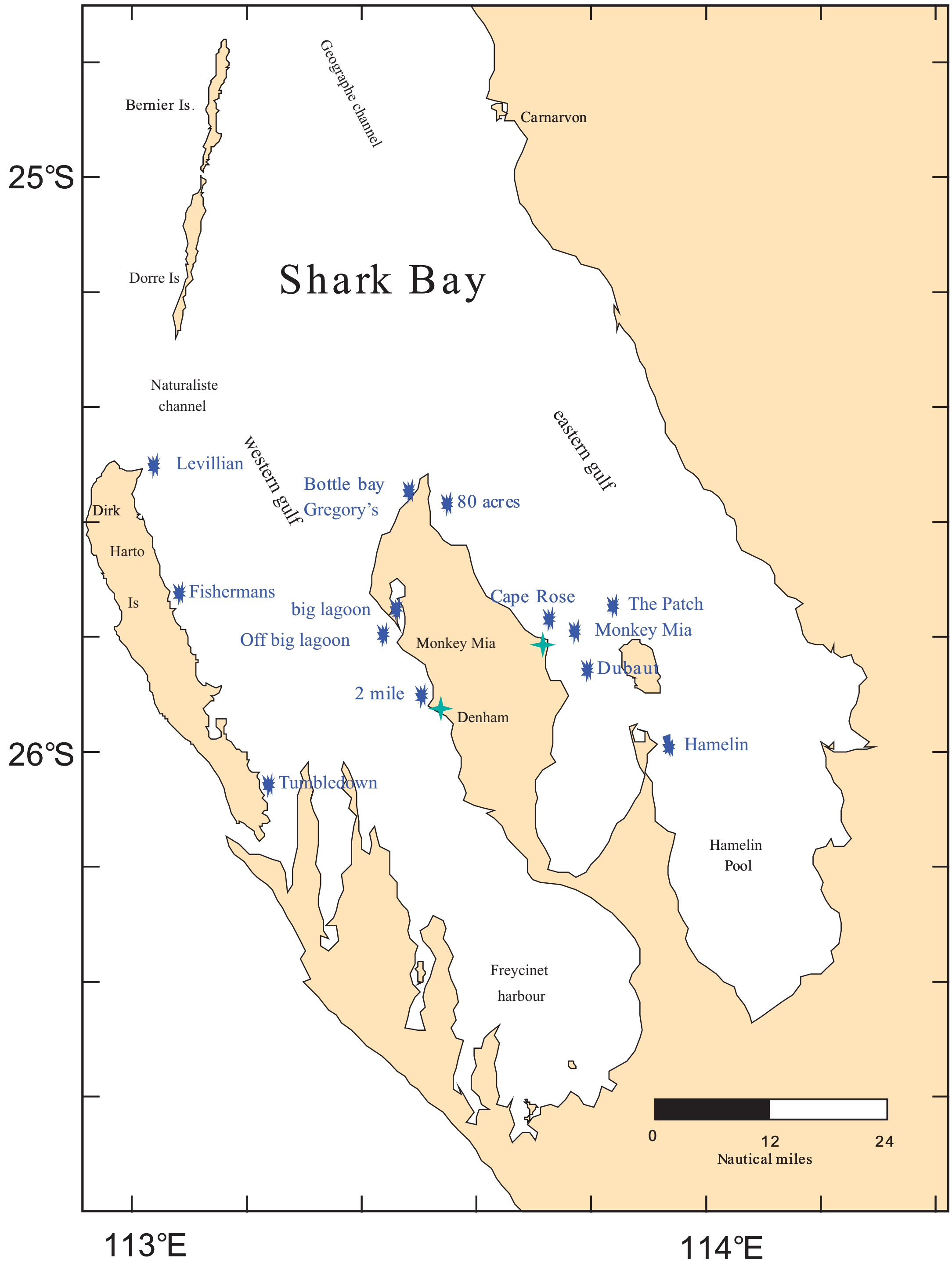


Figure 3.1 Sampling sites for *L. laticaudis* in the eastern and western gulfs of Shark Bay, Western Australia.

Adult whole otoliths

Each whole sagittal otolith was immersed in a 70% glycerol solution and the distal surface examined for opaque zones with a dissecting microscope under transmitted light. To avoid potential bias in identifying rings, all counts were made without knowledge of fish size, sex or date of capture. Two examiners read each whole otolith. The number of rings and marginal increment category (Table 3.1) were recorded for each otolith.

Table 3.1 Otolith margin increment category used in the analysis of the *L. laticaudis* otoliths.

Category	Otolith margin appearance
0	Opaque margin
1	Translucent margin is less than 50% developed as compared to the previous translucent zone
2	Translucent margin is 50% or more developed as compared to the previous translucent zone

Adult sectioned otoliths

One randomly selected sagittal otolith per fish was embedded in an epoxy resin. Transverse sections, each 250–300 μm thick, were cut using a Buehler Isomet low speed diamond saw. To ensure the primordium was included in a section up to three sections were cut from each otolith. Sections were cleaned and mounted in a casting resin and fibreglass catalyst mixture on microscope slides under coverslips. The sections were examined under a dissecting microscope using transmitted light. Rings (opaque zones) were counted along an axis from the primordium. Two readers examined each sectioned otolith. For each otolith the number of rings and marginal increment category (Table 3.1) were recorded.

Comparison of ring counts from whole and sectioned adult otoliths

A regression model of whole otolith ring counts against sectioned otolith ring counts was developed to compare the ring counts obtained from a sample of the whole and sectioned otoliths. Any bias in the two methods was assessed through deviation of the slope from one (Brown and Sumpton 1998).

Precision of ring counts

The precision of the ring counts from sectioned adult otoliths from readers 1 and 2 was calculated as an index of average percentage error (IAPE) (Beamish and Fournier 1981).

Marginal-increment analysis

Up to 20 fish (or as many as were available) were randomly selected from each age group (aged previously) to represent the entire population. One sagittal otolith per fish was sectioned as per the method described in the previous section. In order to ascertain the periodicity of ring formation, distances were measured between successive opaque zones and from the final opaque zone to the outside margin of the otolith along an axis from the primordium to the proximal surface next to the crista inferior. An image-analysis system comprising a dissecting microscope, a video camera, and a computer installed with image analysis software was used to conduct these measurements. For an otolith with one opaque zone, the marginal increment was expressed as a proportion of the distance between the core and the first opaque zone. For an otolith with more than one opaque zone, the marginal increment was expressed as a proportion of the immediately preceding translucent zone and plotted as a function of month of the year. The otolith edge was recorded as either opaque or translucent. If the otolith edge was opaque a ring was counted (Figure 3.2).

Conversion of annuli counts to age

The technique for converting annuli counts to fractional ages was developed using the transverse sections of otoliths. This protocol, which provides greater resolution in subsequent growth analyses than integer ages, required the otolith ring counts, marginal increment category (Table 3.1), a generalised birth date, and the month of capture for each fish (adapted from the technique described for both Spanish mackerel (Mackie et al. 2003) and southern sea garfish (Jones et al. 2002)).

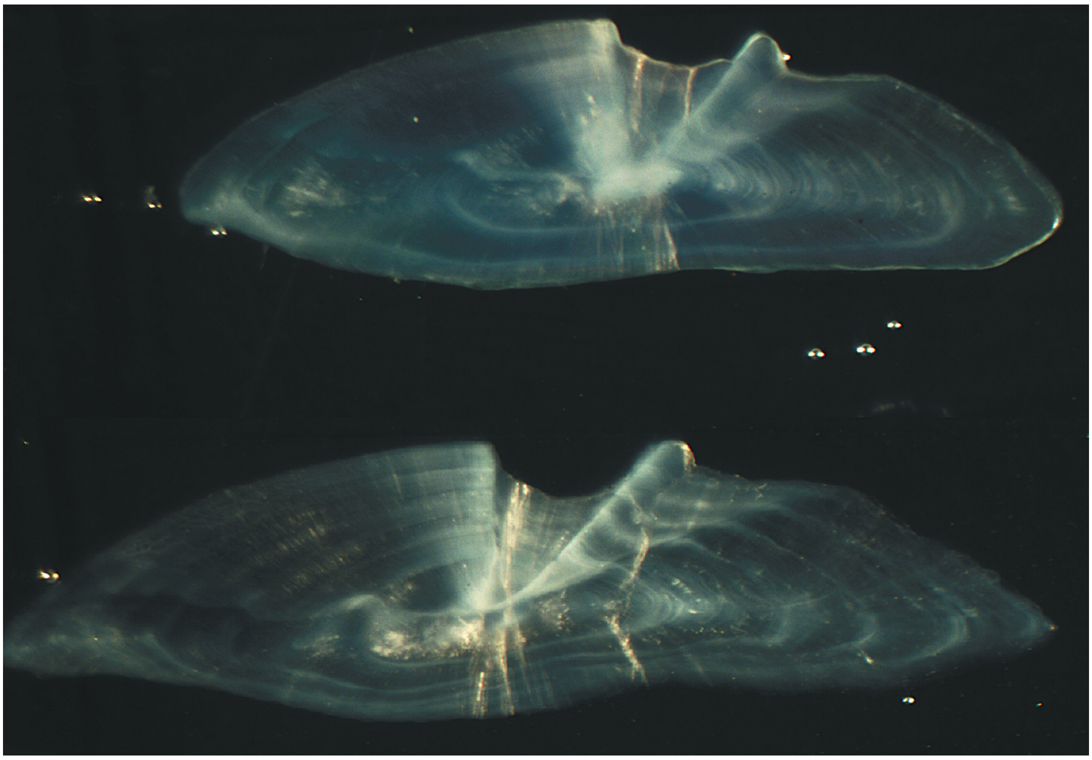


Figure 3.2 Images of a transverse section from a grass emperor sagittal otolith showing zones used for the interpretation of validation of annular ring formation.

Examination of otolith margins showed formation of the annulus between September and December of the first year for grass emperor in Shark Bay. This period coincided with the onset of reproductive maturity and spawning activity (see Chapter 2). Therefore, the generalised birth date was established as January for Shark Bay populations of the grass emperor. It was possible to calculate an adjusted fractional age for each fish using the following algorithm:

If the month of capture was between September and December; and,

If MI = 2, then age in months = (ring count + 1) x 12 (convert to months) - (12 - month of capture).

If MI ≠ 2, then age in months = (ring count) x 12 (convert to months) - (12 - month of capture).

If the month of capture was between January to August and the marginal increment category = 1; then,

Age in months = ring count x 12 (convert to months) + month of capture.

Monthly length frequency analysis

The monthly length frequency data for grass emperor collected from the eastern and western gulfs and the oceanic region using all gear types for the entire study period was compiled to look for trends in the size compositions (representing age classes) by month and region.

Growth model

A von Bertalanffy growth curve was used to model the growth of grass emperor. The model is,

$$L = L_\infty(1 - \exp(-Kt) + \varepsilon,$$

where $\varepsilon \sim N(0,\sigma^2)$, L is the total length of the fish at age t, L_∞ is the asymptotic size and K is the growth constant. This growth equation was used to model the growth of the grass emperor stocks in the gulfs and ocean regions for both sexes.

Growth model with dummy variables

Analysis of regression performed with dummy variables has the same power to detect a difference between two levels within a factor as does analysis of variance. Nonlinear regression with dummy variables was undertaken to test the difference in estimated asymptotic size (L_∞) and estimated growth constant (K) of male and female grass emperor, and to compare growth between each gulf of Shark Bay and the adjacent ocean. The model is,

$$L = (L_\infty + \alpha z)(1 - \exp(-(K + \gamma z)t) + \varepsilon,$$

where, $\varepsilon \sim N(0,\sigma^2)$, z is a dummy variable with the value of 1 equal to the control group and 0 for all other conditions, and α and γ are to be determined. The hypothesis of $\alpha = 0$ can be used to test the whether the difference in L_∞ is significant or not. Similarly, the hypothesis of $\gamma = 0$ can be used to test the whether the difference in K is significant or not.

Relationships between otolith weight and age and body morphometrics

Linear regression analyses were used to investigate relationships between otolith weight, the age of the fish, the morphometrics of total length, fork length and standard weight and body weight. Establishing a relationship between otolith weight and one (or more) body morphometrics would be useful for future monitoring of age from the recreational catch.

3.3 Results

Collection and processing of samples

Between 1999 and 2001, 5,022 grass emperors were caught using five fishing methods from the eastern and western gulfs and ocean waters of Shark Bay (Tables 3.2 and 3.3). The average length of an ocean caught fish was greater than from either gulf, while the western gulf produced a larger average size fish than the eastern gulf. Females comprised more than 50% of the total catch and showed a slightly greater mean length than the males. The greatest number and size distribution of grass emperor were caught by line (Figure 3.3). In addition to the primary fishing techniques (lines and traps), some seine and trawl shots were also undertaken but not on a regular basis; these nets tended to catch the smaller fish. However, despite considerable fishing effort and trials of several fishing techniques in various habitats, we were not able to identify specific nursery areas and collect adequate numbers of juveniles (Table 3.4, Figures 3.4 and 3.5). Trapping effort associated with the grass emperor program was consistent throughout the study with 3 traps used on every trip (except April 2000 and 2001 when 6 traps were used during the sampling for the stock identity project and two boats were used). Only one 0^+ fish was captured in 1999 and none during 2000 and 2001. No 0^+ fish were collected using seine nets, trawls and snorkelling. The Murdoch University blue manna crab sampling program conducted 22 trips to Shark Bay with their trawl nets covering 24,000 m^2 during each trip. They collected six 0^+ fish in 1999 and none in 1998 and 2000. Finally, the Department of Fisheries pink snapper program also conducted an extensive sampling program in Shark Bay that produced one 0^+ fish in both 1998 and 1999.

Table 3.2 Summary statistics for the mean total length of *L. laticaudis* caught from each region of Shark Bay, Western Australia.

Region	Number of fish caught all methods	Minimum length (mm)	Mean length (mm)	Maximum length (mm)
Ocean	132	175	383.4	619
Eastern Gulf	2,685	69	279.6	637
Western Gulf	2,205	68	285.2	573
Total	**5,022**			

Table 3.3 Summary statistics for the mean total length of *L. laticaudis*, by sex, caught from all regions of Shark Bay, Western Australia.

Sex	Number of fish caught all methods	Minimum length (mm)	Mean length (mm) $\pm$ 1 std dev.	Maximum length (mm)
Blank	143	69	242.9 (96.3)	584
Female	2,768	143	292.8 (76.7)	637
Juvenile	220	68	168.6 (30.1)	270
Male	1,871	145	289.3 (75.0)	599
Unknown	20	128	304.8 (131.0)	614
Total	**5,022**			

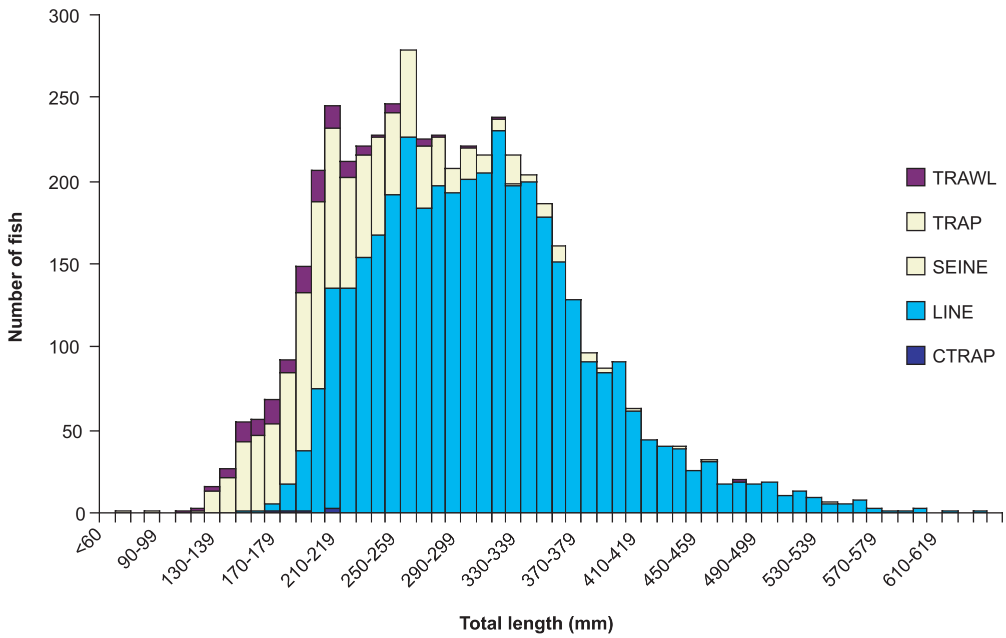


Figure 3.3 The number of fish caught by total length (mm) by the five fishing methods.

Table 3.4 Summary statistics for the number of 0^+ and 1^+ *L. laticaudis* caught by project from all regions of Shark Bay, Western Australia using different gear types between 1998 and 2001, inclusive.

