

## FRDC Project 97/133

## FISHERIES BIOLOGY AND HABITAT ECOLOGY OF SOUTHERN SEA GARFISH (*Hyporhamphus melanochir*) IN SOUTHERN AUSTRALIAN WATERS

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Fisheries biology and habitat ecology of southern sea garfish (*Hyporhamphus melanochir*) in southern Australian waters.

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## NON-TECHNICAL SUMMARY

# 97/133. Fisheries biology and habitat ecology of southern Australian sea garfish (*Hyporhamphus melanochir*) in southern Australian waters.

## PRINCIPAL INVESTIGATOR

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## **OBJECTIVES**

- 1. Determine the extent of genetic discrimination between southern sea garfish stocks of Western Australia, South Australia, Victoria and Tasmania.
- 2. Determine the size and age structure of the commercial catch from the different sectors in southern Australian waters, and improve understanding of the potential impacts of the competing gear-sectors on the South Australian stocks.
- 3. Investigate ways of improving the return to fishers, without increasing overall catches, by improving harvest and post-harvest strategies.
- 4. Investigate the relationship between habitat type, reproduction and productivity in seagrass and other inshore habitats, and determine key aspects of the early life history of garfish.

## NON-TECHNICAL SUMMARY

Southern sea garfish (*H. melanochir*) is a important component of the multi-species commercial and recreational fisheries within the inshore embayments of the southern Australian waters from south western Western Australia (WA), throughout South Australia (SA) and eastwards to Victoria (Vic) and Tasmania. Prior to this investigation, there were significant gaps in our knowledge of its fishery biology, including the number of manageable stocks, impacts on the resource by competing sectors, and its dependence on the seagrass habitat at different levels of its life history, thereby providing some uncertainty as to whether the fisheries were biologically sustainable. Also, for the commercial fisheries in SA there was also a need to determine its economic status and investigate ways for enhancing its economic status. This report, therefore, is the output from a collaborative project between research institutes in SA, WA and Vic aimed to answer these questions, with an outcome to provide scientific advice for improving the sustainable management of this species throughout its geographic range.

*H. melanochir* is managed separately by each state (eg size limits and recreational bag limits) and within each state, there are also differing management policies for each commercial fishery, for example, differing haul-netting depth limits between the SA gulfs. The DNA stock discrimination component of this study, found 4 genetically separate populations existing in WA, western SA, the SA gulfs / Vic bays and Tasmanian waters, respectively. Thus, we now know how likely a management policy in one region may influence the population of sea garfish in an adjacent area.

Samples of fish were collected both independently in SA, and from the fisheries in SA, Vic and WA for investigations on age, growth and reproductive biology in these states. Through age validation techniques (marginal increments, tetracycline marking and calibration between two research laboratories), otoliths were found to form annual rings, and an ageing protocol was successfully developed. Sea garfish was found to be a species which exhibited medium growth rates, with maximum ages for fish in SA, Vic and WA estimated at 6, 6 and 10 years respectively. Although there are slightly differing minimum size limits between the three states (21, 20 and 23 cm, resp), fish in each state reach these lengths at similar ages (13 – 15 months). In only one state (SA), was the size at first sexual maturity similar to the minimum legal length (21cm); in WA and Vic, sizes at first maturity were approximately 3 cm higher.

The effect of the fishery on the garfish stocks were investigated in two ways; a comprehensive analysis of the commercial catch, effort and catch rates and the age structure of the commercially fished component. However, because of differing netting regulations (mesh size and lengths of nets, and the detail of reporting of fishing effort) between states, it was not possible to directly compare catch rates, and hence relative abundances, between states. In SA, the state where the highest commercial landings of garfish occurred, trends in catch rates between 1983/84 and 1999/00 were found to be either stable or increasing in all regions. In Victoria, in contrast, catches over the same period declined due to declining fishing effort and catch rates for haul seines and ring nets in Port Phillip Bay and Westernport. In WA, commercial catches have risen steadily over this period, however, catch rate data were not interpretable.

The potential impact of competing sectors on the SA stocks was investigated by examining the temporal trends in catch, effort and catch rates by the commercial hauling net and dab net fisheries, and it was found that the two sectors showed limited temporal and spatial overlap between these fisheries. In areas where there was some overlap, (eg northern Gulf St. Vincent), highest catch rates by dab net fishers occurred at the same time that highest catches and effort by hauling net fishers took place. There were insufficient temporal data for the recreational fishery, to investigate any impact with the commercial fisheries.

The size and age compositions of commercial catches of garfish were determined from samples collected during fish measuring programs in each state. The average size and ages at capture for fish from SA and Victoria were similar (25.5 and 25. 9cm; 1.9 and 1.6 yrs, resp.), but for WA, the average size and age was higher (28.8 and 2.2 yrs). The overall mean annual survival rates of age groups were estimated at approx. 16, 21 and 38 %, resp. for the three states. In the SA commercial fishery, the average size of fish taken by hauling nets was slightly lower than that for dab nets. Also, in the SA gulfs, the results from garfish fish measuring programs since 1954/55 detected slightly lower survival rates as catches increased over time. There is also a suggestion that the size at first sexual maturity has decreased slightly in the SA gulf population over the same period, and this is believed to be a general response of fish populations to fishing.

Southern sea garfish is a serial batch spawner, producing relatively few but large eggs over its extensive spawning season. In WA and SA, the season occurred from September to April, and in Victoria and Tasmania it was slightly shorter (October – March). SA was the only state where 2 distinct spawning peaks were detected (Nov/Dec and Feb). In the SA hauling net fishery, the sex ratio of the catch was highly biased towards female fish during the spawning season, which were found, from independent surveys, to form large schools in relatively shallow waters.

It is also in these relatively shallow waters of the southern Australian embayments where seagrass productivity is high. The possible connection between seagrass distribution and garfish reproduction was investigated by way of independent SCUBA and beam trawl and neuston surveys of the distributions of eggs and larvae, respectively in Gulf St.Vincent (GSV) and eastern Investigator Strait. Although the egg surveys were unsuccessful, the neuston surveys of larvae in both 1998 and 2000 found highest concentrations of larvae in northern GSV, an area which is almost entirely occupied by seagrass habitat. Analysis of wind speed and directional data for a number of sites around the coast of GSV just prior to the peak garfish spawning period in those years could explain the retention of larvae in the northern waters of that gulf.

To investigate ways how South Australian commercial fishers could improve their economic returns on garfish, firstly, an analysis of data collected on financial performance found that those fishers with high dependence on garfish within the multiple species hauling net and dab net fisheries of that state, generated positive financial returns. Then, an economic model was designed to examine the changes in economic rent and returns per kg from changes in management strategies (ie an increase in size limit to 24 cm, and/or a diminution in the harvesting period – seasonal closure). The greatest economic gains were found to occur with a summer fishing closure in place. Only some economic benefits would occur from a rise in the size limit, and only then, if accompanied by a significant rise in catch. Significant improvements to the analysis would occur if an integrated bio-economic model was developed. Post-harvesting strategies including the potential for increased inter-state and international trade were also investigated and it was found that export market, particularly to Japan, offered some benefits.

Finally, the outcomes of this project will benefit the future assessment and management of the sea garfish fishery in two ways in the near future. Firstly, the key biological parameters, including size frequencies, growth, reproductive seasonality, obtained from this project are currently being incorporated into a stock assessment indicator model for this species(FRDC grant No. 1999/145). Secondly, a management plan for the SA marine scalefish fishery is about to be developed, and there are currently, considerations underway with the SA Marine Scalefish Fishery Management Committee concerning the minimum size limit for garfish in that state.

**KEYWORDS:** Fisheries biology, southern sea garfish, seagrass, early life history, economic improvement

## ACKNOWLEDGMENTS

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#### BACKGROUND

Southern sea garfish (*Hyporhamphus melanochir*) supports valuable commercial and recreational fisheries across its distribution in Western Australia, South Australia, Tasmania and Victoria. The most significant commercial fisheries exist in South Australia where garfish is the fourth most valuable commercial inshore species after King George whiting (*Sillaginodes punctata*), snapper (*Pagrus auratus*) and southern calamary (*Sepioteuthis australis*). In 1999/2000 the total catch was 477 mt and was valued at \$ 1.9 million (Knight *et al.* 2000). In South Australia there is a high demand for garfish in the fresh fish market, restaurant trade and takeaway food outlets, with some potential for the value of the fishery to increase with product and marketing development. Furthermore, garfish is one of the most important target species in the SA recreational fishery, ranking second only to King George whiting in terms of catch by weight and numbers for recreational boat fishers. In 1994/95 the recreational catch was estimated at 64 mt (McGlennon & Kinloch, 1997). In Western Australia the recreational catch is also significant, and has been estimated at 25 mt (DPI Tas, 1996). In Victoria, garfish are a popular recreational target species, especially in Port Phillip Bay, with the catch estimated at 20 mt per annum (DPI Tas, 1996).

In South Australia, garfish is a significant contributor to the multi-species scalefish fishery. The main fishing areas are in Spencer Gulf, Gulf St. Vincent and around Kangaroo Island because of this species' close association with seagrass beds for feeding and possibly spawning (Jones *et al.* 1990). The South Australian commercial catch has been relatively stable at around 300 - 500 mt per annum since the mid 1960's with large fluctuations in monthly landings being common. Power-hauling nets have historically taken much of this catch with dab netting responsible for around 10% of the total catch. However, an increasing harvest share by this sector has occurred in the last 5 years, especially in some regions such as the southern waters of Spencer Gulf (SG) and Gulf St. Vincent (GSV) as well as Kangaroo Island (KI). Also, since 1987, hauling net fishers have been permitted to direct their effort at garfish in the previously depth restricted waters of northern GSV to waters greater than 5 metres depth.

The Western Australian commercial catch, taken by beach seine in the estuaries of the south-west and embayments of the south coast, has risen from 20-30 mt in 1980 to 40 - 60 mt per annum in recent years. In Tasmania, the commercial catch has risen to around 80 mt since the mid-1960's and has traditionally been taken by beach seine, although interest is now growing in other methods such as Lampara /purse seine and push nets. In the three major sea garfish fishing regions in Victoria (Port Phillip Bay, Western Port and Corner Inlet), landings have decreased in recent years.

In July, 1995, a garfish workshop was held at the South Australian Aquatic Sciences Centre, with participants representing research agencies and industry sectors from Western Australia, South Australia and Tasmania. A review of the status of the garfish fisheries in each state, and the biological and fishery information currently available, was followed by an identification of research priorities for future management of the garfish resource across its distribution. In general, the literature available on garfish is very limited. Information on the biology and life history is lacking with reproductive biology and egg and larval development being particularly poorly understood. Whether garfish are serial or batch spawners remains to be determined as does age-related fecundity. Research into the basic biology and ecology of the species was therefore deemed a priority. Monitoring of commercial and recreational effort was also emphasised, to facilitate the development of meaningful assessments of garfish resources in all states. Research priorities for garfish, both biological and fishery in nature, identified by the workshop were wide ranging and included the following:

- Basic biology life history, behaviour and diet
- Reproductive biology spawning behaviour, fecundity, critical habitats
- Early life history larval development, distribution and abundance, environmental factors affecting recruitment
- Age and growth
- Stock structure and migration patterns
- Effects of habitat degradation and environmental variability
- Monitoring of commercial and recreational sectors
- Gear technology
- Economics of fisheries, post-harvest technology and market development

#### NEED

In 1992, following a detailed review of the South Australian Marine Scalefish fishery, South Australian garfish stocks were assessed as being fully exploited. A range of measures was suggested to prevent any future increase in overall catch and to better utilise the available resource (SA Dept. Fisheries White Paper, 1992). Since that time, little dedicated research has been undertaken nor have any of the management options been acted upon, apart from the introduction of a recreational bag limit and some closed netting areas. There has been an increase in the targeting of garfish by the dab net sector to further exert pressure on the resource. Increasing interest in the species is not restricted to South Australia, with similar moves afoot in Western Australia and Tasmania. Victoria is the only state in which the commercial garfish catches have declined, and there is a need to determine the reason for this decline.

A management plan is currently being developed for the South Australian marine scalefish fishery that will require the development of biological reference points to facilitate sustainable resource management into the next century. The management plan requires information on the stock structure of garfish to enable the appropriate spatial management unit to be decided. The paucity of information available for garfish on stock structure and other fisheries biological parameters will impede this process. As a result, the South Australian Marine Scalefish Fishery Management Committee (MSFMC) has identified southern sea garfish as a research priority.

There are also developments towards the management of marine resources at the eco-system level. There will be a clear need to identify areas and/or habitats of particular importance for fishery production, and to be able to assess the impact of environmental loss and degradation on species productivity, including those habitats critical to garfish. Anecdotal information exists suggesting the importance of particular spawning habitats to garfish. A closely related species attaches its eggs to seagrass blades and the eggs of southern sea garfish are known to be adhesive (Jones, 1990). However, the degree of selectivity or reliance on seagrass or other benthic structure is unknown. Substantial seagrass loss has occurred over recent decades particularly in Tasmania, Victoria and South Australia. The impact of such habitat degradation on species closely associated with such habitats, such as garfish, remains to be assessed. The loss of seagrass, and possible effects on garfish spawning success, early life history and adult productivity is of concern in all states where such losses/reduction in habitat quality have been identified.

A yield per recruit model has been developed in SA to assess the effects of different fishing strategies. The model was based upon growth and mortality parameters from earlier studies, which assumed constant recruitment, the validity of which is unknown. The model has been used to establish current size limits in the SA fishery. Spatial differences in age and growth require investigation allowing development of a population model, building on the earlier research. More detailed catch sampling from all sectors would obtain data for such a model and allow comparison of growth and age structure between areas and with existing data obtained 10 - 15 years ago.

As a result of increasing development of the fishery in terms of the increased catching efficiencies and the opening up of new areas to harvesting, information regarding the seasonal movements of adult garfish from their inshore summer habitats to deeper waters in the South Australian Gulfs during the cooler months would allow the extent of potential inter-sectorial conflict to be established. In Gulf St. Vincent, fishing effort in the winter "deep water" fishery does not appear to have adversely affected catch rates in the summer shallow water fishery (Jones *et al.* 1990) and this report highlighted the potential usefulness of tagging experiments to determine the seasonal, inshore-offshore movements of these fish. Such movements may be a feature in the populations in the other states. A better

understanding may help to determine gear-sector interactions and allow some prediction of the effects of one sector (in one season) on others in subsequent seasons. Information quantifying the impacts of the different gear sectors and the level of interactions (between gear-types and seasons) in South Australia have been specifically requested by the MSFMC.

There appears to be general agreement on the need to make better use of the available resource by catching larger fish (White Paper, 1992) and therefore the potential for more specific targeting of larger adult garfish (preferred by the fresh fish buyers) with methods such as dab netting needs to be assessed.

#### **RATIONALE AND APPROACH**

This investigation was designed to spatially examine the fishery biology of sea garfish (stock structure, reproductive biology, age and growth) of sea garfish in the major fishing areas of Western Australia, South Australia and Victoria through a collaborative research program designed between SARDI (Aquatic Sciences), Fisheries Research, Western Australia, the Evolutionary Biology Unit of the SA Museum and the Marine and Freshwater Research Institute, Victoria.

All other research conducted on sea garfish concentrated in the South Australian gulfs and KI waters. These included:

- a) Studies on the early life history and habitat ecology of sea garfish conducted collaboratively through a PhD scholarship (Dept. of Environmental Biology, Adelaide University) and SARDI (Aquatic Sciences) and undertaken at a number of sites throughout GSV and KI.
- b) Studies on the spatial distribution of adult sea garfish in GSV waters during the 1999/2000 spawning season undertaken by SARDI (Aquatic Sciences);
- c) Econsearch Pty. Ltd conducted an economic evaluation of the management strategies for sea garfish in the SA garfish fishery in collaboration with SARDI (Aquatic Sciences).

This report is presented in 7 main chapters, each one dealing with one or more of the objectives. Each chapter begins with the objective and a short abstract. Chapter 3 includes a historic overview of the fisheries in SA, WA and Victoria, and provides a background to the rest of the report.

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## CHAPTER 1. GENETIC DISCRIMINATION BETWEEN SOUTHERN SEA GARFISH (Hyporhamphus melanochir) STOCKS OF WESTERN AUSTRALIA, SOUTH AUSTRALIA, VICTORIA AND TASMANIA

S. Donnellan, L. Haigh, M. Elphinstone, D. McGlennon and Q.Ye.

# Objective: Determine the extent of genetic discrimination between southern sea garfish stocks of Western Australia, South Australia, Victoria and Tasmania.

Nucleotide sequence variation in the mitochondrial control region was examined in the southern sea garfish, *Hyporhamphus melanochir* from southern Australia as a test for stock subdivision. Haplotype diversity, as assayed by temperature gradient gel electrophoresis, was high (82.5%) with 47 haplotypes observed. Phylogenetic relationships among the haplotypes, determined by evolutionary distance and quartet puzzling analyses, showed some phylogenetic structure among the haplotypes, but there was no strong correlation between phylogenetic relatedness and geographic location. Homogeneity tests of haplotype frequencies revealed significant differences between regions but not within regions. While overall  $\Phi_{st}$  was significant (1.83%), It is the smallest value reported for marine fishes to date. Also, few pairwise  $\Phi_{ST}$  values were significant and high levels of gene flow were inferred at all spatial scales. However, analyses of the data after pooling of samples within regions (based on political boundaries) revealed significant between region differentiation for both homogeneity testing and  $\Phi_{ST}$  statistics, except for the South Australian gulfs and Victoria. Pooling of TGGE haplotypes based on their phylogenetic relationships to increase statistical power did not reveal any evidence of genetic differentiation between samples from across southern Australia. The southern sea garfish appears to have a very low level of historical population subdivision and with the present data could be considered to comprise four management units: western Australia, west coast South Australia, the South Australian gulfs and Victoria and Tasmania.

## **1.1 Introduction**

This study is the first attempt at determining the stock structure of southern sea garfish (*Hyporhamphus melanochir*) using genetic discrimination methods. Previously, Collette (1974) compared meristic and morphometric characteristics of samples over its entire geographic range from Western Australia to southern NSW, reported a cline in characteristics, but drew no conclusions regarding the significance of variation from region to region, or likely stock structure.

The approach used in this study centres around phylogeographic and haplotype frequency analyses of haplotypes of the mitochondrial control region. The phylogeographic analysis assesses the phylogeny of individual haplotypes in relation to the geographical distribution of each haplotype (Avise *et al.* 1987). The haplotype frequency analysis mainly addresses recent population processes and short-term management issues but can be confounded by its inability to disentangle past and contemporary gene flow processes (Neigel 1997). Traditional approaches, such as F statistics (Wright 1931, 1943), do not use temporal information on allelic variation, but several new approaches can use the temporal

information made available from phylogenetic analysis of allele nucleotide sequences (eg Slatkin 1989, Templeton *et al.* 1995). The phylogeographic approach can provide a perspective that is relevant to long-term population processes and management issues, but recent analytical approaches also offer the prospect of the phylogeographic approach being able to disentangle the effects of historical and contemporary processes (Templeton *et al.* 1995, Templeton 1998).

Because of the essentially coastal distribution of the southern sea garfish and its habit of spawning in association with shallow sea grass meadows (see Chapter 5, this report), its population structure is likely to approximate a one-dimensional "stepping stone" model in which neighbouring demes are more likely to exchange genes (Kimura and Weiss 1964) rather than an "island model" in which each deme is equally as likely to receive genes from any other deme (Wright 1943). Alternatively at equilibrium between drift and gene flow, an isolation by distance population structure is likely in the absence of historical subdivision of the species range.

#### 1.2 Materials and Methods

#### Samples

Livers from 273 individual southern sea garfish were collected by the South Australian Research and Development Institute (SARDI), Marine and Freshwater Research Institute, Victoria (MAFRI), Fisheries Western Australia and Tasmanian Fisheries from 11 locations across the geographic range of the species in southern Australia. All fish were adult as determined from body length and gonadal maturity according to the criteria of Ling (1958). Locations, collection dates and sample sizes are listed in Table 1.1 and the geographical locations of these sites are shown in Figure 1.1. Temporally replicated samples were collected from GSV (samples GSV1,2) and multiple samples were collected from within each region, i.e. WA, SA, Victoria and Tasmania. Regions are defined on the basis of legislative regions of responsibility (i.e. states) and therefore do not necessarily represent biological entities. Two other species of garfish, the river garfish *Hyporhamphus regularis* and the snub-nosed garfish *Arrhamphus sclerolepis*, were used as outgroups. The tissues were either frozen at -80°C or preserved in ethanol/sodium chloride solution 1:1 at room temperature.

#### DNA extraction, Polymerase Chain reaction (PCR) amplification, nucleotide sequencing

DNA was extracted from the tissues using either phenol/ chloroform (Sambrook *et al.* 1989) or salt extraction (Miller *et al.* 1988) methods, followed by ethanol precipitation then resuspended in nuclease free water. A 350 bp fragment of control region mtDNA was PCR amplified using the primers M252 5'-ACC ATC AGC ACC CAA AGC TAG G3' and H16498 5'- CCT GAA GTA GGA ACC AGA TG -3' (Meyer *et al.* 1990). Amplification conditions were: 50-100 ng target DNA, 10 pmol each primer, 0.2mM each of dATP, dTTP, dCTP and dGTP, 4mM MgCl<sub>2</sub>, 1x Taq dilution buffer and 0.75unit Promega Taq DNA polymerase in a 50µl reaction volume. PCR cycling

conditions were: 94 °C 3', 55 °C 45'', 72 °C 1' for one cycle, 94 °C 45'', 55 °C 45'' 72 °C 1' for 34 cycles and 72 °C 6', 26 °C 10''for one cycle (FTS-320 Thermal Sequencer, Corbett Research). PCR products were purified using Bresa-clean Nucleic Acid Purification Kit (Bresatec). Both strands of the purified PCR product were sequenced with the same primers used for PCR with the Perkin Elmer ABI PRISM<sup>™</sup> Dye Terminator Cycle Sequencing Ready Reaction Kit. Products were run on an ABI 373 or 377 model auto sequencing machine.

Table 1.1. Sample details of southern sea garfish examined for mitochondrial DNA variation

Sample	Location	Latitude	Longitude	Ν	Collection Date
Code		$(^{0}, S)$	(°,E)		
WA1	Cockburn Sound	32 12	115 44	29	10/5/1998
WA2	Oyster Harbour	34 58	117 57	29	28/1/1998, 7/5/1998
WCSA	Thevenard	32 09	133 39	28	18/12/1997
SGSA1	Tickera	33 46	137 42	29	20/1/1998
SGSA2	Arno Bay	33 56	136 35	27	20/1/1999
GSV1	Port Gawler	34 36	138 25	29	16/12/1997
GSV2	Port Gawler	34 36	138 25	28	27/1/1999
VIC1	Western Port	38 25	145 20	24	5/3/1998
VIC2	Corner Inlet	38 45	146 20	24	17/3/1998
TAS1	Marion Bay	42 45	147 55	27	15/1/1998
TAS2	Flinders Island	40 10	148 00	28	10/8/1997

To test whether the mitochondrial DNA sequences were mitochondrial in origin and not nuclear paralogues (Zhang & Hewitt, 1996), we carried out the following procedures outlined in Donnellan *et al.* (1999). MtDNA was enriched from frozen liver of a southern sea garfish and a river garfish by a plasmid DNA isolation method modified from Welter *et al.* (1989). Serial dilutions (eg. neat to  $10^{-6}$ ) of the enriched mtDNA were amplified with three sets of PCR primers: G18S2/G18S3 specific for the nuclear 18S rRNA locus (Monis et al. 1999), L1091/H1478 specific for 12S rRNA (Kocher *et al.* 1989), and the CR primers M252/ H16498 being tested. For each primer pair, we determined the maximum dilution (the endpoint) that produced successful amplification. We observed a thousand fold difference between the endpoints for the two mitochondrial primer pairs and the 18S rRNA primer pair. We also compared the sequence of the product amplified with the *CR* primers from the maximum dilution of the enriched mtDNA with that from total cellular DNA from the same individual. If these sequences were the same, we concluded that the primers only amplified mtDNA.

Figure 1.1. Map showing collection locations of 11 samples of southern sea garfish analysed for variation in mitochondrial DNA.



#### Temperature Gradient Gel Electrophoresis

Temperature gradient gel electrophoresis (TGGE) was performed on the horizontal gel apparatus available from DIAGEN GmbH. Conditions for parallel TGGE were optimised according to the manufacturer's directions with minor modifications (Campbell *et al.* 1995). Heteroduplexes were generated following a denaturing and renaturing protocol on a mixture comprising 10-15 ng of test PCR product and 10 ng of reference PCR product, in total volume of  $10\mu$ l of 1X DR buffer (4 mM urea, 200 mM MOPS, 10 mM EDTA 0.005 % bromophenol blue 0.005 % xylene xyanol FF Ph 8.0). Samples were denatured for 5 min at 95°C, allowed to re-anneal for 15 min at 50°C and then left to return to room temperature before 2-3 µl was run on a 5% polyacrylamide multi-well gel. Based on the melting behaviour of the heteroduplex bands on the perpendicular gel, a temperature gradient of 22°C-52°C was chosen for parallel TGGE/Heteroduplex analysis. All samples were heteroduplexed with a single reference sample (haplotype "x" - see below). The gels were run at 340 V and 35 mA for 3.5 hours and silver stained. Haplotypes were identified by comparison with samples that were repeatedly included on each gel (internal controls) and through critical side-by-side comparisons (line-ups).

#### Analysis

The program Modeltest2 (Posada and Crandell 1998) was used for maximum likelihood (ML) testing of models of molecular nucleotide evolution. Once the optimal model had been identified, iterative bouts of Quartet Puzzling (QP) (Strimmer and Vonhaeseler 1996), a variant of the maximum likelihood method for finding phylogenetic trees, was used to find the optimal value of any parameters of the model that needed to specified. Bouts were repeated until the likelihood of the resulting tree and the value of the parameter to be estimated did not change between bouts. The QP approach was implemented in PAUP\* version 4.0b2 (Swofford 1999) which was also used for constructing trees with the Neighbour Joining (NJ) algorithm (Saitou and Nei 1987) from a matrix of evolutionary distances determined under the same model found with Modeltest 2.

Genetic differentiation between samples and regions was quantified by an analysis of variance approach adapted for molecular data, AMOVA (Excoffier *et al.* 1992). AMOVA yields a statistic  $\Phi_{ST}$  analogous to the conventional  $F_{ST}$  except that the evolutionary divergence between haplotypes is incorporated into the analysis as well as the haplotype frequencies. The evolutionary distance between haplotypes utilised the model of nucleotide substitution found with the Modeltest procedure outlined above. The significance of  $\Phi_{ST}$  was tested by generating null distributions of values from 100,000 random permutations of the data matrix.

Exact tests of population differentiation used an extension of Fisher's exact probability test on contingency tables. It tests the hypothesis of a random distribution of k different haplotypes among r populations as described in Raymond and Rousset (1995). Instead of enumerating all possible contingency tables, a Markov chain is used to efficiently explore the space of all possible tables. During this "random walk" between the states of the Markov chain, the probability of observing a table less or equally likely than the observed sample configuration is estimated under the null hypothesis of panmixia. The table is built using sample haplotype frequencies (Raymond and Rousset 1995). An estimation of the standard error of the p value is done by partitioning the total number of steps into a given number of batches (Guo and Thompson 1992). Tests were performed with the program ARLEQUIN version 1.1 with a Markov chain length of 500,000 steps (Schneider *et al.* 1999). Significance levels ( $\alpha$ ) of pairwise tests were adjusted for multiple comparisons with the sequential Bonferroni procedure of Hochberg (1988).

Tests for isolation-by-distance were made with an approach proposed by Slatkin (1993) in which a significant negative correlation between the log-log regression of  $N_e m_f$  and geographical distance can be taken as evidence for isolation-by-distance. Because of the lack of independence of data points in matrices of pairwise comparisons, Mantel's (1967) test, implemented with NTSYS-pc version 1.70

(Rohlf 1990), was used to assess the significance of correlations after 1000 random permutations of the matrices.

The Tajima (1989) and Fu and Li (1993) tests for the selective neutrality of mutations were implemented in DnaSP, version 3.14.3 (Rozas and Rozas 1999). These statistics test the hypothesis that all mutations are selective neutral (Kimura 1983). For *n* nucleotide sequences,  $\pi$ , the average number of pairwise nucleotide differences between sequences (Nei 1987), *S*, the number of segregating (or polymorphic) sites (Watterson 1975), and the total number ( $\eta$ ) of mutations and the number ( $\eta_e$ ) of mutations in the external branches (Fu and Li 1993) are calculated and used in the  $D_T$  test [Equation 38 in Tajima (1989)], and the *D* and *F* tests (Fu and Li 1993). The tests were computed using  $\eta$ , the total number of mutations and S, the total number of segregating sites. Under the infinite sites model (with two different nucleotides per site) estimates of the three test statistics based on *S* and on  $\eta$  should have the same value. However, if there are sites segregating for more than two nucleotides, values of *S* will be lower than those of  $\eta$ . The tests were performed with the outgroups removed.

#### 1.3 Results

A total of 47 different control region haplotypes were detected by TGGE among the 273 garfish screened. Overall haplotype diversity was  $0.8253 \pm 0.0180$ . The geographic distribution and frequency of each haplotype is presented in Table 1.2. Each of these haplotypes was sequenced and aligned along with the control region sequences of the outgroups, two *Arrhamphus sclerolepis* and a single *Hyporhamphus regularis*. Initial alignment was done with the program ClustalW (Thompson *et al.* 1994) and improved by eye (Appendix 3). A total of 413 sites were in the final alignment. Uncorrected sequence divergence among haplotypes ranged from 12.8 to 16%. Haplotype diversity estimates were similar across all samples (Table 1.3) varying by approximately 20% at the most. Many haplotypes were shared among samples within regions and among regions, resulting in low endemism indices at both spatial scales (Table 1.3). Nucleotide diversity estimates were variable over a twofold range with the greatest difference occurring within the VIC region (Table 1.3).

Table 1.2. TGGE haplotype frequencies in 11 samples of southern sea garfish from southern Australia.

The first three rows show frequencies of TGGE haplotypes pooled according to their phylogenetic relationships (upper case letters). Pooled haplotype designations refer to lineages indicated in Fig. 1.2. The remaining rows show frequencies of individual TGGE haplotypes (lower case letters and numerals). The total number of individuals per sample = N. Superscript numbers show the total numbers of individuals with a TGGE phenotype from a sample that were nucleotide sequenced. All haplotypes found once were sequenced (not indicated). At least one individual for each haplotype was sequenced.

	WA1	WA2	WCS A	SGSA 1	SGSA 2	GSV1	GSV2	VIC1	VIC2	TAS1	TAS2
Ν	29	29	28	29	27	29	28	24	24	27	28
А	27	28	26	27	23	24	26	24	20	25	25
B C	1 1	1 1	2	1 1	4	4 1	2		3 1	2	3
a	1	1	1	6 <sup>1</sup>		$\frac{1}{4^3}$	1	1	1		2
b	-		$\frac{1}{2^{1}}$	Ū		•	-	-			-
с					2			3 <sup>1</sup>		$2^{1}$	
d e	1	$10^{1}$	3 <sup>1</sup>	3 <sup>1</sup>	2 10	$2^{2}$	2	31 91	4	4 4	6 4
f	1	10	1	5	10	2	1	7	4	1	4
g h										1	6 <sup>1</sup>
h						1	1		3 <sup>1</sup>		
i ;			1	$2^1$							
j k	1			$\frac{2}{2^{1}}$	1	3					
1	$17^{2}$	12	$11^{1}$	$2^{1}$ $2^{1}$ $8^{1}$ $1^{1}$ $3^{1}$	4	3 11 <sup>9</sup>	9	$7^{1}$	8 <sup>1</sup>	12	5
m	1	2	2	$1^{1}$			4	2			
n				31	4	$2^{1}$	3	1	$\frac{3}{1^1}$		
o p				1		1		1	1		
q				1	4	$\frac{1}{3^3}$	1			2	1
q r			$1^{1}$					1			
S	$\frac{3}{1^{1}}$	1	$1^1$						1		
t u	1	1				$1^1$					
v	1	1	1		1	1				1	
W									$2^{1}$		
х			1			1			1		
y z			1 1								
1			1								
2			1								
1 2 3 4				1 1							
4 5	1			1							
6	1										
6 7	1										
8 9		1									
9 10		1 1									
		1									1
11 12											1
13									1		1
14 15					1				1		
16					1		1				
16 17							1				
18							1				
19 20							1 1				
20							1				

Phylogenetic relationships among the 47 haplotypes were explored with a number of approaches. The model of sequence evolution found to be optimal through a likelihood ratio test with Modeltest2 was the TrN model (Tamura and Nei 1993) with a specified gamma shape parameter (Г). The gamma shape parameter was estimated to be 0.112346 after three iterative QP rounds of estimation. The NJ distance tree (with the TrN +  $\Gamma$  model) and the maximum likelihood (QP) trees produced the same rooting for the position of the outgroups. As the length of the branch to the outgroups was very long in comparison to the branch lengths among the ingroup haplotypes, we present these trees with the outgroups removed but rooted by the outgroup method so that the details of relationships among the ingroup haplotypes are more easily seen (Fig. 1.2a,b). Both trees are concordant in finding three major lineages: lineage A which includes haplotypes a, c, d, e, f, g, i, j, l, k, m, n, o, p, r, s, t, u, v, w, y, z, 1, 2, 4, 5, 6, 8, 9, 10, 12, 14, 15, 17, 18, 19, 20 and 21; lineage B which comprises haplotypes b, h, q, 11, 13, and 16; and lineage C which includes haplotypes x, 3, and 7. Each lineage is mostly well supported by either NJ bootstrap analysis or QP support values (Fig. 1.2). The minimum spanning network (not shown), without the outgroup haplotypes, showed three major groupings equivalent to the three lineages present in Fig. 1.2. Because we did not aim to make the TGGE procedure sensitive enough to detect all substitutions, we also sequenced several individuals from six haplotypes that were found in more than one individual from lineages A and B, a (N=4), e (N=6), k (N=5), 1 (N=15), n (N=3) and q (N=3) as a test that a TGGE phenotype was indicative of membership of one of these lineages. The position of these multiple sequences in the phylogenetic analysis was consistent with their membership of the same lineage as the representative sequence of that TGGE haplotype shown in Fig. 1.2 (data not presented). All three lineage C haplotypes occurred in single individuals only. Thus we were confident that the TGGE phenotype is representative of phylogenetic affinity.