Year	Murdoch University sampling program		Dept of Fisheries pink snapper sampling program		Dept of Fisheries grass emperor sampling program		
Gear	trawl	trawl	trap	trap	trap	trap	line
	0+	1+	0+	1+	0+	1+	1+
1998			1		ns	ns	ns
1999	6	16	1		1	19	8
2000		3	0	11	0	60	2
2001	ns	ns	ns	ns	0	5	0

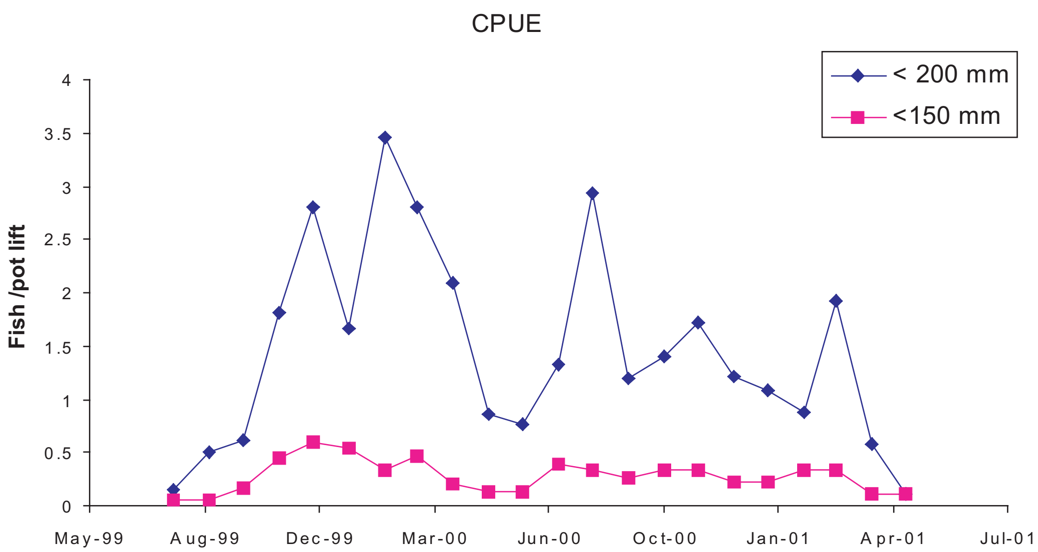


Figure 3.4 The catch per unit effort of juvenile fish caught over time from the eastern and western gulfs of Shark Bay, Western Australia.

Conversion formulae

The total length, fork length and standard length were recorded for all grass emperor, when possible. Conversion formulae were applied to these values and the following statistical relationships established. The conversion ratio from standard length to total length is 1.27 ($p = <0.01$); and the conversion ratio from fork length to total length is 1.09 ($p = <0.01$).

Length – weight relationships

The relationships between length and weight for females, males and both sexes combined from each region in Shark Bay demonstrated that the intercept ($\hat{a}$) and slope ($\hat{b}$) parameter estimates were highly significant, and different between sexes in the eastern gulf and ocean regions, but not in the western gulf (Tables 3.5 and 3.6). There were no significant differences in the length-weight relationships between regions (Table 3.7).

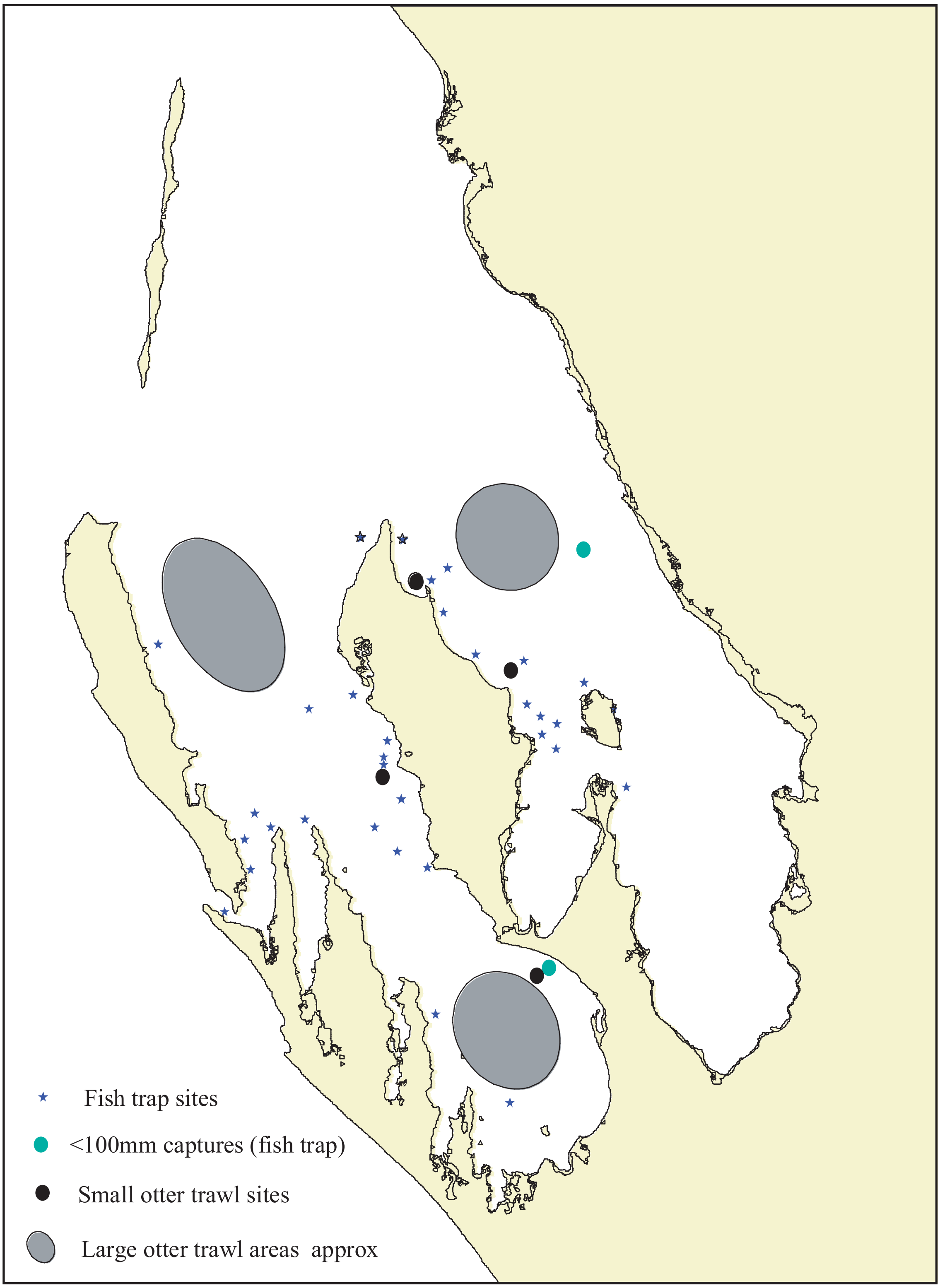


Figure 3.5 Grounds sampled in Shark Bay using various gear types by different sampling programs that produced a small number of young of the year fish between 1998 and 2001.

Comparison of ring counts from whole versus sectioned adult otoliths

A sample of 97 otoliths read by readers 1 and 2 were used to determine if a bias existed between ring counts from whole versus sectioned otoliths. These otoliths spanned a wide range of ring counts from 0 through 11. Regression analysis of whole versus section ring counts indicated the slope ($\hat{b}$ = 0.965) was significantly different from one ($t = 18.08$, $p < 0.0001$) and therefore a bias existed between these two ageing methods. Visualising the bias between methods demonstrated the difference in the ring counts between sectioned and whole otoliths to be minimal until ages greater than 8 (Figure 3.6). Ring counts of nine through 11 showed an increasing trend for the whole otolith ring counts to be less than the sectioned otolith ring count, by up to four rings.

Table 3.5 Total length and weight relationship for *L. laticaudis* by sex and collection region.

Region (sex)	$\hat{a}$ (std. error) (10^{-6})	$\hat{b}$ (std. error)	Number of fish
Eastern Gulf (total)	9.11 (0.35)	3.09 (0.0065)	2,336
Eastern Gulf (female)	8.19 (0.46)	3.11 (0.0093)	1,280
Eastern Gulf (male)	11.84 (0.66)	3.04 (0.009)	903
Ocean (total)	7.71 (1.39)	3.12 (0.029)	21
Ocean (female)	5.19 (1.17)	3.18 (0.036)	11
Ocean (male)	13.71 (2.84)	3.03 (0.034)	10
Western Gulf (total)	9.27 (0.35)	3.09 (0.0063)	1,998
Western Gulf (female)	9.03 (0.49)	3.09 (0.0093)	1,241
Western Gulf (male)	9.15 (0.57)	3.09 (0.010)	608

Table 3.6 Probability (p-value) of a difference in length-weight relationship by sex of *L. laticaudis* collected from Shark Bay, Western Australia.

Region (parameter)	p-value
Eastern Gulf ($\hat{a}$)	**0.00**
Eastern Gulf ($\hat{b}$)	**0.00**
Ocean ($\hat{a}$)	**0.02**
Ocean ($\hat{b}$)	**0.01**
Western Gulf ($\hat{a}$)	0.87
Western Gulf ($\hat{b}$)	0.81

Table 3.7 Probability (p-value) of a difference in length-weight relationship of *L. laticaudis* by region collected from Shark Bay, Western Australia.

Region (parameter)	Ocean ($\hat{a}$)	Ocean ($\hat{b}$)	Western Gulf ($\hat{a}$)	Western Gulf ($\hat{b}$)
Eastern Gulf ($\hat{a}$)	0.39		0.75	
Eastern Gulf ($\hat{b}$)		0.34		0.78
Ocean ($\hat{a}$)			0.28	
Ocean ($\hat{b}$)				0.25

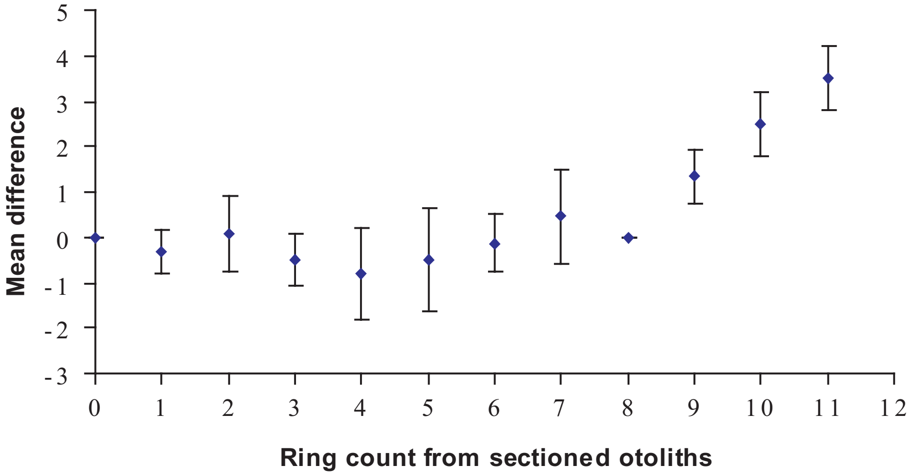


Figure 3.6 A comparison of ring counts between sectioned and whole otoliths in *L. laticaudis* (n = 97). Mean differences (± 1 standard deviation) between ring counts from sectioned minus whole otoliths were plotted against ring count from sectioned otoliths.

Precision of ring counts

An index of average percent error (IAPE) was calculated to determine the precision of ring counts between otolith readers. A subset of 131-sectioned otoliths were chosen as representative of the total sample. In this subset of otoliths the IAPE was 1.79% between readers, indicating a high degree of consistency in the interpretations of otolith internal structures.

Marginal increment analysis

Grass emperor otoliths collected monthly between July 1999 and August 2001 from fish over a wide size range provided a complete time series in order to examine the periodicity of opaque zone formation (Figure 3.7). Grass emperor with one opaque zone were only collected in small numbers and were not available each month. Grass emperor showed between one and 14 opaque zones. All otoliths from fish collected between February and July revealed translucent margins. Note that because the patterns in marginal increments were not always clearly evident in each class of otoliths, the description represents a summary of the most consistent trends. The general pattern for those otoliths with two to five opaque zones was for the marginal increment to increase throughout this same period. This pattern was reversed for those otoliths with more than six opaque zones in 2000. Otoliths formed opaque margins from October to January (Figures 3.7 & 3.8), but this was not evident in otoliths with three opaque zones in 2000. By February, all otoliths had translucent margins, and the formation of the opaque zone had been completed.

The otolith marginal categories followed accordingly, with category 1 (1-50% of previous translucent zone) most prevalent between February and April and category 2 (51-100% of previous translucent zone) common between May and August (Figure 3.8). Otoliths with opaque margins (category 0) were most common between September and January. This pattern was clear for fish with either 2 or 5 opaque rings during 1999 and 2000. During 2001, there were no grass emperor collected with 2 opaque rings and few fish with 5 opaque rings. Examination of the margin condition for all age groups demonstrates the same annual pattern of opaque formation; while the timing is somewhat variable for different ages, opaque formation consistently occurs between September and December. Given this consistency across all age classes examined, and the concurrence between the MI and marginal categories patterns for the younger age classes, it is reasonable to infer that the older age classes also form rings on their otoliths at annual intervals.

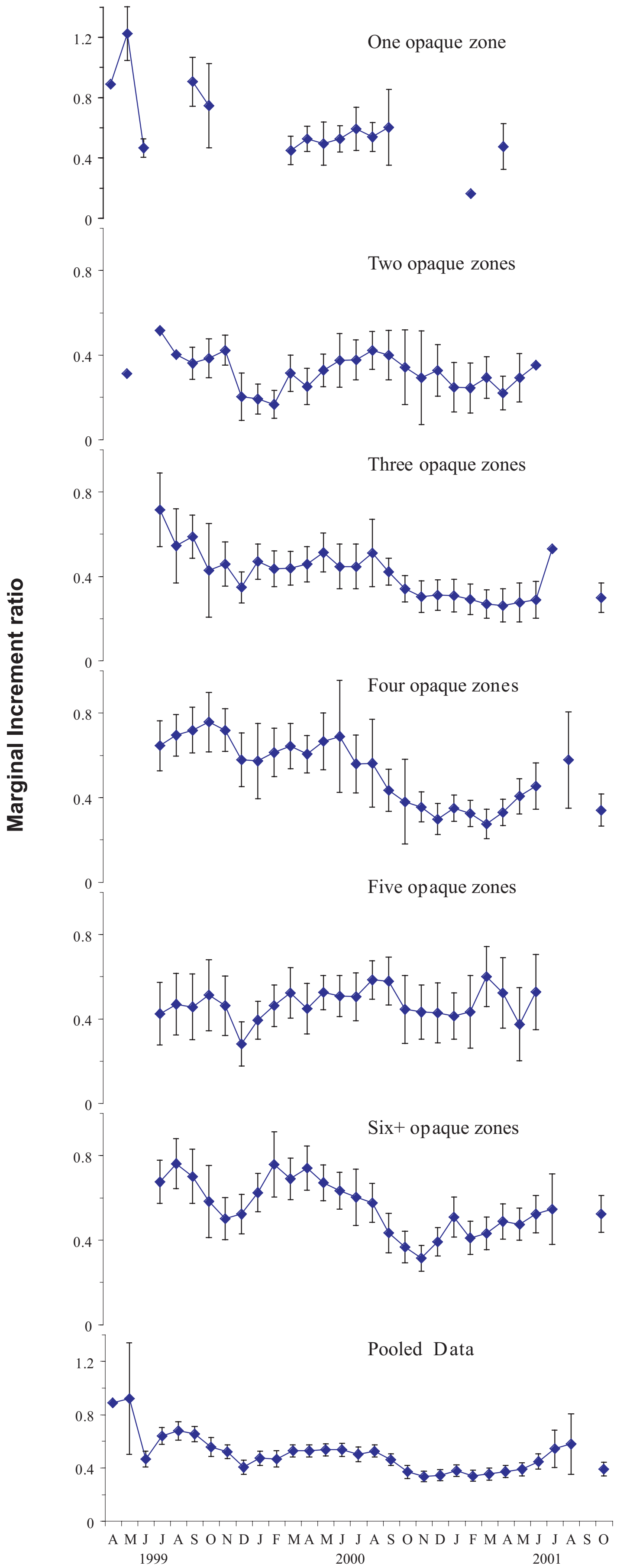


Figure 3.7 Mean monthly marginal increment (number of fish sampled and +1 std error) for sagittal otoliths of *Lethrinus laticaudis* collected from Shark Bay between April 1999 and October 2001.

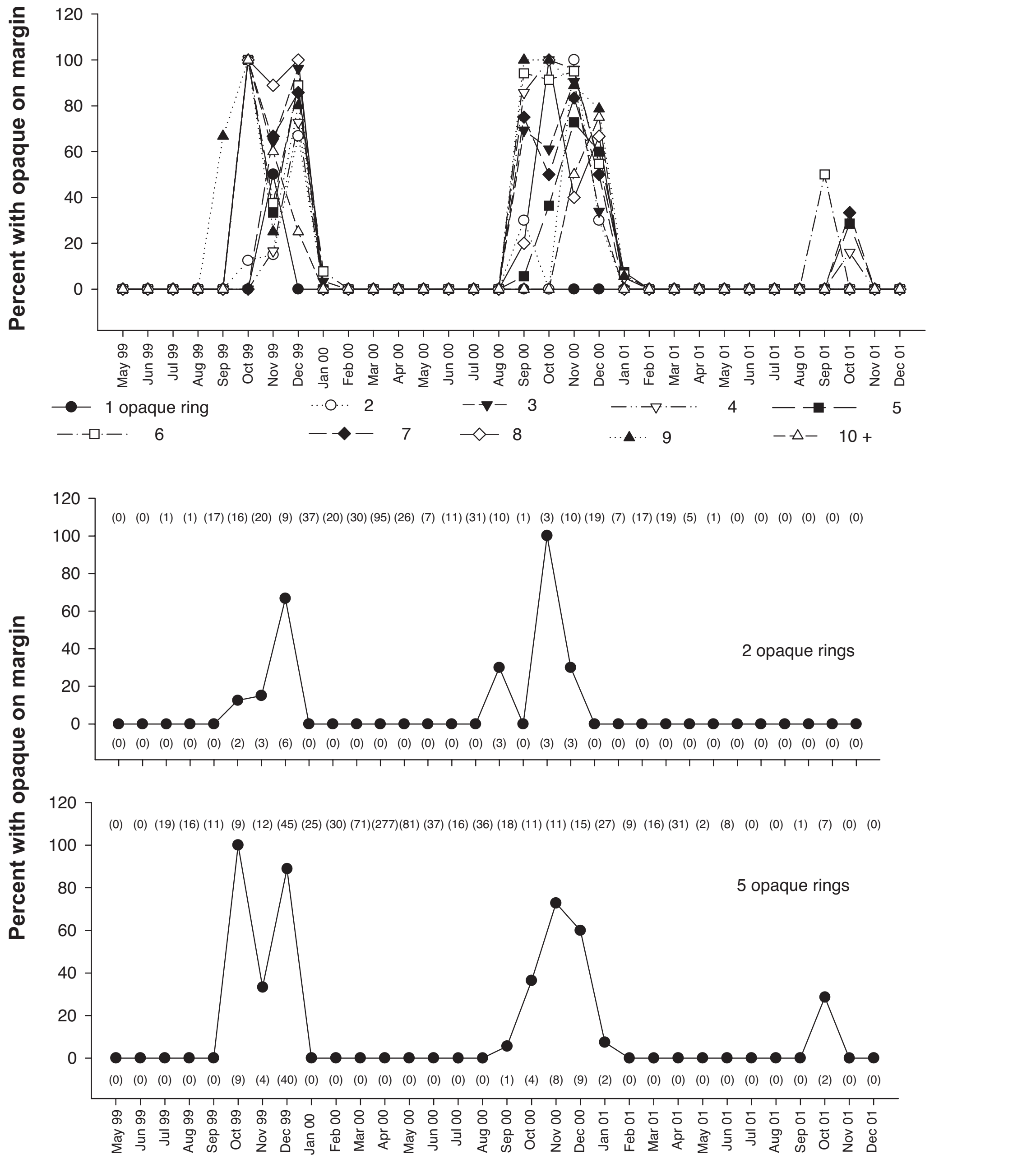


Figure 3.8 Percent of sectioned sagittal otoliths examined with an opaque ring on the outer margin. a) All age classes; b) fish with two opaque rings; c) fish with five opaque rings. In both b) and c) the numbers in parentheses above the line are the sample size and those below are the number with an opaque ring on the outer margin.

Monthly length frequency analysis

There was considerable variation in the numbers of grass emperor caught monthly from each region. Preliminary examination of the length frequency data by month revealed that in many cases there were insufficient numbers of fish caught to produce a reliable length frequency histogram; the monthly data are therefore not presented. Rather, annual summaries of the data are shown, and only for the eastern and western gulfs (Figures 3.9a-f). The length frequency distribution of grass emperor indicated a lack of a discrete young-of-the-year age class recruiting into these sites (Figure 3.9), reflecting the low vulnerability of young fish to capture in this project. Furthermore, even at the annual level there was little consistency in the presence and location of modes between years for either the eastern or western gulfs. This is attributable to the wide range in sizes for any given age, evident from the von Bertalanffy plots for each region sampled (see Figure 3.11). Length frequency data are thus of less value than age composition data, see next, for assessing the age structure of grass emperor stocks.

Age structure and estimation of total mortality

A summary of the annual age compositions (Figure 3.10) clearly illustrates the small number of fish that are 0^+ and greater then 8^+ years old in the samples. An abundant 4^+ year old cohort identified in 1999 can be followed through successive years to 7^+ year old fish in 2002, at which time it was still the dominant age class. The lack of grass emperor from the 0^+ age class makes it difficult to identify settlement period and precluded the use of back-calculation of the daily rings from these newly settled fishes to verify spawning date estimates.

Total mortality (fishing and natural) for an exploited stock can be determined using catch-at-age (or catch curve) analysis, which estimates the rate of decrease in abundance over time of the fully recruited age classes. Recruitment pulses, such as that which resulted in the strong 4+ year class of 1999, invalidate the assumption of constant recruitment (e.g. Haddon, 2001) necessary for generating a relationship between abundance and time. Therefore, estimates of total mortality were made for each year using age composition data that both included and excluded the dominant year class. To ensure that the estimates were based on reasonable sample sizes, the age composition data for the eastern and western gulfs were combined for these regression analyses. The estimates of total mortality fell within the range of 0.41 to 0.57; we had no other evidence against which to decide which end of this range might be more accurate.

Growth of grass emperor

Von Bertalanffy growth curve parameters were estimated for grass emperor length at age for both sexes collected from the eastern and western gulfs and the adjacent ocean sites (Table 3.8, Figure 3.11). The trend in the L_∞ values demonstrated that females from the three regions consistently showed higher values than males. This relationship was statistically significant only for fish collected from the eastern gulf (Table 3.9). There was no statistically significant relationship between the K values for different sexes of fish (Table 3.9). Spatially, the ocean fish had a greater L_∞ than the gulfs, and the eastern gulf produced higher values of L_∞ than the western gulf. There was a highly significant statistical relationship demonstrated between these three regions (Table 3.10). Similarly, there was a highly significant statistical relationship among K values over the different regions (Table 3.10).

Table 3.8 Estimates of von Bertalanffy growth model parameters for *L. laticaudis* from Shark Bay, Western Australia.

Region (sex)	L_∞ (st. error) mm TL	K (st. error)	Number of fish
Eastern Gulf (total)	468.89 (8.24)	0.20 (0.0066)	2,167
Eastern Gulf (female)	494.46 (12.24)	0.19 (0.0084)	1,206
Eastern Gulf (male)	449.55 (13.02)	0.21 (0.012)	837
Ocean (total)	592.23 (23.77)	0.23 (0.017)	128
Ocean (female)	613.08 (40.73)	0.22 (0.026)	69
Ocean (male)	575.77 (38.00)	0.23 (0.032)	42
Western Gulf (total)	403.71 (4.48)	0.27 (0.0065)	2,078
Western Gulf (female)	404.58 (5.58)	0.27 (0.0081)	1,296
Western Gulf (male)	398.93 (8.28)	0.27 (0.013)	649

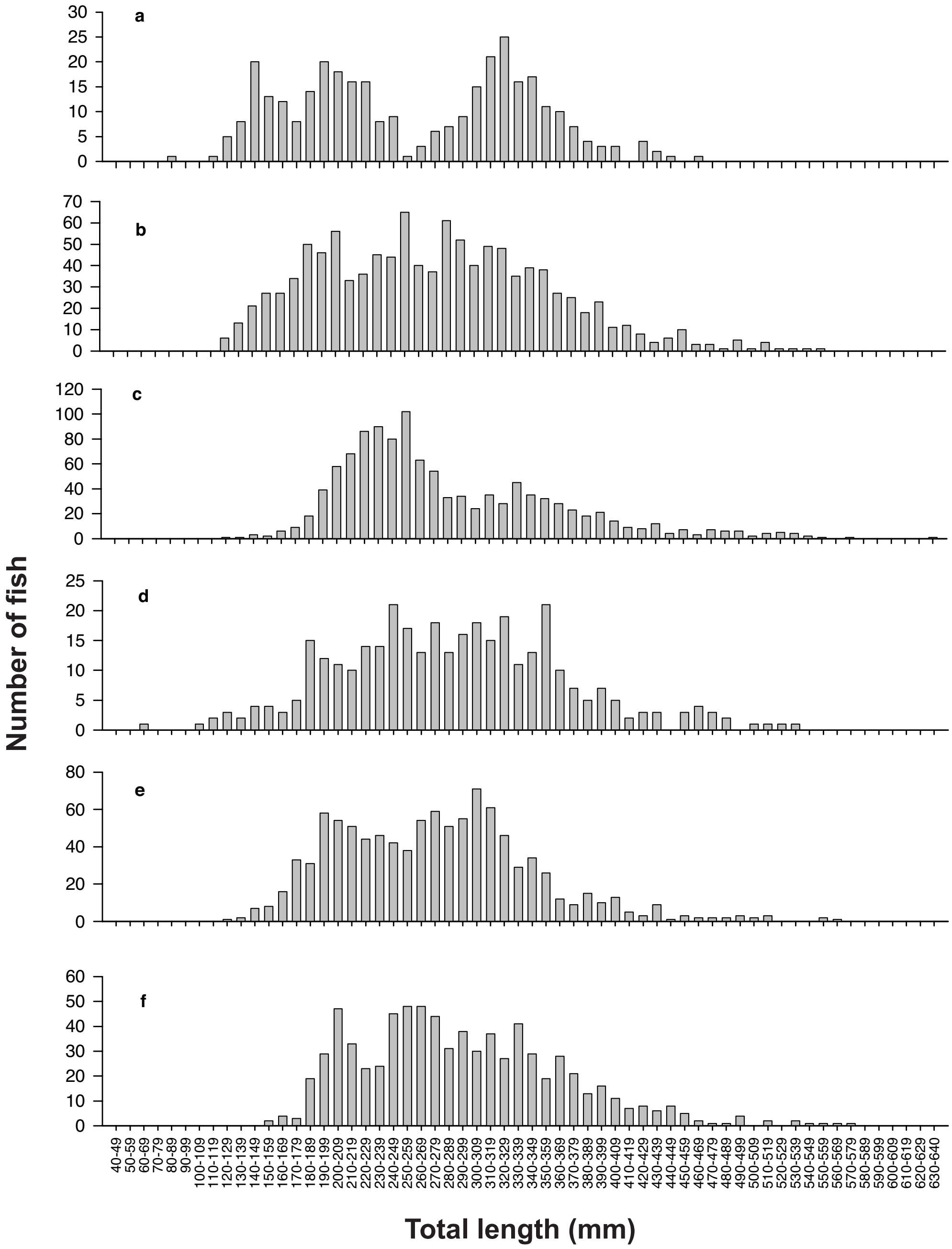


Figure 3.9 Annual length frequency distribution of grass emperor, *Lethrinus laticaudis*, from the eastern (a – 1999, n = 335; b – 2000, n = 1,107; c – 2001, n = 1,132) and western gulfs (d – 1999, n = 354; e – 2000, n = 1,014; f – 2001, n = 760).

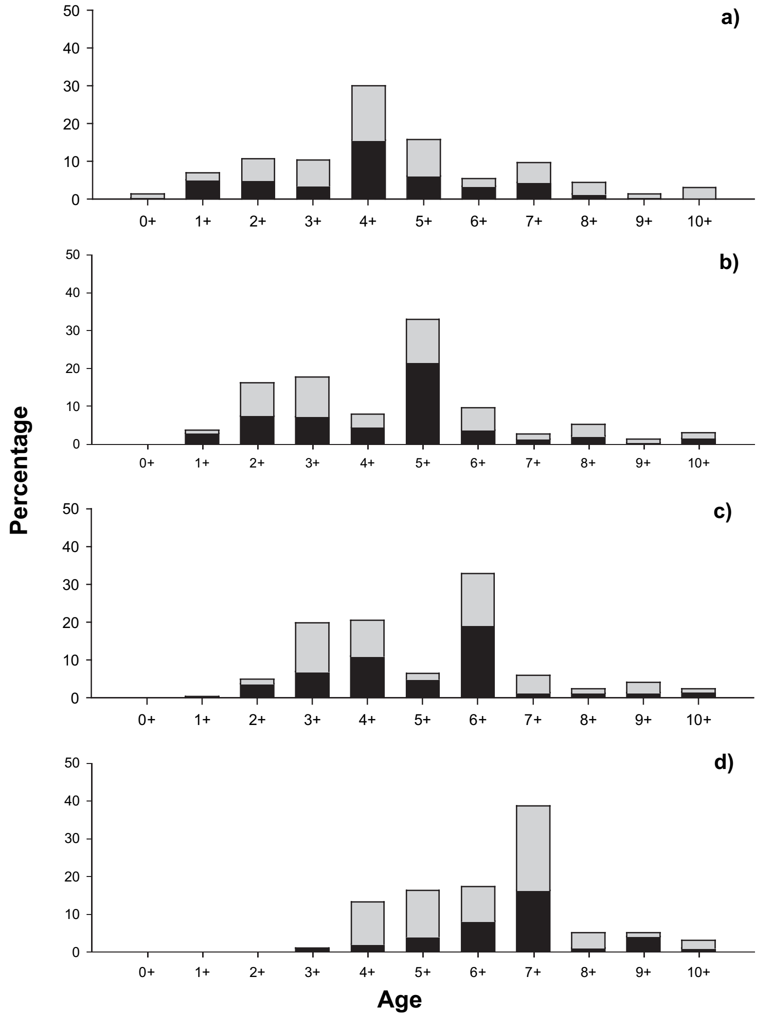
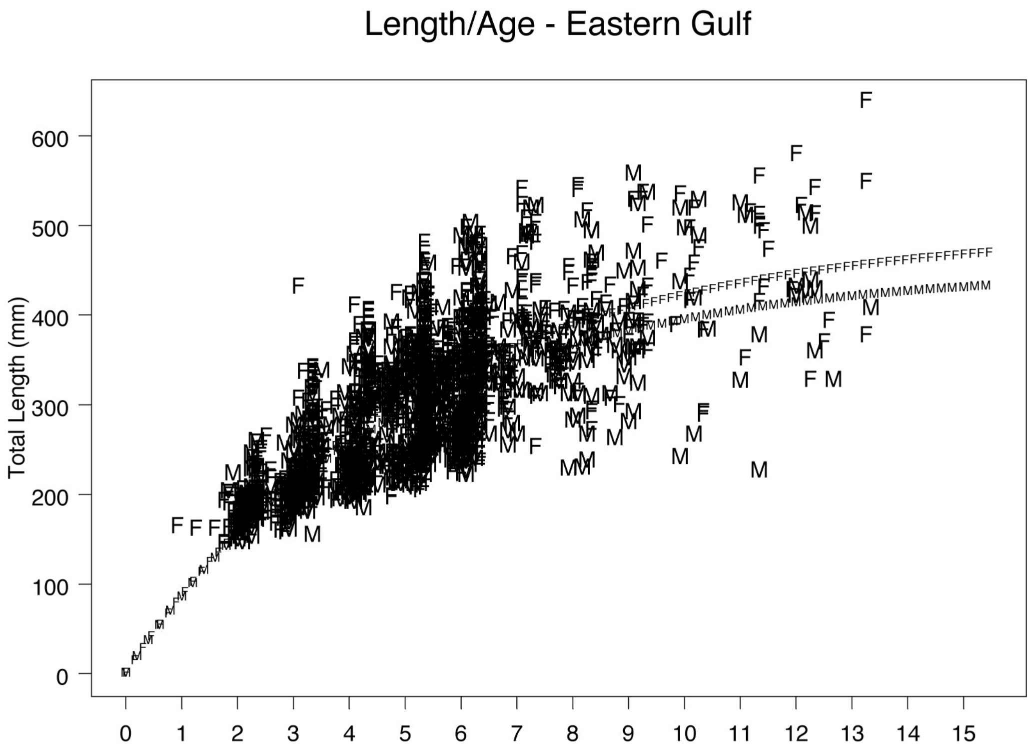


Figure 3.10 The percentage of grass emperor, *Lethrinus laticaudis* caught from the eastern (black shading) and western (grey shading) gulf regions between 1999 and 2002 inclusive from age classes 0+ through 10+ (and greater). a) 1999 (n= 582), b) 2000 (n= 2,096), c) 2001 (n=1,483), d) 2002 (n=98).