Relationships within the three lineages are not well resolved in general. Apart from six pairs or triplets of taxa that receive strong support from either NJ bootstraps or QP support indexes or both, no other node received strong support (Fig. 1.2). The relationships between the geographic distribution of each haplotype and its phylogenetic relationships appear to be unrelated as a comparison of Table 1.2 and the topology of the trees in Fig. 1.2 indicates. The common haplotypes 'e' and 'l' are found in all samples. Of the three major haplotype lineages, A is distributed through all samples, B is found in all regions except WA and C is found in WA and the South Australian gulfs (SG and GSV). In summary there does not appear to be strong phylogeographic structure among these samples.

Table 1.3. Mitochondrial control region diversity within samples and regions sampled for southern sea garfish.

<sup>1</sup>N = sample size. <sup>2</sup>Calculated according to E = e/n, where *e* and *n* are the numbers of putatively endemic haplotypes and the total number of haplotypes detected in each sample respectively. <sup>3</sup>Haplotype diversity =  $(1 - \Sigma x_i^2)/n/(n-1)$ , where *x* is the frequency of the *i*th haplotype (Nei 1987). <sup>4</sup>Nucleotide diversities ( $\pi_n$ ) for TGGE haplotypes, after Nei (1987), based on their nucleotide sequences, standard errors calculated after Nei and Jin (1989).

Region	Sample	$N^1$	No. of	Endemism	TGGE Haplotype	Nucleotide
			haplotypes	Index <sup>2</sup>	diversity <sup>3</sup>	diversity <sup>4</sup>
WA	WA1	29	11	0.27	0.6576 <u>+</u> 0.0992	0.0051 <u>+</u> 0.0033
	WA2	29	8	0.38	0.7241 <u>+</u> 0.0586	0.0047 <u>+</u> 0.0032
Total regi	ion	58	15	0.47	0.7169 <u>+</u> 0.0563	0.0050 <u>+</u> 0.0032
SA	WCSA	28	14	0.43	0.8413 <u>+</u> 0.0646	$0.0065 \pm 0.0041$
	SGSA1	29	11	0.27	0.8744 <u>+</u> 0.0384	0.0074 <u>+</u> 0.0045
	SGSA2	27	8	0.13	0.8177 <u>+</u> 0.0524	0.0072 <u>+</u> 0.0044
	GSV1	29	10	0.10	0.8300 <u>+</u> 0.0562	0.0086 <u>+</u> 0.0051
	GSV2	28	14	0.43	0.8783 <u>+</u> 0.0487	0.0074 <u>+</u> 0.0045
Total regi	ion	141	32	0.50	$0.8679 \pm 0.0200$	$0.0074 \pm 0.0044$
VIC	VIC1	24	7	0.00	0.7790 <u>+</u> 0.0570	0.0041 + 0.0029
	VIC2	24	9	0.22	0.8514 <u>+</u> 0.0505	0.0095 <u>+</u> 0.0056
Total regi	ion	48	13	0.15	0.8271 <u>+</u> 0.0371	0.0070 <u>+</u> 0.0043
TAS	TAS1	27	8	0.13	0.7721 <u>+</u> 0.0688	0.0048 + 0.0032
	TAS2	28	10	0.30	0.8757 <u>+</u> 0.0315	0.0082 <u>+</u> 0.0049
Total regi	ion	55	12	0.42	0.8411 + 0.0293	$0.0660 \pm 0.0040$

In the absence of strong evidence of phylogeographic structure for most of the species range, we proceeded to analyse differences in haplotype frequencies between samples and regions. Population differentiation tests showed significant differentiation only between samples from different regions (Table 1.4A). Both haplotype-based and sequence-based AMOVA analyses partitioned molecular variation in a similar pattern. The haplotype-based analysis, which treated all haplotypes as evolutionarily equidistant partitioned 4.2% of the variation between samples similar to the 4.01% for the sequence-based analysis. Pairwise  $\Phi_{ST}$  values ranged from 0 to 0.17 (haplotype based) and from 0 to 0.11 (sequence based) with only three values being significantly greater than zero after permutation testing (Table 1.4A). Estimated numbers of effective female migrants per generation ( $N_em_f$ ) were calculated from the pairwise haplotype-based  $\Phi_{ST}$  values according to the formula  $N_em_f = \frac{1}{2}[1/\Phi_{ST} - 1]$  which is corrected for mtDNA (Table 1.4B). While estimated levels of  $N_em_f$  vary widely among the samples, the largest estimates occur over all spatial scales e.g. GSV1- SGSA1; GSV2 - TAS1; WA2 - VIC1.

After pooling of haplotypes within regions, population differentiation tests showed significant differentiation between all regional comparisons except for SA with VIC (Table 1.4B). The haplotype-based and sequence-based AMOVA analyses partitioned 2.27% and 1.83% of the molecular variance respectively between samples. The pattern of significant  $\Phi_{sT}$  values differed

between the haplotype and sequence based approaches (Table 1.4B) but both were consistent in not showing significant differences between SA and VIC. Estimated levels of  $N_e m_f$  between regions were uniformly high.

In tests for an isolation-by-distance model, regressions using haplotype and sequence based  $\Phi_{ST}$  were used to test for a relationship between gene flow and geographical distance among sample localities. Mantel's (1967) test showed a negative correlation with variation in gene flow that was not significant in either case: haplotype based - Mantel's matrix r = -0.126, P = 0.18, sequence based - Mantel's matrix r = -0.184, P = 0.12 respectively.

As a large proportion of the haplotypes occurred at relatively low frequency, we also explored an objective method of pooling haplotypes into "classes" so that individual pooled haplotype classes would have higher sample sizes in order to increase the power to detect statistically significant differences in haplotype frequencies. The approach we took was to use the topology of the haplotype trees (Fig. 1.2) to pool haplotypes into lineages. We chose to pool haplotypes into the three major lineages, A, B and C. The frequencies of each of these pooled haplotype classes are summarised in Table 1.2. All pairwise comparisons in the population differentiation test were non-significant (smallest P = 0.11 with 100 000 Markov chain steps). As the haplotype frequencies in this analysis were so similar and there was no evidence of differentiation between samples we did not pool temporal replicates or regional samples.

All three tests for the neutrality of mutations returned non-significant values:  $D_T = -1.30746$ , P > 0.10; D = -1.52194, P > 0.10; F = -1.71751, P > 0.10. The results were identical when tested under the separate comparisons of either total number of mutations or segregating sites, indicating that the southern sea garfish *CR* does not violate the assumptions of the infinite sites model of substitution.



Fig. 1.2 A Neighbour-joining tree showing relationships among southern sea garfish mitochondrial control region haplotypes. The NJ tree was constructed from  $TrN + \Gamma$  distances. Values at the nodes represent bootstrap proportions among 2000 NJ pseudoreplicates (upper value) and QP support indices (lower value).

Table 1.4. Population differentiation tests (lower left matrix) and estimated numbers of female migrants per generation  $N_e m_f$  (upper right matrix) among A) 11 samples of southern sea garfish samples and B) among regions.

\* indicates tests that remained significant after permutation testing (1 000 randomisations) for robustness of sample sizes. \*\*  $N_e m_f$  not calculated as the  $\Phi_{ST}$  value was not significantly different from zero. <sup>h,s</sup> indicate significant haplotype based  $\Phi_{ST}$  (h) or sequence based  $\Phi_{ST}$  (s) values. The  $\alpha$  for all tests of significance was adjusted for multiple testing with the sequential Bonferroni procedure. **A**)

	WA1	WA2	WCSA	SGSA1	SGSA2	GSV1	GSV2	VIC1	VIC2	TAS1	TAS2
WA1	-	10	**	55	$3^{h,s}$	8	24	7	5	65	$7^{\rm h}$
WA2	0.0048	-	30	6	4	$4^{\rm s}$	7	**	4	11	5
WCSA	< 0.0001*	< 0.0001*	-	113	4	13	**	20	8	**	20
SGSA1	0.0058	0.0441	< 0.0001*	-	4	**	**	10	27	**	15
SGSA2	< 0.0001*	< 0.0001*	< 0.0001*	0.0078	-	13	6	5	**	7	6
GSV1	0.1572	0.0272	< 0.0001*	0.8508	0.0094	-	**	6	**	**	21
GSV2	0.0422	0.0054	0.0076	0.2381	0.0052	0.1856	-	10	343	**	27
VIC1	0.0011	0.0003*	0.0013	0.0124	0.0488	0.0043	0.0372	-	7	11	6
VIC2	0.0085	< 0.0001*	< 0.0001*	0.0253	0.0128	0.1261	0.2592	0.0175	-	45	20
TAS1	0.0067	< 0.0001*	< 0.0001*	0.0029	0.0177	0.0046	0.0235	0.0935	0.0089	-	151
TAS2	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	0.0027	0.0005*	0.0095	0.0317	0.0005*	0.1052	-

B)

	WA	SA	VIC	TAS
WA	-	18.76308 <sup>h,s</sup>	22.56337 <sup>s</sup>	10.22523 <sup>h,s</sup>
SA	0.0109*	-	60.23708	20.93691 <sup>h</sup>
VIC	0.0069*	0.1071	-	23.47942 <sup>h</sup>
TAS	< 0.0001*	< 0.0001*	0.0005*	-

#### **1.4 Discussion**

The frequency based analyses detected significant differences in haplotype frequencies and significant partitioning of variance in haplotype frequencies across the species range. However, these differences were not detected at all spatial scales, rather they were pre-eminent in comparisons among regions. The absence of population differentiation within WA, the SA gulfs/VIC regions indicates that each could be considered as a single management unit. Furthermore, with the increased power from larger samples sizes after pooling of samples within regions, we were not able to demonstrate any differentiation between the South Australian and Victorian regional samples, suggesting that at least four management units could be recognised along the Australian southern coastline, namely WA, WCSA, SA Gulfs/VIC and TAS. As garfish samples were pooled on the basis of political and legislatively determined management regions, the units we suggest here do not necessarily represent units consistent with demographically isolated populations. Sampling of further localities across the species range would be required to determine the nature of the low level of population differentiation observed among regions.

A comparison of southern sea garfish  $\Phi_{ST}$  partitioning among regions with other marine species based on mitochondrial *CR* sequences, shows that the southern sea garfish has one of the lowest values (1.83%) reported to date, eg catadromous barramundi 32.8% (Chenoweth *et al.* 1998), catadromous Australian bass 6-14.6% (Chenoweth and Hughes 1997, Jerry and Baverstock 1998), rockfish 15% (Rocha-Olivares and Vetter 1999), snapper (frequency based only) 12.53% (Donnellan & McGlennon, 1996), and oceanic swordfish 15.32% (Rosel and Block 1996).This together with the "shallow" *CR* haplotype tree, star-like structure to the tree and low levels of population differentiation suggest that the southern sea garfish population may not have been demographically stable. This could influence our ability to distinguish an isolation by distance population structure model from panmixia as sufficient time may not have elapsed since a population decline/expansion event for the population to have come into drift/gene flow equilibrium throughout its current geographic range.

As a word of caution it should be noted that several recent studies have reported mtDNA showing no or less evidence of population differentiation than nuclear markers, e.g. Elliott 1996, Smith *et al.* 1997, Ward *et al.* 1994. In two of these cases however, Elliott (1996) and Ward *et al.* (1994), the nuclear differentiation was detected only in a single locus, and could have been influenced by selection rather than overall population history. In Smith *et al.* (1997), orange roughy showed population differentiation with multiple nuclear markers among more pairs of populations than did mtDNA. Birky *et al.* (1989) have shown that under conditions of strongly female biased sex ratios, mtDNA may be less sensitive to historical population differentiation. They suggest that the sex ratio

would need to be at least 7:1 in favour of females. Although there appears to be a female biased sex ratio (approx. 5:1) among fish in shallow inshore waters during the spawning period (October to February), the sex ratio during winter in deeper inshore waters is close to 1:1 (see chapter 5). Alternatively mitochondrial haplotype frequencies could be influenced by stabilising selection maintaining similar frequencies in different regions in the face of limited gene flow, but tests for the neutrality of mutations did not detect evidence of selection on the southern sea garfish *CR*. The potential effect of selection could be also tested with data from nuclear markers.

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Appendix 1.1. Nucleotide sequence alignment of 47 southern sea garfish CR haplotypes, with the two outgroups, *Hyporhamphus regularis* (Hr) and *Arrhamphus sclerolepis* (As).

As1 CGCCCCAR-A-GTA-CATATATGGAC-TATAC-TAACATTTAT-CTAGTACATAAATATATGTATTATCACCATTAATTTATATCAAACATAAT-TGAATGATTAGAGGA As2 CGCCCCA-RA-GTA-CATATATGGAC-TATAC-TAACATTTAT-CTAGTACATAAATATATGTATTATCACCATTAATTTATATCAAACATAAT-TGAATGATTAGAGGA Hr CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGA а CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGA a2 a3 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATTAAACAT-TTATCAATGA-TAGAGGA a4 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGAG b CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGA С d CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATMAATGA-TAGAGGR CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG е CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG e2 e3 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG e4 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG e5 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG eб f CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGA CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG g h CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGAG i CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG j ????CA-AA-STR-CATATATSGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTAKCAATGA-TARAGGG k CGCCCCA-AA-GTAGCATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG k2 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG k3 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG k4 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG k5 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATTAAACAT-TTATCAATGA-TAGAGGG 1 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATNTCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG 12 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATTAAACAT-TTATCAATGA-TAGAGGG 13 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG 14 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATTAAACAT-TTATCAATGA-TAGAGGG 15 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG 16 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG 17 CGCCCCA-AA-GTA-CATATATGGACATATACATAR-RTCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATTAAACAT-TTATCAATGA-TAGAGGG 18 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG 19 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATTAAACAT-TTATCAATGA-TAGAGGG 110 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATTAAACAT-TTATCAATGA-TAGAGGG 111 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG 112 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATTAAACAT-TTATCAATGA-TAGAGGG 113 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATRTCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGA m CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG n n2 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATGTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATTAAACAT-TTATCAATGA-TAGAGRG n3 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG Ο р CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGAG q CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATTAAACAT-TTATCAATGA-TAGAGAG q2 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGAA q3 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATTAAACAT-TTATCAATGA-TAGAGGG r CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG s CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATYTATA-TAGTACATATATCTATGKATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG t CGCCCCA-AA-GTR-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TARAGGG u CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATTAAACAT-TTATCAATGA-TAGAGGG v CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATAAATGA-TAGAGGA W CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATTAAACAT-TTATCAATGA-TAGAGAA х CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGA У SGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGRG Z 1 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATTAAACAT-TTATCAATGA-TAGAGGG 2 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG 3 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATTAAACAT-TTATCAATGA-TAGAGGG 4 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG 5 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGAA б CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG 7 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATSTATA-TAGTACATATATSTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG 8 CGCCCCA-AA-GTA-CATATATGGACATATGCATAA-WTYTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG 9 CGCCCCA-AAAGTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGAA 10 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATYTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG 12 ?GCCCCA-AA-GTA-CATATATGGACATATACATAA-ATYTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG 13 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTATATAAACAT-TTATCAATGA-TAGAGAG 14 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATAAAATGA-TAGAGGA 15 CGCCCCA-AA-GTA-CATATATGGACGTATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTATATAAACAT-TTATCAATGA-TAGAGAG 16 CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGA

CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCATTCATTTATATAACAT-TTATCAATGA-TAGAGGG
CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCATTCATTTATATAACAT-TTATCAATGA-TAGAGGG
CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG
CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGA
CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGA
CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG
CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG
CGCCCCA-AA-GTA-CATATATGGACATATACATAA-ATCTATA-TAGTACATATATCTATGTATTATCCCCATTCATTTATATAAACAT-TTATCAATGA-TAGAGGG

As1	CACACCATTCTTGATCAATAAACTGGT-T-TCTTTCAAACATA-TAACTCCACATAAAGATCCACTCCAA-A-ATA-AAGTGAGA-AAAACAA-AGATA-GACAA
As2	CACACCATTCTTGATCAATAAACTGGT-T-TCTTTCAAACATA-TAACTCCACATAAAGATCCACTCCAA-A-ATA-AAGTGAGA-AAAACAA-AGATA-GACAA
Hr	CATATCATTTTTGAT-AATAAAGAAAAATGTCTAT-AAACATAATAATTCCCCCATATA-ATCCAACAA-ACCATATAAATGAAGAAAACAT-TTAAT-GATATCT
а	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
a2	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
a3	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
a4	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
b	TATATCAATATTTAAACAACT-AAATTAAGACATAGAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
С	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAA-TCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
d	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
е	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AGTATAACAGAACTA-GA-AT-T
e2	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AGTATAACAGAACTA-GA-AT-T
e3	TATATCAGTGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
e4	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AGTATAACAGAACTA-GA-AT-T
e5	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GG-AATATAACAGAACTA-GA-AT-T
еб	TATATCAATGTTTTAAACAACT-AAATTAAGACATAGAAATCCA-T-CAATAC-ATA-AAATG-GA-AGTATAACAGAACTA-GA-AT-T
f	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAA-TCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
g	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GG-AT-T
h	TATATCAATATTTTAAACAACT-AAATTAAGACATAGAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
i	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GG-AT-T
j	TATATCAATGTTTTAAACAACT-AAATTAARACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACARAACTA-RA-AT-T
k	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAAAAGAACTA-AA-AT-T
k2	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GT-AATATAACAGAACTA-GA-AT-T
k3	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GT-AATATAACAGAACTA-GA-AT-T
k4	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAAAAGAACTA-GA-AT-T
k5	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAAAAGAACTA-GA-AT-T
1	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
12	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
13	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
14	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
15	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T

16	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
17	
18	TATATCAATGTTTTAAACAAC-T-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
19	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
110	) TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
	2 TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
-	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
115	5 TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GR-AT-T
m	TATATCAATGTTTTAAACAAC-T-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AACATAACAGAACTA-GA-AT-T
n	TATATCAATGTTTTAAACAAC-T-AAATTAAGACATAGAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
n2	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GG-AT-T
n3	TATATCAATRTTTTAAACAACT-AAATTAAGACATARAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GR-AT-T
0	TATATCAATGTTTTAAACAACT-AAATTAAGACATAGAAATCCA-T-CAATAC-ATA-AAATG-GA-AACATAACAGAACTA-GA-AT-T
p	CATATCAATGTTTTAAACAACT-AAATTAARACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAAAACTA-GA-AT-T
a	TATATCAATATTTTAAACAACT-AAATTAAGACATAGAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
q2	TATATCAATATTTTAAACAACT-AAATTAAGACATAGAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
q3	TATATCAATATTTTAAACAACT-AAATTAAGACATAGAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
r	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AGTATAACAGAACTA-GA-AT-T
S	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AGTATAACATAAC
t	TATATCAATGTTTTAAATAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AGTATAACAGAACTA-GA-AT-T
u	TATATCAATGTTTTAAACAACT-AAATTAARACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACARAACTA-GA-AT-T
v	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
w	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAA-TCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
x	TATATCAATATTTTAAACAACT-AGATTAAGACATAGAAATCCA-T-CAATAC-ATG-AAATG-GA-AATATAACAGAACTA-GA-AT-T
У	CATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAAAACTA-GA-AT-T
z	TATATCAATRTTTTAAACAACT-AAATTAAGACATARAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
1	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
2	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
3	TATATCAGTGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-GATATAACAGAACTA-GG-AT-T
4	TATATCAATGTTTTAAATAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AGTATAACAGAACTA-GA-AT-T
5	TATATCAATATTTTAAATAACT-AGATTAAGACATAGAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAGCAGAACTA-GA-AT-T
6	TATATCAATGTTTTAAATAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AGTATAACAGAACTA-GA-AT-T
7	TATATCAATGTTTTAAASAA?T-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAAYTA-GG-AT-T
8	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T
9	TATATCAATATTTTAAACAACT-AGATTAAGACATAGAAATCCA-T-CAATAC-ATG-AAATG-GA-AATATAACAGAACTA-GA-AT-T
10	TATATCAATGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AACATACCAGAACTA-GA-AT-T
11	ТАТАТСААТGTTTTAAACAACT-AAATTAAGACATAAAAATCCA-T-CAATAC-ATA-AAATG-AT-AATATAACAGAACTA-GA-AT-T

12TATATCAATGTT--T--TAAACAA----C--T-AAAT---TAA--GACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AGTATAACAGAACTA-GG-AT-T13TATATCAATATT--T--TAAACAA----C--T-AAAT---TAA--GACATAGAAATCCA-T-CAATAC-ATG-AAATG-GA-AATATAACAGAACTA-GA-AT-T14TATATCAATGTT--T--TAAACAA----C--T-AAAT---TAA--GACATAGAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T15TATATCAATGTT--T--TAAACAA----C--T-AAAT---TAA--GACATAGAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GA-AT-T16TATATCAATGTT--T--TAAACAA----C--T-AAAT---TAA--GACATAGAAATCCA-T-CAATAC-ATA-AAATG-GA-AACATAACAGAACTA-GA-AT-T17TATATCAATGTT--T--TAAACAA----C--T-AAAT---TAA--GACATAGAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GG-AT-T18TATATCAATGTT--T--TAAACAA----C--T-AAAT---TAA--GACATAGAAATCCA-T-CAATAC-ATA-AAATG-GA-AATATAACAGAACTA-GG-AT-T19TATATCAATGTT--T--TAAACAA----C-T-AAAT---TAA--GACATAAAAATCCA-T-CAATAC-ATA-GAATG-GA-AATATAACAGAACTA-GG-AT-T20TATATCAATGTT--T--TAAACAA----C-T-AAAT---TAA--GACATAAAAATCCA-T-CAATAC-ATA-GAATG-GA-AACATA-CAGAACTA-GG-AT-T21TATATCAATGTT--T--TAAACAA----C-T-AAAT---TAA--GACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AACATA-CAGAACTA-GG-AT-T22TATATCAATGTT--T--TAAACAA----C-T-AAAT---TAA--GACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AGTATAACAGAACTA-GA-AT-T23TATATCAATGTT--T--TAAACAA----C-T-AAAT---TAA--GACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AGTATAACAGAACTA-GA-AT-T23TATATCAATGTT--T--TAAACAA----C-T-AAAT---TAA--GACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AGTATAACAGAACTA-GA-AT-T23TATATCAATGTT--T--TAAACAA----C--T-AAAT---TAA--GACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AGTATAACAGAACTA-GG-AT-T23TATATCAATGTT--T--TAAACAA----C--T-AAAT---TAA--GACATAAAAATCCA-T-CAATAC-ATA-AAATG-GA-AGTATAACAGAACTA-GG-AT-T

AS1 AGTACCATACGTTA-AA-TT-AAGCCCGACCACAAAAACTCATCAGTCTAGATATACCAGGACTCAACACCTCTGGATAAT--CAAATATTTAATGTAGTAAGAGACC-A As2 AGTACCATACGTTA-AA-TT-AAGCCCGACCACAAAAACTCATCAGTCTAGATATACCAGGACTCAACACCTCTGGATAAT--CAAATATTTAATGTAGTAAGAGACC-A Hr T-TGCCAAACG-TATAA-TT-AAGCCCGATCACAATAACTCATCTGTCTAGATATACCAGGACTCAACATCTCT-GAAGAT--CAATTATTTAATGTAGTAAGAGACC-A AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA а a2 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA a3 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA a4 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA b AGTACCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAAACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA С AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGASACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA d е AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAAACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA e2 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA e3 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAC-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA e4 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA e5 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAKTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAKTAAGAG-CCTA AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA e6 f AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA q AGTGCCAAACG--A-AAGTTTAAGACCGATCACAC-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATCTCAAATATTTAATGTAGTAAGAG-CCTA AGTACCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAAACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA h AGTGCCANACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA i j AKTGCCAAACG--A-AAKTTTAARACCGATCACAA-CTCTCATAAKTCTAGTTATACCAARAGACACCATCCCT--AAAATTTCAAATATTTAATGTAKTAAGAR-CCTA k AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA k2 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA k3 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA k4 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA k5 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA

1		AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
1:	2	AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
1	3	AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
1.	4	AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
1!	5	AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
10	6	AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
1.	7	AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
1:	8	AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
1	9	AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
1	10	AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAAACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
1	11	AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
1	12	AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
1	13	AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
11	14	AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
1	15	AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
m		AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
n		AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGANACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
n	2	AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAANAG-CCTA
n	3	AGTRCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGARACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
0		AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
p		AGTGCCAAACGA-AAGTTTAARACCGATCACAA-CTCTCATAAKTCTAGTTATACCAARARACACCATCCCTAAAATTTCAAATATTTAATGTAKTAAGAG-CCTA
q		AGTACCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGGAACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
q	2	AGTACCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAAACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
q	3	AGTACCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAAACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
r		AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCWAATATTTAATGTAGTAAGAG-CCTA
S		AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
t		AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
u		AKTGCCAAACGA-AAGTTTAARACCGATCACAA-CTCTCATAAGTCTAGTTATACCAARARACACCATCCCTAAAATTTCAAATATTTAATGTAKTAAGAG-CCTA
v		AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTYTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
W		AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGGCACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
x		AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAAACACCATCCCTAAAACTTCAAATATTTAATGTAGTAAGAG-CCTA
У		AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
Z		AGTRCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGARACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
1		AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTYTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
2		AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
3		AGTGCCAAACGA-AAGTTTAAGACCGATCACAC-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATCTCAAATATTTAATGTAGTAAGAG-CCTA
4		AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA
5		AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAAACACCATCCCTAAAACTTCAAATATTTAATGTAGTAAGAG-CCTA
б		AGTGCCAAACGA-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCTAAAATTTCAAATATTTAATGTAGTAAGAG-CCTA

7 8 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA 9 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAAACACCATCCCT--AAAACTTCAAATATTTAATGTAGTAAGAG-CCTA 10 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTTTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA 11 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA 12 13 AGTACCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAAACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA 14 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGACACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA 15 AGTACCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAAACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA 16 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA 17 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA 18 AGTACCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAAACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA 19 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTTTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA 20 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA 21 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA 22 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA 23 AGTGCCAAACG--A-AAGTTTAAGACCGATCACAA-CTCTCATAAGTCTAGTTATACCAAGAGACACCATCCCT--AAAATTTCAAATATTTAATGTAGTAAGAG-CCTA

As1 CCATCAGTTGATTTCTTAATGCATACTCTTATTG-ATGGTCAGGGACAGAAATCGTGG--GGGTTTC-ACTTC-TTGAATTAT As2 CCATCAGTTGATTTCTTAATGCATACTCTTATTG-ATGGTCAGGGACAGAAATCGTGG--GGGTTTC-ACTTC-TTGAATTAT Hr CCATCAGTTGATTTCTTAATGCATACTCTTATTG-ATGGTCAGGGACAGAAMTC-GT-GGGGGGTTTC-ACTTC-TTGAATTAT CCATCAGTTGATTCCTTAATGAACACTCTTATTG-AAGGTGAGGGACAAAAATCCGT-GGGGGTTTC-ACTTCCTTGAATTAT а a2 CCATCAGTTGATTCCTTAATGAACACTCTTATTG-AAGGTGAGGGACAAAAATC-GT-GGGGGGTTTC-ACTTC-TTGAATTAT a3 CCATCAGTTGATTCCTTAATGAACACTCTTATTG-AAGGTGAGGGACAAAAATC-GT-GGGGGGTTTC-ACTTC-TTGAATTAT CCATCAGTTGATTCCTTAATGAACACTCTTATTG-AAGGTGAGGGACAAAAATC-GT-GGGGGGTTTC-ACTTC-TTGAATTAT a4 CCATCAGTTGATTCCTTAATGAACACTCTTATTG-AAGGTGAGGGACAAAAATC-GT-GGGGGGTTTC-ACTTC-TTGAATTAT b CCATCAGTTGATTCCTTAATGAACACTCTTATTG-AAGGTGAGGGACAAAAATC-GT-GGGGGGTTTC-ACTTC-TTGAATTAT С CCATCAGTTGATTCCTTAATGAACACTCTTATTG-AAGGTGAGGGACAAAAATC-GT-GGGGGGTTTC-ACTTC-TTGAATTAT d CCATCAGTTGATTCCTTAATGAACACTCTTATTG-AAGGTGAGGGACAAAAA-C-GT-GGGGGGTTTC-ACTTC-TTGAATTAT е e2 CCATCAGTTGATTCCTTAATGAACACTCTTATTG-AAGGTGAGGGACAAAAATC-GTCGGGGGGTTTC-ACTTC-TTGAATTAT e3 CCATCAGTTGATTCCTTAATGAACACTCTTATTG-AAGGTGAGGGACAAAAATC-GT-GGGGGGTTTC-ACTTC-TTGAATTAT CCATCAGTTGATTCCTTAATGAACACTCTTATTG-AAGGTGAGGGACAAAAATC-GT-GGGGGGTTTC-ACTTC-TTGAATTAT e4 e5 CCATCAGTTGATTCCTTAATGAACACTCTTATTG-AAGGTGAGGGACAAAAATC-GT-GGGGGGTTTC-ACTTC-TTGAATTAT CCATCAGTTGATTCCTTAATGAACACTCTTATTG-AAGGTGAGGGACAAAAATC-GT-GGGGGGTTTC-ACTTC-TTGAATTAT eб f CCATCAGTTGATTCCTTAATGAACACTCTTATTG-AAGGTGAGGGACAAAAATC-GT-GGGGGGTTTC-ACTTC-TTGAATTAT CCATCAGTTGATTCCTTAATGAACACTCTTATTG-AAGGTGAGGGACAAAAATC-GT-GGGGGGTTTC-ACTTC-TTGAATTAT q h CCATCAGTTGATTCCTTAATGAACACTCTTATTG-AAGGTGAGGGACAAAAATC-GT-GGGGGGTTTC-ACTTC-TTGAATTAT i CCATCAGTTGATTCCTTAATGAACACTCTTATTG-AAGGTGAGGGACAAAAATC-GT-GGGGGGTTTC-ACTTC-TTGAATTAT j CCATCAGTTGATTCCTTAATGAACACTCTTATTG-AAGGTGAGGGACAAAAATC-GT-GGGGGGTTTC-ACTTC-TTGAATTAT
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# **CHAPTER 2. AGE AND GROWTH RATE DETERMINATION**

Q. Ye, D. A. Short, C. Green, P. Coutin

Objective: Determine the size and age structure of the commercial catch from the different sectors in southern Australian waters, and improve understanding of the potential impacts of the competing gear sectors on the South Australian stocks.

Age and growth of sea garfish *Hyporhamphus melanochir* were studied across WA, SA, and Victoria. The otoliths were assessed for their usefulness in adult ageing. Transverse sectioned sagittae displayed alternating opaque and translucent zones. Both marginal-increment analysis and treatment of fish with tetracycline demonstrated that the first three opaque zones formed annually during the spring/summer, starting from the first year of life. An ageing protocol is recommended based on sectioned otoliths. An algorithm was developed to calculate age in months from otolith counts and edge interpretation based on a fixed birth-date at the mid-point of the spawning season. Age assignment was confirmed by linear regressions of otolith weight against fish age.

To calibrate the age determination for *H. melanochir*, otolith readings were compared between readers, laboratories, i.e. SARDI Aquatic Sciences and the Central Ageing Facility (CAF) at the Marine and Freshwater Resources Institute (Victoria), and two otolith preparation methods (transverse sectioning and breaking and burning) using a combination of age bias plots, age frequency tables, and coefficient of variations. Initial comparisons based on transverse sections indicated consistent age estimation between two readers within SARDI with a mean CV of 2.2% for fish aged 0 to 10, but apparent bias in otolith interpretation between the readers from two organisations with relatively high CV (average about 10%) particularly for age groups of 1 to 3. Therefore a calibration workshop was conducted between SARDI and CAF to standardise the ageing techniques through re-examining otolith structure, comparing readings of the same otolith by three readers, and clarifying reading criteria. The first annulus was clearly defined as the broad opaque zone immediately adjacent to the opaque primordium; false marks were identified as dark zonal structure that could not be traced clearly around the entire potion of the otolith; and edge type should be examined around the whole otolith margin rather than being judged from faster growth area. After the calibration, there was good agreement in age determination for fish aged 1 to 4 and appreciably improved precision (mean CV < 3%) between SARDI and CAF. Paired age comparisons between the two otolith preparation methods suggested that broken/burnt otoliths provided unbiased age estimates for sea garfish relative to the transverse sections. The CV of age estimates by broken/burnt method was averaged at 3.7% across age groups 0 to 10.

The growth of sea garfish is relatively fast particularly in their first 3 years of life across the southern Australian waters. However, there was wide variability in length-at-age data. The maximum age recorded was 6, 6, and 10 for fish from SA, Victoria, and WA, respectively. There were significant differences in growth parameters between females and males from most of the regions, where females reached a higher asymptotic length but males often grew faster. Growth was also variable for combined sexes between regions within SA and WA and among the three states. The von Bertalanffy growth parameters for combined sexes were:  $L_{\hat{A}} = 289.1 \text{ mm SL}$ ,  $k = 0.0618 \text{ month}^{-1}$ ,  $t_o = -1.0$ months from SA,  $L_{\hat{A}} = 327.4 \text{ mm SL}$ ,  $k = 0.0385 \text{ month}^{-1}$ ,  $t_o = -6.9 \text{ months from Victoria,}$ and  $L_{\hat{A}} = 323.8 \text{ mm SL}$ ,  $k = 0.0513 \text{ month}^{-1}$ ,  $t_o = -4.5 \text{ months from WA}$ . The relationships between different length types and between the length and weight were determined for this species from the three states.

# 2.1. Age Validation

#### Methodology

Adult *Hyporhamphus melanochir* were sampled from commercial catches at three sites, and juveniles were caught during research sampling by beach seine and dab nets at *Site 2* in South Australia (Figure 2.1). Each fish was measured for the standard length (SL) (from the tip of upper jaw to the posterior end of the hypural bone), total length (TL) (from the tip of upper jaw to the tip of the longest caudal fin rays), and whole weight. The sagittal otoliths were extracted, cleaned, dried and stored in labelled plastic bags. The term sagittae and otoliths are used synonymously throughout the report.



Figure 2.1. Sampling sites of adult and juvenile garfish *Hyporhamphus melanochir* (*Inset* area relative to Australian coastline).

### Characteristics of Sagittae

Whole otoliths were immersed in water and examined for opaque zones with a binocular microscope at x6 magnification under transmitted and reflected light. The other sagitta was embedded in polyester resin by method used by Anderson *et al.* (1992a). Using a "Gemasta" diamond saw, up to four transverse sections (300-400  $\mu$ ms thick) were cut from each otolith to ensure the primordium was included in one of the sections. Sections were cleaned and mounted in polyester resin on microscope slides under coverslips. They were then examined and counted for opaque zones at x10-16 magnification on the section closest to the

primordium. Terminology follows Kalish *et al.* (1995). To avoid potential bias, all counts were made without knowledge of fish size, sex or date of capture.

### Periodicity of Opaque Zone Formation

## Marginal-increment analysis

Fish were collected monthly from the commercial fishery at *Site 1* (Figure 2.1) during October 1998 to September 1999. Five adults were randomly sampled from each of four size classes (...209 mm, 210 to 229 mm, 230 to 249 mm, —250 mm SL). One otolith from each fish was sectioned transversely and examined at x6 magnification. The distances were measured between consecutive opaque zones and also from the last opaque zone to the outside edge of the otolith along the axis from the otolith centre to the proximal surface next to the crista superior (Figure 2.2) using an image-analysis system, which was comprised of a dissecting microscope, a video camera (Panasonic wv-GL 700), and a computer installed with an image analysis software VideoPro. Because the radius of the first opaque zone varied greatly for garfish otoliths, the marginal increment for an otolith with one opaque zone, the marginal increment was expressed as a proportion of the immediately preceding annulus, and plotted as a function of month of the year. Additionally, the appearance of the otolith margin was recorded as opaque or translucent. An edge ring was counted when the opaque margin was seen.



Figure 2.2. A transverse section of *Hyporhamphus melanochir* otolith for marginal increment analysis. Red marks show opaque zones. Black arrows show measurements between consecutive opaque zones and from the last opaque zone to the outside edge of the otolith.

#### Tetracycline marking

Live fish were netted using beach seine from *Site 2* (Figure 2.1), the Port River-Barker Inlet estuary near Adelaide, on 27 October 1998 and transported in 60-litre insulated containers (60 cm length x 40 cm width x 20 cm depth) containing aerated seawater to the South Australian Aquatic Sciences Centre. They were maintained in a large tank for acclimation for about a week. Tetracycline in the form of Terramycin/MA injectable solution (oxytetracycline hydrochloride at 100 mg/ml) was diluted into five concentrations (0.5, 2, 5, 10 and 20 mg/ml) with 5% saline. Appropriate volumes were determined based on estimated weights by garfish length-weight relationship for Gulf St. Vincent (GSV)  $\log_{10} W = 3.2991 \log_{10} SL - 5.8921$  (Chapter 4). The volume was used to provide a dosage of 50 mg/kg body weight.

On 3 November 1998, fish were anaesthetised in 40 ppm benzocaine and then injected with tetracycline into the coelomic cavity using a 1ml syringe and a 29 gauge needle (0.33 x 12.7 mm). As the garfish is a fragile species, extra care was taken throughout the treatment to minimise the potential damage to fish. They were maintained in a 30,000-litre large tank. Due to high mortality suffered by fish less than 100 mm (TL) during first week of acclimation, on 3 December 1998, more juveniles were collected and injected using the same method. They were held separately in a 600-litre smaller tank.

Throughout the experiment, fish were maintained in the 30,000-litre (large) and the 600-litre (small) tank with flow-through seawater, a natural day/night cycle, and a normal seasonal temperature-cycle for up to 16 months. Fish were generally fed once a day with aquarium feed ("marine green" and brine shrimp) at a rate of 5% of the total live body weight.

On 12 August 1999, all fish from both the large and small tanks were re-injected with tetracycline to double mark the otoliths. Fish were sampled from both tanks on 26 March and 12 August 1999, and then harvested on 28 February 2000. Their otoliths were transverse-sectioned, examined, and photographed under both UV and transmitted white light, and the relative positions of the fluorescent bands and the opaque zones were identified.

#### First increment formation

Juveniles were collected on 12 occasions from the Port River-Barker Inlet Estuary using beach seines and/or dab net between September 1997 and March 1999. Sampling was concentrated at the Quarantine Station within the Port River system after January 1998 (Figure 2.2).

Every month, one otolith from each of ten fish was collected, sectioned and examined for the first opaque zone formation, except for December 1997 when only two sagittae were checked due to limited sample size. In order to obtain thinner transverse sections, the small juvenile otoliths were prepared by grinding and polishing techniques in the following manner: the otolith was first mounted on a microscope slide using thermoplastic glue (Crystalbond) such that its anterior half protruded beyond the edge of the slide. Using the slide to hold and orientate the otolith, the anterior half was hand ground away using 600 grit wet/dry sand paper. Upon approaching the level of the otolith's primordium two grades of imperial lapping film (9 $\mu$  and 3 $\mu$ ) were used to finely polish the ground face. The slide was then heated and the remaining otolith half removed and remounted in the centre on another microscope slide polished face down. The posterior half of the otolith was approximately 250 $\mu$  thick and contained the primordium. The sections were smeared with immersion oil when read to clear surface irregularities.



Figure 2.2. Map of the Port River-Barker Inlet estuary showing juvenile sampling location for *H. melanochir* (*Inset* area relative to South Australian coastline).

## **Otolith Growth**

The fish collected from Barker Inlet and Kingscote displayed the greatest range in size and age (Figure 2.1). Their sagittae were weighed (to the nearest milligram) and sections were measured for otolith thickness (the minimum distance from otolith centre to the proximal surface). Best-fit relationships were determined between otolith weight and thickness and fish age estimates.

### Results

# **Otolith Characteristics**

Whole sagittae of *H. melanochir* have the typical shape and orientation of those of most teleost fishes (Pannella 1980; Smale *et al.* 1995). When examined whole in aniseed oil or water, annuli were very indistinct especially for older fish. Therefore, the whole otolith method was abandoned for our age determination studies.

The transverse-sections displayed alternating opaque and translucent zones under transmitted light (Figure 2.3). The otolith primordial area was distinctly opaque, as were the thin zones out towards the proximal surface from the centre. Immediately adjacent to the opaque primordium, there was a broad opaque band fainter than the following opaque zones, which was identified as the first opaque zone. The radius of the first zone varied significantly (Figure 2.3 a and b). Outside the first zone, the structure differed with all sagittae having a consistent narrow opaque ring, beyond which there were distinctive opaque zones with decreasing width towards the margin.

The zonal pattern was usually clearest at the vicinity of sulcus acusticus towards the proximal surface, and became less distinct near the dorsal and ventral margin. In many otoliths, there appeared to be some zonal microstructure (false mark) between real opaque zones. As these zones could not be traced clearly around the entire portion of the otolith, they were distinguished from true annuli. For sea garfish, the increment was relatively easy to read in older fish despite the narrowing of translucent zones.



Figure 2.3. Transverse sections of *H. melanochir* otoliths. a and b having one opaque zone and wide edge; c having four opaque zones and a narrow edge; d having seven opaque zones and a narrow edge. Red marks show opaque zones. **V** ventral. **D** dorsal.

# Periodicity of Opaque Zone Formation

# Marginal-increment analysis

Fish from four size classes, collected monthly over 12 months, displayed one to four opaque zones. All otoliths collected between January and August had translucent margins, with the marginal increment increasing through the period (Figure 2.4). There was a distinct group with new edge growth occurring in early spring. Otoliths formed opaque margins during September to December. By January, all otoliths had translucent margins, and the formation of the new opaque zone had been completed.



Figure 2.4. Marginal increment analysis showing relative widths of marginal increment for *H. melanochir* sampled over 12 months. ( otoliths with opaque margins otoliths with translucent margins)

#### Tetracycline marking

Fish treated with tetracycline in November 1998 (large tank) were from three size classes (Figure 2.5). About 54% of those from the smallest size class (<100 mm TL) died within the first week of capture and treatment. Additional small fish brought in and treated in December 1998 (small tank) also had a 51% mortality within several days after treatment. Otoliths of most these small fish displayed one clear opaque zone near the margin. After the initial mortality, the survival rates of garfish throughout the 16-month experiment were 69% and 71% in the large and small tanks, respectively.



Figure 2.5. Size-frequency distributions of *H. melanochir* in the large and small tanks for the tetracycline validation study. a. fish size at initial capture (*open bars* fish that died soon after capture; *maroon bars* survivors); b. fish size at time of sampling in August 1999 (*yellow bars* fish sampled on 12 August 1999; *maroon bars* fish remained in tanks); c. fish size at harvest on 28 February 2000.

All fish sampled between March 1999 and February 2000 displayed between 1 and 3 opaque zones, as well as 1 or 2 fluorescent bands (Figure 2.6). Most of the fish formed an opaque zone right before the first fluorescent band (marked in November and December 1998) and all formed the next opaque zone shortly after the second fluorescent band (marked in August 1999) (Figure 2.7). All otoliths sampled in March and August 1999 showed completely translucent edges. The results indicated annual deposition of an opaque zone, which occurred during spring and completed by early summer.



Figure 2.6. Fluorescent bands in transverse section of sagitta from *H. melanochir* injected with tetracycline in November 1998 and August 1999.



Figure 2.7. *Hyporhamphus melanochir*. Photomicroscopy analysis of otoliths from juvenile and adult fish treated with tetracycline and maintained for different periods between 4 to 16 months after treatment. Fish in large and small tanks were injected in November and December 1998, respectively, and all fish were re-injected in August 1999. Each horizontal bar represents relative radius of one otolith, measured between otolith centre (*left hand axis*) and proximal surface (*dark segments* locations of opaque zones; *arrows* tetracycline bands; *fish codes* month and year of death: *M/99* March 1999; *A/99* August 1999; *F/00* February 2000).

### First opaque zone formation

All juveniles collected on different occasions originated either from the previous spawning season (September to April) or current spawning season (Figure 28). In September 1997, 20% of the otoliths had a distinct opaque edge, and the proportion increased in the following two months (Figure 2.9). By December 1997, each otolith had formed one clear opaque zone. The gradual deposition of the opaque zone was also evident from samples in 1998. It is of note that in January 1998, January and March 1999, all otoliths from the smaller size class were completely translucent whilst those from the bigger size group invariably showed one distinct opaque zone (Figure 2.9). It is most likely that the smaller size juveniles were newly born in the early period of current spawning season, and began to recruit to the research sampling gear from January. Fish from the bigger size classes in January and March had apparently originated from the previous spawning season. All these results suggest that the first opaque zone is initiated during September to December for garfish born from the previous spawning period. It was decided to set the mid spawning season (1 January) as the universal birthday, and therefore, formation of the first opaque zone occurred during their first year of life.

## **Otolith Growth**

*H. melanochir* otolith weight was related significantly linearly with age estimates in months (Figure 2.10). The relationship was: otolith weight (g) = 0.0008 age (months) + 0.015 ( $R^2 = 0.8625$ , n = 157, p<0.001). The otolith thickness had a power relationship with age in months, and the equation was: otolith thickness = 0.1002 age (months)<sup>0.4978</sup> ( $R^2 = 0.8359$ , n = 157, p<0.001). Regressions showed that garfish otoliths continued to grow through the life of the fish at a rate that allowed us to distinguish the zonal structure, despite the fact that each increment became progressively narrower.



Figure 2.8. Monthly size frequency distribution of juvenile *Hyporhamphus melanochir* from research sampling in the Port River-Barker Inlet system (*open bars* fish born in the current spawning season; *shaded bars* fish born in the previous spawning season).



Figure 2.9. Formation of first increment in otoliths from fish less than 12-month old. *Open bars* otoliths without an opaque zone; *grey bars* otoliths with an opaque edge; *black bars* otoliths with a complete opaque zone and a translucent edge.



Figure 2.10. Relationships between otolith weight and thickness and age of H. melanochir

### Ageing Protocol Development

An ageing protocol was developed using transverse sections of sagittae. The process of age determination involved analysis of otolith structure, interpretation of opaque zone counts and description of edge types. Edge types were judged either to be narrow (0-30%), medium (30-70%) or wide (>70%) based on the distance from the last opaque zone to the otolith margin relative to the width of the immediately preceding increment. An opaque zone at the margin was included in the ring counts with a "narrow" edge recorded.

The relationship between otolith zone formation and life history illustrated that sea garfish deposited the first opaque zone during September to December of the first year, 5 to 12 months after the time of spawning, and then formed new opaque zones around the same season in the following years (Figure 2.11). A universal birth date of 1 January (the middle of spawning season) was assigned. Based on this relationship, the following algorithm was developed to estimate the age of fish in months:

$$Age_m = N^* \ge 12 + M_c$$

where

 $Age_m$  = age in months  $M_c$  = number of months from start of year (nominated birth date) to capture month  $N^*$  = number of opaque zones (N) modified by edge width and capture month

The value of N\* is the age class, which is determined using the model shown in Figure 2.12. If a fish is caught between August and December with a narrow otolith edge, N\*=N-1 as the last opaque zone has only recently formed. This also applies for the fish caught in November/December with a medium edge. Alternatively, for fish captured in January but having a wide otolith edge, N\*=N+1 as they should have deposited a new opaque zone by this time of the year. For fish captured between January and July, January and October, and February and December, with narrow, medium, and wide otolith edges, respectively, age class N\* equals to N, the opaque zone counts. Such ageing estimation allows appropriate age-class (year-class) to be determined for each fish considered (Williams and Bedford 1974).



Figure 2.11. Relationship between opaque zone formation, life history and time of year for *H. melanochir*.



Figure 2.12. Model used for determining the value of N\* for use in ageing algorithm.

# Discussion

## **Otolith Characteristics**

The sagittae of *Hyporhamphus melanochir* were examined both as whole and transverse sections under transmitted light. Due to the thickness, whole otoliths do not reveal clear zonal structure except for some juveniles. Therefore, it is almost impossible to age garfish through reading the whole otolith.

Using whole otoliths for age determination of sea garfish, Ling (1958) suggested the difficulties in annuli interpretation for otolith with obscure central regions, and the possibility of erroneous or dubious readings. He further analysed size distribution by Peterson's method of length frequency polygons to check on age groups. However, the lack of prominent size frequency modes for older year classes often makes age interpretation difficult (Jones 1990). St. Hill (1996) also found whole sagittae having ambiguous annuli were unsuitable for ageing sea garfish from Tasmanian waters.

Although whole otoliths have been utilised in ageing young individuals of some species (Fowler and Short 1998), this method could seriously underestimate the true age for larger fish whose otoliths are thicker (Beamish 1992; Beamish and McFarlane 1995).

In contrast to whole otoliths, the transverse sections of otoliths for sea garfish displayed relatively clear opaque-translucent zones, which were easy to count. Transverse preparation usually reveals the clearest zonal structure of otoliths in ageing studies (Beamish 1992). Jordan *et al.* (1998) applied the same technique for age determination of *H. melanochir* in the study of Tasmanian populations. Their interpretations for otolith structure, first opaque zone, and the following annuli were comparable to those from our current study. However, we also found that the clarity and interpretability of the otolith increments for sea garfish was not as good as for species such as snapper (*Pagrus auratus*) (Francis *et al.* 1992), black bream (*Acanthopagrus butcheri*) (Morison *et al.* 1998), King George whiting (*Sillaginodes punctata*) (Fowler and Short 1998), and Murray cod (*Maccullochella peelii peelii*) (Anderson *et al.* 1992b). In addition to the presence of false marks, extra care is needed in annulus identification for garfish. To be a real opaque zone, it should trace clearly around the entire portion of the otolith.

An alternative technique using broken/burnt otoliths for age estimates has also been applied in age-growth studies for *H. melanochir* (Jones1990; St. Hill 1996). The broken/burnt method

revealed similar zonal structure to transverse sections (Section 2.2). Nevertheless, the major disadvantage with burning was the possibility of overheating, whereby the otolith often fractured or crumbled (St. Hill 1996). Transverse sections are ideal for long-term storage in case re-reading becomes necessary in the future.

## Periodicity of Opaque Zone Formation

Both marginal increment analysis and tetracycline marking methods were used to assess this criteria.

The first method indicated the deposition of one sequence of opaque/translucent zone each year. The opaque zone occurred in spring and early summer whilst the translucent material formed throughout the remainder of the year. For otoliths with one opaque zone (Figure 2.4), although opaque edge ones were only occurred in November and December, the significant drop of marginal increment in October suggested that the deposition of opaque zone should have begun in September.

Tetracycline is often used as a time-marker in otoliths of adult fish for field-based tagging programs (e.g. Beamish and Chilton 1982; Fowler 1990; Francis *et al.* 1992; Ferreira and Russ 1992; Beamish and McFarlane 1995) as well as tank-based validation experiments (Ferrell *et al.* 1992; Ferreira and Russ 1994; Fowler and Short 1998). As sea garfish is a relatively fragile species, a preliminary study was conducted to compare different tetracycline marking methods of emersion, feeding, and injection, where the last technique was the most effective and provided otoliths with a clear tetracycline mark (Ye, unpublished data). Therefore, the injection method was adopted in the current validation study. During the experiment, although sea garfish were confined in unnatural conditions, they still experienced natural water temperature and day-length regimes. Their otoliths had comparable zonal structure to those of wild fish. Temporal shifts in size frequency distributions also indicated that one opaque zone was formed each year in the spring and early summer, and thereby corroborated the conclusion from the marginal increment analysis.

Using two independent techniques, validation of the periodicity of opaque-zone formation was only achieved for the first three opaque zones. This is mainly due to the difficulty in catching big and relatively old individuals, as most of the fish from the Gulfs of SA were less than four-year old (Chapter 3). However, Jones (1990) suggested that sea garfish could live up to 10 years at the Baird's Bay, SA. Counts of up to 9 were also obtained for the population

from Wilson Inlet, WA (Section 2.3). Thus otolith zones after the third remained unvalidated. Nevertheless, indirect evidence also suggested their annual formation. Firstly, such opaque zones were identical in appearance to those previous zones, suggesting their similar causation and underlying nature. Secondly, the linear relationship between otolith weight and age indicated a constant rate of the addition of new material to the otolith surface.

Due to the protracted spawning season (September to April) of sea garfish, fish from a same cohort had a great size variation. Juveniles collected in November between 50 to 189 mm SL, invariably demonstrated one clear opaque zone near the margin (Figures. 2.8 and 2.9). This explains the significant difference in the first ring diameter for this species. In contrast, the samples from the small size group had completely translucent otoliths in January and March, which indicated that sea garfish did not initiate their first ring until the following spawning season during their first year of life.

#### **Otolith Growth**

For the otoliths to be useful in age determination, they must continue to grow through the lives of the fish at a rate that allows us to distinguish the zonal structure (Fowler 1990). The otolith growth rate was analysed by examining weight and thickness across a broad range of ages, which has been done in similar studies (Fowler and Doherty 1992; Fowler and Short 1998; Morison *et al.* 1998). The linear relationship between weight and age indicated the addition of otolith material at a constant rate. On average, each opaque/translucent sequence added 9.4 mg weight to the otolith. Despite the progressive decrease in growth rate of the annual thickness, the zonal increment were clearly distinguishable for fish up to ten years old, the life span of this species (Jones 1990).

#### **Ageing Protocol Development**

Age determination must refer to the time of otolith zone-formation and life history of the fish (Fowler and Short 1998). An algorithm has been developed to calculate garfish age in months, which is determined by three factors: opaque ring counts, edge interpretation, and time of fish captured.

As the opaque zone for *H. melanochir* can form during a protracted period (between September and December), their age class is not always equal to the number of ring counts. The edge interpretation is equally essential in age estimates. Francis *et al.* (1992) indicated that ageing error was caused both by incorrectly identifying annual rings and incorrectly

interpreting the otolith edge, and recommended that edge type be explicitly and clearly addressed in validation studies. Insufficient attention has been paid to this aspect in many previous age validation studies. For sea garfish captured during the spring and summer, there is often an edge interpretation problem, which requires subjective judgement to decide whether the last opaque zone was deposited in the current or previous year. The chart given in Figure 2.12 assists in converting opaque ring count to age class. For example, for a fish caught in November with two opaque zones and a narrow edge, the last opaque zone has only recently formed, N = 2,  $N^* = N - 1 = 1$ , Mc = 11, giving an estimate of  $age_m$  of 23 months and year class of 1+. Instead, for a fish caught in November with two opaque zone has formed at the end of previous year, N = 2,  $N^* = 2$ , Mc = 11, giving an estimate of  $age_m$  of 35 months and year class of 2+. Alternatively, for a fish caught in January with two opaque zones and a wide edge, an opaque zone is assessed as having been laid down recently, N = 2,  $N^* = N + 1 = 3$ , Mc = 1, giving an estimate of  $age_m$  of 37 months and year class of 3+.

# 2.2. Ageing Calibration

## Methodology

For the calibration of age determination for *Hyporhamphus melanochir*, otolith readings were compared between readers, laboratories (SARDI Aquatic Sciences and Central Ageing Facility (CAF) at the Marine and Freshwater Resources Institute, Victoria), and two otolith preparations methods (transverse sectioning and breaking and burning).

Sagittal otoliths were extracted from 80 randomly chosen fish per state from WA, SA, and Victorian samples. Either the left or right otolith from each fish was prepared as transverse sections by the method described in Section 2.1, whilst the other otolith of the same fish was broken and burnt. This was done by breaking the otolith in half along the dorsal ventral axis through the primordium using a scalpel blade. The exposed face of one half was ground smooth using 600-grit wet/dry sand paper and 9 $\mu$  imperial lapping film, and then heated over the flame of a Bunsen burner until it turned a honey brown colour. Only 8 broken/burnt otoliths fractured during preparation and fail to provide readings. The remaining 232 broken/burnt ones and 240 transverse sections were used in study.

For transverse sections, increment counting techniques followed those in Section 2.1. Alternatively, for the examination of broken/burnt otoliths, the heated half was mounted with the polished face up using plasticine. A thin layer of immersion oil was smeared over the face and the otolith was read under a dissecting microscope using reflected light. Broken/burnt otoliths displayed alternating light (white) and dark (honey brown) zones under reflected light, where the light zone corresponded to the opaque zone in the transverse sections under transmitted light (Figure 2.13).

Edge type was interpreted for each otolith based on the criteria described in Section 2.1. In addition, a confidence index (CI) was assigned according to otolith clarity and/or interpretability. The CI was classified to 4 categories defined as follows:

- 4 = increments were clear and provided unambiguous counts and relatively clear edge type;
- 3 = counts and edge type were relatively clear but some interpretation was needed (eg. rings were not clear all around the otolith or edge type was unclear in some parts but not others);
- 2 = counts and/or edge type were uncertain, and considerable interpretation was required;
- 1 = increments and/or edge type were unclear, and unable to be interpreted.



Figure 2.13. A pair of sagittae of *Hyporhamphus melanochir*. **a** the transverse sectioned otolith under transmitted light. **b** the broken/burnt otolith under reflected light. Red marks show the 5 annuli.

The algorithm developed in the ageing validation study (Section 2.1) was applied for age determination for each fish.

All otolith sections were interpreted independently by two readers at SARDI (reader 1 and reader 2) and one at CAF (reader 3) without referring to the size, sex and time of capture of the fish. Pairwise comparisons were made among three readers for ring counts, edge interpretation, and age determinations using a combination of age/count bias plots, age frequency tables, and coefficient of variation (CV) estimates (Campana *et al.* 1995).

Age/count bias graphs plotted one reader's age/counts versus another, where the readings of reader Y were presented as the mean age/counts and 95% confidence intervals corresponding to each of the age/ring number categories reported by reader X. The confidence intervals allowed informed interpretation of any difference between the observed line and the equivalence line reader X = reader Y. Either parallel but separated lines or increasing divergence as the lower or upper age range is approached indicated systematic differences between the two age readers. The selection of reader for the abscissa was arbitrary. As reader 2 was an experienced age reader for sea garfish, we believed reader 2 provided unbiased age estimates; thus comparisons were generally made against reader 2.

CV was used for the estimates of precision and defined as (Chang 1982):

$$CV_{j} = 100 \times \frac{\sqrt{\sum_{i=1}^{R} \frac{(X_{ij} - X_{j})^{2}}{R - 1}}}{X_{j}}$$

Where  $X_{ij}$  is the *i*th age determination of the *j*th fish, Xj is the mean age of the *j*th fish, and *R* is the number of times each fish is aged. Thus *CVj* is the CV of the age estimate for a single fish (*j*th fish), which can be averaged across fish to produce a mean CV.

Broken/burnt otoliths were read by reader 2, and a similar comparison was made with match pair readings from transverse sections by the same reader at SARDI.

Following the comparisons, a calibration workshop was conducted to bring all readers from two laboratories together to standardise the ageing techniques from otolith sections for sea garfish if any age bias existed. This was done by re-examining the microscopic structure of otoliths, comparing readings of the same otolith, identifying the false mark, and clarifying the criteria for opaque zones and edge type interpretation.

After the calibration, a new set of randomly sampled otoliths from 106 Victorian sea garfish were sectioned and read by the previous three readers in a similar manner. The results were then compared for pair readings using the same method.

As sea garfish is relatively a difficult species in terms of otolith interpretation (Section 2.1), only those otoliths with a clarity/interpretability CI greater than 2 were used in pairwise comparisons.

# Results

## **Comparisons for Transverse Sections**

#### Before calibration

### Detection of bias

There was no apparent systematic error between readers 1 and 2 in ring counts within SARDI (Figure 2.14). However, there were very obvious biases between reader 1 and reader 3, as well as between reader 2 and reader 3 from different laboratories, where both comparisons indicated increasing divergence from the equivalence line as the lower ring count was approached, and a fish counted 9 rings by both readers 1 and 2 was interpreted as 10 rings by reader 3. Reader 3 appeared to over count the opaque ring numbers compared to the other two readers. The inconsistency could have been caused by the differences in either interpretation of otolith zonal structure or edge type identification between two the laboratories.

Edge interpretations by reader 2 were generally agreed by reader 1, with 96%, 78%, and 76% consensus for narrow, medium, and wide edge types, respectively (Figure 2.15). Nevertheless, the percentage agreement between reader 3 and readers 1 or 2 were much lower, especially for the edge type of narrow or wide. For example, for the otoliths classified as medium edge by reader 1, only 38% was agreed by reader 3 whilst the rest of 53% and 10% was interpreted as narrow and wide edge, respectively; for those identified as wide edge by reader 2, only 32% was agreed by reader 3 whilst the remaining 54% and 14% was read as narrow and medium edge, respectively. These variations were considerable because different edge interpretation can result in different age estimates ( $\pm$  1 year) in addition to different opaque ring count if any. For example, a fish captured in October with 3 opaque rings and a wide edge was 3-year old, whilst age 2 would have been assigned if the edge was identified as narrow for the same fish.

Age frequency tables for each of three pairwise age comparisons are presented in Table 2.1. The percent agreement of age estimates was high (>90%) between reader 1 and reader 2 within SARDI, whereas significantly lower (generally ranging from 65-83%) between reader 3 and readers 1 or 2. The extremes of consensus (0% or 100%) for age 7- and 10-year-old were only based on one fish sample. Close inspection of Table 3.1 suggests some higher ages

for most of the age groups (1-6) by reader 3 from CAF relative to the readings of readers 1 and 2 from SARDI.

Age bias plots indicated very obvious systematic differences between readers 3 and 1, as well as readers 3 and 2, but none between readers 1 and 2 (Figure 2.16). For fish aged from 1 to 6, reader 3 consistently gave higher estimates than did readers 1 and 2.

## Estimates of precision

CVs for age comparisons between reader 3 and readers 1 or 2 were substantially higher than those between readers 1 and 2 (Figure 2.17), which indicated the most consistent age estimates between the two readers from the same laboratory (SARDI). The mean CV were 2.2%, 9.7%, and 10.2% for three pairwise age comparisons between readers 1 and 2, readers 3 and 1, and readers 3 and 2, respectively.



Figure 2.14. Ring count bias graphs for three pairwise comparisons of opaque ring counts from otolith transverse sections of *H. melanochir* between three readers before the ageing calibration workshop. Readers 1 and 2 from SARDI. Reader 3 from CAF. Each error bar represents the 95% confidence interval about the mean ring count assigned by one reader for all fish assigned a given count by a second reader. The 1:1 equivalence (black solid line) is also indicated.



Figure 2.15. Graphical comparisons of edge interpretation for otolith transverse sections of *H. melanochir* between three readers before the ageing calibration workshop. Readers 1 and 2 from SARDI. Reader 3 from CAF.



Figure 2.16. Age bias plots for three pairwise comparisons of age determination from otolith transverse sections of *H. melanochir* between three readers before the ageing calibration workshop. Readers 1 and 2 from SARDI. Reader 3 from CAF. Each error bar represents the 95% confidence interval about the mean age assigned by one reader for all fish assigned a given age by a second reader. The 1:1 equivalence (black solid line) is also indicated.

Table 2.1. Age frequency tables summarising pairwise comparisons of age estimates from otolith transverse sections of *Hyporhamphus melanochir* by three readers before the ageing calibration workshop. Readers 1 and 2 from SARDI. Reader 3 from CAF. Data are numbers or percentage of fish.