Table 3.9 Probability (p-value) of a difference in von Bertalanffy growth model parameters by sex of *L. laticaudis* collected from Shark Bay, Western Australia.

Region (parameter)	p-value
Eastern Gulf (L_∞)	**0.01**
Eastern Gulf (K)	0.19
Ocean (L_∞)	0.50
Ocean (K)	0.70
Western Gulf (L_∞)	0.56
Western Gulf (K)	0.91

(a)



b)

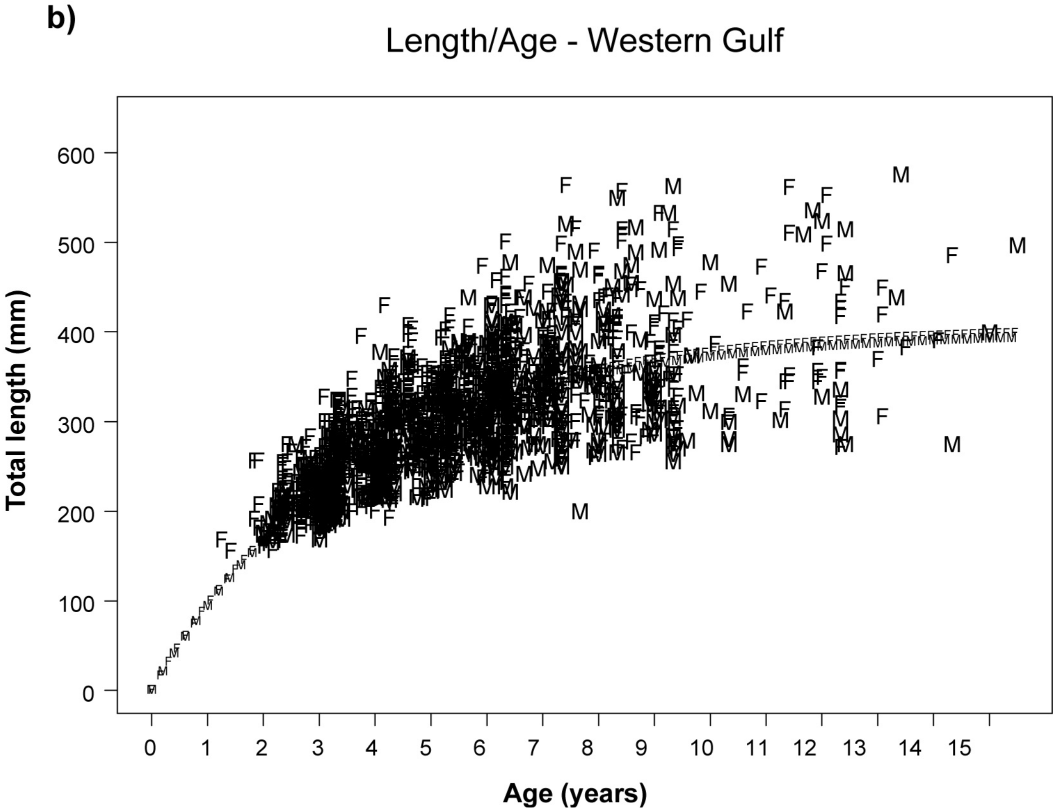


Figure 3.11 ***continued.***

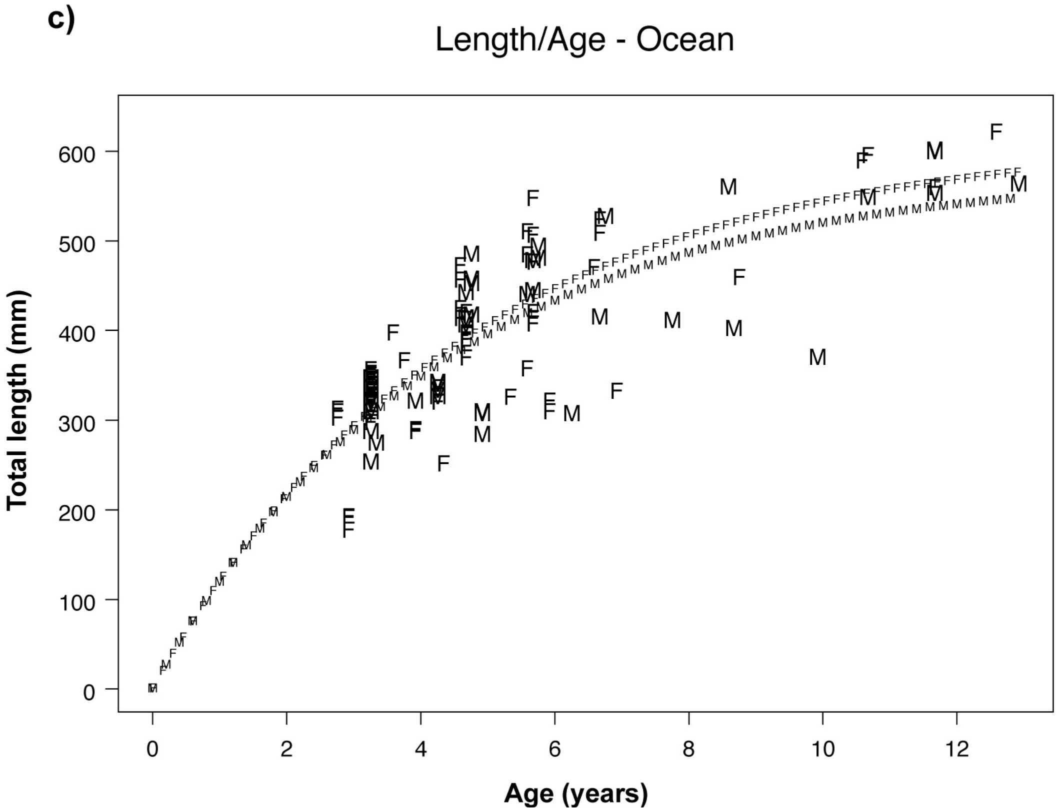


Figure 3.11 Von Bertalanffy growth curve of *Lethrinus laticaudis* from; a) the eastern gulf, b) the western gulf of Shark Bay, and c) the adjacent oceanic regions, using total length at age.

Table 3.10 Probability (p-value) of a difference in von Bertalanffy growth model parameters of *L. laticaudis* by region collected from Shark Bay, Western Australia.

Region (parameter)	Ocean (L_∞)	Ocean (K)	Western Gulf (L_∞)	Western Gulf (K)
Eastern Gulf (L_∞)	0.00		0.00	
Eastern Gulf (K)		0.02		0.00
Ocean (L_∞)			0.00	
Ocean (K)				0.01

Relationships between otolith weight and age and body morphometrics

Prior to establishing a relationship between otolith weight and other variables, a linear regression was performed between the right and left otolith weights to determine if biases existed. A significant linear relationship was shown between the left and right otoliths (Table 3.11). The remainder of the regressions models were developed using the left otolith weight. There was a significant and positive relationship between otolith weight and the age of the fish; variation in otolith weight increases with increasing age (Table 3.11, Figure 3.12a). There was a statistically significant relationship between otolith weight and the three measures of body length. Otolith weight increases with total (Figure 3.12b), fork and standard length in a similar manner, with increasing variation in otolith weight with increases in the length measures (Table 3.11, Figure 3.12). Lastly, the relationship between otolith weight and total body weight was statistically significant, however the relationship was non-linear. Applying a square root transformation to the values of total weight linearised the relationship with otolith weight (Table 3.11, Figure 3.12c).

Table 3.11 Statistical relationship between the weight of the otolith and fish age and body morphometrics for

***L. laticaudis* from Shark Bay. The number of fish used in the analyses was 4,038 except in the case of left otolith weight vs. total weight when the number of fish was 3,716.**

Variables	R^2	F	p	$\hat{a}$	$\hat{b}$
Left *vs*. right otolith weight	0.995	848,235.3	**0.0**	6.95×10^{-5}	1.00
Left otolith weight *vs*. fractional age	0.724	10,625.0	**0.0**	-5.7×10^{-3}	2.48×10^{-3}
Left otolith weight *vs*. total length	0.894	34,325.1	**0.0**	-1.0×10^{-1}	8.69×10^{-4}
Left otolith weight *vs*. fork length	0.895	34,497.6	**0.0**	-1.0×10^{-1}	9.36×10^{-4}
Left otolith weight *vs*. standard length	0.894	34,117.4	**0.0**	-9.0×10^{-2}	10.71×10^{-4}
Left otolith weight *vs*. square root total weight	0.892	30,896.5	**0.0**	-2.1×10^{-2}	8.51×10^{-3}

a)

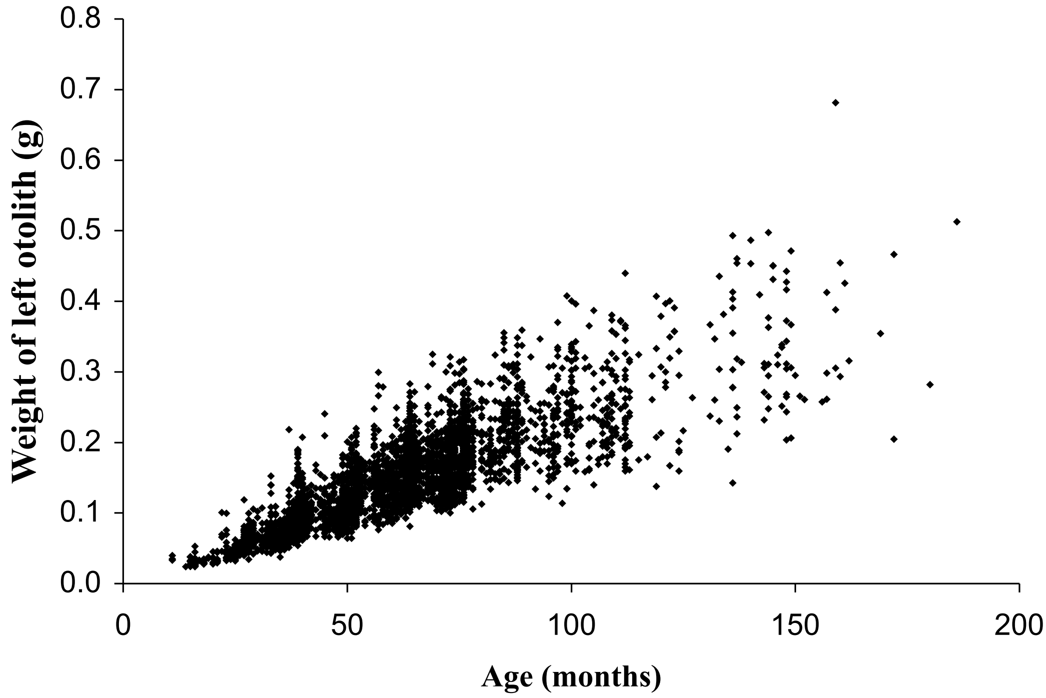
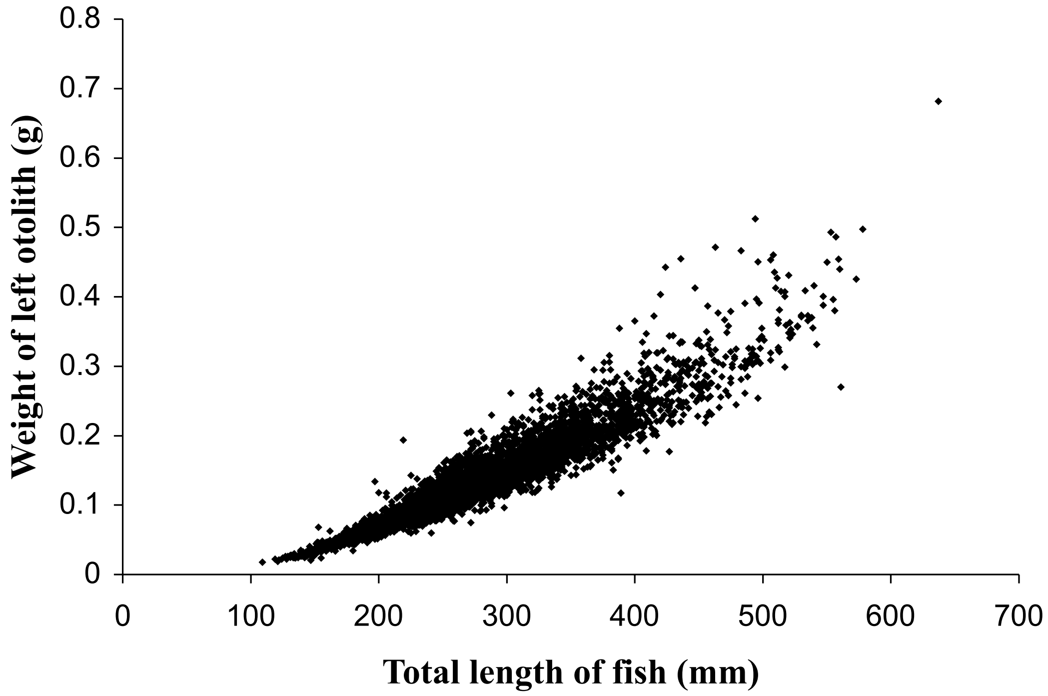


Figure 3.12 ***continued.***

b)



c)

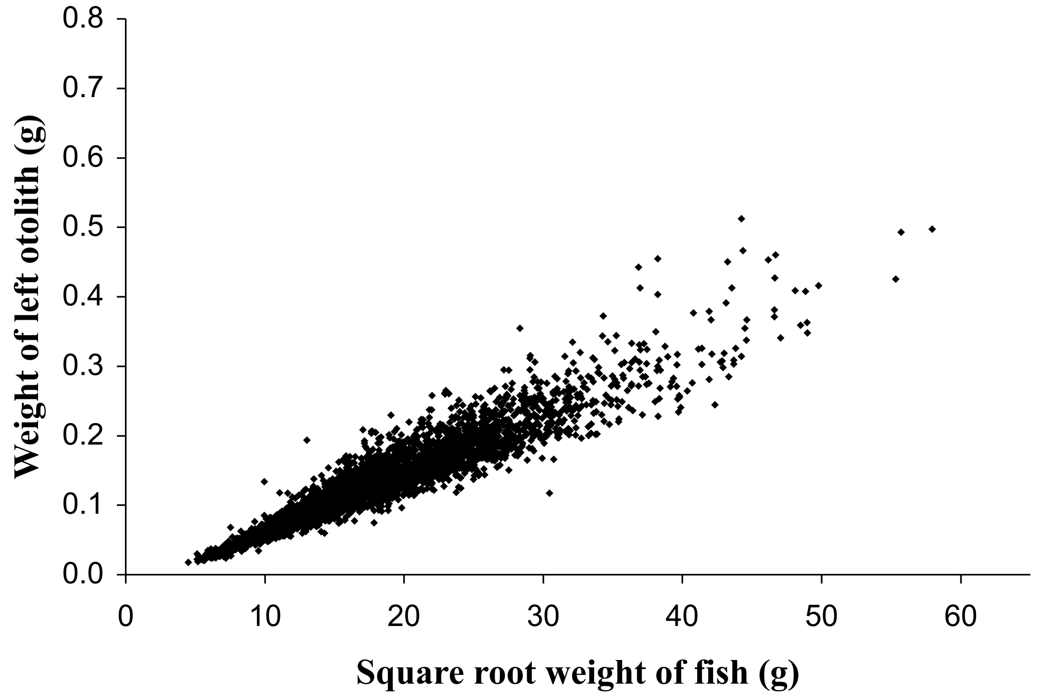


Figure 3.12 Relationship between a) weight of the left otolith versus fractional age (n = 4,038), b) weight of the left otolith versus total length of the grass emperor (n = 4,038) and c) weight of the left otolith versus square root transformation of the whole weight of the grass emperor (n = 3,716).

3.4 Discussion

Consistency of age determination

The otoliths can be used for age determination of *L. laticaudis*. There is however a statistically significant bias between estimates derived from whole versus sectioned otoliths. The whole otoliths for fish older than approximately 8 years consistently underestimated the age as compared to the sectioned preparations. This result is in agreement with other researchers (Loubens 1978, Boehlert 1985, Brown and Sumpton 1998) who propose that as an otolith grows, its thickness will obscure the internal markings making it difficult to read the annuli. Although not statistically significant, Laursen et al. (1999) indicated a discrepancy between whole versus sectioned otoliths, with the latter producing higher ages. Brown and Sumpton (1998) have supported this finding by determining that otolith thickness in *L. miniatus* develops at a faster rate than length. While either whole or sectioned otoliths may be used for age determination to age 8, from age 9 onwards there is a discrepancy between the age-estimate from the two techniques. Sectioned otoliths provide a more accurate appraisal of the age structure.

The index of average percent error (IAPE) calculates the precision, or consistency, between age determinations. This method is superior to other percent agreement methods as the IAPE is not independent of the age structure of the species (Beamish and Fournier 1981). The IAPE of 1.79% indicated very consistent results between readers. This may be explained, in part by the clearly defined opaque and transparent zones on sectioned otoliths. This value is lower than that reported by Brown and Sumpton (1998) for sectioned otoliths of *L. miniatus* (3.83%) and by Ferreira and Russ (1994) for coral trout *Plectropomus leopardus*, collected from the Great Barrier Reef (6.7%). Brown and Sumpton (1998) tracked changes in the IAPE values over time and found that the value decreased over time, signifying increasing expertise of the otolith readers.

Validation of the annuli

The opaque zones on the otoliths of *L. laticaudis* collected from the eastern and western gulfs and ocean regions of Shark Bay can be equated to annular rings and can be used for age determination. Validation of these annular ring(s) was derived from the regular and consistent seasonal development of the otolith margin observed from sectioned otoliths with various ring counts. Wide bands of translucent material (under reflected illumination) are deposited between February and October, representing a period of fast growth through the autumn, winter and early spring. The opaque bands or annual rings are laid down between November and January, signifying slow growth during the late spring and summer months. In this study, grass emperor otoliths exhibited between one and 14 annular rings.

This study represented the first attempt to age grass emperor in Western Australian waters and validate the ageing method using marginal increment analysis. Previously a preliminary ageing study using less than 50 grass emperor in Northern Territory found fish up to 10 years of age and approximately 50 cm fork length. The authors caution against extrapolating this information across a broader spatial and temporal scale (Knuckey et al. 1996). Apart from this study, there have been several age and growth studies involving commercially valuable lethrinids throughout the Indo-Pacific region.

Morales-Nin (1988) provides validation of ageing for a small sample of *Lethrinus nebulosus* from New Caledonia, using three methods. Each method verified that the otolith rings may be considered annuli. This result is of particular interest because it has been suggested that due to seasonally modest environmental fluctuations, tropical fish may not produce discernable annular rings (Toor 1968).

Laursen et al. (1999) indicated that bands on the otolith of *Gymnocranius audleyi* collected from the Great Barrier Reef (GBR), Queensland are identical in form to those verified as annuli using tetracycline marking in *L. nebulosus* (see review in Williams and Russ 1994), and *L. harak* and *L. lentjan* from the GBR (Hilomen 1997). Tetracycline marking has been a successful technique for age validation in many tropical finfish families (see review in Laursen et al. 1999), but is relient on recapture of marked individuals over time, which is not always feasible (Newman 1995).

Brown and Sumpton (1998) investigated validation of age estimation of *L. miniatus* using marginal increments. A clear pattern in the periodicity of annuli formation was evident for fish from a wide variety of ages, leading the researchers to conclude otolith rings represented annuli. The single periodicity of annuli formation was similar to *L. laticaudis* with the opaque zone (slow growth) occurring during the summer months (November-December).

Growth rates of grass emperor

The highest vales of L_∞ were produced by ocean caught *L. laticaudis* (L_∞= 59.2 cm), followed by the eastern gulf (L_∞= 46.9 cm), and then the western gulf (L_∞= 40.3 cm). The oceanic fish were collected both just at the head of Shark Bay and to the north of Shark Bay. Growth may have been greater at the ocean sites due to several factors such as water temperature, salinity, food and habitat availability. The difference in L_∞ between the eastern and western gulfs results, in part, from the large grass emperor collected from Hamelin Pool and the 'patch' within the eastern gulf. Hamelin Pool is within a conservation area renowned for its speciose seagrass meadows reef patches and stromatolites. The region has a history of lower fishing pressure due to relative remoteness from launching facilities. The 'patch', offshore from Monkey Mia, was a favourite recreational fishing location for the take of pink snapper, *Pagrus auratus*, the premier fishing species from Shark Bay. The closure of the "patch" in the late 1990s was designed to protect pink snapper spawning aggregations. Subsequently, the whole of the eastern gulf was closed to pink snapper fishing in 1999. This closure resulted in few anglers fishing the eastern gulf area for a number of years. As well as its intended benefits for pink snapper, the closure also provided a default refuge for grass emperor, which may account for the higher numbers of larger fish.

Dalzell et al. (1987) review estimates of von Bertalanffy parameters for 12 lethrinid species (from eight studies) within the Indo-Pacific region. The values of L_∞ within a species, from multiple studies, vary considerably (e.g. *L. miniatus* from the Gulf of Aden = 106.5 to 58.9 cm total length) as well as between species (e.g. *L. nematacanthus* from New Caledonia at 15.0 cm total length to *L. miniatus* from the Gulf of Aden at 106.5 cm). This variation may result from inherent differences in ageing, sampling and/or computational techniques leading to either under or over estimates of the parameter values. Dalzell et al. (1987) then interpret the previous studies and suggest the lethrinids are a relatively slow growing and long-lived species. Brown and Sumpton (1998) present differences in the growth parameters for *L. miniatus* from two locations on the Great Barrier Reef. *L. miniatus* collected from Swain Reef had an L_∞ = 51.73, K = 0.188, while at Capricorn-Bunker Group L_∞ = 52.06, K = 0.229. These authors postulate the existence of difference stocks; availability of resources (i.e. food) and/ or collection protocols may explain observed variation. Further, differences among comparative estimates of the growth parameters within the species in the western Pacific Ocean (Walker 1975, Church 1985, Loubens 1980 in Brown and Sumpton 1998) highlights the complexity involved in developing rigorous estimates. Within this complex of growth parameter estimates, the grass emperor values of L_∞ and K between oceanic and gulf sites in Shark Bay is similar to that reported for other lethrinids and is within the range of variation noted for other species collected from difference locations (see Dalzell et al. 1987).

Otolith weight and age and body morphometrics

A statistically significant correlation was demonstrated for otolith weight versus age and four body morphometric measures. The strong correlation suggests otolith weight may subsequently act as a proxy for ageing the grass emperor, providing an alternative to reading sectioned otoliths. This would provide a more cost-effective method of monitoring age structure. Otolith weight also showed a high correlation with fish body size measurements (TL, FL, SL and weight) suggesting a physiological basis for this positive relationship. Laursen et al. (1999) described otolith weight as a good indicator of age for *Gymnocranius audleyi* collected from the GBR, as opposed to other otolith dimensions that were measured. Similarly, Francis et al. (1992) determined that otolith weight and not otolith radius provided a better age estimate.

3.5 Summary

The findings from this study suggest that whole and sectioned otoliths produce similar age estimates until approximately 7 to 8 years of age at which time the whole otoliths begin to underestimate age. The marginal increment analysis validated the annual ring structure on the otoliths. The clearly visible movement of a strong year class of fish through the population (4+ in 1999 to 7+ in 2002) provides supporting evidence that the ageing work was robust. Very low numbers of young-of-the year grass emperor obtained in the study may reflect the low vulnerability of young fish to capture in this project. Alternatively, if grass emperor populations only have infrequent strong recruitment events, it is possible that very few young-of-the-year fish were available for capture between 1999 and 2001. The growth rate and average maximum size of grass emperor depended on capture location within the greater Shark Bay region. Ocean regions showed the highest average maximum size and a moderate rate of growth followed by the eastern and the western gulfs. Lastly, it appears otolith weight is a good age estimator, however further statistical analyses would need to be conducted prior to adopting otolith weight as the proxy for a complete ageing study.

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CHAPTER 4.0 Evaluation of the carbon and oxygen stable isotope ratio of the grass emperor, *Lethrinus laticaudis*, otolith carbonate as a possible stock delineation tool

Suzanne Ayvazian, Lionel Glendenning, Ian Keay, Brenton Chatfield, Monty Craine and Dan Gaughan

Objective: Determine the stock structure of grass emperor in Shark Bay using the isotopic composition of otolith carbonate.

The isotopic composition of adult grass emperor were examined from six locations spread through both of the inner gulfs of Shark Bay; results indicate that grass emperor are not panmictic in this region but display some degree of site fidelity. This was displayed most clearly by the distinct isotope signatures between the sites at Hamelin Pool and Dubaut Point, the two closest sites examined in this study. The microchemistry technique demonstrated that there is minimal movement between fish residing at reef habitats separated by non-reef habitat. Thus, in parts of the Shark Bay where habitat suitable for adults is patchily distributed, grass emperor were not mixing to any significant degree, if at all, over the lifespan of individual fish. It was therefore assumed that grass emperor from other sites would also show a high degree of site fidelity unless "patches" of suitable habitat were sufficiently close to warrant movement of individual fish. A high degree of site fidelity indicates that isolated reefs are more susceptible to localised depletions of grass emperor.

4.1 Introduction

The Lethrinidae (commonly known as the emperors) are coastal marine fishes common throughout the western Indo-Pacific oceans. *Lethrinus laticaudis* or grass emperor, is distributed throughout northern Australia, southern Indonesia, Papua New Guinea and the Solomon Islands. Along the Western Australia coastline the southern extent of the species is Shark Bay, approximately 1,000 km north of Perth. In the more northern Kimberley region, this species is an important member of the inshore finfish assemblage (Newman et al. 2003). Research in the Northern Territory of Australia has shown the juveniles prefer seagrass and mangrove habitats while adults were found to occur over hard substrate such as coral reef (Knuckey et al. 1996). In Shark Bay adult grass emperor were caught over hard substrate; however despite considerable sampling effort the young of the year fish were not found (see Chapter 3 for juvenile sampling description).

In Shark Bay, grass emperor is a popular fish targeted by recreational anglers. Shark Bay stocks have come under increased fishing pressure in the last five years as a result of restrictions of catches of pink snapper, the traditional focus of angling effort, and an increase in the level of recreational fishing in this region. A recreational angling survey of the Gascoyne region conducted between 1998-99 revealed that 22 tonnes of grass emperor were landed by recreational fishers in Shark Bay, making this the second most common recreational species (Sumner and Malseed 2002). The commercial catch for this species in Shark Bay is only of the order of a few tonnes annually.

Currently, there is no biological information available on this species from the inner gulfs of Shark Bay on which to develop models of stock status and evaluate current management regulations. In addition, it is desirable to determine whether grass emperor consists of one or several discrete stocks within the gulfs of Shark Bay and whether any stock structure evident is consistent with current management units. Recent accounts of the use of carbon and oxygen stable isotope analyses of otolith carbonate found in pink snapper (*Pagrus auratus*) and tailor (*Pomatomus saltatrix*) in Shark Bay, demonstrate the potential of this technique as a stock delineation tool (Edmonds et al.

1999 and Bastow et al. 2002). The $^{18}O/^{16}O$ and $^{13}C/^{12}C$ ratios in the otolith carbonate from pink snapper stocks indicate no significant degree of movement between oceanic and inner Shark Bay waters, however, tailor stocks appeared less site specific with evidence of considerable mixing between oceanic and inner bay waters.

Stable isotope analyses of teleost otolith carbonate has been studied to establish relationships between isotopic composition and environmental variables (Kalish 1991 a, b). Typically, oxygen isotopes are deposited in equilibrium or close to equilibrium with ambient seawater (Devereux 1967, Kalish 1991a, b, Thorrold et al. 1997). The carbon isotopes are not deposited in equilibrium with ambient seawater (Kalish 1991b), but rather as a result of metabolic rates, diet, and age (Kalish 1991b, Thorrold et al. 1997). Finally it should be noted that a complete understanding or detailed knowledge of the underlying causes and mechanisms of formation of stable isotope composition of fish otolith carbonate does not preclude using the measured differences as an aid in delineation of fish stocks (Edmonds and Fletcher 1997, Edmonds et al. 1999; Stephenson et al. 2001; Bastow et al. 2002).

The marine environment of Shark Bay is unique along the Western Australian coastline. The salinity gradient includes normal oceanic seawater at the entrance to the bay to metahaline waters in Freycinet Estuary (< 50‰) at the head of the western gulf and hypersaline waters in Hamelin Pool (< 65‰) at the head of the eastern gulf. Areas of elevated salinity result from low rainfall levels, little runoff from rivers and high evaporation rates (Logan and Cebulski 1970). These environmental factors result in a partitioning of the isotopes, with the loss of the lighter isotopes to the atmosphere. The heavier isotopes are thereby concentrated in the remaining waters of increased salinity.

The present study addresses the use of isotopic composition of otolith carbonate analysis as a tool for stock structure delineation of *Lethrinus laticaudis* within Shark Bay. In this instance a ‘stock’ is considered to be a group of fish that remain separate, without suggesting that these groups are reproductively isolated. Understanding the population structure, including spatial considerations (i.e. mixing between gulfs and between gulfs and adjacent oceanic waters) is fundamental to the population modelling and subsequent fisheries management approach.

4.2 Material and methods

Collection of L. laticaudis

Grass emperor (*Lethrinus laticaudis*) were caught from six locations in Shark Bay during April 2000 and 2001, using rod and line and baited fishing traps (diameter of 110 cm by 48 cm high with 2.4 cm and 5.5 cm mesh used on the outside). The six locations were chosen to be representative of the various Shark Bay salinity gradients in both the western and eastern gulfs; Fisherman’s Bay (western gulf) and 80 Acres (eastern gulf) - slightly above normal ocean salinity, 2 Mile (western gulf) and Dubaut Point (eastern gulf) - above normal ocean salinity, Freycinet Estuary (western gulf) - metahaline and Hamelin Pool (eastern gulf) - hyersaline (Figure 4.1). Samples from each location in Shark Bay except Freycinet estuary were taken by research staff from patchy reef habitat within an area of diameter <200m; thus, for any one site the same patch was returned to within and between years, which provides spatially restricted samples. In contrast, grass emperor from Freycinet Estuary were taken by recreational fishers over a much broader area of approximately 15 km. Additionally, one sample was taken from Thevenard Island in 1999, outside of the Shark Bay region, to provide a potentially contrasting data point from distant oceanic conditions.

Chemical Preparation of the Otoliths for Isotope Ratio Analysis

Both sagittal otoliths were extracted from the fish as soon as possible after capture, washed with distilled water and placed in a paper envelope prior to physical and chemical degradation. For each individual fish, one of the pair of otoliths was selected at random, cleaned ultrasonically with Milli-Q high purity water, dried (40°C) and powdered using a mortar and pestle. Approximately 100 mg of powder was transferred to a labeled 5 ml plastic vial. Each portion was treated carefully with ~10% sodium hypochlorite (NaOCl) solution to remove residual organic material from the mixture. After approximately 5 minutes, the exhausted sodium hypochlorite was removed and a further portion of fresh sodium hypochlorite added. This process was continued with progressively longer reaction times 10, 60 and to ~240 minutes. During the initial applications of sodium hypochlorite the vials were immersed in a cold water bath to minimise any effects of the heat generated by the rapid exothermic reaction which ensues.

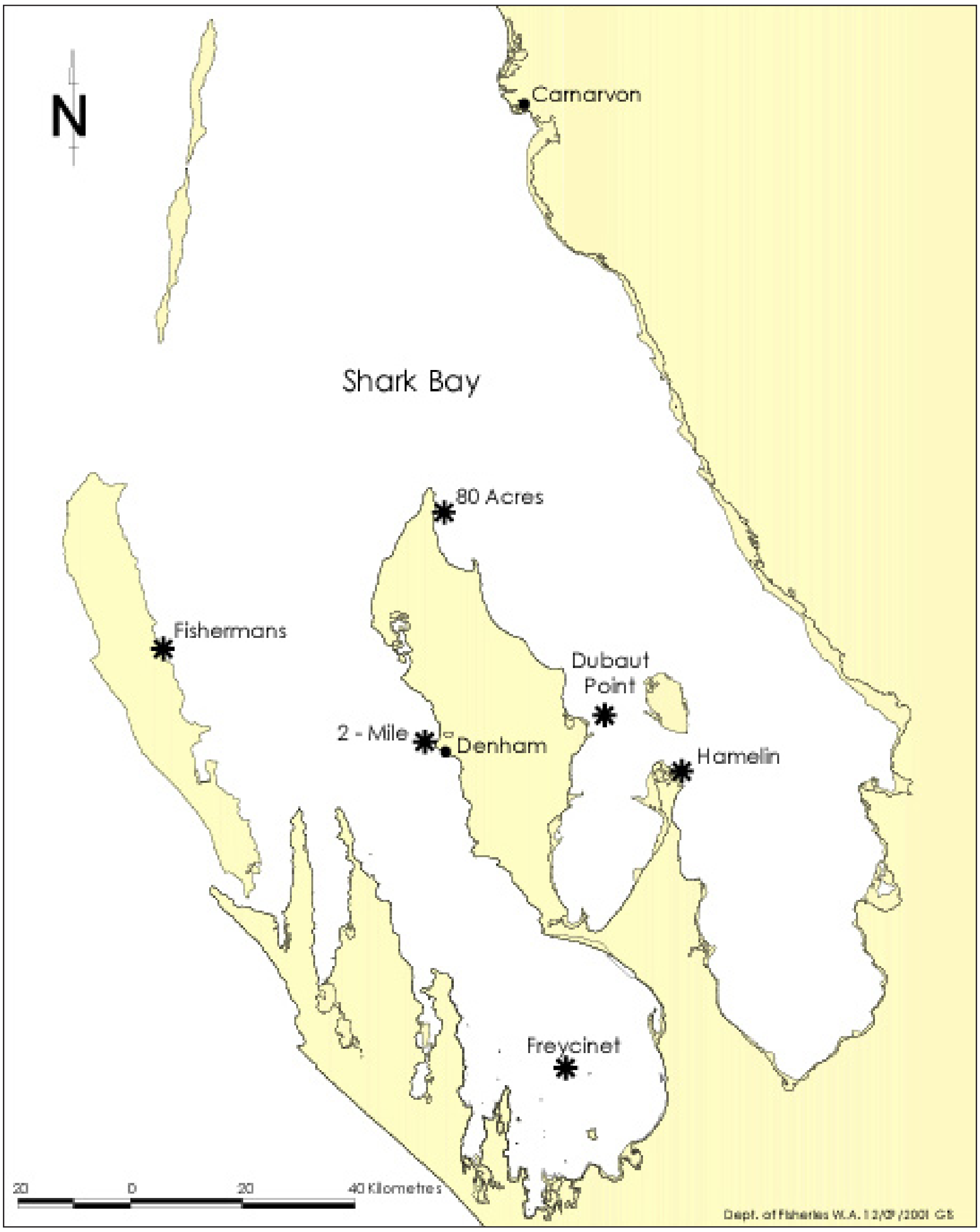


Figure 4.1 Sampling loctaions at Shark Bay.