No. frequency				-		y reade		-		<b>-</b>
Age reader 2	0	1	2	3	4	5	6	(	10	Tota
0	3	4.0	4							-
1		48	4	0						5
2		4	78	3						8
3				47	45					4
4 5					15	2				1
5						3	6			
6							6	1		
10								I	1	
% frequency				Age esti	mated b	y reade	r 1			
Age reader 2	0	1	2	3	4	5	6	7	10	Tot
0	1 <b>00</b> %									100
1		92%	8%							1009
2		5%	92%	4%						100
3				1 <b>00</b> %						1009
4					100%					100
5						100%				100
6							100%			1009
7								1 <b>00</b> %		100
10									100%	1000
10				Ago osti	matod b	w roado	• 3		100%	1009
No frequency	0	1	2	Age esti 3	mated b	<del>y reade</del> 5	<b>r 3</b> 6	7	<u>100%</u> 10	
No_frequency	0	1		0				7		Tot
No_frequency	0	1		0				7		Tota
No_frequency Age reader 1 0 1 2			2	0				7		Tot: 3
No_frequency Age reader 10 1		27	2 9	3				7		Tota 3 4
No frequency Age reader 1 0 1 2 3 4		27	2 9 33	<u>3</u> 13	4	5 2		7		
No frequency Age reader 1 0 1 2 3 4 5		27	2 9 33	<u>3</u> 13	4	55	61			
No frequency Age reader 1 0 1 2 3 4 5 6		27	2 9 33	<u>3</u> 13	4	5 2	6 1 5	7		
No frequency Age reader 1 0 1 2 3 4 5 6 7		27	2 9 33	<u>3</u> 13	4	5 2	61			
No frequency Age reader 1 0 1 2 3 4 5 6		27	2 9 33	<u>3</u> 13	4	5 2	6 1 5			
No frequency Age reader 1 0 1 2 3 4 5 6 7 10		27	9 33 1	3 13 24	4 11 8	5 2	6 1 5 1			
No_frequency Age reader 1 0 1 2 3 4 5 6 7 10 % frequency		27	9 33 1	3 13 24	4 11 8	222	6 1 5 1			Tot: 3 4 3 1
<u>No_frequency</u> Age reader 1 0 1 2 3 4 5 6 7 10 <u>% frequency</u> Age reader 1 0	0	27 3	2 9 33 1	3 13 24	4 11 8 mated b	2 2 2 by reade	6 1 5 1	1	1	
<u>No frequency</u> Age reader 1 0 1 2 3 4 5 6 7 10 <u>% frequency</u> Age reader 1 0 1	1	27 3 1 73%	2 9 33 1 2 2 24%	13 24 Age esti	4 11 8 mated b	2 2 2 by reade	6 1 5 1	1	1	Tot: 3 4 3 1 1 
<u>No_frequency</u> Age reader 1 0 1 2 3 4 5 6 7 10 <u>% frequency</u> Age reader 10 1 2	0	27 3	2 9 33 1 2 2 4% 67%	3 13 24 Age esti 3 27%	4 11 8 mated b	2 2 2 by reade	6 1 5 1	1	1	Tot: 3 4 3 1 1 Tot: 100°
No_frequency	0	27 3 1 73%	2 9 33 1 2 2 24%	13 24 Age esti	4 11 8 mated b 4 31%	2 2 2 <b>y reade</b> 5	6 1 5 1	1	1	Tot: 3 4 3 1 1 Tot: 100º 100º 100º
<u>No_frequency</u> Age reader 1 0 1 2 3 4 5 6 7 10 % frequency Age reader 1 0 1 2 3 4	0	27 3 1 73%	2 9 33 1 2 2 4% 67%	3 13 24 Age esti 3 27%	4 11 8 mated b	2 2 2 yy reade 5 20%	6 1 5 1 <b>r 3</b> 6	1	1	Tot: 3 4 3 1 1 100 100 100 100 100
<u>No frequency</u> Age reader 1 1 2 3 4 5 6 7 10 <u>% frequency</u> Age reader 1 1 2 3 4 5 5	0	27 3 1 73%	2 9 33 1 2 2 4% 67%	3 13 24 Age esti 3 27%	4 11 8 mated b 4 31%	2 2 2 <b>y reade</b> 5	6 1 5 1 r 3 6 33%	1	1	Tota 3 4 3 1 1 1 00° 100° 100° 100° 100°
<u>No frequency</u> Age reader 1 1 2 3 4 5 6 7 10 <u>% frequency</u> Age reader 1 1 2 3 4 5 6 7 5 6 7 6	0	27 3 1 73%	2 9 33 1 2 2 4% 67%	3 13 24 Age esti 3 27%	4 11 8 mated b 4 31%	2 2 2 yy reade 5 20%	1 5 1 <b>r 3</b> 33% <b>83%</b>	7	1	Tot: 3 4 3 1 1 100° 100° 100° 100° 100° 100°
<u>No frequency</u> Age reader 1 1 2 3 4 5 6 7 10 <u>% frequency</u> Age reader 1 1 2 3 4 5 5	0	27 3 1 73%	2 9 33 1 2 2 4% 67%	3 13 24 Age esti 3 27%	4 11 8 mated b 4 31%	2 2 2 yy reade 5 20%	6 1 5 1 r 3 6 33%	1	1	

# Table 2.1. Continued.

No. frequency		Age estimated by reader 3								
Age reader 2	0	1	2	3	4	5	6	7	10	Total
0										0
1	1	27	9							37
2		4	33	13	1					51
3			1	23	10					34
4					8	2				10
5						2	1			3
6							5	1		6
7							1			1
10									1	1

<u>% frequency</u>		Age estimated by reader 3								
Age reader 2 _	0	1	2	3	4	5	6	7	10	Total
0										
1	3%	73%	24%							100%
2		8%	65%	25%	2%					100%
3			3%	68%	29%					100%
4					<b>80</b> %	20%				100%
5						67%	33%			100%
6							83%	17%		100%
7							100%	0%		100%
10									100%	100%



Figure 2.17. Coefficient of variation (CV) for the pairwise comparisons of age determination from *H. melanochir* otolith sections between three readers before the calibration workshop. Readers 1 and 2 from SARDI. Reader 3 from CAF.

#### Calibration workshop

As significant biases were detected for age estimates between two laboratories (SARDI and CAF), a calibration workshop was conducted at CAF in August 1999. The aim of this workshop was to standardise the ageing techniques from otolith sections of *H. melanochir* between SARDI and CAF, who were responsible for age determination of large numbers of sea garfish in the population study. Three readers were trained together for the interpretation of otolith microstructure. The first three opaque zones were found to be relatively more difficult to interpret, which was also reflected in the higher CV for the younger age groups (Figure 2.17). There were often false marks shown as dark zonal structure between the real opaque zones. Nevertheless false marks could not be traced clearly around the entire portion of the otoliths, which differentiated them from true annual rings. Therefore, ring counts should be made by examining the whole otolith. The opaque rings counted were standardised as the sharp brown rings (Figure 2.13).

Additionally, edge growth might be inconsistent sometimes at different portion of an otolith. For instance, it usually grew faster at the crista superior (bump along sulcus) and along the long axis of a transverse section (Figure 2.13). Hence reading criteria were further clarified not to judge edge type simply based on these "faster" growth area but by examining the edge appearance around the whole otolith, i.e. a defined edge type needed to be seen around 80% of otolith margin.

#### After calibration

#### **Bias detection**

There was no obvious bias detected between any of the three pairwise comparisons of opaque ring count, which indicated achievement of relatively consistent interpretation for the otolith zonal structure of *H. melanochir* among three readers and between SARDI and CAF (Figure 2.18).

The level of consensus for edge interpretation between readers 1 and 2 was similar to that before the calibration workshop, however, it increased substantially between SARDI (readers 1 or 2) and CAF (reader 3) after the workshop (Figure 2.19). The percent agreement between reader 3 and reader 1 were 90%, 73%, and 78% for narrow, medium and wide edge, respectively; and those between reader 3 and reader 2 were 89%, 73%, and 75% for the relative edge types. These agreement levels were comparable to those from the comparison

between readers 1 and 2 within SARDI. A certain level of variation should be allowed as edge type classification was sometimes a subjective judgement in otolith reading.

Age frequency tables documented considerably higher percent agreement (mostly > 90%) in age estimates for the inter-laboratory comparisons between readers 3 and 1, as well as readers 3 and 2 (Table 2.2). The increased consensus was achieved both through improvement of opaque ring identification and more consistent edge interpretation. The comparable age estimates among three readers were also demonstrated in age bias plots (Figure 2.20). Consequently, there was no apparent systematic error in age determination between any two of the three readers after the calibration.

# Estimates of precision

After calibration, CV reduced significantly to below 5% across all age groups (1 to 4) for age comparisons between reader 3 and readers 1 or 2, which indicated a significant improvement in precision for age determination between two organisations (Figure 2.21). The mean CV were 2.6%, 2.8%, and 2.5% in the age comparison between readers 1 and 2, readers 3 and 1, and readers 3 and 2, respectively.



Figure 2.18. Ring count bias graphs for three pairwise comparisons of opaque ring counts from otolith transverse sections of *H. melanochir* between three readers after the ageing calibration workshop. Readers 1 and 2 from SARDI. Reader 3 from CAF. Each error bar represents the 95% confidence interval about the mean ring count assigned by one reader for all fish assigned a given count by a second reader. The 1:1 equivalence (black solid line) is also indicated.



□ Narrow □ Medium ■ Wide

Figure 2.19. Graphical comparisons of edge interpretation for otolith transverse sections of *H. melanochir* between three readers after the ageing calibration workshop. Readers 1 and 2 from SARDI. Reader 3 from CAF.

Table 2.2. Age frequency tables summarising pairwise comparisons of age estimates from otolith transverse sections of *H. melanochir* by three readers after the calibration workshop. Readers 1 and 2 from SARDI. Reader 3 from CAF. Data are numbers or percentage of fish.

No. frequency			e estimated			
Age reader 2		1	2	3	4	Total
	1	34	3			37
	2	1	40	1		42
	3		1	13		14
	4				3	3
% frequency			e estimated			
Age reader 2	. —	1	2	3	4	Total
	1	92%	8%			100%
	2	2%	95%	2%		100%
	3		7%	93%		100%
,	4				100%	100%
No. frequency		Ag	e estimated	-		
Age reader 1		1	2	3	4	Total
	1	19	1			20
	2	1	19	1		21
	3		1	10		11
	4				2	3
% frequency		Δa	e estimated	by reader 3		
Age reader 1		1	2	3	4	Total
	1	95%	5%			100%
	2	5%	90%	5%		100%
	3	070	9%	91%		100%
	4		0,0	0170	100%	100%
No. frequency		Aa	e estimated	by reader 3		
Age reader 2		1	2	3	4	Total
-	1	20	1			21
	2		17	1		18
	3		2	10		12
	4				2	2
<u>% frequency</u>		<u>Ag</u> 1	e estimated	-		
Age reader 2			2	3	4	Total
	1	95%	5%			100%
	2		94%	6%		100%
	3		17%	83%		100%
						10070

100%

100%

4


Figure 2.20. Age bias plots for three pairwise comparisons of age determination from otolith transverse sections of *H. melanochir* between three readers after the calibration workshop. Readers 1 and 2 from SARDI. Reader 3 from CAF. Each error bar represents the 95% confidence interval about the mean age assigned by one reader for all fish assigned a given age by a second reader. The 1:1 equivalence (black solid line) is also indicated.



Figure 2.21. Coefficient of variation (CV) for three of the pairwise comparisons of age determination from *H. melanochir* otolith sections between three readers after the calibration workshop. Readers 1 and 2 from SARDI. Reader 3 from CAF.

# Comparison Between Transverse Sections and Broken/burnt Otoliths

# **Bias** detection

Ring count bias plots indicated some systematic error between the two otolith preparation methods (Figure 2.22). The broken/burnt otoliths tended to give a lower opaque ring number relative to matched pair transverse sections for otoliths having 3 to 5 rings. The underestimated ring count from the broken/burnt otolith was often caused by the edge interpretation problem, i.e. in the spring or early summer, the newly formed edge ring was usually clearly visible in a transverse section but not its broken/burnt half otolith. Consequently, the transverse section was read as N ring with a narrow edge whilst its broken/burnt counterpart was interpreted as N-1 ring with a wide edge. Such a phenomenon was evident in the comparison of edge interpretation (Figure 2.23). Among all narrow edge transverse sections, only 47% were also interpreted as narrow whilst 14% and 39% were classified as medium and wide edge, respectively, in their broken/burnt counterparts. Similarly, 38% of the medium edge sections were grouped as narrow edge instead in broken/burnt otoliths. These discrepancies in edge interpretation probably derived from several reasons. Firstly, when an otolith was broken and heated over a flame, the width of it's opaque zones could vary with intensity of heating. Secondly, as these opaque zones were shown as white colour under reflected light, they were easily missed when just formed on the margin. Thirdly, there was often an edge effect that confused the visibility of the edge ring.

Fortunately, the algorithm of age determination can mitigate the edge ring problem by taking the formation of opaque ring into account. For example, a fish captured in November with 3 opaque rings and a wide edge should give an age estimate of 3 even if it was read as having 4 rings and a narrow edge.

Therefore, the age bias plot demonstrated consistent age estimates from two otolith preparation methods (Figure 2.24). This was also reflected in the age frequency tables. The level of agreement between broken/burnt and sectioned otoliths was comparable to those between two readers for transverse sections.

#### Estimates of precision

CV for the estimate of precision between breaking and burning and transverse sectioning methods was shown in Figure 2.25, with an average of 3.7% across all age groups. Similar to age comparison between readers from the otolith sections, there was a gradual decline of CV with age estimates. This indicated the relative ease for ageing older fish, thus having higher precision. The overall CV for broken/burnt method was low (<6%), nevertheless, their average level was slightly higher than those between readers using transverse sections.



Figure 2.22. Graphical comparisons for matched pair opaque ring counts between transverse sectioned and broken/burnt otoliths of *H. melanochir* by reader 2 (SARDI). Each error bar represents the 95% confidence interval about the mean ring count assigned using the broken/burnt method for all fish assigned a given count by the transverse sectioning method. The 1:1 equivalence (black solid line) is also indicated.



Figure 2.23. Graphical comparisons of edge interpretation for transverse sectioned and broken/burnt otoliths of *H. melanochir* by reader 2 (SARDI).

Table 2.3. Age frequency tables summarising the comparison of age estimates between transverse sections and broken/burnt otoliths of *H. melanochir* by reader 2 (SARDI). Data are numbers or percentage of fish.

No. frequency	Age estimated from broken/burnt otoliths													
Transverse sections	0	1	2	3	4	5	6	7	10	Total				
0	1									1				
1	1	40	2							43				
2		4	66	4						74				
3			5	40						45				
4					13	1				14				
5						3				3				
6							6			6				
7								1		1				
10									1	1				

% frequency			Age est	imated	from b	roken/l	ournt of	toliths		
Transverse sections	0	1	2	3	4	5	6	7	10	Total
C	100%									100%
1	2%	93%	5%							100%
2	2	5%	89%	5%						100%
3	5		11%	89%						100%
4	Ļ				93%	7%				100%
5	5					100%				100%
6	;						1 <b>00</b> %			100%
7	•							100%		100%
10									100%	100%





Figure 2.24. Age bias plot for the comparison of matched pair age determination of *H. melanochir* from transverse sectioned and broken/burnt otoliths by reader 2 (SARDI). Each error bar represents the 95% confidence interval about the mean ring count assigned using the broken/burnt method for all fish assigned a given count by the transverse sectioning method. The 1:1 equivalence (solid line) is also indicated.



Figure 2.25. Coefficient of variation (CV) for the pairwise comparison of age determination from broken/burnt and transverse sectioned otoliths of *H. melanochir* before the calibration workshop.

## Discussion

For determining the consistency of age estimation, measures of both systematic difference (bias) and precision are required. A variety of graphical and statistical approaches can be used for these purposes (Boehlert and Yoklavich 1984; Baker and Timmons 1991; Campana and Moksness 1991; Kimura and Lyons 1991). Campana *et al.* (1995) indicated that parametric and nonparametric matched-pair tests, regression analysis, analysis of variance, and age difference plots were all capable of detecting systematic over- or under-ageing, however, only the age bias plot was sensitive to both linear and nonlinear biases.

Consequently, age bias plots were used in our age comparison study, which allowed clear visual detection of systematic error between SARDI and CAF before the calibration, but not between two readers from SARDI. These initial differences were likely due to the fact that readers 1 and 2 had received substantial training together specifically on interpreting otolith structure of *H. melanochir* prior to the exercise, whilst such training was not experienced by the reader from CAF until the calibration workshop. In addition, age interpretations from otoliths were validated for this species at SARDI by various approaches (Section 2.1). Although the type of microscope or image analysis system might have varied among age readers, or between laboratories, such differences were minor and inconsequential in the context of our analysis. The consistent age estimates achieved among three readers after calibration further indicated trivial differences between the two laboratory facilities.

Different kinds of age samples have been used in determining the consistency of age determination. Comparison based on matched pairs, whereby the same structure is interpreted by each age reader, always provides the highest statistical power (Campana *et al.* 1995). Our study focused on the analysis of paired comparisons between readers, laboratories, and otolith preparation techniques. Sea garfish can live up for 10 years (Jones 1990). It is of note that the data set used after our calibration was solely from Victorian fish with age ranging between 1 to 4 years old. However, our study showed that the otolith structure and their average clarity and interpretability were comparable among sea garfish from WA, SA, and Victoria. Also, above 90% of the fish captured were aged between 1 and 4 years in our large-scale population study across southern Australia. Furthermore, our initial comparisons indicated the bias and imprecision was most problematic for the young age groups, which were mainly caused by the inconsistent identification of the first opaque ring and false marks. Therefore, achieving ageing consistency for age 1- to 4-year-old groups was most crucial for our population study of sea garfish.

Francis *et al.* (1992) indicated three conditions necessary for accurate ageing: the existence of a periodic mark in some body part; the ability to identify these marks reliably (which includes distinguishing them from "false" marks); and the ability to accurately convert a count of mark to an age (i.e. to solve the edge interpretation problem). There has been insufficient attention paid to these separate aspects of age determination, particularly for mark identification and edge interpretation. Discrepancies in both aspects had occurred during our initial ageing comparisons between the two laboratories. In the calibration workshop, we found that the inconsistent counting of opaque rings was mainly attributed to the problems in the first ring identification and false marks differentiation, meanwhile, the serious variability in edge interpretation (Figure 2.15) was generally due to the presence of faster growth area on an otolith and the subjective nature in categorising edge types. Both problems had contributed various degrees of error to all age determination. Therefore, standardisation was necessary in otolith reading between SARDI and CAF. In the absence of a known-age reference collection, ageing consistency is the best that can be achieved (Campana *et al.* 1995).

For the estimates of precision, the coefficient of variation (CV) was used, which provided similar values to the average percent error (APE) (Beamish and Fournier 1981). When the number of times each fish is aged equals two, such as in our matched pair comparisons, APE =  $CV/\sqrt{2}$ ; however, CV was statistically more rigorous and thus more flexible (Chang 1982). Although percent agreement was also used in age comparisons, several authors have clearly documented the dangers of the percent agreement statistic and the superiority of both APE and CV as measures of precision (Beamish and Fournier 1981; Chang 1982; Kimura and Lyons 1991; Campana *et al.* 1995). In our calibration study, the age frequency tables were presented with numbers and percent agreement only on the purpose to document the matched observations.

There was a significant reduction in CV between SARDI and CAF from about 10% to between 2.5 and 2.8% after calibration. This indicated much more precise age estimates between the two laboratories with the CV comparable to the intra-laboratory level (2.6%). The average CV among the three readers was equivalent to 1.8 to 2.0% of APE. Morison *et al.* (1998) reported the precision of age estimates of black bream (*Acanthopagrus butcheri*) from similar otolith transverse sections, having an APE of 0.41% between readers. Despite the different experience levels between age readers, these APE, to a certain degree, reflect that *H. melanochir* is a relatively difficult species in terms of ageing from otoliths, which sometimes provide unclear and ambiguous increments. Kimura and Lyons (1991)

demonstrated various CV levels for different species. Nevertheless, measures of precision are relative values only; no one value can be considered an "acceptable level" for all species (Campana *et al.* 1995). Furthermore, many other factors may influence ageing precision, such as the experience of readers, age of fish, type of bony structures used and their preparation methods, etc.

Age estimates were also compared between transverse sections and broken/burnt otoliths, as the later were used in age-growth studies for *H. melanochir* populations from SA (Jones 1990) and Tasmanian (St. Hill 1996) waters. Reader 2 was experienced in terms of interpretation for otoliths prepared by both techniques. Therefore, readings by reader 2 were compared between preparation methods. Despite the slightly lower precision level, broken/burnt otoliths were found to provide un-biased age estimates relative to the transverse sections.

# 2.3. Growth Rate Determination

# Methodology

# Study Area and Sample Collection

Broad scale fish sampling was conducted between August 1997 and September 2000 across WA, SA, and Victoria. Adults were purchased from the commercial markets in each state; and juveniles were collected from the research sampling using small mesh beach seine or dab net. In total, there were 8453 sea garfish collected from three states. Sampling localities were shown in Figure 2.26.



Figure 2.26. Sampling sites and regions of *Hyporhamphus melanochir* for growth study across Western Australia, South Australia, and Victoria. Inset areas show sampling locations for each state relative to Australian coast line.

Whole fish samples from WA and Victoria were sent regularly in frozen condition to SARDI Aquatic Sciences for biological processing. All fish were measured for the standard length (SL) (to the nearest millimetre), weighed (to the nearest gram), and dissected for the study of reproductive biology (Chapter 4). Samples collected in August and September 1997 from SA were also measured for the caudal fork length (CFL) and total length (TL), which allowed to determine the morphometric relationships between different length measurements. Pairs of sagittae were extracted from each fish, rinsed in water, dried with fine tissue paper, and stored in sealed plastic bags for subsequent ageing.

#### **Otoliths Preparation and Examination**

A sub-sample of 3588 pairs of sagittae were prepared as transverse sections, and examined for opaque zones and edge type with a microscope under transmitted light as fully described in Section 2.1. Otoliths of fish from SA and WA were read by SARDI whilst those from Victoria were interpreted by CAF. These two laboratories provided unbiased age estimates for *Hyporhamphus melanochir* with relatively high precision after calibration (Section 2.2). Only 2814 otoliths with confidence indices of reading of more than 2, which gave relatively clear and unambiguous readings (Section 2.2), were used for the production of growth curves. The locality, month and year of collection and number of otoliths used are shown in Table 3.4.

An age was assigned to each fish based on otolith reading and month captured using the algorithm described in Section 2.1.

Table 2.4. Information on locality, month and year of collection and number of *Hyporhamphus melanochir* otoliths used for age growth study. GSV = Gulf St. Vincent, SG = Spencer Gulf, KI = Kangaroo Island, WC = West Cost, CI = Corner Inlet, PPB = Port Phillip Bay, WP = Western Port, SC = South Coast, BI = Barker Inlet, MB = Middle Beach, PW = Port Wakefield, QS = Quarantine Station, BS= Bay of Shoal, KC = Kingscote; AB = Arno Bay; CC = Chinaman's Creek, CP = Corny Point, EC = Eight Mile Creek, PL = Port Lincoln, PP = Port Pirie, TK = Tickera, WH = Whyalla, DC = Davenport Creek, VB = Venus Bay, IB = Israelite Bay, OH = Oyster Harbour, PB = Peaceful Bay, PRH = Princess Royal Harbour, WI = Wilson Inlet, CB = Cockburn Sound, EA = Eagle Bay, KB = Koombana, PH = Peel Harvey Inlet, WB = Warnbro.

State						ę	Sout	h Aus	trali	а		-				Victo	oria				We	sterr	n Au	stralia		
Regio	n/site	GS	V				S	G					KI		WC	СІ	РРВ	WP			SC				WC	
Year	Month	BI MB	PW	QS	AB	cc d	CP E	C PL	PP	тк	WН	BS	KC	DC	VB	СІ	PPB	WP	IB	ОН	PB	PRH	WI	СВ ЕА	KB P	'H WE
97	8										15				56											
	9	26 26			9		4	9	14		19			8	33											
	10	7	5				9			37	15		38						1						9	3
	11	10 22			25		3			36																
	12	2 22			_		10			30												00			16	
98	1	9	12				3	-		28					05							28	11			
	2	17	14		9 9		14 6	7	10	3 7	18		27		25 27	7	26	11					5 3		57	29
	3 4		14		9 21		6		10	5	10		27		27 19		20						3 7		2	29
	4 5	14	11		17		11	13		2	19		20		19	14				20	18		6		1	1
	6		10		19		•••		14	2						15				22	10		Ŭ	10		
	7		14	11					14	1	16		30		29								2			
	8		10		17		12	13	13	2	8					12							3			
	9	20	13	20	18		11		13	1	7					18	5						5			
	10		3	10						51						17	5						6			
	11		1	10						63													5			
	12		8	11			2			82						18							5			
99	1	21		11	11					88						24				26			4			
	2		4				7			88					30									13		
	3			12						27						14							29			
	4					11				28		13				13	13						7			
	5 7						1	~															36			
	9					15	1	0									5									
	9 11					15						7					5									
	12											Ĺ														
2000	1																									
	2																									
	3											5														
	8												6													
	9															15										
Regio	n subte	otal		437							1261		154		227	200	72	11					249			203
_	total														2079			283								452

# Data Analysis

To calculate the relationships between SL, CFL, and TL, a linear relationship in the following form was fitted to the length data using the GLM procedure in SAS, a linear least squares procedure (Anon 1989).

y = ax + b

Where *x* and *y* are the paired length types, *a* and *b* are constants.

To determine the relationship between SL and weight, a power curve of the following form was fitted to the weight at length data using the NLIN procedure in SAS, a non-linear, least squares procedure (Anon 1989).

$$y = ax^b$$

Where y is the whole weight and x is the SL, a and b are constants.

When calculating the length-weight relationships for each sex, juveniles were allocated alternatively to either male or female samples, which assumed that the length-weight relationships for juvenile males and females were not significantly different.

Differences in the fitted length-weight curves between the sexes and states were tested using likelihood ratio test (Kimura 1980).

$$\mathbb{O}^2 = [-N \times \ln (\int_{-\infty}^2 / \int_{-\infty}^2)]$$

Where N is the number of samples,  $\int_{1}^{2}$  and  $\int_{2}^{2}$  are the variances for the hypotheses H i, that all parameters are equal, and H that all parameters are not equal, respectively.

Once age estimates were completed, the ageing data were combined with information on fish length, sex, and location and date of capture for subsequent analyses.

The von Bertalanffy growth function was fitted to the length (SL) at age data using the NLIN procedure in SAS, a non-linear, least squares procedure (Anon 1989).

$$L_t = L_{\Re} (1 - e^{-k(t+t_0)})$$

Where  $L_t$  is the length at age t, t is the age estimate in month,  $L_{\Re}$  is the asymptotic length (mean length fish would reach if left to grow indefinitely), k is a growth constant on monthly basis describing how rapidly this length is achieved, and  $t_o$  is the hypothetical age of fish at length zero.

From a grid search over a range of possible values for  $L_{\Re}$ , K and  $t_o$ , the combination with the lowest residual sum of squares was selected as the starting point for iterations. The Gauss-Newton iterative method was used and solution with the lowest sum of squares selected. Juvenile fish were allocated alternatively to either male or female samples in order to keep the two data sets completely independent. Growth functions were fitted to data for each sex separately and for the sexes combined (including samples of males, females and juveniles). This assumes that the growth of juvenile male and female fish is not significantly different.

Differences in the fitted growth curves between the sexes, regions, and states were tested using likelihood ratio test (Kimura 1980).

#### Results

## Morphometric Relationships

The relationships between SL, CFL, and TL were determined for sea garfish with sexes combined from SA with high  $R^2$  values (Table 2.5).

Table 2.5. The relationships between different length types for *Hyporhamphus melanochir* from SA. Samples were collected in August and September 1997.

Parameter	Relationship	R <sup>2</sup>	n
SL-CFL (mm)	CFL = 1.0704 SL + 2.4887	0.997	388
SL - TL (mm)	TL = 1.1423 SL + 0.7732	0.995	388
CFL - TL (mm)	TL = 1.0671 CFL - 1.8593	0.998	388

The relationships between SL (mm) and weight (g) for males, females and both sexes were presented in Table 2.6 and Figure 3.27 for sea garfish from WA, SA, and Victoria. There were significant differences in the length-weight relationships between males and females for fish from SA and Victoria, but not from WA (Table 2.7). These power relationships were also found to be significantly different among three states for sexes combined populations (Table 2.7). However, the parameters were very similar between three states (Table 2.6), the three relationship curves were almost congruent (Figure 2.27 d), and the mean weight of the same length fish differed less than 20 g between three states for fish up to 380 mm SL. The statistically significant differences might have arisen from the preciseness of the fitted curves for each state, and thus they may not be biologically important.

Table 2.6. The parameter estimates of the power relationships between the standard length and the whole weight of *Hyporhamphus melanochir* from South Australia, Victoria, and Western Australia.

State	Sex	N	a ± SE (E-06)	b ± SE	R <sup>2</sup>
South Australia	Female	2381	$6.996 \pm 0.705$	2.982 ± 0.0182	0.924
	Male	962	1.636 ± 0.291	$3.252 \pm 0.0323$	0.926
	Both	3343	5.578 ± 0.489	3.025 ± 0.0159	0.923
Victoria	Female	463	$4.082 \pm 0.773$	$3.073 \pm 0.0340$	0.951
	Male	286	1.354 ± 0.455	3.283 ± 0.0614	0.912
	Both	749	4.896 ± 0.746	3.043 ± 0.0275	0.943
Western Australia	Female	884	7.475 ± 1.719	$2.979 \pm 0.0404$	0.889
	Male	461	2.179 ± 0.635	3.198 ± 0.0518	0.899
	Both	1345	5.627 ± 0.992	3.029 ± 0.0310	0.895
All states	Both	5437	3.806 ± 0.251	3.095 ± 0.0118	0.927



Figure 2.27. The relationships between the standard length and weight for the male, female, and sexes combined *Hyporhamphus melanochir* from WA, SA, and Victoria.

Table 2.7. The comparisons of the length-weight relationships using likelihood ratio test between the male and female *Hyporhamphus melanochir* and among the sexes combined populations from South Australia, Victoria, and Western Australia.

State	Comparison	N	Chi-square	Р
South Australia	female & male	3343	37.62	<0.005
Victoria	female & male	749	19.58	<0.005
Western Australia	female & male	1345	3.89	0.15
Between states	SA & Victoria	4092	23.47	<0.005
	SA & WA	4688	21.41	<0.005
	Victoria & WA	2094	25.20	<0.005

# Growth

There was wide variability in the length-at-age determination for *Hyporhamphus melanochir* throughout southern Australia (Table 2.8). For example, 0-year-old fish from South Australia (SA) ranged between 49 and 217 mm SL; and the lengths of 6-year-old fish from Western Australia (WA) varied from 252 to 363 mm SL. Similarly, the age of fish of the same centimetre class was also highly variable. For example, fish of 260-269 mm SL from SA varied from 1 to 4 years old and those of 300-309 mm SL from WA ranged from 2-6 years of age.

The maximum size of sea garfish collected was 345, 282, and 320 mm SL for males, and 377, 329, and 378 mm SL for females from SA, Victoria, and WA respectively. The oldest fish sampled was 6, 4, and 4 years old for males, and 6, 6, and 10 years old for females from SA, Victoria, and WA, respectively.

Growth parameters were presented in Table 2.9 for male, female, and sexes combined from different regions of South Australia, Victoria, and Western Australia. No growth curve was fitted to the data from Western Port of Victoria because the samples contained too few large and small fish to adequately define a growth curve. Results of likelihood ratio test for growth curves between sexes for each region and state, between regions within state, and between states were summarised in Table 2.10.

**Region Sex** State Age class (years) 0 2 3 4 5 6 7 10 1 mean s.d. n GSV SA 25 103 125 J 91 17 11 37 18 М 148 8 209 21 25 228 49 252 18 7 265 1 F 162 18 13 203 25 70 231 21 123 271 25 25 313 1 377 1 All 102 35 124 196 34 106 230 20 172 267 24 32 289 34 2 377 1 ΚI 99 29 19 164 13 J 6 Μ 218 1 230 15 7 247 1 252 1 F 208 14 30 239 16 72 264 11 13 291 2 3 276 1 All 29 19 201 16 79 263 19 4 276 1 99 21 37 239 12 14 281 SG J 194 16 2 Μ 103 48 26 212 14 142 235 12 99 258 15 45 286 33 6 312 1 320 1 F 149 62 49 216 18 461 246 17 335 279 15 22 316 323 11 2 16 69 307 1 All 133 61 75 215 17 605 243 17 434 271 19 114 303 22 28 314 3 2 322 8 3 26 8 WC J 99 Μ 228 15 8 231 13 44 270 9 11 279 1 294 1 F 12 12 242 17 112 284 16 23 307 10 6 320 1 234 All 99 26 8 231 13 20 239 16 156 279 15 34 303 14 7 307 18 2 Overall 112 47 226 212 22 768 239 18 841 271 19 194 300 21 41 316 34 6 322 83

Table 2.8. Mean length-at-age (mm SL), standard deviation (s.d.) and sample size (n) of *Hyporhamphus melanochir* from different regions of Western Australia (WA), South Australia (SA), and Victoria, by sex and for juveniles and both sexes combined (All). GSV = Gulf St. Vincent, SG = Spencer Gulf, KI = Kangaroo Island, WC = West Coast, CI = Corner Inlet, Port Phillip Bay, SC = South Coast.

Table 2.8. Continued.

State	Regio	n Sex	C									Ag	ge cla	ss (y	ears)												
			0			1			2			3			4			5			6			7		10	
			mean	s.d.	n	mean	s.d.	n	mean	s.d.	n	mean	s.d.	n	mean	s.d.	n	mean	s.d.	n	mean	s.d.	n	mean s.d.	n n	nean s.c	J.
lictori	a Cl	J	143	9	15																						
		М				204	13	41	241	11	37	265		1	282		1										
		F				209	18	33	257	14	44	273	8	18	302	13	6	304	8	2	322	6	2				
		All	143	9	15	207	15	74	250	15	81	273	8	19	299	14	7	304	8	2	322	6	2				
	PPB	J	114	15	5																						
		М				207	11	6	226	15	13	252	1	2													
		F				203	16	10	246	19	31	288	7	2	305	7	2	329		1							
		All	114	15	5	204	14	16	240	20	44	270	21	4	305	7	2	329		1							
	WP	М							210		1																
		F							243	14	9	270		1													
		All							239	17	10	270		1													
	Overal	1	136	17	20	206	15	90	246	17	135	272	11	24	301	13	9	312	16	3	322	6	2				
VA	SC	J	107		1	155		1																			
		М				210	28	12	271	23	36	292	23	11	291	41	2										
		F				228	19	47	269	38	69	300	32	43	308	18	16	311	13	3	329	44	6	377	1	378	
		All	107		1	223	24	60	270	33	105	299	31	54	306	20	18	311	13	3	329	44	6	377	1	378	
	WC	J	98	18	68	148		1																			
		М				224	16	5	252	13	26	262	14	20	285	16	3										
		F	177	22	3	229	13	17	259	15	37	278	12	14	289	12	8	290		1							
		All	102	24	71	225	21	23	256	15	63	269	15	34	288	13	11	290		1							
	Overal	I	102	24	72	224	23	83	265	29	168	287	30	88	299	20	29	306	15	4	329	44	6	377	1	378	
tates'	Average	e	111	42	318	213	22	941	244	22	1144	276	23	306	300	20	79	312	25	13	326	31	11	377	1	378	_

Table 2.9. Estimates of von Bentalanffy growth parameters (with standard errors) for *Hyporhamphus melanochir* from different regions of South Australia (SA), Victoria (VIC), and Western Australia (WA). GSV = Gulf St. Vincent, SG = Spencer Gulf, KI = Kangaroo Island, WC = West Cost, CI = Corner Inlet, PPB = Port Phillip Bay, SC = South Coast.

State	Region	Sex	Ν	$L_{\infty} \pm SE (mm)$	K ± SE (per month)	To ± SE (month)
SA	GSV	Female	290	339.6 ± 20.6	0.0364 ± 0.0052	-2.5 ± 1.0
		Male	147	332.0 ± 34.9	$0.0359 \pm 0.0082$	-2.1 ± 1.3
		Both	437	337.7 ± 17.7	$0.0365 \pm 0.0043$	-2.2 ± 0.7
	SG	Female	941	311.0 ± 5.9	$0.0441 \pm 0.0031$	-7.3 ± 1.1
		Male	320	263.7 ± 3.0	$0.0897 \pm 0.0048$	1.5 ± 0.4
		Both	1261	282.7 ± 2.7	$0.0678 \pm 0.0030$	-1.6 ± 0.5
	KI	Female	132	285.5 ± 7.1	$0.0593 \pm 0.0056$	-2.1 ± 0.8
		Male	22	288.2 ± 32.9	0.0437 ± 0.0140	-4.1 ± 2.1
		Both	154	285.6 ± 7.4	0.0563 ± 0.0051	-2.3 ± 0.7
	WC	Female	157	327.1 ± 12.5	$0.0437 \pm 0.0062$	$-3.3 \pm 2.0$
		Male	70	270.7 ± 7.9	0.0795 ± 0.0110	2.9 ± 1.1
		Both	227	302.0 ± 7.4	0.0571 ± 0.0056	0.03 ± 1.1
	STATE	Female	1520	296.8 ± 3.5	0.0564 ± 0.0025	$-2.3 \pm 0.4$
		Male	559	280.6 ± 4.6	$0.0630 \pm 0.0036$	-0.1 ± 0.4
		Both	2079	289.1 ± 2.6	0.0618 ± 0.0021	-1.0 ± 0.3
VIC	CI	Female	112	325.7 ± 9.6	$0.0432 \pm 0.0050$	-5.3 ± 1.6
		Male	88	263.8 ± 8.6	0.0724 ± 0.0130	-2.7 ± 2.0
		Both	200	329.3 ± 11.4	$0.0369 \pm 0.0044$	-8.1 ± 1.7
	PPB	Female	49	340.6 ± 23.4	$0.0399 \pm 0.0086$	-3.7 ± 2.6
		Male	23	231.3 ± 4.7	0.221 ± 0.0460	$6.3 \pm 0.7$
		Both	72	319.3 ± 20.6	0.0451 ± 0.0096	$-3.0 \pm 2.4$
	STATE	Female	161	329.4 ± 9.3	0.0421 ± 0.0044	-4.9 ± 1.4
		Male	111	250.8 ± 5.0	0.101 ± 0.014	0.8 ± 1.2
		Both	272	327.4 ± 10.3	0.0385 ± 0.0042	-6.9 ± 1.4
WA	SC	Female	188	348.7 ± 12.8	$0.0397 \pm 0.0074$	-9.5 ± 3.7
		Male	61	296.2 ± 11.4	0.0939 ± 0.0270	$2.4 \pm 2.9$
		Both	249	342.1 ± 10.5	$0.0434 \pm 0.0068$	-7.6 ± 2.8
	WC	Female	114	306.6 ± 9.3	0.0541 ± 0.0058	-4.5 ± 0.7
		Male	89	300.3 ± 13.3	$0.0499 \pm 0.0074$	-5.1 ± 0.9
		Both	203	302.9 ± 7.6	0.0528 ± 0.0046	-4.7 ± 0.5
	STATE	Female	302	329.6 ± 6.2	0.0507 ± 0.0035	-4.6 ± 0.7
		Male	150	302.8 ± 9.2	0.0575 ± 0.0064	-4.1 ± 0.7
		Both	452	323.8 ± 5.3	0.0513 ± 0.0030	$-4.5 \pm 0.5$

Table 2.10. Comparison of von Bentalanffy growth curves between sex, region, and state using Kimura's (1980) likelihood ratio test. GSV = Gulf St. Vincent, SG = Spencer Gulf, KI = Kangaroo Island, WC = West Cost, CI = Corner Inlet, PPB = Port Phillip Bay, SC = South Coast. N = sample number. Degree of freedom = 3. \* significant difference (P < 0.05).

State	Comparison	Ν	Chi-square	Р
South Australia	GSV female & male	437	3.28	0.37
	SG female & male	1261	49.45	<0.005*
	KI female & male	154	5.58	0.15
	WC female & male	227	16.28	<0.005*
	GSV & SG	1698	142.90	<0.005*
	GSV & KI	591	12.71	0.006*
	GSV & WC	664	16.76	<0.005*
	SG & KI	1415	15.22	<0.005*
	SG & WC	1488	12.59	0.007*
	KI & WC	381	8.53	0.037*
	Female & male	2079	43.74	<0.005*
Victoria	CI female & male	200	17.77	<0.005*
	PPB female & male	72	11.35	0.01*
	CI & PPB	272	3.99	0.28
	Female & male	272	24.00	<0.005*
Western Australia	SC female & male	249	20.75	<0.005*
	WC female & male	203	3.58	0.38
	SC & WC	452	29.13	<0.005*
	Female & male	452	8.88	0.032*
Between States	SA & WA	2531	147.41	<0.005*
	SA & Vic	2351	8.94	0.03*
	Vic & WA	724	40.34	<0.005*

# South Australia

The von Bertalanffy growth functions for male and female sea garfish were significantly different for Spencer Gulf (SG) ( $^{\odot}$ <sup>2</sup> = 49.45, p < 0.005) and West Coast waters (WC) ( $^{\odot}$ <sup>2</sup> = 16.28, p < 0.005), but not for Gulf St. Vincent (GSV) ( $^{\odot}$ <sup>2</sup> = 3.28, p = 0.37) and Kangaroo Island (KI) ( $^{\odot}$ <sup>2</sup> = 5.58, p =0.15) using Kimura's likelihood ratio test (Table 2.10). The growth curves were almost congruent for males and females from GSV and KI whilst they were widely separated for fish from SG and WC (Figure 2.28 a to d). For fish from SG and WC, the estimates of L<sub>R</sub> for females were considerably higher than those for males, whereas males grew faster than females.



Figure 2.28. Growth curves for male and female *Hyporhamphus melanochir* from different regions of South Australia (a to d), Victoria (e and f), and Western Australia (g and h).

Combining both sexes, there were significant differences in the von Bertalanffy growth functions between populations from any two regions of GSV, SG, KI, and WC within South Australia (Table 2.10, Figure 2.29 a). Fish from GSV had the highest  $L_{\Re}$  of 337.7 mm SL, but the slowest growth rate. Those from SG and KI had similar  $L_{\Re}$ , but SG fish tended to grow faster. However, the predicted lengths at age for the two fitted curves differed by less than 14 mm for fish between 0 and 120 months (Figure 2.29 a). Fish from WC grew at a comparable rate to those from KI, whereas the former population reached a greater asymptotic length.

Figure 2.29. Growth curves for *Hyporhamphus melanochir* (sexes combined) from different regions of South Australia, Victoria, and Western Australia.



Combining fish from all regions, a significant difference was also detected between the growth curves for males and females ( $@^2 = 43.74$ , p < 0.005) (Table 2.10, Figure 2.30 a). The estimate of L<sub>R</sub> for females was higher whilst males grew slightly faster.



Figure 2.30. Growth curves for male and female *Hyporhamphus melanochir* (regions combined for each state) from South Australia, Victoria, and Western Australia.

## Victoria

The growth functions for males and females from both Corner Inlet (CI) ( $^{\odot}^2 = 17.77$ , p < 0.005) and Port Phillip Bay (PPB) ( $^{\odot}^2 = 11.35$ , p = 0.01) in Victoria were found to be significantly different using Kimura's likelihood ratio test (Table 2.10). Two growth curves diverged substantially with males having a faster growth rate and females reaching a much

higher asymptotic length (Table 2.9, Figure 2.28 e and f). It should be noted that the estimates of growth parameters for males from PPB were based on a limited sample size (Table 2.9).

For sexes combined, there were no significant differences between the growth curves for fish from CI and PPB ( $^{\odot}$ <sup>2</sup> = 3.99, p = 0.28) (Table 2.10). Growth parameters (L<sub>R</sub> and K) were similar, and two curves were nearly congruent (Table 2.9, Figure 2.29 b). The different t<sub>o</sub> and relatively larger differences in the predicted lengths at age for younger fish were probably due the very small sample size for juveniles (less than 12 months) from both regions in Victoria.

Combining data from CI and PPB, the growth functions were also significantly different between males and females from Victoria ( $^{\odot}$ <sup>2</sup> = 24.00, p < 0.005) (Table 2.10). The apparent differences were also evident in the growth curves (Figure 2.30 b).

# Western Australia

The von Bertalanffy growth functions were significantly different between males and females from the south coast (SC) of Western Australia ( $^{\odot}^2 = 20.75$ , p < 0.005) (Table 2.10, Figure 2.28 g). Estimate of I<sub>37</sub> for females was higher, but males grew much faster. Such differences between sexes was not found for fish from the western coast waters (WC) ( $^{\odot}^2 = 23.58$ , p = 0.38) (Table 2.10, Figure 2.28 h).

For sexes combined, the growth curves for fish from SC and WC differed significantly ( $\mathbb{O}^2 = 29.13$ , p < 0.005) (Table 2.10, Figure 2.29 c). The asymptotic length of fish from SC was about 40 mm longer than those from WC although the growth rate of WC fish appeared to be slightly faster (Table 2.9).

Combining both regions, the von Bertalanffy growth curves also differed significantly between female and male sea garfish from WA ( $^{\odot}$ <sup>2</sup> = 8.88, p = 0.032) (Table 2.10, Figure 2.30 c).

#### Comparison between SA, Victoria, and WA

In general, there were significant differences in growth curves between the populations (sexes combined) from SA, Victoria, and WA using Kimura's likelihood ratio test (Table 2.10, Figure 2.31). Fish from SA had the fastest growth rate, but the smallest asymptotic length

compared to those from Victoria and WA (Table 2.9). The  $L_{\Re}$  were similar between Victorian and WA fish, whereas the latter tended to grow more rapidly with a higher growth constant K.



Figure 2.31. Growth curves for *Hyporhamphus melanochir* (sexes and regions combined) from South Australia, Victoria, and Western Australia.

# Discussion

All three forms of measuring sea garfish (SL, CFL, and TL) have been broadly used in our study across the southern Australian waters. For instance, when working with length data from the commercial fishery it is often more practical to obtain size data in the form of TL. Indeed, all management regulations associated with the size of garfish are represented in total length measured. Consequently, relationships were determined which allowed the conversion between SL, CFL, and TL. Although only fish from South Australia were used in this study, we assume that the morphometric relationships remain identical for the same species.

The length-weight data of sea garfish fitted well to the power curves with high  $R^2$ . However, data for WA indicated more variability than those for SA and Victoria (Figure 2.27). Despite the statistically significant differences in the length-weight relationships between sexes for SA and Victoria and between the three states, the parameters of the model were similar and the curves were almost congruent, particularly for fish less than 300 mm SL (Figure 2.27).

The age and growth determined for *Hyporhamphus melanochir* from otoliths indicated that this species had variable but relatively fast growth rates across the southern mainland states of Australia. They progressed to an average length of 160 mm, 170 mm, and 185 mm SL at the age of 1 year (12 months) and reached the current legal minimum sizes of 21, 20, and 23 cm TL (183, 174, and 201 mm SL) at about 15, 13, and 14 months old in SA, Victoria, and WA,

respectively. Using the same ageing technique based on transverse sections of otoliths, Jordan *et al.* (1998) reported an average size of 145 mm SL for the 1+ age-class sea garfish from eastern Tasmania. Despite the fact that the given birthday was set for Tasmanian garfish on 1 December, one month earlier than that for the fish from other states, the growth of eastern Tasmanian population was relatively slower during their first three years of life. This was likely due to the lower average water temperature and shorter summer season although the differences in the number and size range of juvenile samples might also have contributed to the differences in the growth estimation. Overall, comparison of the growth parameters between states show that sea garfish from WA, Victoria and Tasmania approached similar higher asymptotic lengths than those from SA, whereas fish grew most rapidly in SA (Table 2.11).

Table 2.11. Comparisons of growth parameters between different populations of *Hyporhamphus melanochir*. GSV = Gulf St. Vincent, SG = Spencer Gulf, WC = West Coast waters.

State	Region	Data source	Sex	l ∞ (mm)	K (per month)	To (month)
SA	GSV	Current study	Female	339	0.0364	-2.5
			Male	332	0.0359	-2.1
		1950s Ling's raw data	Female	324	0.0523	
		(in Jones 1990)	Male	326	0.0506	
	SG	Current study	Female	311	0.0441	-7.3
			Male	264	0.0897	1.5
		1950s Ling's raw data	Female	342	0.0643	
		(in Jones 1990)	Male	306	0.0550	
	WC	Current study	Female	327	0.0437	-3.3
			Male	271	0.0795	2.9
		Baird Bay	Female	338	0.0450	3.6
		(Jones 1990)	Male	321	0.0423	1.4
SA		Current study	Both	289	0.0618	-1.0
Victoria		Current study	Both	327	0.0385	-6.9
WA		Current study	Both	324	0.0513	-4.5
Tasmania		Eastern population Jordan <i>et al.</i> (1998)	Both	318	0.0450	2.8

Growth of sea garfish was rapid for the first 3 years of life until about 270 mm SL and then slowed considerably. Similar characteristics were also described for the garfish populations by Jones (1990) and Jordan *et al.* (1998). Jones (1990) studied the growth of a lightly fished population of sea garfish in Baird Bay, SA using broken/burnt otoliths for age determination.

There was good agreement in growth parameters with our present data for females from the west coast of SA, but not for males (Table 2.11). At present, males had a significantly lower  $L_{\Re}$  and faster growth rate. Nevertheless samples from Baird Bay contained more larger and older males, and the oldest fish sampled were 10 and 8 years old for males and females, respectively. Comparison between the historical growth data from Ling (1958) from the two gulfs of SA (Jones 1990) and the present data indicate that the  $L_{\Re}$  remained comparable for both males and females from GSV over time, whereas their growth rates were appreciably lower over 40 years after the first study (Table 2.11). Such a reduction was also shown in the growth constant for females from SG but not for males. In addition, significant decreases had occurred in the asymptotic lengths for both sexes from SG over years. Although the sampling time and localities in Ling's study differed slightly with our study for each gulf (Ling 1958), the reasons for the differences in growth remain unknown. They may be either due to climatic variability, food availability, difference in size/age composition of samples, or errors in otolith readings. Ling (1958) aged sea garfish by counting the annuli on cleaned whole sagittae with the aid of a hand lens. Our study found this technique difficult and likely to under estimate the age of older fish and hence over estimate growth rates, such as the case for many other species (Beamish 1992; Beamish and McFarlane 1995).

The von Bentalanffy growth curves differed between sexes for most of the regions and between regions within each state except for Victoria. These variations, at least partially, reflect the differences in the size range of fish collected for each sex and from different regions (Figure 2.32). There were few juvenile samples from Port Phillip Bay and the south coast of WA. Samples from Kangaroo Island and Port Phillip Bay only included limited numbers of males and their size tended to be relatively small. Similarly, there were no samples of large males from Corner Inlet. Samples from the south coast tended to include more large fish than those from the west coast of WA. Furthermore, the temporal differences in fish sampling at different regions may have added to the variability in growth curves (Table 3.4). In SA, fish from Gulf St. Vincent and Spencer Gulf were collected approximately monthly between August 1997 and April 1999, whereas samples from Kangaroo Island and West Coast waters were patchy in terms of months, being mainly dependent on highly seasonal fisheries in these two areas. Some juveniles collected in November 1999, and March and August 2000 were also included in growth analyses for the KI population. In contrast, all Victorian samples were collected between March 1998 and April 1999 except that some juveniles were sampled additionally later. In WA, adult sampling began in January and February 1998 on the south coast and west coast, respectively; however, samples from the SC were more regular throughout the months to May 1999, most of which were collected from Wilson Inlet.



No. frequency



Standard length (mm)

The variability in lengths-at-age of fish were likely to be indicative of variation in spawning times, sample localities (sites), and growth rates of individuals, and was particularly evident among samples from Gulf St. Vincent, Spencer Gulf, and the south coast of WA (Figures 2.28 and 2.29). As sea garfish have a protracted spawning season (September to April) throughout the southern Australia, setting a universal birthday of 1 January for ageing purposes could have introduced up to  $\pm$  4 months difference in an age estimate from the true age. Collected in December 1998 from the Port River-Barker Inlet system in SA, fish with the same age of 12 months varied from 68 to 182 mm SL. Also, there may be inter-annual differences in growth when samples from different cohorts were combined. This was addressed previously. However, comparisons between years were not conducted because most of the samples were collected throughout 1998, and samples from other years were patchy and having relatively small numbers, also the time frames of sampling were slightly different between regions and states.

For growth analyses, each state was broadly divided into regions, which often covered an extensive range of waters (Figure 2.26). Within each region, there were different sampling sites, where growth rate of fish can vary greatly. For example, the mean size at age for fish from Princess Royal Harbour was significantly smaller than those from Wilson Inlet and Oyster Harbour along the south coast of WA. These spatial differences might be attributed to the differences in food, temperature, and other environmental factors.

The maximum ages of *H. melanochir* found in the present study for both sexes from SA and Victoria and for males from WA were lower compared to those from the Baird Bay, SA (Jones 1990) and eastern Tasmania (Jordan *et al.* 1998). However, the 10-year-old fish (378 mm SL) caught from Wilson Inlet, WA was the oldest female sea garfish ever reported. The variability may reflect either spatial or temporal variations in the age structure, selectivity of gear, or the small sample size of fish, particularly for males in this current study. The hypothesis that the size/age structure is dependent on the level of fishing effort is investigated fully in Chapter 3.

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# CHAPTER 3. DESCRIPTION OF THE SOUTHERN SEA GARFISH FISHERIES, THEIR CATCHES, EFFORT AND CATCH PER UNIT EFFORT

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Objective: Determine the size and age structure of the commercial catch from different sectors in southern Australian waters, and improve understanding of the potential impacts of the competing gear sectors on the South Australian stocks.

This chapter provides a description of the gear and regions of three states where there are commercial and recreational fisheries for sea garfish. Annual and seasonal trends in commercial catch, fishing effort and CPUE data in each state have been analysed by location and gear type. For South Australia, peaks in catch, effort and CPUE of hauling nets and dab netting were compared to determine if there were gear interactions. Commercial and recreational fisheries interactions could not be determined because of lack of recreational fishery data.

The commercial garfish fishery is part of the multi-species inshore fisheries in the gulfs and bays and inlet of the three states. Haul seining is the main method of capture, but there are differences between states in fisheries regulations; reporting of fishing effort and targeting of garfish. This makes it problematic to compare the relative abundances of garfish using CPUE data across southern Australia. Even in the SA dab net fishery, where garfish is the only species targeted, the observed increase in CPUE is likely to be related to an increase in catching efficiency rather than an increase in stock abundance. Consequently, the fishery trends in each state are different. However, the garfish catch in SA, which is the largest of all the states, has remained stable over the past 15 years (average: 450 tonnes) and trends in CPUE are either stable or gradually increasing for all methods in all regions where there has been a decline in fishing effort (boat-days). In Victoria, there has been a declining trend in garfish catches over the same period associated with lower market prices, lower fishing effort and declining trends in CPUE of haul seines and ring nets in Port Phillip Bay and Westernport Bay. In Western Australia, as the fishery for garfish is largely an opportunistic one, we have no confidence in providing meaningful CPUE trends, and all that can be said is that there has been a slight increase in total catch in the past ten or so years.

Based on the commercial catch and effort data, there is no evidence of any interactions between the haul and dab netting operations in SA waters. This is shown by a) stable or increasing CPUE's for both methods in all regions of the state; b) similar seasonal peaks in CPUE of hauling and dab nets, and c) no pronounced increase in dab netting annual CPUE's following a haul netting closure in Kangaroo Island waters in 1996.

# 3.1 Introduction

Southern sea garfish (*H. melanochir*) supports valuable commercial and recreational fisheries across its distribution in Western Australia, South Australia, Tasmania and Victoria. The commercial fishery for southern sea garfish in SA is the highest of all states, with approximately 60% of the national catch derived from S.A. waters (381 - 516 tonnes; Figure 3.1). This chapter concentrates on an assessment of the trends in catches, fishing effort and catch rates (CPUE) in the SA commercial garfish fishery, with an aim at understanding the potential impacts of competing gear sectors on the South Australian stock. Implicit in this assessment is the assumption that CPUE's are a satisfactory indicator of relative stock abundance, and this chapter provides advice on which gear type is the best such indicator. For the sake of completeness, similar information on these parameters for the Western Australian and Victorian fisheries have been included.

The paucity of similar long-term data on the recreational fishery precludes investigation on the effect of the commercial fishery on catch rates by recreational fishers; however, again, for the sake of completeness the information available on the recreational sea garfish fishery is included here.



Figure 3.1. Annual catches (tonnes) of southern sea garfish in SA, Victoria, WA and Tasmania between 1982/83 and 1999/00.

#### **3.2 Methods**

This chapter is solely dependent on the interpretation of the catch, fishing effort and CPUE's available in the garfish fishery in each state.

For South Australia, all marine scalefish (MSF) commercial fishers are required by legislation to provide data on monthly catch and effort for all species taken in the MSF fishery on a spatial scale (fishing block). They are required to indicate a target species for each method on each day of fishing.

These data are collated by SARDI (Aquatic Sciences) using the GARFIS software. Data specific to sea garfish (species code 712) were summarised by financial year for the period 1983/84 - 99/00. Sea garfish were categorised as to whether they were targeted or caught whilst "any" species (which included garfish) were targeted. Fishing effort for both target categories was expressed as fisher-days, and CPUE's as kg/fisher-day for each major gear type. All data were summarised on a regional basis, with five main regions delineated throughout the state, Northern Spencer Gulf (NSG), southern Spencer Gulf (SSG), Gulf St. Vincent (GSV), west coast (WC) and Kangaroo Island (KI).

To determine if there are any seasonal interactions between commercial hauling and dab netting operations in the South Australian fishery, the four months of the year when highest catches, targeted effort and CPUE's occurred for both gear types were compared for each of the regions. Also, the effects of a seasonal closure to hauling nets, implemented in 1996 in a bay on Kangaroo Island, was investigated by comparing the CPUE's of the dab netters before and after the closure.

In Victoria, commercial fishers report their catch and effort every month. These data are entered into the Catch and Effort (CandE) data base held at MAFRI. Landed catches are mostly reported by species, but *H. regularis* and *H. melanochir* are rarely distinguished. It has been assumed that *H. regularis* are only caught in the Gippsland Lakes whereas *H. melanochir* are caught in PPB, WPB and CI. Catch and effort are reported on a shot by gear type for each day of the month, but targeting is not specified. For reporting the spatial distribution of catch and effort, the Victorian coast is divided into grids with higher resolution for bays and inlets. Summaries of the garfish statistics from the CandE data base have been prepared for each bay, by month and financial year.

Commercial fishers in Western Australia complete a monthly compulsory fishing return recording their catch by method and species and area fished. This is entered into the Fisheries Western Australia Catch and Effort Statistics System (CAESS). Between 1975-1976 and 1989-1990 catches of sea garfish and river garfish (*Hyporhamphus regularis*) were recorded under separate numeric codes. However, inaccurate identification leading to improper coding meant that it is not possible to determine what proportion of the sea garfish catches are river garfish. Since that time, a change in the coding system has meant that catches of the two species have been recorded under one code.

# 3.3 Results

# The garfish fishery in South Australia

## Description of the commercial fishery

The garfish fishery of South Australia has previously been described by Jones *et al.* (1990), Rohan *et al.* (1991) and Jones (1995). The SA commercial fishery for garfish is primarily a haul netting fishery, with the proportion of the total commercial catch landed by this method ranging from 81 - 94% since 1983/84 (84% in 1999/2000). During this last year, there were 110 MSF licence holders with hauling nets endorsed on their licences. In general, haul netting is restricted to waters of less than 5 metres in depth. An exception to the 5 metre restriction exists in northern Gulf St Vincent where a number of fishers have exemptions to net in deeper water, and is utilised predominantly during winter months. There are no regional restrictions on the number of hauling net fishers.

Garfish nets are 3.0 - 3.2 cm mesh size and up to 600 metres in length. The minimum mesh size is currently 3 cm; although prior to metrication in 1966, the minimum size was set at 1.25 inches, which equated to 3.175 cm mesh. Mesh selectivity experiments undertaken with garfish caught in hauling nets resulted in 50% selection occurring at almost 24 cm for 3.2 cm mesh size (Jones, 1982). The ply size of the netting material used varies in different parts of the net. In the wings, 18 ply size is employed, whereas the 15 ply size (heavier and thicker than 18), is used in the pocket of the net. This practice has traditionally been used by garfish net fishers to reduce the chances of damage to undersize King George whiting (Sillaginodes punctata), which seasonally are found in the same habitats as those for sea garfish (Kumar et al. 1995). Haul netting for garfish can take place either at night or during daylight hours. In the inshore areas, the nets are deployed on the outgoing tide as the fish swim off the shallow banks. The placement of the net is decided by the fisher, following active searching for the surface schools, using spot lights at night or during the day, by standing on the bow of the fast planing tunnel hull vessel. The net is deployed in a full circle ("ring shot"), with the pocket of the net ending up adjacent to the vessel. The net is then towed using the reverse power of the vessel and manually packed onto the vessel's stern by the skipper and deckhand. Often, if schools are not sighted, the net is deployed as "a blind shot" covering the potentially larger area of a semicircle. It is then "power-hauled" to close off the end of the net, with the remainder of the hauling proceeding in the same manner as the ring shot. As the net is hauled the diminishing area of the shot herds the fish into the pocket. All sorting of the fish (sea garfish and by-catch) is carried out whilst the pocket remains in the water alongside the vessel (SAFIC, 1998).

Haul netting is concentrated in the northern regions of Spencer Gulf and Gulf St Vincent, although some activity also takes place in the southern Gulfs and Kangaroo Island waters, Coffin Bay and Venus Bay. Commercial hauling net fishing is subject to a complex array of area and seasonal closures in many parts of the state (Net Review Committee Report, 1995).

A second commercial method used for taking garfish is dab nets. Dab netting is conducted at night using spotlights to locate the fish. Dab netting primarily takes place in northern Gulf St Vincent waters in winter, southern Gulf St Vincent and Kangaroo Island in summer, and western Spencer Gulf throughout the year. There are no restrictions on the use of dab nets by commercial fishers, except that the minimum mesh size of the dab nets must be 3.0 cm. A legal minimum size of 21 cm total length applies to all garfish landings in South Australia

#### Catch, effort and CPUE's in the commercial fishery

# State overview

The State commercial garfish catch has remained relatively stable since the early 1980s, with an average of 460 tonnes since 1983/84 (Figure 3.2). Catches have been even more regular in the last decade, with a mean annual catch of 477 tonnes. The annual catch has varied less than 8% in eight out of the last ten years, except for 1994/95 and 1998/99 with a lower catch of 392 and 421 tonnes, respectively. Currently the catch in 1999/2000 is 477 tonnes.



Figure 3.2. Total annual catch (1951/52 - 1999/2000), value and average price of garfish in the South Australian commercial fishery from 1983/84 - 1999/2000.

The average landed price of garfish (\$/kg) rose steadily from \$2.72 in 1989/90 to a peak of \$4.45 in 1995/96 (Figure 3.2). Although the price dropped back to \$3.21 in 1997/98, but again increased to \$4.00 in 1999/2000. It is of note that the peaks in prices in 1987/88 and 1994/95 coincided with low catches. The value of the total catch has generally remained steady in the last nine years at about
\$1.6–1.9m (Figure 3.2). 1995/96 was an exception when high prices resulted in a total value of \$2.27m.

The State garfish catch is largely determined by the landings from hauling nets (Figure 3.3), with 81 - 94% of the catch taken by this method. The hauling net catch has remained relatively stable since 1983/84 with mean landings during that period of 408.2 tonnes. The catch has varied by more than 50 tonnes from the mean in only 4 years during the 17 year period. Catch for 1999/2000 was within the 50 tonne range around the long term average.

The remainder of the State catch is mostly taken by dab nets (Figure 3.3). Total landings rose from levels of 22 - 40 tonnes during the 1980s to 60 - 100 tonnes in the early 1990s, and have since remained at those levels. The catch in 1999/2000 is about the average (70.9 tonnes) since 1992/93.



Figure 3.3. Annual commercial garfsh catch by fishing method in SA from 1983/84 - 1999/2000.

Targeted effort by haul netters declined by 36% from 1983/84 to 1992/93 to a level of 4,100 fisherdays (Figure 3.4). Effort has since remained relatively stable between 3,700 and 4,700 fisherdays.

However, targeted effort (as reported in catch and effort records) does not adequately reflect total effort in the haul net fishery. For example, targeted haul net catch accounts for only 55.2% of the garfish landings by this method in 1999/2000 (having declined from 65.9% in 1983/84). And yet garfish are an important target species in their own right and the remaining catch could not reasonably be considered to be bycatch of fishing activity targeting other species.

Most of the remaining garfish catch has been landed when fishers have recorded their target species as 000 (or "ANY"). To incorporate this effort, catch and effort records listing target species as 000 which produced average garfish catches (including average from monthly report) exceeding 20 kg/boatday are described separately in each regional analysis. Note that this only refers to haul net fishing.

Targeted dab netting effort doubled from pre-1991/92 levels of about 1,000 fisherdays to 1,990 fisherdays in 1992/93 (Figure 3.4). Effort since 1992/93 has fluctuated between 1,100 and 2,300 fisherdays.