After this reaction sequence, the resultant calcium carbonate ($CaCO_3$) was rinsed with distilled water four times, with thorough mixing, leaving the sample to stand for at least 5 minutes between rinses. The samples were then dried under reduced pressure in a desiccator for at least 24 hours. A small portion of the dried powder was dissolved in hydrochloric acid (2M) to check that no organic components remained. Should any remaining organic material be detected in the solution, further treatment of the powdered sample with NaOCl for a further ~240 minutes was performed.

The samples were submitted for stable carbon and oxygen (δ ^{13}C and δ ^{18}O) isotope analyses. Stable isotope ratios, ^{13}C:^{12}C and ^{18}O:^{16}O were acquired using standard mass spectrometric techniques after the carbonate was decomposed to CO_2 with 100% phosphoric acid (CSIRO Division of Water Resources, Perth). Values are reported in standard δ notation relative to PDB-1 standard (Pee Dee Belemnite, a specific limestone that is used as a standard for carbonates) (Epstein et al. 1953).

Analysis of covariance (ANCOVA) was used to determine the relationships between δ ^{13}C and δ ^{18}O values and the factors location and otolith weight. This analysis was undertaken for individuals with an otolith weight range of 100–300 mg, and the Figures likewise only show the data for this range of otolith weights. Restricting the otolith weight range improved normality and homogeneity of the data. Otolith weight was treated as a proxy for fish age and was considered a covariate in the analysis to account for the differences in age between individuals within the sample. Type III sums of squares were used to test hypotheses of differences in the population means attributed to each factor, followed by an a posteriori multiple comparisons of means ($\alpha = 0.05$) as required (Day and Quinn 1989, Bastow et al. 2002). These analyses did not include the Thevenard Island data because our goal in this study was to determine whether there were fine-scale stock structure patterns within Shark Bay.

4.3 Results

A total of 520 fish ranging from 143 to 350 mm total length and 1–14 years old were collected from all locations and years (Table 4.1). Carbonate from sagittal otoliths was analysed for δ ^{18}O and δ ^{13}C composition from 336 grass emperor collected from five locations during April 2000 and 184 fish collected at six locations during April 2001 throughout Shark Bay, Western Australia. A sample of sagittal otoliths from eight grass emperor collected from Thevenard Island (oceanic site north of Shark Bay) in 1999 was used as a reference location.

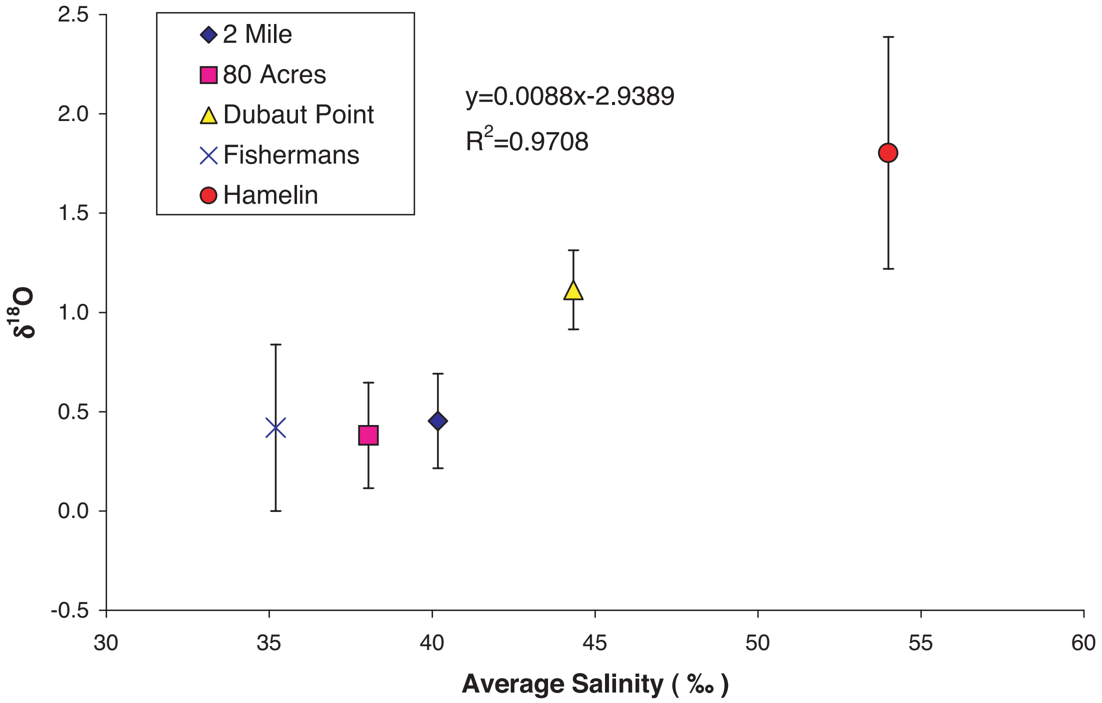
The range of δ ^{18}O values was 0.23 at Fishermans to 1.80 at Hamelin Pool for 2000 and 0.23 at Fishermans to 1.57 at Hamelin Pool for 2001. The range of δ ^{13}C values was -2.79 at Dubaut Point to -1.85 at Hamelin Pool for 2000 and -2.98 at Dubaut Point to -1.64 at Hamelin Pool for 2001 (Table 4.1). Grass emperor mean otolith isotopic composition from Thevenard Island fish were outside the range for the Shark Bay fish for both isotopes; the average δ ^{13}C value was -3.50 and the average δ ^{18}O value was -0.91. The salinity values at the time of collection ranged from a low of 35.2 ppt at Fishermans in 2000 to 57.7 ppt at Hamelin Bay in 2001. The SST values (^{o}C) calculated over the period from January 1998 to April 2001 ranged from 26.3°C at Thevenard Island to 23.8°C at Freycinet Estuary, Dubaut Point, Fishermans and 80 Acres and 21.9°C at Hamelin Pool (Reynolds and Smith 1994).

The positive, linear relationship between δ ^{18}O values and location followed the salinity gradient within Shark Bay (Figure 4.2a and b). The greatest δ ^{18}O values were found at the hypersaline Hamelin Pool, followed by Freycinet Estuary and Dubaut Point, with the lowest values recorded at the more oceanic sites of Fishermans, 80 Acres and 2 Mile (Figure 4.2). However, while 2 Mile was similar to these other two sites in 2000, in 2001 this former site had an elevated mean δ ^{18}O value. The relationship between δ ^{18}O and temperature showed the opposite broad pattern to that with salinity. Thus, δ ^{18}O values were highest at the cooler sites (Hamelin Pool, Freycinet estuary and Dubaut Point) and lower from the warmest waters at the northern boundary of the bay (Table 4.1).

Table 4.1 Summary of the collection sites, grass emperor, *Lethrinus laticaudis* sampled and results of isotopic analyses of otolith carbonate. Water temperature and salinity recorded at the time of fish collection. SST = monthly mean sea surface temperatures, averaged monthly means for period 1989-2001 (Reynolds and Smith 1994).

Site	Year	*N*	Fish total length (mm) mean (range)	Otolith weight (mg) mean (SD)	δ^{13} C mean (SD)	δ^{18} O mean (SD)	Temp (ºC)	Salinity (ppt) mean (range)	SST (ºC)
2 mile	2000	75	272.5 (172-342)	144.0 (56.9)	-2.44 (0.76)	0.49 (0.32)	26.0	40.2 (40.1-40.2)	23.8
	2001	40	291.8 (270-350)	183.8 (26.2)	-2.65 (0.56)	0.57 (0.26)	25.2	39.1 (39.1-39.1)	
80 acres	2000	112	278.9 (133-350)	129.6 (50.5)	-2.75 (1.04)	0.40 (0.33)	26.2	38.1 (37.0-38.6)	23.8
	2001	45	326.4 (270-349)	167.7 (23.4)	-1.94 (0.74)	0.32 (0.23)	25.1	36.8 (36.8-36.8)	
Dubaut	2000	81	268.2 (143-349)	129.3 (48.3)	-2.79 (0.73)	1.04 (0.37)	25.8	44.2 (41.4-46.6)	23.8
	2001	19	297.5 (270-345)	172.5 (20.2)	-2.98 (0.58)	1.04 (0.22)	23.1	45.1 (44.9-45.3)	
Fishermans	2000	46	303.5 (270-340)	148.2 (25.9)	-2.05 (0.84)	0.23 (0.42)	27.3	35.2 (35.2-35.2)	23.8
	2001	39	321.9 (271-347)	175.3 (29.2)	-2.02 (0.69)	0.23 (0.33)	24.5	35.7 (35.7-35.7)	
Freycinet	2001	21	320.8 (285-349)	155.7 (23.4)	-2.54 (0.75)	1.15 (0.38)			23.8
Hamelin	2000	22	293.3 (150-345)	122.8 (39.1)	-1.85 (0.92)	1.80 (0.56)	25.8	54.0 (51.6-54.6)	21.9
	2001	20	312.5 (280-348)	139.7 (20.8)	-1.64 (0.62)	1.57 (0.39)	23.0	57.7 (55.5-58.9)	
Thevenard Is	1999	8	347.1 (297-457)	214.8 (93.4)	-3.50	-0.91			26.3

a)



b)

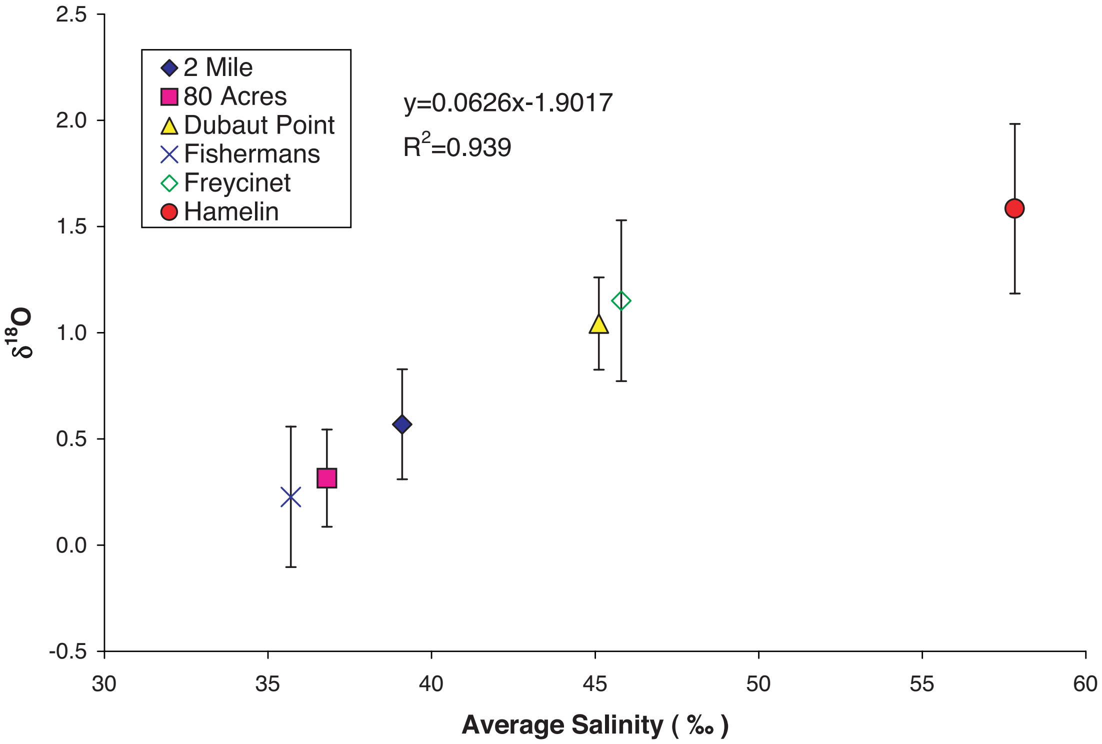
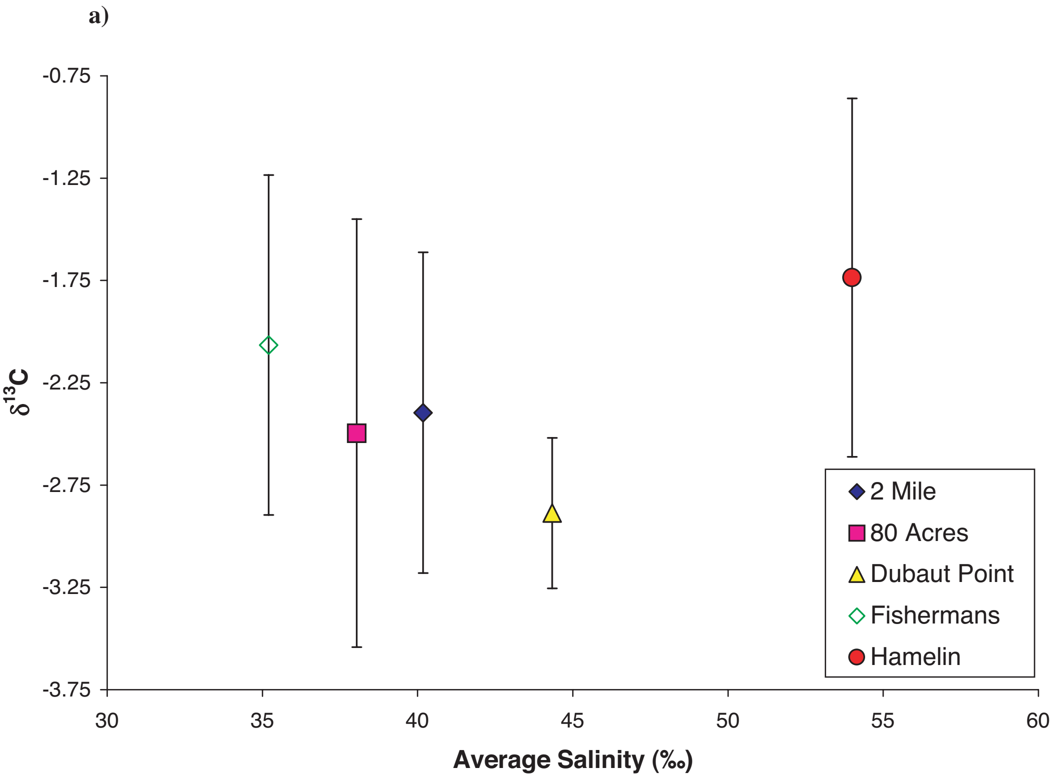


Figure 4.2 The δ [18]O values versus salinity for the grass emperor, *Lethrinus laticaudis* at each sampling location in Shark Bay during (a) 2000 and (b) 2001.

There was no consistent trend for δ ^{13}C values versus salinity (Figure 4.3). While the highest δ ^{13}C values in Shark Bay were recorded from the hypersaline Hamelin Pool the remaining locations showed a high degree of temporal and spatial variation, with substantial overlap in the δ ^{13}C values between locations.



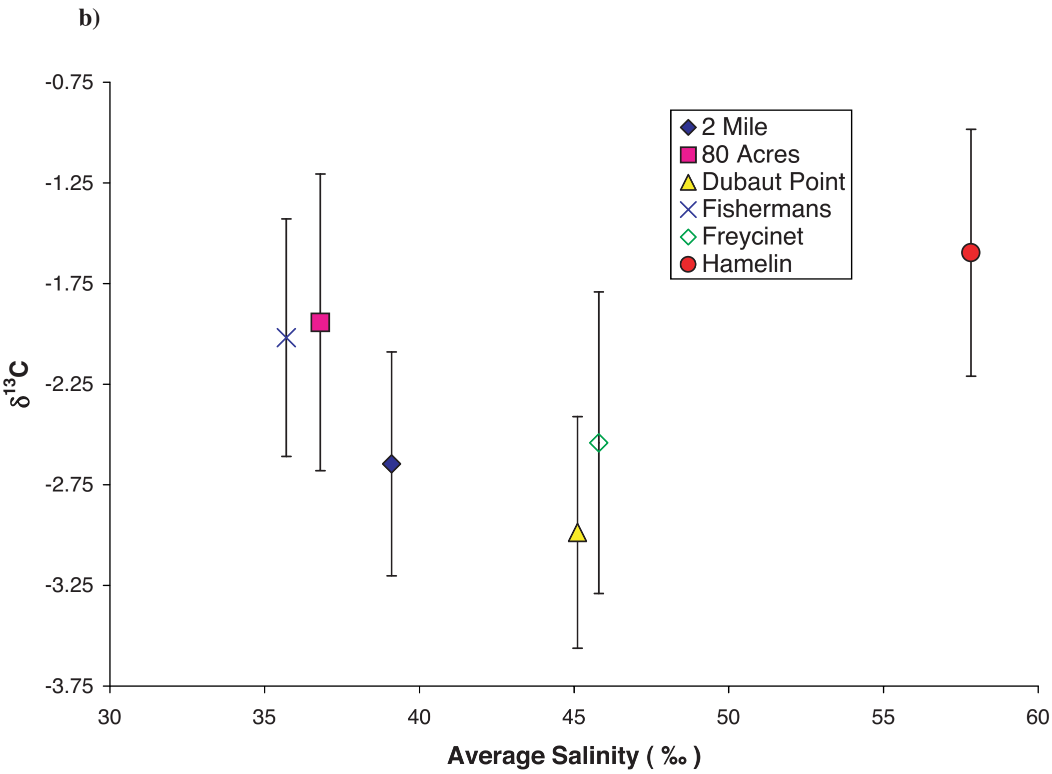


Figure 4.3 The δ ^{13}C values versus salinity for the grass emperor, *Lethrinus laticaudis* at each sampling location in Shark Bay during (a) 2000 and (b) 2001.

Analysis of covariance (ANCOVA) for $\delta^{18}O$ of otolith carbonate and the factors location and otolith weight demonstrated a statistically significant relationship with location ($P < 0.001$) but not with otolith weight for both collection years. The ANCOVA results demonstrated that 67% of the variation in observed $\delta^{18}O$ values could be explained by the factor location. Tukey multiple pairwise comparisons tests of $\delta^{18}O$ indicated the following differences between sites:

Both Gulfs:

Fishermans < 80 Acres < 2 Mile < Freycinet < Dubaut Point < Hamelin Pool.

Eastern gulf only:

< 80 Acres < Dubaut Point < Hamelin Pool.

Western Gulf only:

Fishermans < 2 Mile < Freycinet

ANCOVA results for carbon isotope values of otolith carbonate and the factors location and otolith weight also revealed a statistically significant relationship for location ($P < 0.001$), but in this case the location factor accounted for only 16% of the variation, reflecting the high levels of variation and lack of consistent trends for $\delta^{13}C$ values observed in Figure 4.3. There was a significant difference in 80 acres $\delta^{13}C$ values between 2000 and 2001. Tukey multiple pairwise comparisons tests of $\delta^{13}C$ indicated the following differences between sites:

Both Gulfs:

Dubaut Point < 2 Mile < 80 Acres < Fishermans < Hamelin Pool, and
Freycinet < Hamelin Pool.

Eastern gulf only:

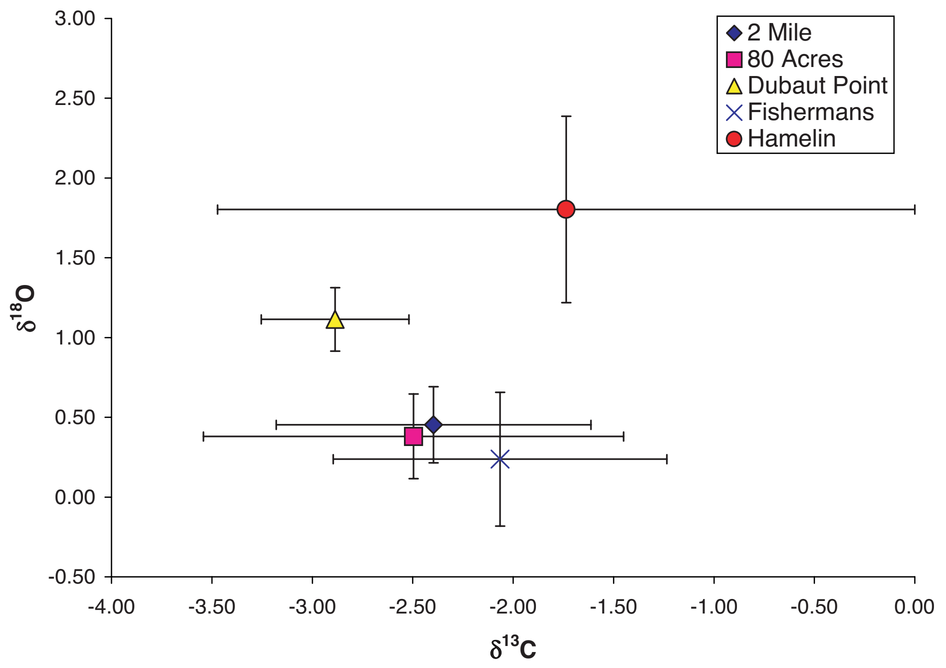
Dubaut Point < 80 Acres < Hamelin Pool, and

Western Gulf only:

2 Mile < Fishermans (with Freycinet Estuary not significantly different from either of these.)

The combined oxygen and carbon stable isotopes plots demonstrated that there was a separation of the stable isotope signatures in each gulf (Figure 4.4 a and b). The data for each gulf are initially described individually because of their geographical separation and the temperature-salinity gradient common along the longitudinal axis of both. The hypersaline Hamelin Bay was distinct from its nearest geographical neighbour, Dubaut Point, which in turn was separate from 80 Acres at the northern end of the western gulf. Only two sites were sampled in the eastern gulf in 2000; the mean isotopes signatures from 2 Mile and Fishermans were separate but the variation around these means for both sites clearly overlapped. In 2001 Freycinet Estuary was also sampled and in this year the three sites showed different isotope signatures. The isotope signature for 80 Acres was very similar to that for 2 Mile in 2000, but was close to that for Fishermans in 2001. When the data was pooled over both years, three groupings of sites within Shark Bay were apparent (Figure 4.5). Firstly, Hamelin Pool constituted a distinct site. Secondly, Dubaut Point and Freycinet estuary had similar isotope signatures. Finally, the outer most sites of the Shark Bay gulfs formed a close group that, nonetheless, exhibited a trend for decreasing $\delta^{18}O$ and $\delta^{13}C$ values that reflected the decreasing salinities (2 Mile>80 Acres>Fishermans). As expected, Thevenard Island was very different from all of the Shark Bay sites, indicating no mixing between these distant regions over the life span of individual fish (Figure 4.5).

a)



b)

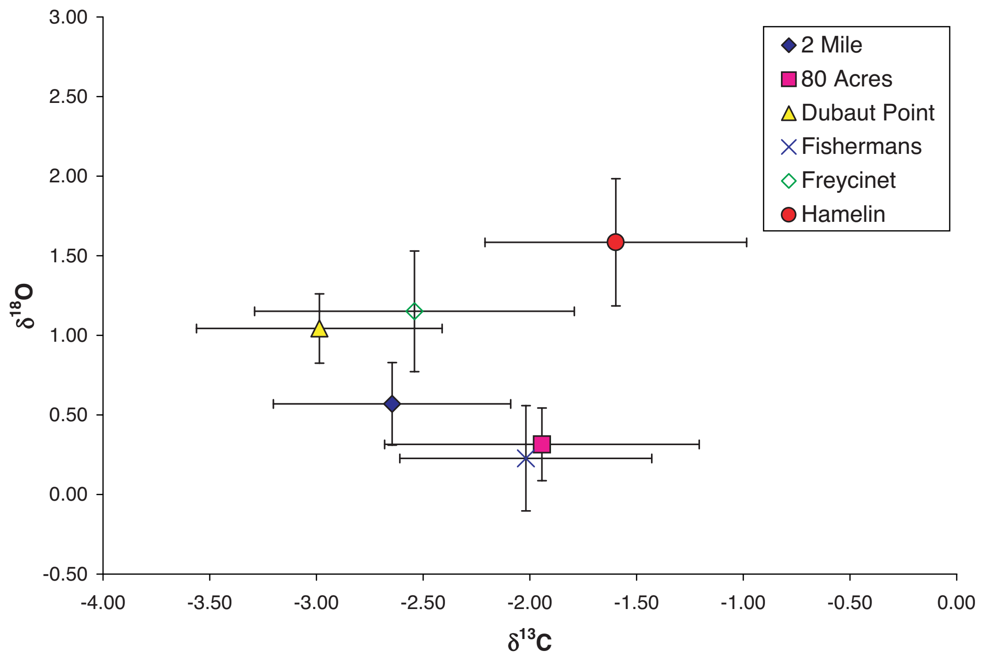


Figure 4.4 Carbon versus oxygen isotope ratios from grass emperor, *Lethrinus laticaudis*, otolith carbonate at each sampling location in (a) 2000 and (b) 2001.

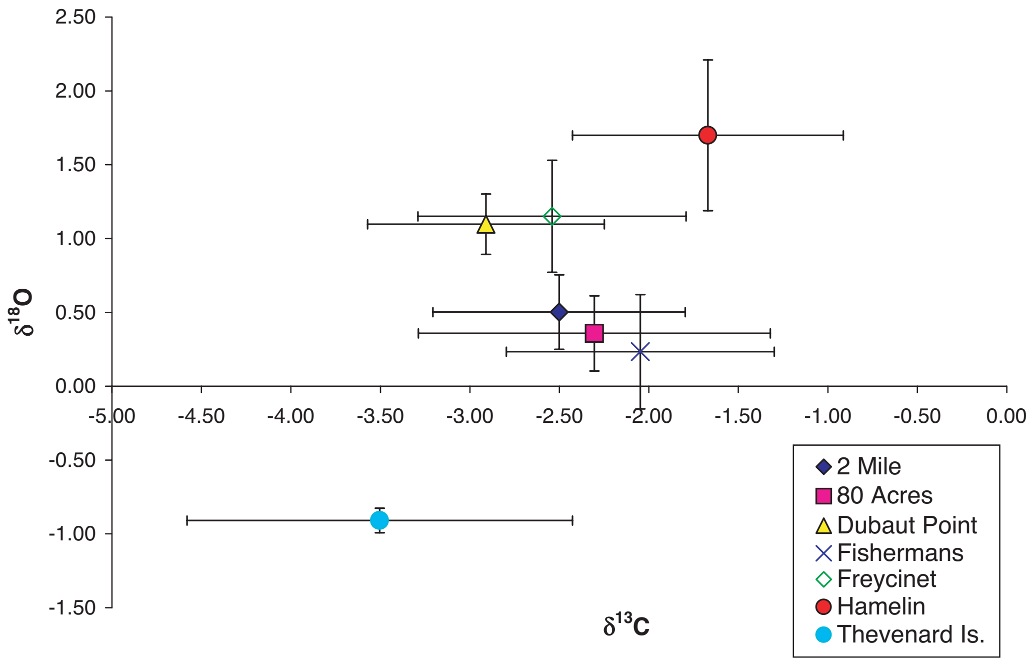


Figure 4.5 Carbon versus oxygen isotope ratios for data pooled over 2000 and 2001 from grass emperor, *Lethrinus laticaudis*, otolith carbonate at each sampling location in Shark Bay, and for samples collected at Thevenard Island in 1999.

4.4 Discussion

Stable isotope analysis of otolith carbonate has been used successfully to study fish migration patterns (Campana 1999, Rubenstein and Hobson 2004), habitat affiliations (Arai et al. 2004) and stock delineation (Edmonds et al. 1999, Stephenson et al. 2001; Bastow et al. 2002) for many species. Within the unique environmental regimes of Shark Bay, the sea surface temperature and salinity profiles have produced a complex pattern of stable isotope signatures. The otolith carbonate analysis of the grass emperor from the eastern and western gulfs of Shark Bay, demonstrated that oxygen isotope values provide a significant measure of differentiation between some sites. Additionally, the pattern of the variation in oxygen isotope ratios appears related to salinity and sea surface temperature (SST) within the bay although the geographical resolution for the SST was not at a scale to permit differentiation between several mid-bay sites. The Reynold's SST data are designed for open water systems; however they will provide a good approximation of the water temperatures for the area.

The differences discerned in the otolith carbonate oxygen isotope composition at six locations in Shark Bay suggest that grass emperor are not panmictic in this region but display some degree of site fidelity. This was displayed most clearly by the distinct isotope signatures between the sites at Hamelin Pool and Dubaut Point, the two closest sites examined in this study. Fishing for grass emperor at these two sites was conducted on relatively small patches of reef in each case; because of this high level of spatial resolution we can be confident that grass emperor were not mixing to any significant degree, if at all, over the lifespan of individual fish, across the relatively short distance of 20 km. Indeed, a straight-line movement between the fishing locations at Hamelin Pool and Dubaut Point would be unlikely by fish the size of grass emperor because of an extensive, shallow (depth <0.3 m) sand bank between them, that on spring tides is exposed. Although the fishing locations are connected by depths >1.5 m over a distance of 40 km, this is nonetheless a very small scale over which to find distinct isotope signatures in otolith carbonate.

The consistent difference between grass emperor isotope signatures over such a short distance is a key outcome of this study and can be used to help interpret the remaining results. Thus, while the sampling sites of 80 Acres, Fishermans and 2 Mile form a group with similar isotope signatures, albeit with interannual inconsistency, these similarities may well be driven by the similarities in the salinity-temperature regime the fish experienced at each site. Within each gulf, all sites had significantly different $\delta\ ^{18}O$ signatures, while the lack of difference in the $\delta\ ^{13}C$ for grass emperor from Freycinet Estuary may have been due to the much wider spatial area over which grass emperor were caught for this study. Thus, the high level of variation in $\delta\ ^{13}C$ for Freycinet Estuary may have caused the non-significant result. This contention is support by the fact that $\delta\ ^{18}O$ did differ markedly between Freycinet Estuary and the other western gulf sites. Given the lack of mixing across a 40 km distance in the eastern gulf, the similarity in isotope signatures of grass emperor in from eastern and western gulf sites has most likely resulted from the environmental influences rather than there being mixing of fish. Such a scenario is highlighted by the fact that grass emperor from Freycinet Estuary and Dubaut Point have similar signatures, but would not be expected to mix at higher rates than with fish from the intervening sites at 80 Acres and 2 Mile.

The salinity-temperature gradients in both gulfs of Shark Bay have allowed identification of a high degree of residency by grass emperor that may not have otherwise been possible.

These results can be contrasted with those for pink snapper, *Pagrus auratus*, in Shark Bay (Edmonds et al. 1999, Bastow et al. 2002). This species is also relatively location-specific within the Shark Bay gulfs once recruited. Tailor (*Pomatomus saltatrix*) otolith carbonate isotope signatures from Shark Bay indicate a greater degree of mixing between locations than was the case for pink snapper or the grass emperor (Edmonds et al. 1999). These species demonstrate the usefulness of this technique to discern stock structure for management purposes. Similarly, results from the present study show site fidelity for the hypersaline Hamelin Pool stock which showed unique oxygen and carbon signatures. The remainder of the oxygen and carbon isotope values indicated grass emperor do mix within their respective gulfs, particularly the mid- and oceanic areas, as supported by variable and overlapping oxygen and carbon otolith signatures. These results have implications for the design of fisheries management strategies.

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CHAPTER 5.0 Develop a stock assessment model for grass emperor for the inner gulfs of Shark Bay

P. Stephenson, D. Gaughan and S. Ayvazian

Objective: Development of preliminary stock assessment models.

Stock assessment was performed by developing a yield per recruit (YPR) model and assessing the rate of fishing mortality. The YPR model determined that the most appropriate age at first capture was 5.1 years, which corresponds closely to the current LML of 32 cm TL. Combining the results of the YPR model with the estimates of fishing mortality indicated that exploitation levels were likely to have been too high during the study period and that some degree of over exploitation may have occurred.