Other methods (line fishing, gill nets) are very minor in terms of the overall catch. Due to confidentiality requirements, targeted effort and CPUE for these gear types cannot be presented for 1983/84, 1985/86, 1987/88, 1993/94, 1994/95, 1996/97, and 1997/98 (Figures 3.4 & 3.5).



Figure 3.4. Targeted effort (fisherdays) for garfish by method in SA from 1983/4 - 1999/2000.

Targeted catch per unit effort (CPUE) in the haul net sector has slowly risen since 1983/84 to 61 kg/fisherday in 1997/98, a level 50% higher than 1983/84 (Figure 3.5). Although it decreased slightly in 1998/99, the CPUE has recovered to 58 kg/fisherday in 1999/2000. However, the increase has not been consistent, showing a number of fluctuations. The last four years provide momentum to the increase whereas CPUE had been relatively stable for the previous seven years. Dab net targeted CPUE has more than doubled since 1983/84 to 46 kg/fisherday (Figure 3.5). This increase was consistent to 1993/94, however, the rate of increase has diminished over the last six years.



Figure 3.5. Targeted catch per unit of effort (kg/fisherday) for garfish by method in SA from 1983/84 - 1999/2000.

The trends in catch and effort show distinct regional differences and further results will therefore be presented regionally (see Table 3.1). Please note that Coffin Bay has been included in West Coast waters in this chapter. Previous reports have reported Coffin Bay and Spencer Gulf together.

REGION	MSF Blocks					
Northern Spencer Gulf (NSG)	11, 19, 20, 21, 22, & 23					
Southern Spencer Gulf (SSG)	29, 32, 30, 31, & 33					
Gulf St. Vincent (GSV)	34, 35, 36, 40, 43 & 44					
West Coast (WC)	7, 8, 9, 10, 15, 16, 17, 27, & 28					
Kangaroo Island (KI)	41 & 42					

Table 3.1. Marine Scalefish Fishery Blocks for regional division of garfish catch.

Table 3.2. Regional catches and percentage of annual catch (both methods combined) for garfish in<br/>SA from 1983/84 to 1999/2000.

YEAR		83/4	84/5	85/6	86/7	87/8	88/9	89/90	90/1	91/2	92/3	93/4	94/5	95/6	96/7	97/8	98/9	99/00
REGION																		
NSG	tonnes	199.8	182.9	196.5	201.3	171.7	234.4	252.8	225.7	252.7	212.1	204.6	158.6	203.5	230.3	215.8	161.0	180.1
	%	45.8	42.7	44.7	51.8	45.1	50.6	49.0	49.8	49.2	41.2	43.3	40.5	39.8	44.9	42.8	38.3	37.7
SSG	tonnes	31.4	24.8	40.1	43.1	42.1	40.8	50.8	43.4	56.8	50.0	36.3	27.9	40.9	67.1	76.1	55.3	68.6
	%	7.2	5.8	9.1	11.1		8.8	9.8	9.6	11.0	9.7	7.7	7.1	8.0	13.1	15.1	13.2	14.4
GSV	tonnes	157.4	182.2	164.8	96.6	111.9	135.7	157.3	118.8	142.7	175.7	173.5	149.2	218.0	160.8	152.2	158.1	189.5
	%	36.1	42.5	37.5	24.8	29.4	29.3	30.5	26.2	27.8	34.1	. 36.8	38.1	42.7	31.4	30.2	37.6	39.7
wc	tonnes	35.2	28.8	22.7	30.6	32.8	32.9	34.4	33.0	32.2	32.7	24.5	30.4	28.9	28.8	31.8	28.4	20.5
	%	8.1	6.7	5.2	7.9	8.6	7.1	6.7	7.3	6.3	6.3	5.2	7.7	5.7	5.6	6.3	6.7	4.3
кі	tonnes	10.6	7.3	12.3	12.8	15.8	18.7	20.1	28.2	28.1	42.4	32.2	22.8	17.8	23.3	26.7	17.3	17.0
	%	2.4	1.7	2.8	3.3	4.2	4.0	3.9	6.2	5.5	8.2	6.8	5.8	3.5	4.5	5.3	4.1	3.6
OTHER	tonnes	1.7	2.6	2.7	4.4	6.1	1.0	0.6	4.4	1.6	1.8	0.9	3.0	1.8	2.6	1.4	0.6	1.5
	%	0.4	0.6	0.6	1.1	1.6	0.2	0.1	1.0	0.3	0.4	0.2	0.8	0.3	0.5	0.3	0.1	0.3
TOTAL		436.0	428.7	439.1	388.9	380.5	463.5	515.8	453.6	514.1	514.8	472.0	391.9	510.9	512.9	503.9	420.6	477.1

#### Northern Spencer Gulf (NSG)

Northern Spencer Gulf represents the most important region in terms of the State garfish catch, generally producing 40 - 50% of annual commercial landings until 1997/98 (Table 3.2). In the last two years, the percentage catch in this region was reduced slightly to about 38%. The catch in this region is almost entirely caught by haul nets, with dab netting only a minor component of the fishery (generally < 10 tonnes) (Figure 3.6).

The haul net catch has averaged 199 tonnes since 1983/84, with a range between 168 and 248 tonnes (Figure 3.6). While subject to these cyclical patterns, the catch appears to be stable.



Figure 3.6. Trends in annual garfish catches by method in northern Spencer Gulf from 1983/84 - 1999/2000.

<u>Haul net fishery</u> Targeted haul net effort peaks in February and March in this region before declining to a low in June and July (Figure 3.7). However, some effort is maintained all year. The pattern of targeted catches lags behind effort with peak catches occurring between March and May. The lowest catches are taken during the early part of the spawning season between October and December.



Figure 3.7. Seasonality of average monthly targeted catch and targeted effort in the haul net fishery of northern Spencer Gulf from 1983/84 - 1999/2000.

Targeted CPUE peaks in the late autumn and winter (between May and August) before gradually declining to the lowest level (less than half of the peak level 80 kg/fisherday) in the summer (between October and February), which coincides with most of the spawning season of garfish (Figure 3.8).



Figure 3.8. Seasonality of average monthly targeted CPUE in the haul net fishery of northern Spencer Gulf from 1983/84 – 1999/2000.

Annual targeted haul net catch and effort have shown large fluctuations in the last 16 years, with effort ranging from 2,200 to 3,350 fisherdays (Figures 3.9 & 3.10). Whilst catch where target species have not been specified (but garfish catches > 20 kg/boatday) has been relatively stable at about 53 tonnes (Figure 3.9). The non-targeted effort had been around 1,800 fisherdays before 1991/92, but has since declined to about 1,300 fisherdays (Figure 3.10). There are no apparent long-term trends to these patterns.



Figure 3.9. Trends in annual catch of garfish in the haul net fishery of northern Spencer Gulf from 1983/84 – 1999/2000.



Figure 3.10. Trends in annual effort for garfish in the haul net fishery of northern Spencer Gulf from 1983/84 – 1999/2000.

Targeted CPUE has been relatively stable for the past 16 years, averaging 50 kg/fisherday (Figure 3.11). CPUE in 1997/98 was the highest recorded during this period at 61 kg/fisherday, and corresponded with low targeted effort. Targeted CPUE in 1999/2000 was slight below the average level. CPUE for target = "ANY" is lower (generally 30 - 40 kg/fisherday). Although it steadily increased between 1987/88 and 1992/93, it has been relatively stable for the last seven years.



Figure 3.11. CPUE (kg/fisherday) for haul nets in northern Spencer Gulf from 1983/84 – 1999/2000.

<u>Dab net fishery</u> The dab net fishery in northern Spencer Gulf is minor in terms of production. Targeted effort has averaged less than 18 fisherdays per month and catches have averaged less than 1 tonne per month (Figure 3.12). Seasonality of catch and effort is not pronounced although effort is lowest in the period from June to September. The CPUE reaches the lowest level in June and July (Figure 3.13).



Figure 3.12. Seasonality of average monthly targeted catch and targeted effort in the dab net fishery of northern Spencer Gulf from 1983/84 - 1999/2000.



Figure 3.13. Seasonality of average monthly targeted CPUE in the dab net fishery of northern Spencer Gulf from 1983/84 – 1999/2000.

Annual targeted effort has fluctuated substantially between 100 and 240 fisherdays since 1983/84, and the effort has declined dramatically during the last five years (Figure 3.14). CPUE was consistently 15 - 30 kg/fisherday until 1991/92, after which it increased dramatically to 118 kg/fisherday in 1993/4 (Figure 14). CPUE then decreased just as dramatically, but had since climbed steadily to 76 kg/fisherday in 1997/98. However, it dropped significantly to 55 kg/fisherday in 1998/99. Catch and effort can not be provided for 1999/2000 as there were less than five fishers active in the fishery.



Figure 3.14. Trends in annual targeted catch and effort for garfish in the dab net fishery of northern Spencer Gulf from 1983/84 - 1999/2000 (catch and effort cannot be provided for 1999/2000 due to confidentiality requirements).



Figure 3.15. Trends in annual targeted CPUE for garfish in the dab net fishery of northern Spencer Gulf from 1983/84 – 1999/2000.

## Southern Spencer Gulf (SSG)

The contribution of southern Spencer Gulf to the State's commercial garfish catch has been slowly rising in recent years (Table 3.2). The 1999/2000 catch of 68.6 tonnes represented 14.4% of the State's catch and was the highest recorded for the region except for 1997/98. The catch was largely taken by haul nets during the 1980s and early 1990s, but the catches by dab nets have become increasingly important in recent years (Figure 3.16).

The haul net catch has generally fluctuated between 30 and 45 tonnes but the 1997/98 catch was 58.7 tonnes and shows a strong increase over recent years (from a relatively low base) (Figure 3.16). The

dab net catch was generally less than 8 tonnes prior to 1994/95 but has more than doubled in recent years (Figure 3.16).



Figure 3.16. Trends in annual garfish catches by method in southern Spencer Gulf from 1983/4 - 1999/2000.

<u>Haul net fishery</u> The haul net fishery of southern Spencer Gulf is strongly seasonal with average targeted effort peaking from February to April at 44 - 56 fisherdays (Figure 3.17). All other months recorded less than 30 fisherdays per month. The pattern of monthly targeted catch closely follows effort (Figure 3.17).



Figure 3.17. Seasonality of average monthly targeted catch and targeted effort in the haul net fishery of southern Spencer Gulf from 1983/84 – 1999/2000.

The CPUE generally peaked in April and May and dropped to a low in October and November (Figure 3.18). The peak CPUE was about 30% less than that from the haul net fishery in the NSG.



Figure 3.18. Seasonality of average monthly targeted CPUE in the haul net fishery of southern Spencer Gulf from 1983/84 – 1999/2000.

Annual targeted effort in the haul net fishery has slowly declined from its peak in the mid 1980s of about 500 fisherdays to 100 - 220 fisherdays in the last six years (Figure 3.20). Targeted catch has also been reduced to about 10 tonnes since the early 1990's, except for 1997/98 (Figure 3.19). However, haul netting where no target species is nominated but where garfish catches are significant has risen considerably – particularly in the last four years (Figures 3.19 & 3.20). Both non-targeted catch and effort has exceeded targeted catch and effort since 1991/92.



Figure 3.19. Trends in annual catch of garfish in the haul net fishery of southern Spencer Gulf from 1983/84 – 1999/2000.



Figure 3.20. Trends in annual effort for garfish in the haul net fishery of southern Spencer Gulf from 1983/84 – 1999/2000.

Until 1995/96, targeted CPUE had fluctuated between 30 and 60 kg/fisherday but has increased to a peak of 94 kg/fisherday in 1997/98 (Figure 3.21). This rise had resulted in the increase in overall haul net catch in this region. In the last two years, CPUE has dropped back to the level of 60 kg/fisherday. Non-targeted CPUE has also fluctuated widely but shows no long-term trend.



Figure 3.21. CPUE for targeted haul netting and non-targeted haul netting where garfish catches > 20 kg/boatday in southern Spencer Gulf from 1983/84 – 1999/2000.

<u>Dab net fishery</u> Dab netting is highly seasonal peaking between January and April and secondarily in November (Figure 3.22). However, targeted effort is relatively low with less than 35 fisherdays on average per month. Targeted monthly catches closely follow effort. The average CPUE is generally high in the winter and low in the summer (Figure 3.23).



Figure 3.22. Seasonality of average monthly targeted catch and targeted effort in the dab net fishery of southern Spencer Gulf from 1983/84 – 1999/2000.



Figure 3.23. Seasonality of average monthly targeted CPUE in the dab net fishery of southern Spencer Gulf from 1983/84 – 1999/2000.

Annual targeted effort has shown a general increase in recent years, rising from about 200 fisherdays in the mid 1980s to 300 - 470 fisherdays in the last five years (Figure 3.24). However, the pattern shows considerable fluctuations with effort in 1997/98 declining about 32% from the previous year. CPUE has shown a strong increase with the 1999/2000 rate of 59 kg/fisherday the highest yet recorded (Figure 3.25).



Figure 3.24. Trends in annual targeted catch and effort for garfish in the dab net fishery of southern Spencer Gulf from 1983/84 – 1999/2000.



Figure 3.25 Trends in annual targeted CPUE for garfish in the dab net fishery of southern Spencer Gulf from 1983/84 - 1999/2000.

## Gulf St Vincent (GSV)

Gulf St Vincent is the second most important region for garfish production in South Australia, producing 30 - 40% of the commercial catch (Table 3.2). Average production from 1983/84 to 1999/2000 was 155.5 tonnes, and the catch for 1999/2000 was 189.5 tonnes.

The overall catch was mostly taken by haul net through the 1980s but dab net catches have become increasingly important during the 1990s (Figure 3.26).



Figure 3.26. Trends in annual garfish catches by method in Gulf St. Vincent from 1983/84 - 1999/2000.

<u>Haul net fishery</u> The haul net fishery of Gulf St Vincent is strongly seasonal with peak targeted effort from January to May of 130 - 250 fisherdays per month, followed by a significant decline to a low of about 50 fisherdays per month between July and November (Figure 3.27). Targeted catch is only loosely related to effort from January to May (peak monthly effort) but more closely coincides during the remaining months (Figure 3.27).



Figure 3.27. Seasonality of average monthly targeted catch and targeted effort in the haul net fishery of Gulf St. Vincent from 1983/84 – 1999/2000.

The seasonal pattern of CPUE in GSV is similar to that in the NSG. The catch rates peak between May and August at the level of 80 kg/fisherday and decline to an average of 40 kg/fisherday during the rest of the months (Fig. 3.28).



Figure 3.28. Seasonality of average monthly targeted CPUE in the haul net fishery of Gulf St. Vincent from 1983/84 – 1999/2000.



Figure 3.29. Trends in annual catch of garfish in the haul net fishery of Gulf St. Vincent from 1983/84 - 1999/2000.

Annual targeted catch dropped significantly from 118 tonnes in 1984/85 to 50 tonnes in 1986/87, but has since stabilised with most of the years catches between 45 to 75 tonnes (Figure 3.29). Targeted effort was also halved in the mid 1980s from a high of 2,813 fisherdays in 1984/85 to 1,405 fisherdays in 1986/87 (Figure 3.30). Effort has averaged 1117 fisherdays in the last ten years. However, haul netting where no target species is nominated but garfish catches have been significant increased at about the time when targeted effort declined. Overall effort therefore appears to have been stable since 1986/87.



Figure 3.30. Trends in annual effort for garfish in the haul net fishery of Gulf St. Vincent from 1983/84 - 1999/2000.

Targeted CPUE fluctuated between 35 and 55 kg/fisherdays until 1994/95 but has since increased generally to 80 kg/fisherday in the last two years (Figure 3.31). Non-targeted CPUE shows a trend of a slow increase from 20 kg/fisherday in mid 1980's to about 40 kg/fisherday in the last four years.



Figure 3.31. CPUE for targeted haul netting and non-targeted haul netting where garfish catches > 20 kg/boatday in Gulf St. Vincent from 1983/4 – 1990/00.

*Dab net fishery* The dab net fishery of Gulf St Vincent shows two separate peaks in targeted fishing effort (Figure 3.32). The summer peak from November to February (but principally November to December) includes fishing activity in the south-eastern Gulf while the winter peak in May and June is predominantly fishing activity in the northern Gulf. Targeted catches closely follow seasonal patterns of effort.



Figure 3.32. Seasonality of average monthly targeted catch and targeted effort in the dab net fishery of Gulf St. Vincent from 1983/84 - 1999/2000.

The CPUE is the lowest (less than 20 kg/fisherday) in August and September before significantly increasing to more than double in November and December (Figure 3.33). The catch rate remained between 25-40 kg/fisherday for the rest of the months.



Figure 3.33. Seasonality of average monthly targeted CPUE in the dab net fishery of Gulf St. Vincent from 1983/84 – 1999/2000.

Annual targeted dab net effort in Gulf St Vincent increased strongly in the early 1990s to a peak of 1,317 fisherdays in 1995/96 or about four times the levels of the mid 1980s (Figure 3.34). However, effort declined dramatically in the following three years to 313 fisherdays in 1998/99, but then increased slightly to 484 fisherdays in 1999/2000. This may (at least in part) be due to the transfer of effort to blue crab hoop netting by fishers who acquired quota. Targeted catch closely follows effort (Figure 33). Targeted CPUE increased steadily to about 40 kg/fisherday in 1991/92 and has since remained above that level (Figure 3.35).



Figure 3.34 Trends in annual targeted catch and effort for garfish in the dab net fishery of Gulf St. Vincent from 1983/84 – 1999/2000.



Figure 3.35. Trends in annual targeted CPUE for garfish in the dab net fishery of Gulf St. Vincent from 1983/84 – 1999/2000.

# West Coast (WC)

West Coast waters have produced an average of 29.9 tonnes per year since 1983/84, with little variation until 1998/99 (Table 3.2). The total catch dropped about 30% to 20.5 tonnes in 1999/2000. More than 70% of the annual catch was taken by haul net (principally Venus and Coffin Bays) before 1997/98 with the remaining catch taken by dab nets (Figure 3.36). Nevertheless, the proportion of dab net catch has increased greatly in the last three years and reached 50% of the total catch.

From 1983/84 to 1997/98, the haul net catch had been relatively stable and fluctuated between 19 and 33 tonnes before the substantial decline in the last two years (Figure 3.36). The dab net catch was generally less than 7 tonnes until 1997/98 when it reached 9.9 tonnes, and remained at that level.



Figure 3.36. Trends in annual garfish catches by method in West Coast waters from 1983/84-1999/2000.

<u>Haul net fishery</u> Monthly targeted effort is strongly seasonal with more than 50 fisherdays expended monthly from March to May (Figure 3.37). Targeted catch follows effort closely. The catch rate also peaks in May with a value of 86 kg/fisherday (Figure 3.38).



Figure 3.37. Seasonality of average monthly targeted catch and targeted effort in the haul net fishery of West Coast waters from 1983/84 – 1999/2000.



Figure 3.38. Seasonality of average monthly targeted CPUE in the haul net fishery of West Coast waters from 1983/84 – 1999/2000.

Annual targeted catch declined from 32 tonnes in 1983/84 to 17 tonnes in 1985/86 (Figure 3.39), then fluctuated around 23 tonnes until 1999/2000 when it dropped to 9.6 tonnes. The targeted effort also declined significantly from 1983/84 to 1985/86 but then slowly increased to 427 fisherdays in 1988/89 (Figure 3.40). Effort has then averaged about 400 fisherdays until 1997/98 before declining to the lowest level of 180 fisherdays in 1999/2000. The impact of non-targeted haul netting is minor in this region. Their detailed catch and effort data from 1983/84 to 1985/86, and since 1992/93 can not be presented as there were less than five fishers active in the fishery during those years.



Figure 3.39. Trends in annual catch of garfish in the haul net fishery of West Coast waters from 1983/84 – 1999/2000 (non-targeted catch from 1983/84 to 1985/86, and since 1992/93 are confidential data).



Figure 3.40. Trends in annual effort for garfish in the haul net fishery of West Coast waters from 1983/84 – 1999/2000 (non-targeted effort from 1983/84 to 1985/86, and since 1992/93 are confidential data).

Targeted CPUE has fluctuated between 40 and 77 kg/fisherday since 1983/84 and there appears to be no long-term trend (Figure 3.41). Non-targeted CPUE is more variable and is only presented for the period of 1986/87-1991/92 due to confidentiality requirements.



Figure 3.41. CPUE for targeted haul netting and non-targeted haul netting where garfish catches > 20 kg/boatday in West Coast waters from 1983/84 – 1999/2000.

## Dab net fishery

Targeted effort in the West Coast dab net fishery peaks in April and May with a secondary peak in August (Figure 3.42). However, effort is low with an average of less than 25 fisherdays per month. Monthly catch follows effort and are less than one tonne per month. The CPUE varies between 23-46 kg/fisherday without any significant seasonal pattern (Figure 3.43).



Figure 3.42. Seasonality of average monthly targeted catch and targeted effort in the dab net fishery of West Coast waters from 1983/84 – 1999/2000.



Figure 3.43. Seasonality of average monthly targeted CPUE in the dab net fishery of West Coast waters from 1983/84 – 1999/2000.

Annual targeted effort increased strongly during the 1980s to a peak of 237 fisherdays in 1988/89 (Figure 3.44). Effort then declined to about 100 fisherdays per year until 1997/98 when it increased rapidly back to 224 fisherdays. In 1999/2000, the effort has further increased to the highest level of 325 fisherdays. The increase of dab net effort in the last few years has mainly occurred in Coffin Bay, which was probably due to the net closure in 1995. Targeted catch loosely follows effort. The annual catches in the last three years have doubled from pre-1997/98 level of 5 tonnes to about 10 tonnes. CPUE has fluctuated between 15 and 33 kg/fisherdays until 1991/92 but had since consistently stayed between 44 and 53 kg/fisherdays until 1999/2000 when the CPUE dropped significantly back to 34 kg/fisherday (Figure 3.45).



Figure 3.44 Trends in annual targeted catch and effort for garfish in the dab net fishery of West Coast waters from 1983/84 – 1999/2000.



Figure 3.45 Trends in annual targeted CPUE for garfish in the dab net fishery of West Coast waters from 1983/84 – 1999/2000.

## Kangaroo Island (KI)

The commercial garfish catch in Kangaroo Island waters steadily increased during the 1980s and early 1990s to a peak of 42.4 tonnes in 1992/93 (Table 3.2). Total catches then declined and have averaged 22.4 tonnes since that time (range 16 - 32 tonnes). Until the early 1990s, the catch was largely taken by haul net but dab net catches have contributed a relatively greater proportion since that time (Figure 3.46).

Catches by haul and dab nets increased steadily until their peaks in 1992/3 (Figure 3.46). While dab net catches appear to have stabilised at about 7 - 10 tonnes since then, haul net catches have fluctuated more widely (7 – 25 tonnes), and in 1999/00, they were at their lowest level since 1984/85.



Figure 3.46. Trends in annual garfish catches by method in Kangaroo Island from 1983/4-1999/2000.

## Haul net fishery

Average targeted haul net effort in Kangaroo Island waters peaks in May but is generally low (10 - 25 fisherdays) throughout the year (Figure 3.47). Targeted catch follows effort and is generally less than 1 tonne per month. There are two peaks of CPUE, one in September and the other in April (Figure 3.48).



Figure 3.47. Seasonality of average monthly targeted catch and targeted effort in the haul net fishery of Kangaroo Island from 1983/84 – 1999/2000.



Figure 3.48. Seasonality of average monthly targeted CPUE in the haul net fishery of Kangaroo Island from 1983/84 – 1999/2000.

Detailed annual catch, effort and CPUE for the Kangaroo Island haul net fishery cannot be provided as there are less than five fishers active in the fishery. Together with a seasonal netting closure during the early 1990's, reporting practices have changed over the last decade (primarily nomination of target species vs "ANY"), and trends in effort and CPUE are therefore difficult to interpret.

## Dab net fishery

The dab net fishery is highly seasonal and is most active from November to February (Figure 3.49). Effort in those months has averaged 20 - 35 fisherdays with other months generally less than 10 fisherdays. Targeted catch closely follows effort, and is about 1 tonne per month in the peak season. The highest catch rates generally occur between October and April (Figure 3.50). The relatively high CPUE in June is probably due to the low effort at this time of the year.



Figure 3.49. Seasonality of average monthly targeted catch and targeted effort in the dab net fishery of Kangaroo Island from 1983/84 – 1999/2000.



Figure 3.50. Seasonality of average monthly targeted CPUE in the dab net fishery of Kangaroo Island from 1983/84 – 1999/2000.

Annual targeted dab net effort rose steadily to 274 fisherdays in 1992/93 but has declined about 30% in 1993/94 and has since remained between 140 and 200 fisherdays (Figure 3.51). Targeted catch generally follows effort and has stabilised between 7 and 10 tonnes in the last seven years. Targeted CPUE has risen steadily to 54 kg/fisherday in 1999/2000 (Figure 3.52).



Figure 3.51. Trends in annual targeted catch and effort for garfish in the dab net fishery of Kangaroo Island from 1983/84 – 1999/2000.



Figure 3.52. Trends in annual targeted CPUE for garfish in the dab net fishery of Kangaroo Island from 1983/84 – 1999/2000.

# **Other State waters**

Commercial catches of garfish in other State waters are very minor, accounting for 0.5% of the total catch (Table 3.2). The catch has been less than 5 tonnes for the last 12 years and has been caught by a mixture of haul and dab nets (Figure 3.53). Most of the catch and effort by haul net and other method cannot be presented here due to confidentiality reasons.



Figure 3.53. Trends in annual garfish catches by method in other State waters from 1983/84-1999/2000 (catch by haul net and other methods for most of the years and by dab net in the last two years can not be presented due to confidentiality requirements).

## Summary of SA commercial fishery.

In general, State garfish landings have been relatively stable since 1983/84, with a mean annual catch of 460 tonnes. The 1999/2000 catch of 477 tonnes was therefore about 4% above the long term average.

### Haul net fishery

The catch continues to be dominated by haul net landings ( $\cong$  90% total catch) which have averaged 407.4 tonnes over the entire period. Catches have been slightly below this long term average for the last two years.

Targeted effort in the haul net fishery declined about 36% from 1983/84 to 1992/93 but has apparently stabilised since that time (average = 4,110 fisherdays since 1992/93). The 1999/2000 targeted effort was about 6% below this average.

In all regions, CPUE (kg/fisherday) for the haul net fishery is either stable or has been slowly increasing since 1983/84.

However, reporting practices in the catch and effort returns in the haul net fishery make interpretation of targeted effort and CPUE difficult. Targeted catch only accounts for about 56% of the total haul net catch, down from 66% in 1983/84. Most of the remainder of the garfish has been reported as target = "ANY". In this document, "ANY" fishing effort where garfish catches exceeded 20 kg/boatday were reported separately and, in some regional cases, this effort was equivalent in magnitude to or even exceeded targeted effort.

Appropriate analyses of these data require further consideration, while the reporting for unspecified targeting haul netting requires resolution. It is important to note, however, that for the two areas where highest hauling net catches occur (ie NSG and GSV), both targeted and unspecified targeted CPUE's show similar increasing trends over time. In SSG, the only other area where there is comparative information, although there are opposing temporal fluctuations for the garfish targeted and "any species" targeted CPUE's, (which may be due to temporal changes in the reporting methods by fishers in this area), there is no overall downward trend in either CPUE.

#### Dab net fishery

Dab net landings increased in the early 1990s and now vary between 50 and 100 tonnes per year. The 1999/2000 catch of 69 tonnes was about 2% below the average (70.8 tonnes) since 1992/93.

Targeted dab net effort also increased in the early 1990s to a peak in 1995/96. The last two years have seen an average decrease of 43% from that level.

CPUE (kg/fisherday) in the regional dab net fisheries have generally been increasing through the 1990s, in some cases quite rapidly. Several regions have now stabilised at levels significantly higher than the CPUE's of the 1980s.

A seasonal (November - March) closure to hauling nets in one of the traditionally important garfish hauling net areas of Kangaroo Island was implemented in 1996. Inspection of the annual dab net CPUE's throughout the entire period (1983/84 - 99/00) has shown that the rate of increase has been steady, almost linear, with no evidence of any rise in the rate of increase after the hauling net closure. It is concluded that commercial dab netting catch and effort data on its own have not provided any evidence for an improvement in the availability of garfish to commercial dab netting since the hauling net closure.

# Comparison between seasonal trends in catch, effort and CPUE in haul net and dab net fishery

The information presented above has shown that garfish catch and effort by the hauling net fishery is significantly higher than dab netting in all regions except, recently for KI. The season when these levels of fishing intensity are at their highest should be the best time when any potential impacts of competing gear-sectors may be seen. For both gear types in most areas there exists strong seasonal fluctuations in catch, effort and CPUE (Figures 3.54, 55).



Figure 3.54. Summary of seasonal peaks (4 month highs) in catch and effort on sea garfish by region and by gear type.



Figure 3.55. Summary of seasonal peaks (4 month highs) in CPUE of sea garfish by region and gear type.

For SSG and WC regions, the period of high catch and effort by the hauling net sector corresponds with the highest CPUE's for the dab net fishery. In NSG and GSV, the two regions of highest hauling

net activity, seasonally high catch and effort occurred from Jan - June, and dab net CPUE's were peaked before or during the beginning of this period (for both areas), and, in the case of GSV, mid way through this period (April). The differences in timing may be due to differences between the locations of high hauling net effort and dab netting within these regions, as inspection of catch and effort in individual fishing blocks within each of these regions indicate that most of the hauling net effort occurred in the more northern blocks, whereas, dab netting catch and effort was higher in the more southern blocks. The main area where there was some overlap in fishing block occurred in the northern GSV, and here the dab net CPUE's peaked in April, at the same time that hauling net catch and effort was at its highest. Actual figures cannot be presented here for reasons of confidentiality.

In KI, the peaks in catch and effort by hauling and dab netting generally occurred at different times, partly because of a seasonal (November - March) closure to hauling nets since 1995 in a previously important garfish haul netting area, and so any seasonal effects on the dab net CPUE's cannot be determined.

### Description of the The South Australian Recreational Fishery.

Recreational fishing for sea garfish is a traditional recreational past-time for SA anglers; the species is classed as one of the four "bread and butter species" (others include tommy ruffs, salmon trout and mullet). The most popular method of catching garfish is by rod and line, often with a large wooden or plastic hollow float, filled with "berley", which is made up of bread soaked in fish oil. Up to 3 small hooks (size 8 - 12) are used, and these are baited with blowfly larvae (commonly known as "gents"), which are either marketed through the fishing tackle trade or are raised in home-made plants by the more passionate of the garfish fishers. Fishing occurs both during daylight hours and at night, and can occur off most platforms, including boats and jetties and by some specialist fishers from the shores of sheltered bays and inlets. Summer months tend to be most commonly fished period of the year.

The other form of recreational fishing for sea garfish is carried out at night by dab netting, and involves the use of high wattage lights to search for aggregations of garfish at the surface of the water, generally on the "dark" of the moon. The lights are either hand held or are attached underwater to the fishing vessel. This form of recreational fishing occurs in the both the shallow and deeper waters of the more northern waters of Gulf St. Vincent, Kangaroo Island bays, southern and northern Spencer Gulf. The garfish harvest by recreational fishers is regulated by a bag limit of 80 garfish per person per day and a boat limit of 240 garfish per person per day.

During the period 1980 - 90, a number of comprehensive creel surveys on recreational catch and effort in the marine scalefish fishery were conducted at a number of coastal areas of South Australia. These surveys concluded that southern sea garfish comprised a significant proportion of the total catch by anglers using rod and line from both boats and jetties (see Table 3.3). Garfish were not taken by recreational shore based gill nets, because the minimum mesh size of 5 cm was too brge (Jones, 1986). There are no catch and effort data specific to the recreational dabbing for garfish; however, in a 1982/83 study on levels of recreational fishing participation throughout SA (Philipson et al, 1986), dab net fishing (predominantly for garfish) usually received the second or third highest percentages of participation levels, after line fishing (6 - 10% of the total population, depending on the season). The most comprehensive survey of recreational boat fishing catch and effort throughout the SA gulfs, KI and west coast waters was carried out during 1994/96, and estimated the annual catch of southern sea garfish by this sector of the fishery to be 64.1 tonnes per year (13.1 % of the total catch; McGlennon & Kinloch, 1996). The areas where highest catches occurred were central and southern Gulf St. Vincent and south-eastern Spencer Gulf. Catch rates (1.5 - 3 fish per boat-hr) were seasonal with peaks generally occurring over the summer months (January - March). The size composition of sea garfish caught during the survey peaked between 26 and 28 cm. About 8% of fish caught were less than 24 cm.

Date of study	Area	Fishing	Garfish catch as a	Reference
		platform, gear	% of the total	
		type	catch of all species	
			(importance)	
Jan - Dec, 1980	Adelaide	Boat, rod and line	21.1 % (2 <sup>nd</sup> )	Jones, 1981
	metropolitan			
Jan - Dec, 1980	Adelaide	Jetty, rod and line	18.3 % (2 <sup>nd</sup> )	Jones, 1981
	metropolitan			
Jan - Dec, 1980	Adelaide	Shore, rod and line	19.6 % (3 <sup>rd</sup> )	Jones, 1981
	metropolitan			
March - May, 1985	Pt. Hughes -	Boat, rod and line	$13 - 22\% (2^{nd}),$	Hill, 1987
	Wallaroo		41% Easter, $(1^{st})$	
March - May, 1985	Pt. Hughes -	Jetty, rod and line	$11 - 22\% (2^{nd})$	Hill, 1987
	Wallaroo		49%, Easter (1 <sup>st</sup> )	
Jan - Dec, 1986	Pt. Lincoln Bays	Boat, rod and line	$1\%, (3^{rd})$	Jones, 1986
Jan - Dec, 1986	Pt. Lincoln Bays	Shore, recreational	None	Jones, 1986
		net		
1977 - 80	Coffin Bay	Boat, rod and line	$9.2\% (2^{nd})$	Jones, 1987
Easter, 1981	Coffin Bay	Boat, rod and line	29.4% (2 <sup>nd</sup> )	Jones, 1983
Easter, 1981	Coffin Bay	Shore and jetty, rod	30.8% (1 <sup>st</sup> )	Jones, 1983
		and line		
Easter, 1981	Coffin Bay	Estuary fishing	18.5 % (3 <sup>rd</sup> )	Jones, 1983
		competition, rod		
		and line		
Jan - June, 1990	Coffin Bay	Boat, rod and line	10.9% (3 <sup>rd</sup> )	Staniford and Siggins,
				1992

Table 3.4. Summary of results of recreational creel surveys of catch and effort (1980 - 90).

## The Garfish fishery in Victoria.

#### Description of the commercial fishery.

In Victoria, the bulk of the commercial catch of southern sea garfish is taken in Port Phillip Bay, Western Port Bay and Corner Inlet. Different types of haul nets of varying dimension have been modified catch this species, according to the fishing grounds in each location. Four main types of nets are used in the multi-species net fisheries of these bays and inlets - gar seines, beach seines, estuary seines and ring nets (Knuckey et al, 2000). In Port Phillip Bay, beach seines are about 350 m in length whereas in Westernport Bay they are smaller at about 200 m. In Corner Inlet, gar seines are small usually 150 - 200 m in length, whereas the ring nets and estuary seines are larger at over 400 m length.

Garfish seines are haul nets that have been developed to specifically target southern sea garfish. These specialised nets have high float to weight ratios so that the net floats at the surface and the garfish are retained by the relatively small mesh sizes in the wings (40 mm) and pockets (25 mm) of their nets. However, this traditional form of targeting garfish has been replaced and garfish are now mostly caught with ring nets, beach seines and estuary seines as part of the multi-species fisheries in Victorian bays and inlets.

## The commercial catch, effort and CPUE's.

#### State overview.

During the 1980's, the annual garfish catch was stable fluctuating between 100 and 200 tonnes, but during the 1990's catches dropped and remained stable at lower levels fluctuating between 60 and 100 tonnes (Fig. 3.56). When garfish catches dropped below 100 tonnes in 1995/96 and 1996/97, there was a sharp rise in the average market price to more than \$ 6 / kg. During this period, King George whiting provided better returns to Victorian haul seine fishers. Haul seine fishers increasingly targeted King George whiting because the market price was much higher compared to southern sea garfish and because King George whiting were abundant due to high recruitment (*reference ?*). As a result of this change in fishing practices, the commercial catch of King George whiting in Victoria increased from less than 128 t between 1993/94 and 1995/96 to more than 226 t in 1996/97 and 1997/98. Since then, most beach seines have been modified to reduce by-catches of undersized King George whiting. The larger meshes that have recently been adopted also allow a proportion of the garfish to escape with the undersized King George whiting. This change in gear selectivity has further reduced the fishing effort targeted at garfish in Victoria.



Figure 3.56. Total annual catch, value and average price of southern sea garfish in the Victorian commercial fishery between 1982/83 - 1999/00.

Most of the sea garfish caught commercially in Victoria is traditionally sold for local human consumption, and in recent years, with the decline in these catches, sea garfish caught in Tasmania have also been sold on the Melbourne market (Jordan, pers comm), indicating that the consumer demand for this species has not diminished.

#### Pt. Phillip Bay

In most years, the catch taken by gar seines has been higher than those by the other seines and ring nets (hence called B,E,H seines and ring nets) (Fig. 3.57). During the 1980's and early 1990's, total catch fluctuated between 70 and 45 mt; however, over the last 5 years, catches dropped by about 50%.



Figure 3.57. Annual catches (tonnes) of southern sea garfish by gar seines and beach, estuary and hauling seines and ring nets in Port Phillip Bay, 1982/83 - 1999/00.



Figure 3.58. Annual fishing effort (boat-days) directed at southern sea garfish by gar seines and beach, estuary and hauling seines and ring nets in Port Phillip Bay, 1982/83 - 1999/00.

Fishing effort (boat-days) with BEH seines and ring nets was higher than fishing effort gar seines. All fishing methods showed similar annual trends in fishing effort which fluctuated between 1800 - 3200 boat days during the period 1982/83 and 1997/98, with lower levels of effort in the last two years (Figure 3.58).



Figure 3.59. Average and annual catch per unit effort (CPUE, kg/ boat-day) of southern sea garfish by gar seines and beach, estuary and hauling seines and ring nets in Port Phillip Bay, 1982/83 - 1999/00.

The average CPUE for gar seines was higher (44.2 kg / boat-day) and showed larger inter-annual fluctuations than for the BEH seines and rings nets (average: 10. 7 kg / boat-day) (Figure 3.59). The two gear types showed different trends in CPUE during the 1980's with a declining CPUE for gar seines and stable CPUE for BEH seines and ring nets between 1982/83 and 1987/88. Between 1988/89 and 1992/93, the CPUE for both methods increased, but then fell to their lowest levels on record. The lowest CPUE was recorded in 1995/96 for gar seines and in 1996/97 for BEH seines and ring nets. Since then the CPUE for gar seines have recovered and for the last three years they have been higher than the long term average. The CPUE for the BEH seines and ring nets did not show the same fluctuations, but have increased slightly since 1996/97 remaining at a lower level than the average for the for the rest of the period.

### Westernport Bay

Between 1982 /83 - 95/96, the catch of sea garfish fluctuated in Westernport Bay between 10 - 35 tonnes. During this period, catches taken with gar seines were lower than those taken by other fishing gears. Over the last 4 years, there has been a sharp decline in the catch (Fig. 3.60), particularly those taken with BEH seines and ring nets that is related to the decrease in fishing effort and CPUE. (Figures 3.61,62).



Figure 3.60. Annual catches (tonnes) of southern sea garfish by gar seines and beach, estuary and hauling seines and ring nets in Westernport Bay, 1982/83 - 1999/00.



**Figure 3.61.** Annual fishing effort (boat-days) directed at southern sea garfish by gar seines and beach, estuary and hauling seines and ring nets in Westernport Bay, 1982/83 - 1999/00.



**Figure 3.62.** Average and annual catch per unit effort (CPUE, kg/ boat-day) directed at southern sea garfish by gar seines and beach, estuary and hauling seines and ring nets in Westernport Bay, 1982/83 - 1999/00.

## Corner Inlet.

The multi-species fishery in Corner Inlet has operated for more than 100 years. Since 1982/83, southern sea garfish catches have fluctuated between 10 - 70 mt due to changes in the abundance of commercial species and associated targeted fishing effort with different fishing gears. During the 1990's, there have been large fluctuations with garfish catches declining from a peak of 58 mt in 1990/91 to 17 mt in 1996/97 (Fig. 3.63). This decrease in garfish catches was related to changes in species targeting and King George whiting catches in Corner inlet increased from 58 to 116 mt between 1990/91 and 1996/97. Since 1997/98 the fishing gears have been modified to target both

species and ring nets have now mostly replaced gar seines. Over the last two years, fishing effort with ring nets has risen (Figure 3.64) and garfish catches were the highest for the last 18 years.



Figure 3.63. Annual catches (tonnes) of southern sea garfish by gar seines and beach, estuary and hauling seines and ring nets in Corner Inlet, 1982/83 - 1999/00.

There has been an increasing trend in fishing effort (boat-days) (Figure 3.63). During the 1980's, fishing effort more than doubled, but has remained at about the same level (1500 - 1900 boat-days) throughout the 1990's, reaching its highest level in 1998/99. The long term average CPUE for garseines and other nets were very similar (26.9 kg / boat-day for garseines and 25 kg / boat-day for BEH seines and ring nets (Figure 3.65).



Figure 3.64. Annual fishing effort (boat-days) directed at southern sea garfish by gar seines and beach, estuary and hauling seines and ring nets in Corner Inlet, 1982/83 - 1999/00.


**Figure 3.65.** Average and annual catch per unit effort (CPUE, kg/ boat-day) directed at southern sea garfish by gar seines and beach, estuary and hauling seines and ring nets in Corner Inlet, 1982/83 - 1999/00.

The CPUE for gar seines between 1995/96 and 1998/99 were well below the long term average, but increased to former levels in 1999/00. This period of lower CPUE has resulted in a declining CPUE trend for gar seines over the last 10 and 18 years. For the other gear types, there were similar years of low and high CPUE, but although CPUE fluctuated, there was no distinct trend.

## The recreational sea garfish fishery in Victoria.

Sea garfish is a popular species taken by recreational fishers in Port Phillip Bay and Corner Inlet. Surveys of recreational catch and effort during the early 1990's indicate that in Port Phillip Bay, annual catches of sea garfish at 20 mt were taken (DPI, Tas, 1996).

# The Garfish Fishery in Western Australia.

# Description of the commercial fishery.

Historically, garfish catches in Western Australia have been one component of a multi-species coastal and estuarine fishery. Between 1983-1984 and the present, 12 different fishing methods have been recorded against garfish commercial catches. These methods include: beach haul net, beach seine net, beam tide trawl, gill net, hand line, haul net, lift net, purse seine, trap net, trawling. Prior to 1989-1990, beach haul was the fishing method category that included both beach seines and haul nets. After 1990, this category was replaced by the two categories of beach seines and haul nets. Not all methods produced significant catches of garfish. The greatest proportion of the annual garfish catch can be

attributed to, beach seine and haul net (=beach hauling) methods combined and gill netting. The details of these important fishing methods are:

Gill nets: These nets vary in length from 140 to 3000 metres. They are set overnight in sheltered nearshore waters and estuaries on both the west and south coasts. Historically the catches of garfish from gill nets has not been greater than 5 tonnes for either the west or south coasts until 1994-1995 on the west coast. A lawful garfish set net has been defined in the Fisheries Western Australia Fisheries Management Act for particular locations. Examples along the west coast include the Mandurah Estuary where a 'garfish net' is a net not more than 55 metres in length having meshes throughout of not less than 28 mm, the Leschenault Estuary where a net used to take garfish must not be more than 60 metres in length with mesh no less than 28 mm and no more than 100 meshes deep. In Wilson Inlet, on the WA south coast, a lawful set net has been described as having meshes throughout of not less than 44 mm, a length of not more than 500 metres and a depth of not more than 50 meshes, and used or intended to be used for catching garfish during the period from May 1 to 31 October in each and every year.

Beach seine and haul nets (beach hauls): Beach seines are hauled by netting teams of two or more people, and are set from the beach using a small rowing boat. There is a wide range of seine net lengths and lengths between 60 and 800 metres have been recorded. The depth of the net depends on the depth of water fished. Mesh size varies from 25 to 50 mm. Beach seines are used on both the west and south coasts along the beach front and in estuaries. Haul nets are modified beach seines that are operated from a boat. These are used in Geographe Bay. The haul net teams work these nets over the near shore waters and sheltered portions of the coast over sand and seagrass meadows.

## Commercial catch and effort.

#### State overview.

Commercial fishers in Western Australia complete a monthly compulsory fishing return recording their catch by method, species and area fished. This is then entered into the Fisheries Western Australia Catch and Effort Statistics System (CAESS). Between 1975-1976 and 1989-1999 catches of sea garfish (*Hyporhamphus melanochir*) and river garfish (*Hyporhamphus regularis*) were recorded under separate numeric codes. However, inaccurate identification and improper coding have meant that it is not possible to determine what proportion of the sea garfish catches are river garfish. Since that time, a change in the coding system has meant that catches of the two species have been recorded under one code.

The annual total catch of garfish in the southern half of the state was 16.5 tonnes during 1975-1976. The catch increased the following year to 27.4 tonnes and subsequently declined to its lowest level of 7.7 tonnes in 1981-1982. With some variation, the total catch of garfish rose to a high of 64.3 tonnes in 1991-1992. The annual total catch declined from that peak figure and fluctuated between 39 and 54 tonnes during the mid 1990's. The annual catch reached a second peak in 1998-1999 of 63.4 tonnes. Currently, the 1999-2000 annual total catch is 36.6 tonnes (Figure 3.66). The legal minimum size limit for garfish caught from the commercial sector is 23 cm. These commercial catches are produced from the west and south coast regions of WA.



Figure 3.66. Total annual catch, value and average price of garfish caught in the combined west and south coast regions of the Western Australian commercial fishery from 1983/84 to 1999/00.

Garfish are one of a suite of coastal and estuarine species caught as a part of multi-species fishery. In general, catches of garfish are not targeted, but opportunistic. For example, in the Cockburn Sound region, between 1983/1984 and the present, garfish have been landed with Australian herring in nearly equal proportions and smaller quantities of yellow-eye mullet and yellowtail scad. Along the south coast in the Albany region, garfish have been taken with Australian herring, leatherjackets, squid and King George whiting.

The bulk of the garfish landed in the commercial fishery in WA is for human consumption. The product is sold as whole fresh fish in the local Perth fish markets. A smaller quantity is sold for commercial and recreational bait.

The fluctuations in the total catch of the west and south coast garfish catches, value and the average price have been examined since 1983-1984 (Anon. 1979, 1982, 1985, 1988, 1991, Fisheries Western Australia unpublished data; Figure 3.66). The annual average price (per kg) of garfish based on market prices in Western Australia, has increased from \$0.64 in 1975-1976 to a high of \$3.59 in

1999-2000. The total catch and total value for the fishery have varied together with a generally increasing trend over time, with peaks in the catch and value occurring during 1987-1988, 1991-1992 and 1998-1999. The 1999-2000 catch has declined to 1996-1997 levels.

#### Regional Catch and Effort

The garfish fishery (sea and river garfish) in Western Australia is focused on the west and south coasts of the state. The west coast sector of the fishery extends from Jurien Bay (30°S) to Augusta (116°E) and includes coastal habitats as well as the Swan River Estuary, Peel Inlet-Harvey Estuary and Leschenault Estuary, although commercial fishing in the Lechenault Estuary ceased in December 2000. Garfish catches from the west coast have been prepared from 1983-1984 to the present and include both sea and river garfish (Figure 3.67). Garfish catches from 1983-1984 to the present have been less than one tonne from ocean blocks north of Perth and adjacent to Augusta and from the Peel Inlet-Harvey Estuary and Leschenault Estuary. The small catches (less than 300 kg) reported from the Peel Inlet-Harvey Estuary have increased since the Dawesville Channel was opened in 1994. This follows the same pattern as with other marine fish species. The greatest proportion of the west coast catches has been reported from Cockburn Sound and the adjacent ocean block. Catches comprise between 39% and 93% of the annual catch (average=76%). Geographe Bay and the adjacent ocean block reported the second highest catches representing between 3% and 36% of the west coast catch.



Figure 3.67. Total annual commercial catch and the Cockburn Sound and Geographe Bay catches from the west coast of Western Australia from 1983/84 to 1999/2000.

The fishing effort (in boat days) in Cockburn Sound for the main six commercial garfish fishers demonstrates an increase in effort from 382 boat days in 1983-1984 to 592 boat days in 1989-1990

(except for the decline to 389 boat days in 1986-1987). This was followed by a slight but steady decline in the past decade to the present effort of 270 boat days (Figure 3.68).



Figure 3.68. Effort in boat days for the six main garfish fishers in Cockburn Sound, Western Australia from 1983/84 to 1999/00.

The commercial catch from these areas along the west coast were taken initially by gill nets in 1983-1984 until the mid 1980's when beach haul, beach seine and haul net methods were the primary means of capture. This changed abruptly in 1994-1995 when gill net catches rose and exceeded the beach haul catches. This was attributed to one additional fisher gill netting, as well as continuing with beach haul fishing. Since 1983-1984, 50% of the total west coast commercial catch has been attributed to beach haul, beach seine and haul net, 45% from gillnets and the remainder to other fishing methods (Figure 3.69).



Figure 3.69. Total annual commercial catch of garfish from the west coast of Western Australia by fishing method from 1983/84 to 1999/2000.

The south coast sector of the fishery extends from east of Augusta (116°E) to the South Australian border (129°E). Garfish catches examined from 1983-1984 to the present show the majority of landings have been identified from the greater Esperance, Albany to Bremer Bay, Albany Harbours (Princess Royal, Oyster and King George Harbours) and the Wilson Inlet regions. The garfish landings from the Albany to Bremer Bay area and the Albany Harbours constitute between 22% and 87% (average =57%) of the total south coast annual commercial catch. While this catch accounts for most of the total south coast catch in most years, during 1989-1990, 1991-1992 and 1996-1997 the Esperance area commercial catch was responsible for a large proportion of the total south coast commercial catch (Figure 3.70).



Figure 3.70. Total annual commercial catch, and the Albany, Esperance and Wilson Inlet catches from the south coast of Western Australia from 1983/84 to 1999/2000.

Along the south coast the predominant fishing method reported since 1983-1984 has been beach haul, (beach seine and haul net) which accounts for 80% of the total annual south coast commercial catch. Landings from gill nets have comprised only 15% of the catch during this period. In the Esperance region during the early 1990's a special fishing method and endorsement was given to fishers interested in fishing for garfish by surface trawl. Fisheries WA records show only two fishers carry the endorsement. The reported contribution over this period by surface trawling is 3% of the total catch (Figure 3.71).



Figure 3.71. Total annual commercial catch of garfish from the south coast of Western Australia by fishing method from 1983/84 to 1999/2000.

# The Recreational Fishery for Garfish in Western Australia

#### **Overview**

Garfish are a very popular species with Western Australian anglers. There is no legal minimum length for the recreational sector and the daily bag limit is 40 fish per person per day. Garfish are considered both a top quality table fish and as bait for sportfish ranging from tailor to billfish. Southern sea garfish usually appear in the Perth metro region about February and can be caught in numbers until the first major winter storms break up schools in May or June.

Shore based anglers fish for garfish with the same fishing tackle they use for herring: a beach rod and reel, 5-7 kg fishing line, a wooden float or blob, and a 3-4 kg nylon trace of about 1.8m to a long shanked Mustad Carlisle hook (size 8 for big fish and 10 for smaller garfish). The best baits for successful fishing are maggots, prawn, squid or octopus. However, garfish are opportunists and will eat excess bait without hooking up. The best time to catch garfish is at first light. The use of berley is contentious amongst garfish anglers. The common complaint is that a mixture of bread, fish oil and pollard will interest garfish, however they often follow the oil slick out beyond casting range. Boat anglers may try a different tack by throwing berley into the water to attract the fish to the side of the boat then fishing for them with handlines and light lines (Cusack and Roennfeldt 1987).

The best shore based spots in the Perth metropolitan area are the Fremantle Moles, Grant Street, North Street, and the Hillary's Boat Harbour.

#### Estimates of recreational catch and effort

The Western Australian salmon and Australian herring anglers survey conducted between 1994 and 1995 interviewed fishers from Perth to east of Esperance. The survey demonstrated that garfish were the third most popular species caught. There were 7,138 garfish caught during the survey period that comprised 7.1% of the catch. Partitioned by fishing method, garfish ranked second comprising 9.4% of the catch for shore based anglers while for boat based anglers, garfish ranked 7<sup>th</sup> and comprised 3.9% of the catch (Ayvazian unpublished data).

Subsequent to the anglers' survey, a 12-month boat based anglers survey was conducted during1996-97, to estimate fish catches from Kalbarri to Augusta along the WA west coast. The estimated boat catch number of garfish was 77,868 fish for trailered boats and 1,323 fish kept for non-trailered boats. The estimated total number of fish kept was 79,191 (s.e. 11%) (7,600 kg). The garfish catch was highest in the Perth North, Perth South and Mandurah districts. More fish were caught in the autumn than other seasons. There was a high proportion of fish caught between 27 and 30.9 cm total length, although a considerable number of larger fish were caught. Limitations of the survey to be noted are; the survey was conducted between 8am and 4pm only and garfish are caught outside this survey area so the catch estimate will be an underestimate of total recreational catch (Sumner and Williamson 1999).

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# CHAPTER 4. SIZE AND AGE STRUCTURE OF THE COMMERCIAL FISHERIES AND MORTALITY RATES

# Q. Ye, G.K. Jones, D. McGlennon, S. Ayvazian and P.C. Coutin

Objective: Determine the size and age structure of the commercial catch from the different sectors in southern Australian waters, and improve understanding of the potential impacts of the competing gear sectors on the South Australian stocks.

The size and age structures of the garfish commercial catches were determined across WA, SA, and Victoria based on a market measuring study between February 1998 and June 1999. Otoliths were used to develop age length keys for fish sampled from different seasons and regions.

The overall mean size and age of the SA fisheries was 25.5 cm TL and 1.6 years, respectively, with age ranging from 0 to 6 years. There was significant spatial and temporal variation in the size and age structures between gear sectors, regions, seasons and years. The sizes of fish in catches by haul nets were smaller than those by dab nets particularly for fish from SG during the summer. The mean size of garfish from haul net fisheries were 25.6, 25.1, 27.2, and 28.5 cm for GSV, SG, KI, and WC, respectively, with 2 year old fish dominant except for SG, where 1 year old was the most abundant. Fish were generally smaller and younger in summer than in winter for the haul net fisheries from both gulfs, but not for those from KI and the dab net catches from SG.

There has been a substantial decline in the size of fish from the SA commercial fisheries since 1954/55, which is consistent with the general responses of fish populations to exploitation. The mean size of fish from GSV decreased from 27.8 cm in 1954/55 (Ling 1958) to 27.2 cm in 1986/87 (Jones *et al.* 1990) and 25.6 cm in 1998. A similar reduction also occurred in SG and was the most significant between 1954/55 and 1977/78 (Jones 1979) with the mean size declining from 28.5 to 25.3 cm. Compared with the size compositions of the haul net catches in 1994 (Jones 1995), 1998 samples were about 1 cm smaller in both gulfs. Using the present age length keys, age compositions also showed relative decrease in the means over years. However, the age structure of SG catch did not change between 1977/78 and 1998.

The mean size and age of the Victorian commercial catch were 25.9 cm TL and 1.7 years, respectively, with age ranging from 0-6 years. The size and age compositions differed significantly between regions with the length distributions showing a single mode in PPB and two distinct modes in CI, and the catch was dominated by 2 year old fish in PPB but both 1 and 2 year old in CI. For both regions, fish from the winter catches were generally bigger and older.

Fish from the WA commercial fisheries had the highest mean size of 28.8 cm TL and the oldest age of 2.2 years, with 11 age classes represented (0-10 years). Compared to those from the WC, catches from the SC had a broader size and age range with the mean size 3 cm bigger. The seasonal size and age structures were also variable along both coasts of WA, e.g. along the SC, the catch in the winter was 0.5 year younger than in the summer; and along the WC, there was a single dominant age class for the summer catch but not for the winter fishery.

Regional and temporal reference mortality rates of the populations from the three states were estimated by Chapman-Robson's method using the catch curves. The overall instantaneous mortality rates were 1.85, 1.55, and 0.98 for populations from SA, Victoria and WA, respectively. The mortality estimates increased significantly over years for populations from GSV and SG, SA due to increasing exploitation.

# **4.1. Introduction**

The previous chapter summarised the catch and effort in the commercial and recreational fisheries of South Australia (SA), Victoria, and Western Australia (WA). Despite the commercial catch and effort showing a stable fishery in SA, temporal trends in catch per unit of effort data were concluded to be relative poor indicators of fluctuations in relative abundance, because of differences in regulations between states and gear types, and undetermined temporal changes in gear efficiencies.

This chapter uses a second more reliable biological performance indicator of stock status, the size and age composition of the fished component of the stock in each state. It not only compares these parameters between states during 1998/99, but also uses temporally collected data from the SA fishery over the past 45 years to investigate if there are any population changes in the size/age structure over this period.

# 4.2. Methodology

## Study Area and Market Measuring

Broad scale market measuring study was conducted between February 1998 and June 1999 to determine the size and age composition of the commercial catches of sea garfish across SA, Victoria, and WA. Commercial fisheries from 12 sites in SA, 2 sites in Victoria, and 7 sites in WA were targeted approximately on a monthly basis at the local markets of each state (Figure 4.1). These chosen sites were also the main ports for garfish production from the three states, which fall into four regions in SA (Gulf St. Vincent (GSV), Spencer Gulf (SG), Kangaroo Island (KI), and the west coast (WC)); two regions in Victoria (Port Phillip Bay (PPB) and Corner Inlet (CI)); and two regions in WA (the west coast (WC) and the south coast (SC)). The locality, month and year of market measuring and the number of fish measured are shown in Table 4.1.

In SA, the sampling was conducted at the Adelaide Central Fish Market (SAFCOL), where fish arrive from around SA each morning and are auctioned to Adelaide fish retailers and/or wholesalers each weekday. At the market, garfish arrive in boxes of approximately 20 kg. These boxes thus provide a basic sampling unit. Sampling was undertaken 1 to 2 mornings per week, and measurement started usually at 5:00 am when the market opened, till 6:30 am when the auction began, in order to try and get samples from all sites each month. On each

occasion, the total number of boxes of fish caught by a fisher from a targeted site were counted and the average weight per box was calculated by weighing 3 to 5 boxes to estimate the total catch on the sampling date from the particular site. After that a number of boxes were randomly sampled as following: if there were < 4 boxes from a particular site, all boxes from that site were sampled; if there were 430 boxes, every second one from that site was sampled; and if there were > 30 boxes, every 6th box from that site was sampled. Then from each box, a subsample of 1 kg (determined from weighing it on a scale) was randomly chosen and each fish in that kg subsample was measured for total length. The total weight of measured fish was recorded. Due to the time constrain, on each morning when sampling fish from a particular site, we must bear in mind the objective of covering all of the sites in each month.

As all or part of the garfish caught from Venus Bay, Port Lincoln, Whyalla, and Kangaroo Island, SA were often sold locally, personnel were hired for measurement of these fish using the same methods as those we applied at the Adelaide SAFCOL. The overall length sampling protocol for WA and Victoria was similar to that for SA.



Figure 4.1. Sampling sites and regions of the sea garfish commercial fisheries from South Australia, Victoria, and Western Australia. Inset areas show sampling locations for each state in respect to Australian coastline. Symbols for SA: MB = Middle Beach, PW = Port Wakefield, CJ = Cape Jervis, PV = Port Vincent, KC = Kingscote, AB = Arno Bay, CP = Corny Point, PL = Port Lincoln, PP = Port Pirie, TK = Tickera, WH = Whyalla, and VB = Venus Bay.

		,	Year/mo	onth															
State	Region	Site	1998											1999					
			2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	
SA	Gulf St.	Cape Jervis		84															
	Vincent	Middle Beach	272	373	121					43	89	37	49	38					
		Port Vincent	207					82					33						
		Port Wakefield	66	251	83	187	208	79				7							
	Kangaroo Island	Kingscote		33	337			26			38	51							
	Spencer Gulf	Arno Bay	81	86	83	128	33	14	108	13				29					
		Corny Point	41	75	196	136			45	30		18	21						
		Port Lincoln	17	26	155	142	77	48	29	129	9	36	48						
		Tickera	74	405	208	416	44	151	84	122	141	102	107	186					
		Port Pirie	124		89	141	48	66	108	10	25		24	158					
		Whyalla		132	69				209	44		16							
	West Coast	Venus Bay			734	1421													
Victoria	Corner Inlet	Corner Inlet		82	205	73	310	187	145	141	69		170	168		115	102		
	Port Phillip Bay	Port Phillip Bay	60	92	207	148	385	102	185	138	224			160	141	114			
WA	South Coast	Cheynes Beach						30											
		Oyster Harbour				20	65	45			55				81	84		50	
		Peaceful Bay				90		60											
		Princess Royal Harbour									15								
		Wilson Inlet	60			124		30	65	114	130	26	147	80	106	32	11	31	2
	West Coast	Cockburn Sound	74	30	30	60													
		Quindalup															100		

Table 4.1. Information on locality, month and year of market measuring for Hyporhamphus melanochir and number of fish measured.

## **Otoliths Collection, Preparation and Examination**

A total of 8453 sea garfish were sampled between August 1997 and September 2000 from South Australia, Victoria, and Western Australia for the study of age and growth and reproductive biology (Chapter 2 and Chapter 5). Almost all of the adult fish were purchased from the local markets in each state where market measuring was conducted. Fish greater or equal to 170 mm TL, collected between October 1997 and May 1999 from WA, between August 1997 and February 1999 from SA, and between March 1998 and April 1999 from Victoria were also used to develop the age-length keys. The sampling sites of these fish approximately corresponded to the targeted ports of each state for the market measuring study.

A sub-sample of 3297 pairs of sagittae were prepared as transverse sections, and examined for opaque zones and edge type with a microscope under transmitted light as fully described in Chapter 2 Section 2.1. Otoliths of fish from SA and WA were read by SARDI whilst those from Victoria were interpreted by CAF. Only 2552 otoliths with confidence indices of reading of more than 2, which gave relatively clear and unambiguous readings (Chapter 2 Section 2.2), were used for the production of age-length keys. An age was assigned to each fish based on otolith reading and month captured using the algorithm described in Chapter 2 Section 2.1. The locality, month and year of collection and the number of otoliths used are shown in Table 3.2.

#### Data Analysis

#### Size and age composition

Length frequency data from market measuring were pooled for each site and each month. We assume that sampling is random and thus representative at the levels of fish in each box, boxes from each fisher-day, and fisher-days in each site. For data analysis, the months between October and March were grouped into the summer season whilst those between April and September were grouped into the winter season. The total monthly catch of the garfish commercial fisheries from the relative sites during the corresponding period of market measuring were obtained from the Fisheries Statistics for each state.

For the sea garfish from South Australia, the length frequency distributions were weighted based on the relative contributions to the total catch from each site to each region by season. The weighting factor was calculated as follow:

State	Region	Site	Year/Mo	nth																				
			1997					1998												1999				
-			8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	ţ
South	Gulf St.	Barker Inlet		6	4	3		3																
Australia	Vincent	Middle Beach		26		22	22		17	19	14					20				21				
		Port Wakefield		5	5	5	7	12		14	13	11	10	14	10	13	22	17	23	22	24			
		Quarantine Station																2						
	Kangaroo Island	Kingscote			38					27	28			30										
	Spencer Gulf	Arno Bay		9		25			9	9	21	17	19	7	17	18				11				
		Corny Point		4	9	3	10	3	14	6	6	11			12	11			2		7			
		Port Lincoln		9					7	7	15	13	12	11	13	7								
		PortPirie		14	14	17	17	13		10	17	9	14	14	13	13								
		Tickera		2	37	36	30	28	3	7	5	2	2	1	2	1	51	63	82	88	88			
		Whyalla	15	19	15					18	19			16	8	7								
	WC	Davenport Creek		1																				
		Venus Bay	56	33					25	27	19			29							30			
Victoria	Corner Inlet	Corner Inlet								7	20	14	15	13	12	18	17		18	24		14	13	
	Port Phillip Bay	Port Phillip Bay								26	3	3	5	3	4	5	5						13	
Western	South Coast	Oyster Harbour										20	22							26				
Australia		Peaceful Bay										18												
		Princess Royal Harbou	ır					28																
		Wilson Inlet						11	5	3	7	6		2	3	5	6	5	5	4		29	7	37
	West Coast	Cockburn Sound							10		10	8	10	14							13			
		Eagle Bay																					13	
		Koombana			9		7		1															
		Peel Harvey Inlet							-	29		11												
		Warnbro			3																			

Table 4.2. Information on locality, month and year for and number of otoliths used in age-length key for *Hyporhamphus melanochir*.

$$F_{SA} = C_{ss} / (C_t \ge W_{ss})$$

Where  $C_{ss}$  = catch from the site by weight in a given season;  $C_t$  = total catch by weight from the region; and  $W_{ss}$  = total weight measured at the market for the site in the given season.