5.1 Introduction

This document develops a stock assessment model to assess the status of the grass emperor (*Lethrinus laticaudis*) stock in Shark Bay, Western Australia. The time series of catch and particularly effort data collected for grass emperor in this study are insufficient to develop an age-structured model. Reliable catch and effort details for the commercial sector are not available as this is not a targeted species by the line and gill net fishers in Shark Bay. Although catch and effort figures for the recreational sector are available for three years (Sumner and Malseed, 2002; Sumner et al., 2002), the complex and dynamic nature of the recreational fishery in Shark Bay currently precludes the use of the recreational fishing effort data from being rigorously applied to stock assessment models.

A yield-per-recruit (YPR) model was developed using the biological data collected in the study. However, data on fecundity were insufficient (Chapter 2) to enable an egg-per-recruit model to be developed. The YPR model uses the biological parameters for age and growth for determining the level of fishing effort and age at first capture that will maximise yield. The assessment involves the determination of target and limit Biological Reference Points (BRPs) for the fishery and an evaluation of the current status of the fishery with respect to these BRPs.

5.2 Materials and methods

From a fishery management perspective, and in consideration of data-limitations, the Shark Bay grass emperor population will be modeled as one biological stock. The data required for YPR model was acquired during the studies on reproductive biology (Chapter 2) and age and growth (Chapter 3). Means for L_∞, the length-weight relationship, sex ratio etc. for the whole Shark Bay region were derived from the regional estimates.

The values used in the model were as follows

1. von Bertalanffy parameters

	L_∞	K	t_0
Male	424 mm	0.24	0 mm
Female	449 mm	0.23	0 mm

2. Length-weight relationship parameters.
   a = 0.0000000092; b = 3.09.

3. Maximum age (t_λ) = 20 years.

4. Natural mortality (M, based on an estimated maximum age of 20 years) = 0.21

5. Proportion of males = 0.39

6. Age at recruitment (t_r) = 1.0

7. Age at vulnerability to the fishery (t_c) = 1.7

The YPR model was implemented using Mathcad (version 11.2a, 2003). The model structure was as follows:

$$YPR(F, t_c, t_\lambda) := F \cdot \exp[-M \cdot (t_c - t_r)] \cdot \int_{t_c}^{t_\lambda} [W(L(t,1)) \cdot p + W(L(t,2)) \cdot (1-p)] \cdot \exp[-(M+F) \cdot (t - t_c)]\, dt$$

where F is fishing mortality, W is weight, L is length and t is age. W(L(t,1)) and W(L(t,2)) define the weight-at-age for males and females, respectively, given the length-weight relationship and the L_∞ and K for both sexes.

The search for an optimal fishing effort to maximise yield is carried out for a range of age classes and a range of potential fishing mortalities. A graphical depiction is used to assess which combination of age and fishing mortality (F) give the best yield. The graphical analysis allows determination of the optimal age (or size) at first capture for a given level of F.

Once the optimal age at first capture has been determined, a target fishing mortality is determined that maximises yield (i.e. given the optimal age at first capture). Two management reference points will be examined, each of which lies below F_{max}, the level of F that theoretically maximises yield but, in reality has a history of leading to overexploitation when used as a management reference point (e.g. Haddon, 2001). The first step is to determine $F_{0.1}$, the point of the YPR-F relationship where the slope is 0.1 of that at the origin of the relationship. This represents the Limit BRP and has been adopted simply as a conservative alternative to F_{max} because of the levels of uncertainty with YPR models, and the fact that they cannot assess risk to sustainability of a stock, given the various assumptions such as equilibrium status with constant recruitment. The $F_{0.1}$ BRP has thus gained widespread acceptance for those fisheries where no other stock assessment is possible, but nonetheless remains an ad hoc choice (Haddon, 2001) that is not without risk. An even more conservative management reference point, $F_{0.2}$, is also determined to provide a Target BRP.

5.3 Results

The plot of F against YPR (Figure 5.1) indicates that maximum YPR occurs when the exploitation first occurs at an age of 4 or 6 years. Iterative runs of the YPR model were able to determine that within this range of 4–6 years, 5.1 years was the optimum age at first exploitation (tc).

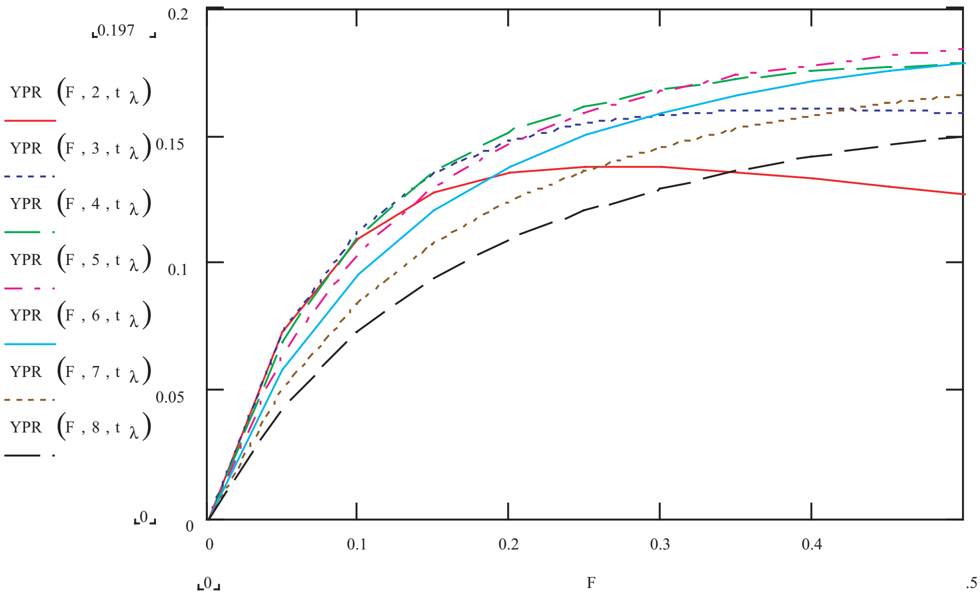

Figure 5.1 Yield per recruit (kg per recruit) for a range of Fs from 0 to 0.5 and for ages at first vulnerability from 2 to 8.

The limit and target Biological Reference Points of $F_{0.1}$ and $F_{0.2}$ respectively were then estimated from the YPR model using tc = 5.1, with the following outcome:

- $F_{0.1} = 0.28$,
- $F_{0.2} = 0.18$

5.4 Discussion

Size at first capture

The size at age at first capture that maximises yield (i.e. 5.1 years) corresponds closely to the minimum legal length of 32 cm; the YPR model thus support the recent increase in LML from 28 to 32 cm TL. Prior to this model being developed it had been though that the LML should be as high as 35 cm, but the YPR model now indicates that 32 cm TL as size at first capture will provide a better yield for the fishery and hence that a further increase in LML is not required at this time.

Fishing mortality

The estimates of total mortality fell within the range of 0.41 to 0.57 (Chapter 3). Because we had no other evidence against which to decide which end of this range might be more accurate, both ends of this estimated range will be used in assessing whether or not fishing mortality during the study period was at an acceptable level. Given this range of total mortality, removing natural mortality of 0.21 leaves a range of fishing mortalities of 0.20 to 0.36. Even the lower end of this range exceeds the Target BRP of $F_{0.2}$ (i.e. M = 0.18), while the upper estimate of F is double that for $F_{0.2}$. Given that even the more conservative BRP was exceeded, the YPR model indicates that fishing mortality between 1999 and 2001 was higher than what should have been applied to the stock of grass emperor in Shark Bay. The pessimistic scenario (i.e. F as high as 0.36) is that a significant degree of over exploitation of grass emperor was occurring.

$F_{0.2}$ appears to be a reasonable target BRP as it keeps F<M, which is a minimum requirement when setting mortality-based BRPs. Two factors may have decreased fishing mortality of grass emperor since that time. Firstly, the increase in LML will have provided a degree of increased protection for the stock assuming that, because Shark Bay is a relatively shallow system, released grass emperor have a high survival rate. Secondly, bag limits for emperors have also recently been reduced.

Finally, as the pink snapper stock in the region has recovered, some of the recreational fishing effort has now switched back to that icon species of the region.

The recreational landings of grass emperor at the primary boat ramps was 17,073 (15.9 tonnes) in 1998-99 compared to 10,042 (11.6 tonnes) during 2000-01 and 7,357 (7 tonnes) during 2001-02 (Table 5.1). Although Sumner and Malseed (2002) suggested that the reduction in catch may not have been due to changes in stock size but rather because of a reduction in fishing effort following from management changes to the pink snapper fishery, the decrease in catches supports the results from the stock assessment that F was too high during this period. As mentioned above, problems with determining the actual effort directed towards grass emperor from the estimates of recreational effort preclude our ability to make definitive statement on catch rates. Nonetheless, catch rates were stable between 1999 and 2000, but then exhibited a 38% decrease in 2001. Thus, both catch and catch rates underwent substantial declines during the study period, which does not contradict our contention that excessive fishing mortality may have been overexploiting the stock. Recreational fishing effort in Shark Bay has previously contributed to the overexploitation of pink snapper, the dominant angling species in the region. Therefore, the potential for recreational fishing effort to overexploit a less abundant but long-lived species is a distinct possibility.

Table 5.1. Annual recreational catches of grass emperor from Shark Bay WA from 1999 – 2001. The 1999 survey included non-boat ramp landing points, whereas the latter two surveys examined only the 3 primary boat ramps in Shark Bay. Given that the 1999 survey showed that 29.1% of grass emperor catch was landed at secondary landing sights, a scaled total catch for all years is also shown in the table.

Year	Effort (days)	Number kept	Number released	Weight kept (tonnes)	Scaled weight (tonnes)
1998 - 1999	49,321	17,073		15.9	22.0
2000 - 2001	34,816	10,042	18,272	11.6	16.0
2001 - 2001	34,037	7,357	15,470	7.0	9.7

5.5 References

Haddon, M. (2001). Modelling and quantitative methods in fisheries. Chapman and Hall/CRC, Boca Raton, Florida. 406 pp.

Sumner, N.R., Williamson, P.C. and Malseed, B.E. (2002). A 12 month survey of recreational fishing in the Gascoyne bioregion of Western Australia during 1998-99. Dept. of Fisheries WA research Report No. 139, 54 pp.

Sumner, N. and Malseed, B. (2002) Quantification of changes in recreational catch and effort on inner Shark Bay snapper species following implementation of responsive management changes, Final report, FRDC project 2000/139, 46 pages.

CHAPTER 6.0 Benefits and adoption

The beneficiaries of this project are the recreational fishing community of Shark Bay, fisheries managers and the general community. The overarching goal of this research programme is to conserve the grass emperor stock while optimising recreational fishing at sustainable levels.

Fisheries managers will benefit from the increased knowledge of the biology of this important, though previously unstudied species. In particular this study has addressed issues of the reproductive biology; such as the length at 50% maturity, and sex ratio at length. These data are critical to establishing minimum legal lengths for retained fish and understanding whether this species, in Shark Bay, exhibits sex change.

The general community will benefit from these results through increased understanding of the biology of this species, leading to increased conservation and species diversity in Shark Bay. The results have provided the basis for the recreational fishing sector agreeing to an increase in the LML of grass emperor.

CHAPTER 7.0 Further development

There are some aspects of the biology of grass emperor in Shark Bay that warrant further investigation and development.

The age and length data from this study could be set as baseline information and periodic research surveys/angler surveys to intensively sample the stock could be conducted to monitor any changes in the distribution of age and length of fish taken from the recreational sector. Observed deviations might provide an early warning of a change in recreational fishing behaviour or identify poorly represented age classes in the age-structure, which would be indicative of recruitment failures in some years. New assessments of total mortality could then be examined with respect to the outputs from the yield per recruit model to update the stock assessment, and in particular assess whether fishing mortality rates continued to exceed the target reference point ($F_{0.2}$).

Targeted angler surveys within Shark Bay should be repeated periodically to document and verify the decline in catches from the recreational sector. A better estimation of the effort specifically directed towards grass emperor would also allow catch rates to be formally incorporated into stock assessments.

The efficacy of intensive “one-off” surveys as a monitoring tool for age compostion etc. of grass emperor in Shark Bay will be tested during 2005.

CHAPTER 8.0 Planned outcomes

The biological data collected and the yield per recruit model provide a robust basis for implementing management changes, if required.

Grass emperor can be managed as gonochoristic, i.e. not exhibiting sex change. This simplifies the implementation of LMLs, which would have been otherwise complicated if one sex dominated the larger size classes and the other dominated the smaller size classes.

The reproductive biology and YPR model indicate that the recent increase in LML from 28 to 32 cm TL was fully warranted.

Stock assessment indicated that the increased pressure on grass emperor, following implementation of severe restrictions on the take of pink snapper, did in fact result in a higher risk to the stock, with fishing morality having likely been high enough to over-exploit the stock. The potential for this to occur was the primary reason this study was undertaken and indicates that any future changes in the management of pink snapper, the icon angling species in Shark Bay, should not be made without also considering the flow-on effects of transferring effort to other angling species in the region.

CHAPTER 9.0 Conclusions

OBJECTIVE 1. Objective 1 was met with the following outcomes. Stable isotope analysis of otolith calcium carbonate from Shark Bay grass emperor demonstrated the positive relationship between isotopic oxygen values and location. Further investigation indicated that the greatest isotopic oxygen values were associated with hypersalinity and cooler water temperatures (i.e. Hamelin Pool, Freycinet estuary and Dubaut Point). There was no consistent trend for isotopic carbon values between sampling locations in Shark Bay, due to a high degree of temporal and spatial variability between locations. The combined oxygen and carbon isotope plots revealed separation of the stable isotope signatures in each gulf, associated with the temperature-salinity gradient present along the longitudinal axis of both gulfs. Therefore it was possible to delineate stocks based on the physiochemical properties of their environment. This analysis suggest some fidelity to a particular location (physiochemical regime). Movement patterns of adult grass emperor are hypothesized to be strongly linked to availability and distribution of suitable habitat, with less movement in those cases were suitable habitat is sparsely distributed. This is support by limited tagging information; recaptures were all taken close to the original tagging site.

OBJECTIVE 2. Objective 2 was met with the following outcomes. Aging the 4 259 grass emperor samples was made possible after thorough examination of the sagittal otolith internal structure and establishing that rings are laid down on an annual basis. The ages of the sampled fish, collected by various fishing methods throughout Shark Bay, was 0^+ to 16^+ years old. However the 3^+ to 7^+ age class dominated the recreational take of this species. In keeping with similar species, it is likely that the species lives to at least 20 years.

OBJECTIVE 3. Objective 3 was met with the following outcomes. Growth rates for the grass emperor were estimated using the von Bertalanffy growth curve parameters. The L_∞ values were consistently greater for females than males for each region, although this difference was only statistically significant for the eastern gulf. Ocean caught fish had a significantly greater L_∞ than either gulf, while the eastern gulf had a greater L_∞ than the western gulf. The K values were also significantly different between regions. Both growth parameters were within the range for other lethrinid species. While we do not have a measure of growth for ocean stocks to the north of Shark Bay, the fact that the growth rate parameters are within those reported for other lethrinids suggests the unique environment of Shark Bay has not unduly diminished growth rates.

OBJECTIVE 4. Objective 4 was met with the following outcomes. The reproductive biology of grass emperor in Shark Bay did not demonstrate sequential hermaphroditism as reported from the Northern Territory. The Western Australia LML of 28 cm TL has recently been revised upwards to 32 cm TL, which is well above the sizes at 50% maturity of 23 cm for females and 19 cm for males. This corresponds to a grass emperor that is around 5 years old. Spawning period, as described from the monthly gonadosomatic index, was between December and March, with peak spawning in January. During this spawning period, female grass emperor released eggs every two or three days (batch spawning).

OBJECTIVE 5. Objective 5 was met with the following outcomes. A complex population dynamics model could not be developed with data available from this research alone. Because this species is almost exclusively a recreationally caught fish there are no monthly commercial catch data from which a catch history could be developed. Therefore a yield per recruit (YPR) model was the most appropriate type of assessment. The YPR model determined that the most appropriate age at first capture was 5.1 years, which corresponds closely to the current LML. The YPR model also assessed whether $F_{0.1}$ was an appropriate Limit BRP and whether $F_{0.2}$ was an appropriate Target BRP. $F_{0.1}$ was clearly not acceptable as it was much higher than M. $F_{0.2}$ was reasonable in that it gave an F that was less than M. However, estimates of total mortality indicated that exploitation levels were likely to have been too high during the study period and that some degree of over exploitation may have occurred.

Appendices

Appendix 1 Intellectual property

There is no intellectual property arising from this project.

Appendix 2 Staff

S. Ayvazian, J. Brown, B. Chatfield, Y. W. Cheng, M. Craine, D. Gaughan, E. Jones, L. Glendenning, I. Keay, G. Nowara, P. Stephenson.