For the fish from Victoria, the two sampling sites, Port Phillip Bay and Corner Inlet, were also the two studied regions. The length frequency distributions were weighted based on the relative contributions to the total catch by month for each region. The weighting factor was calculated as follow:

$$F_{VIC} = C_{rm} / (C_t \ge W_{rm})$$

Where  $C_{r m}$  = catch from the region by weight in a given month;  $C_t$  = total catch by weight from the region; and  $W_{r m}$  = total weight measured at the market for the region in the given month.

As the commercial fishery of sea garfish in Western Australia was relatively small compared to the other two states, the sample from targeted sites was patchy in terms of sites and months. Only the sites of Cockburn Sound and Quindalup from the west coast and Wilson Inlet and Oyster Harbour from the south coast provided regular commercial garfish catches during the study period. Therefore, the length frequency distributions were only weighted based on the relative contributions to the total catch from each region by season. The weighting factor was calculated as follow:

$$F_{WA} = C_{rs} / (C_t \ge W_{rs})$$

Where  $C_{rs}$  = catch from the region by weight in a given season;  $C_t$  = total catch by weight from the region; and  $W_{rs}$  = total weight measured at the market for the region in the given season.

The age-length keys were applied to the seasonal and regional size frequency distributions to develop the relative age compositions of the commercial fisheries for each state. The age-length keys are presented in Appendices 4.1 to 4.11.

For South Australia, the size compositions of sea garfish from the present study at GSV and SG were also compared with historical length frequency data, 1954/55 for both GSV and SG (Ling 1958), 1977/78 for SG (Jones 1979), 1986/87 for GSV (Jones *et al.* 1990), and 1994 for

both gulfs by haul net only (Jones 1995). Assuming that there was no significant temporal difference in growth rates of sea garfish, the age-length keys developed in the present study for fish from GSV and SG were applied to the historical data to produce the age structures of the historical catches.

The spatial and temporal differences in the size and age structures were tested by Chi-square  $(^{\circ})$  test using the Proc Freq procedure in SAS (Anon 1989).

## Mortality rates

The annual survival rates were estimated from the catch-curves using the Chapman-Robson method (Chapman and Robson 1960). This method provides the estimates with lower root mean square error and lower bias than most regression estimators (Dunn *et al.* 1999).

The regional catch-curves of the haul net fisheries in SA were used in the mortality estimation as the haul net landings have dominated the state garfish catch for more than 20 years (about 90% of the total catch), and the gear selectivity of the secondary dab net fishery is unknown. In contrast, a combination of gear type was used in the garfish fisheries in WA and Victoria (Chapter 3). This confounds the gear selectivity problem. Consequently, the regional catchcurves of the total catches were used to estimate the mortality rates in WA and Victoria. In addition, historical mortality rates for populations from GSV and SG, SA were also estimated and compared using catch curves by haul net and by all gears.

We assumed that sea garfish were fully recruited to the fisheries at 2 year old, which corresponded to the peak abundant age group on the catch curves for most of the regions. Ages were coded so that the age was equal to 0 at the point of 2-year-old when fish were fully vulnerable to the fishing gear. In addition, catch curve analysis assumes closed population (no immigration and emigration), constant year-class strength and survival rate, as well as equal vulnerability to the gear by different age classes.

The Chapman-Robson estimator for the annual survival rate (s) was calculated as:

$$s = \frac{\sum a_i N_i}{\sum N_i + \sum a_i N_i - 1}$$

Where  $a_i$  is the i<sup>th</sup> coded age (in years),  $N_i$  is the number of fish in the age class  $a_i$ . Also, we assume that there was no ageing error by using the age information with CI > 2.

Instantaneous mortality (Z) =  $-\log_e s$ , where Z = F (fishing mortality) + M (natural mortality).

The variance was calculated from:

$$s\left(s - \frac{\sum a_i N_i - 1}{\sum N_i + \sum a_i N_i - 2}\right)$$

The standard error (SE) is the square root of the variance.

# 4.3. Results

## 4.3.1. Size composition

## South Australia

# Haul net

Sea garfish sampled from the SA commercial haul net fishery ranged from 18 to 38 cm TL (Figure 4.2). The size frequency distribution consisted of a single mode with a mean of 25.4 cm TL. The size range of fish was narrow with 80.7% of fish between 22 and 28 cm. Only 7.6% of the fish were more than 30 cm TL.



Figure 4.2. Size frequency distribution of *Hyporhamphus melanochir* from the SA haul net commercial fishery between February 1998 and January 1999. N is the sample size. Mean is the average size. \*Note: values are not observable due to comparatively small sample sizes at 18 and 38 cm.

The size frequency distributions of the haul net fisheries differed significantly between regions in SA ( $\mathbb{O}^2 = 2480.1$ , p < 0.0001) (Table 4.3). They demonstrated a single mode except for Kangaroo Island (Figure 4.3). Fish caught from WC and KI were considerably larger than those from GSV and SG. However, the WC sample was patchy with data only from April and May. The overall means of the fish from GSV, SG, KI, and WC were 25.6, 25.1, 27.2, and 28.5 cm TL, respectively.



Figure 4.3. Regional size frequency distributions of *Hyporhamphus melanochir* from the SA haul net commercial fisheries between February 1998 and January 1999. N is the sample size. Mean is the average size.

State	Fishing method	Region	Comparison	Ν	Chi-square	P
South Australia	Haul net	Gulf St. Vincent	Summer & winter	2146	176.0	<0.0001
		Spencer Gulf	Summer & winter	4361	249.2	<0.0001
		Kangaroo Island	Summer & winter	485	72.2	<0.0001
		-	GSV, SG, KI & WC	9140	2480.1	<0.0001
	Dab net	Spencer Gulf	Summer & winter	817	148.5	<0.0001
	Both		GSV & SG	7464	71.3	<0.0001
Victoria	All	Port Phillip Bay	Summer & winter	1955	371.6	<0.0001
		Corner Inlet	Summer & winter	1768	81.7	<0.0001
			PPB & CI	3724	230.5	<0.0001
Western Australia	All	South Coast	Summer & winter	1574	49.0	<0.0001
		West Coast	Summer & winter	324	30.4	0.0007
			SC & WC	1896	191.8	<0.0001
Among states	All		SA, VIC & WA	15727	2585.4	<0.0001

Table 4.3. The comparisons of the length frequency distributions between the seasons, regions, and states for *Hyporhamphus melanochir* from the commercial fisheries of SA, Victoria, and WA using Chi-square test.

The length frequency distributions also showed a significant difference between the seasons for the haul net fisheries from each region (Table 4.3) (Figure 4.4). The overall mean sizes of the summer catches from GSV and SG were smaller than those of the winter fisheries; whilst the summer catch from KI contained more large fish with 61.5% ranging between 28 and 31 cm TL. Additionally, our study of the reproductive biology found that fish collected from the commercial fisheries varied greatly in sex ratio between seasons (Chapter 5). About

90% of summer samples were females whilst the sex ratio was more even during the winter. Seasonal variation in sex ratio might explain the size structure difference for the population from KI, but not for fish from both gulfs as evidence showed that females were actually bigger in the mean size than males from all regions in SA (Chapter 5). Therefore, the differences in the size structures of the gulf populations more likely resulted from other reasons such as: a) difference in fisheries operation between seasons, e.g. in GSV, some net fishers were allowed to fish in the water deeper than 5 metres during the winter; b) possible movement of large fish into deeper water during the summer, becoming un-accessible for the fisheries; and c) spawning behaviour, perhaps large mature males being more segregated during the summer (spawning season) and becoming less vulnerable to the haul net fisheries.



Figure 4.4. Seasonal size frequency distributions of *Hyporhamphus melanochir* for each region from the SA haul net commercial fisheries between February 1998 and January 1999. N is the sample size. Mean is the average size.

# Dab net

Sea garfish sampled from the commercial dab net fisheries in SA ranged from 19 to 38 cm with a size distribution of a single mode, having a mean of 26.2 cm TL (Figure 4.5). There were 75.3% of the fish between 22 and 28 cm and 12.3% of the fish more than 30 cm TL. It was obvious that fish from the dab net fishery were bigger than those from the haul net fishery (Figure 4.2). As the dab net fishery was relatively small in SA (10% of the state catch), the market measuring data were patchy with most of the samples from Arno Bay and Port Lincoln along the west coast of SG and some fish from Cape Jervis and Port Vincent in GSV (Figure 4.1); however, as these areas were also the areas where the largest catches by dab netting were made, it is considered that these size frequency data are representative of this part of the fishery.



Figure 4.5. Size frequency distribution of *Hyporhamphus melanochir* from the SA dab net commercial fishery between February 1998 and January 1999. N is the sample size. Mean is the average size. \*Note: the value is not observable due to a comparatively small sample size at 19 cm.

For the summer dab net fishery of the GSV, the size ranged between 20 and 32 cm with 84.6% of the fish between 22 and 28 cm (Figure 4.6). The size frequency distribution showed a single mode with a mean of 25.4 cm TL, which was slightly larger than that (24.8 cm) of the summer haul net fishery in the same gulf.

There was a significant difference in the size compositions between the summer and winter dab net fisheries from SG ( $\mathbb{O}^2 = 148.5$ , p < 0.0001) (Table 4.3). In contrast to the haul net fisheries, the size of the summer dab net catch was considerably bigger than that of the winter catch. The mean lengths of the summer and winter dab net fisheries were 28.7 and 26.1 cm TL, respectively. The dab net fisheries in SA were not subject to the 5-metre fishing restriction as the haul net fisheries. Also anecdotal evidence indicated that dab netters did not necessarily target large schools of garfish but tended to select larger fish (see Chapter 6).



Figure 4.6. Seasonal size frequency distributions of *Hyporhamphus melanochir* from the dab net commercial fisheries in GSV and SG between February 1998 and January 1999. N is the sample size. Mean is the average size.

## Both methods

The size frequency distribution of the sea garfish sampled from the SA commercial fisheries (both method combined) showed a single mode with a mean of 25.5 cm TL (Figure 4.7). Most (80.4%) of the fish ranged between 22 and 28 cm, and only 7.9% were more than 30 cm.



Figure 4.7. The size frequency distribution of *Hyporhamphus melanochir* from the SA commercial fisheries between February 1998 and January 1999. N is the sample size. Mean is the average size. \*Note: values are not observable due to comparatively small sample sizes at 18 and 38 cm.

The size compositions of the commercial fisheries differed significantly between GSV and SG ( $^{\odot}$  = 71.3, p < 0.0001) (Table 4.3) with the average lengths of 25.6 cm and 25.2 cm TL, respectively (Figure 4.8). The mode was 25 cm for fish from the GSV and 24 cm for those from the SG.



Figure 4.8. The size frequency distributions of *Hyporhamphus melanochir* from the GSV and SG commercial fisheries (haul net and dab net combined) in SA between February 1998 and January 1999. N is the sample size. Mean is the average size. \*Note: the values are not observable due to comparatively small sample sizes.

#### Temporal variation in size compositions

Temporal trends in the size compositions of sea garfish from both GSV and SG demonstrate a progressive modal reduction between 1954/55 and 1998 (Figure 4.9). The length frequency distributions differed significantly among the years for each gulf using Chi-square test (Table 4.4).

In GSV, the mean size was 27.8 cm in 1954/55 with 56% of the fish above 28 cm TL. In 1986/87, the average size decreased to 27.2 cm and the percentage of large fish ( $\geq$  28 cm) also declined to 46%. In 1998, the overall mean size further decreased to 25.6 cm with only 21% of the fish more than 28 cm.

Region	Fishing method	Comparison	N	Chi-square	P
Gulf St. Vincent	All	1954/55, 1986/87, and 1998	9346	1078.5	<0.0001
	All	1954/55 and 1998	7039	960.2	<0.0001
	All	1986/87 and 1998	4614	411.5	<0.0001
	All	1954/55 and 1986/87	7039	213.5	<0.0001
	Haul net	1994 and 1998	7189	324.8	<0.0001
Spencer Gulf	All	1954/55, 1977/78, and 1998	68400	7461.4	<0.0001
	All	1954/55 and 1998	11334	2789.9	<0.0001
	All	1977/78 and 1998	62222	152.0	<0.0001
	All	1954/55 and 1977/78	63244	7085.7	<0.0001
	Haul net	1994 and 1998	18703	602.1	<0.0001

Table 4.4. The comparisons of the South Australian size frequency distributions of *Hyporhamphus melanochir* from the historical catches with data from the present study (1998). N is the sample size.

A similar reduction also occurred in the SG and was the most dramatic between 1954/55 and 1977/78, when the mean length declined from 28.5 to 25.3 cm (Figure 4.9). Since 1977/78, the average size has been relatively stable despite the significant statistical difference in size composition between 1977/78 and 1998 ( $^{\circ}$ <sup>2</sup> = 152.0, p < 0.0001) (Table 4.4).

The modal shift of the size compositions in both gulfs is most likely due to that the sea garfish population in 1954/55 was much closer to an unfished population whilst the exploitation rate had increased, especially during the 1960s and 1970s. The state commercial landing rose from 168 tonnes in 1954/55 to 651 tonnes in 1981/82, and since then has remained relatively stable with an average annual catch of 460 tonnes (Chapter 3). Most of the catches (average 88.2% 1983/84-1997/98) were from the GSV and SG. The increase in annual landings was gradual in GSV but more rapid in SG. The catches from GSV increased from 97 tonnes in 1986/87 to 145 tonnes in 1998; whilst the catches from SG were 190 tonnes in 1977/78 and 244 tonnes in 1998.



Figure 4.9. Comparison of the historical size frequency distributions of *Hyporhamphus melanochir* from the Gulf St. Vincent and Spencer Gulf commercial fisheries (all methods combined). N is the sample size. Mean is the average size.

There was also slight reduction in the modes of the size compositions for the commercial haul net fisheries from both the GSV and SG between 1994 and 1998 (Figure 4.10). The mean size decreased from 26.6 to 25.6 in GSV, and it declined from 26.0 to 25.1 cm in SG.



Figure 4.10. Comparison of the size frequency distributions of *Hyporhamphus melanochir* between 1994 and 1998 (present study) from the haul net commercial fisheries in Gulf St. Vincent and the Spencer Gulf of SA. N is the sample size. Mean is the average size.

## Victoria

The sea garfish sampled from the Victorian commercial fisheries ranged from 17 to 47 cm TL with a mean of 25.9 cm TL (Figure 4.11). The size frequency distribution showed two modes at 23 and 27 cm with 68.5% of the fish having a size between 22 and 28 cm. There were 13.4% of the fish larger than 30 cm.



Figure 4.11. The size frequency distribution of *Hyporhamphus melanochir* from the Victorian commercial fisheries between February 1998 and April 1999. N is the sample size. Mean is the average size. \*Note: the values are not observable due to comparatively small sample sizes at 42, 43, and 47 cm.

There was a significant difference in the size composition of the garfish commercial fisheries from PPB and CI in Victoria (Table 5.3) (Figure 4.12). The length frequency distribution of fish sampled from PPB had a single mode with about 50% of the fish ranging between 24 and 28 cm TL. However, the size distribution of fish from CI showed two distinct modes at 23 and 27 cm with a broader size range. There were considerably more large fish ( $\geq$  30 cm TL) from CI (17.7%) than from PPB (8.4%).



Figure 4.12. The size frequency distributions of *Hyporhamphus melanochir* from the commercial fisheries of Port Phillip Bay and Corner Inlet in Victoria between February 1998 and April 1999. N is the sample size. Mean is the average size.

There was also a significant seasonal difference in the length frequency distributions of garfish from both PPB and CI (Table 4.3) (Figure 4.13). The mean sizes of the summer catches were generally smaller than those of the winter landings, particularly in PPB. Fish from the summer catch in PPB had a broader size range with most of the fish approximately evenly distributed between 20 and 29 cm; whilst more than 60% of the winter catch ranged narrowly between 25 and 28 cm TL. In CI, the size composition of the winter fishery showed two distinct modes whilst that of the summer fishery was more complex. Similar to the fisheries from SA, most of the samples were dominated by females during the summer (spawning season) particularly for the population from PPB (see Chapter 5).



Figure 4.13. The seasonal size frequency distributions of *Hyporhamphus melanochir* from the commercial fisheries of Port Phillip Bay and Corner Inlet in Victoria between February 1998 and April 1999. N is the sample size. Mean is the average size.

#### Western Australia

Sea garfish from WA commercial fisheries ranged between 21 and 43 cm TL with a much bigger mean size of 28.8 cm TL and significant difference in length frequency distribution compared to those from SA and Victoria (Table 4.3) (Figure 4.14). In WA, fish larger than 30 cm accounted for 41% of the total catch. The complex size structure is probably due to the combination of gear types used in the commercial fisheries in WA, as well as differences between regions.



Figure 4.14. The size frequency distribution of *Hyporhamphus melanochir* from the WA commercial fisheries between February 1998 and June 1999. N is the sample size. Mean is the average size. \*Note: the values are not observable due to comparatively small sample sizes at 41, 42, and 43 cm.

There was a significant difference in the size distribution for garfish sampled from the SC and WC commercial fisheries in WA (Table 5.3) (Figure 5.15). In general, the fish from the SC were bigger and had a much broader size distribution than those from the WC. The length frequency distribution of the SC fish consisted of a single mode skewed to the left with a mean of 31.5 cm, and 26.2% of the fish were bigger than 35 cm TL. In contrast, the size composition was more complex for the fish from WC with a mean of 28.5 cm, and fish larger than 35 cm accounted for only 1.8%.



Figure 4.15. The size frequency distributions of *Hyporhamphus melanochir* from the commercial fisheries of the south coast and west coast of WA between February 1998 and June 1999. N is the sample size. Mean is the average size.

There were significant differences in the size compositions between the summer and winter catches from either coast of WA (Table 4.3) although the overall mean sizes were similar (Figure 4.16). The difference in the size distributions was more distinct for the fisheries from the WC of WA. The length frequency distribution of the fish from the summer fishery in the WC had a much narrower distribution with 81.7% of the fish ranged between 27 and 31 cm whilst only 51.6% of the fish from the winter catch were between this size range. Similar to the commercial fisheries from SA and Victoria, the sex ratio was biased toward females in the summer but more even in the winter.



Figure 4.16. The seasonal size frequency distributions of *Hyporhamphus melanochir* from the commercial fisheries of the south coast and west coast of WA between February 1998 and June 1999. N is the sample size. Mean is the average size.

## 5.3.2. Age composition

# South Australia

#### Haul net

The age composition of the sea garfish from the SA haul net commercial fishery between February 1998 and January 1999 indicated that a maximum of 7 age-classes occurred in the catches, dominated by 1 and 2 year old fish, which together made up 88.8% of the sampled population (Figure 4.17). There were only 0.2% of the fish aged 5 and 6 year old, which are relatively too small to be observable in Figure 4.17.



South Australia (haul net)

Figure 4.17. Age composition of *Hyporhamphus melanochir* from the SA haul net commercial fishery between February 1998 and January 1999. N is the sample size. \*Note: the values are not observable due to comparatively small sample sizes at 5 and 6 years of age.

The age structures varied significantly among the haul net fisheries from different regions in SA (Table 5.5) (Figure 4.18). A maximum of 6 age-classes occurred in the regional fisheries except for SG, where 7 age-classes were represented. The ages where there are low numbers are not clearly seen in Figure 4.18. Nevertheless, there were less than 5% of the fish from each region ranging between 4 and 6 year old. The modal age of the fish from haul net landings was 2 year old in GSV, KI, and WC whilst 1 year old in SG. The younger fish from SG was probably attributed to the combination effect of the use of the smaller mesh size haul nets and the faster growth rate of fish in this region. There was no indication of stronger recruitment in 1997 for fish from SG than those from other regions.

Table 4.3. The comparisons of the age structures between the seasons, regions, and states for *Hyporhamphus melanochir* from the commercial fisheries of SA, Victoria, and WA using Chi-square test.

State	Fishing method	Region	Comparison	Ν	Chi-square	Р
South Australia	Haul net	Gulf St. Vincent	Summer & winter	2144	111.0	<0.0001
		Spencer Gulf	Summer & winter	4356	217.5	<0.0001
		Kangaroo Island	Summer & winter	485	54.6	<0.0001
			GSV, SG, KI & WC	9142	2045.0	<0.0001
	Dab net	Spencer Gulf	Summer & winter	799	46.4	<0.0001
	Both		GSV & SG	7467	546.8	<0.0001
Victoria	All	Port Phillip Bay	Summer & winter	1957	47.9	<0.0001
		Corner Inlet	Summer & winter	1722	165.4	<0.0001
			PPB & CI	3723	191.3	<0.0001
Western Australia	All	South Coast	Summer & winter	1575	99.7	<0.0001
		West Coast	Summer & winter	293	32.3	0.0007
			SC & WC	1868	16.9	0.005
Among states	All		SA, VIC & WA	15695	1614.7	<0.0001



Figure 4.18. Regional age compositions of *Hyporhamphus melanochir* from the SA haul net commercial fisheries between February 1998 and January 1999. N is the sample size. \*Note: the relatively low numbers are not clearly seen.

The age structures also varied significantly between the seasons for each region (Table 4.5) (Figure 4.19). In GSV, fish  $\geq 2$  year old accounted for 75.6% of the winter catch, but only 56.5% of the summer fishery. Fish of 0 and 1 year classes consisted of 24.4% and 43.5% of all fish in the winter and summer, respectively. The difference in age structures was likely due to the fact that a number of fishers had exemptions to net in deeper water (>5m) in northern GSV during winter. In SG, despite the statistical difference (Table 4.5), the age structures were similar between the seasons except that there was no fish less than 1 year old in the winter fishery. In KI, the summer catch had the most abundant 2 and 3 year old fish whilst the winter catch was only dominated by 2 year old ones. The lack of summer samples from the WC precludes a seasonal comparison in age structures. The winter haul net fishery was dominated by 2 year old fish in the WC.



Figure 4.19. Seasonal age compositions of *Hyporhamphus melanochir* for each region from the SA haul net commercial fisheries between February 1998 and January 1999. N is the sample size. \*Note: the relatively low numbers are not clearly seen.

# Dab net

Sea garfish sampled from the SA dab net commercial fishery between February 1998 and January 1999 consisted of 6 age-classes, with the 2 and 3 year old fish the most abundant (85.2% of all fish). All samples were from GSV and SG.



Figure 4.20. Age composition of *Hyporhamphus melanochir* from the SA dab net commercial fishery between February 1998 and January 1999. N is the sample size. \*Note: the value is not observable due to a comparatively small sample size at 5 years of age.

Fish from the summer dab net fishery in GSV only included a maximum of 5 age-classes (0 to 4 year old), dominated by 1 and 2 year old fish, which made up 88.7% of the catch (Figure 4.21). The age structure was similar to that of the summer haul net landings (Figure 4.19). There were no winter dab net samples from GSV in the present study. In the SG, the age structures of the dab net fisheries differed significantly ( $^{\circ}$ <sup>2</sup> = 46.4, p < 0.0001) between the seasons with a 2 year old modal age in the summer but 1 year old in the winter (Table 4.5). The SG age compositions were similar for both methods of capture in the winter, but significantly different in the summer with dab net catches being about 1 year older.



Figure 4.21. Seasonal age compositions of *Hyporhamphus melanochir* from the dab net commercial fisheries in GSV and SG between February 1998 and January 1999. \*N is the sample size. Note: the relatively low numbers are not clearly seen.

## Both methods

Combining both haul net and dab net samples, the age composition of the SA fisheries showed a maximum of 7 age-classes with 1 and 2 year old fish the most abundant (88.5% of all fish) (Figure 5.22). Fish  $\geq$  4 year old accounted for only 1% of the total catch.



Figure 4.22. The age composition of *Hyporhamphus melanochir* from the SA commercial fisheries between February 1998 and January 1999. N is the sample size. \*Note: the relatively low numbers are not clearly seen at 5 and 6 years of age.

The age structures of the commercial fisheries from GSV and SG (both methods combined) indicated that the modal age was one year younger in SG ( $^{\odot}$ <sup>2</sup> = 546.8, p < 0.0001) (Table 4.5). The catches were dominated by 1 year old fish in the SG and the 2 year old class in the GSV.


Figure 4.23. The age compositions of *Hyporhamphus melanochir* from the GSV and SG commercial fisheries in SA between February 1998 and January 1999. N is the sample size. \*Note: the relatively low numbers are not clearly seen at 4 and 5 years of age for GSV, and at 5 and 6 years of age for SG.

## Temporal variation in age compositions

The age structures of the commercial catches (methods combined) from GSV and SG were compared with historical data (Figure 5.24), indicating significant temporal variations except for the comparison between the samples in 1977/78 and 1998 (present study) from SG (Table 4.6).

In the GSV, although the commercial catches continued to be dominated by 2 year old classes, the proportion of 3 year old was reduced significantly from 1954/55 to 1998 with the increasing numbers of younger fish (1 year old) caught.

In the SG, there was a substantial change in the age structures between 1954/55 and 1977/78 ( $\mathbb{C}^2 = 4392.3$ , p < 0.0001) (Table 4.6). In the former year fish were dominated by 1 to 3 age classes with a modal age of 2 year old; whilst in the later year the most abundant age class was the 1 year old group. The age structure remained stable between 1977/78 and 1998 ( $\mathbb{C}^2 = 4.7$ , p = 0.5773) (Table 4.6).

As discussed in the size compositions, the temporal shift of the age structures over years was likely due to the fact that the population fished in 1954/55 was less heavily exploited and fishing mortality had increased during the following two decades, especially in the SG.



Figure 4.24. Comparison of the historical age compositions of *Hyporhamphus melanochir* from the Gulf St. Vincent and Spencer Gulf commercial fisheries (all methods combined). N is the sample size. \*Note: the relatively low numbers are not clearly seen.

Region	Fishing method	Comparison	N	Chi-square	P
Gulf St. Vincent	All	1954/55, 1986/87, and 1998	9351	287.1	<0.0001
	All	1954/55 and 1998	7042	266.7	<0.0001
	All	1986/87 and 1998	4619	113.2	<0.0001
	All	1954/55 and 1986/87	7041	43.8	<0.0001
	Haul net	1994 and 1998	7189	85.2	<0.0001
Spencer Gulf	All	1954/55, 1977/78, and 1998	68403	4511.9	<0.0001
	All	1954/55 and 1998	11336	1717.0	<0.0001
	All	1977/78 and 1998	62224	4.7	0.5773
	All	1954/55 and 1977/78	63246	4392.3	<0.0001
	Haul net	1994 and 1998	18705	177.5	<0.0001

Table 4.6. The comparisons of the age compositions of *Hyporhamphus melanochir* from the historical catches with data from the present study (1998). N is the sample size.

The age structures of the fish from haul net fisheries in 1994 in both GSV and SG were also compared with the haul net data from the present study (Figure 4.25). The age compositions were comparable between the years despite the statistical differences (Table 4.6).



Figure 4.25. Comparison of the age compositions of *Hyporhamphus melanochir* between 1994 and 1998 (present study) from the haul net commercial fisheries in Gulf St. Vincent and the Spencer Gulf of SA. N is the sample size. \*Note: the relatively low numbers are not clearly seen.

## Victoria

Garfish samples from the Victorian commercial fisheries between February 1998 and April 1999 consisted of 7 age-classes with 1 and 2 year old fish the most abundant (91.2% of all fish) (Figure 4.26). Fish  $\geq$  4 year old only accounted for 3.6% of the state total catch.



Figure 4.26. The age composition of *Hyporhamphus melanochir* from the Victorian commercial fisheries between February 1998 and April 1999. N is the sample size. \*Note: the relatively low number is not clearly seen.

There was a significant difference between the age structures of fish from Corner Inlet and Port Phillip Bay ( $\mathbb{O}^2 = 191.3$ , p < 0.0001) (Table 5.5) (Figure 4.27). Catches from both regions were dominated by 1 and 2 year age classes, but in CI the modal age was 1 year old whereas it was 2 year old in the PPB. There were considerably more older fish ( $\geq 3$  year old) from CI (12.8%) than from PPB (4.0%). The relatively higher proportion of 6 year olds in the sample from CI, representing the 1992 year-class, might indicate that strong recruitment occurred in that year. However, it was more likely due to the sampling variation as the sample size for the larger fish was very limited. Additionally, there have been temporal changes in gear types with different mesh selection characteristics used in the garfish fisheries in Victoria, which might account for these older fish occurring in the catches (Chapter 3).



Figure 4.27. The age compositions of *Hyporhamphus melanochir* from the commercial fisheries of Port Phillip Bay and Corner Inlet in Victoria between February 1998 and April 1999. N is the sample size. \*Note: the relatively low numbers are not clearly seen.



Figure 4.28. The seasonal age compositions of *Hyporhamphus melanochir* from the commercial fisheries of Port Phillip Bay and Corner Inlet in Victoria between February 1998 and April 1999. N is the sample size. \*Note: the relatively low numbers are not clearly seen.

There was a significant seasonal difference in the age compositions for fish from each region (Table 4.5) (Figure 4.28). In CI, the dominant age was 1 year old in the summer whist 2 year old in the winter. In PPB, both the summer and winter age structures peaked at 2 year old, but there was a higher proportion of 1 year old fish in the summer catch (42.4%) than that in the winter (27.9%).

### Western Australia

The age structure of the fish sampled from the WA commercial fisheries between February 1998 and June 1999 was the most complex among the three states ( $@^2 = 1614.7$ , p < 0.0001) (Table 4.5). Numerous age classes from 0 to 10 years were represented with 1, 2, and 3 year old fish the most abundant (87.1% of all fish). Fish  $\ge$  5 years of age only accounted for 2.6% of the state total catch.



Figure 4.29. The age composition of *Hyporhamphus melanochir* from the WA commercial fisheries between February 1998 and June 1999. N is the sample size. \*Note: the relatively low numbers are not clearly seen.

There was a significant spatial difference in the age structures of sea garfish from the south coast and west coast of WA ( $^{\odot}^2 = 16.9$ , p = 0.005) (Table 4.5) (Figure 4.30). At the former locality, fish ranged from 1 to 10 years with 2.6% of the fish  $\geq 6$  year old. At the latter locality, the age range was only between 0 and 5 years. However, age classes of 1 to 3 years dominated the fisheries from both SC (87.6%) and WC (87.1%).



Figure 4.30. The age compositions of *Hyporhamphus melanochir* from the commercial fisheries of the south coast and west coast of WA between February 1998 and June 1999. N is the sample size. \*Note: the relatively low numbers are not clearly seen.

There was a significant seasonal difference between the age compositions for sea garfish from the south coast and the west coast waters of WA (Table 4.5) (Figure 4.31). In the SC waters, there was a broader age range in the summer (1-10 years) than in the winter (1-6 years) ( $^{\odot}^2$  = 99.7, p < 0.0001) (Table 4.5). In the WC waters, the 2 year old fish clearly dominated the summer catch whilst the age composition was evenly distributed among 1, 2, and 3 year age classes, which together made up 85.5% of the winter fishery.



Figure 4.31. The seasonal age compositions of *Hyporhamphus melanochir* from the commercial fisheries of the south coast and west coast of WA between February 1998 and June 1999. N is the sample size. \*Note: the relatively low number is not clearly seen.

### **4.3.3.** Mortality rates

The estimates of the annual survivorship and instantaneous mortality rates are presented in Table 5.7. These estimators provide, as a first approximation, benchmark mortality estimates from the catch curves of a single season (1998/99) for *Hyporhamphus melanochir* throughout the southern Australia waters. The overall means of the annual survival rates were 15.7%, 21.3%, and 37.7% for fish populations from SA, Victoria, and WA, respectively. The lowest survival rate for the SA population was attributed to the highest fishing mortality of all three states.

State	Region	S	SE of s	Z	N
South Australia	Gulf St. Vincent	0.117	0.008	2.15	1399
	Kangaroo Island	0.264	0.019	1.33	403
	Spencer Gulf	0.170	0.009	1.77	1543
	West Coast	0.203	0.008	1.60	1897
	State overall	0.157	0.005	1.85	4434
Victoria	Corner Inlet	0.312	0.012	1.16	959
	Port Phillip Bay	0.082	0.007	2.50	1247
	State overall	0.213	0.008	1.55	2184
Western Australia	South Coast	0.408	0.011	0.90	1297
	West Coast	0.374	0.026	0.98	220
	State overall	0.377	0.010	0.98	1413

Table 4.7. Estimates of the annual survivorship (s) with standard error (SE) and the instantaneous mortality rates (Z) for *Hyporhamphus melanochir* from different regions of SA, Victoria, and WA. N is the sample size.

In SA, the survival rates of garfish were the lowest in GSV and SG for fish of 2 years and older, which could be explained by the highest exploitation rates of the commercial fisheries in these two gulfs. The survival rates were 11.7% and 17.0%, which corresponded to the instantaneous mortality rates of 2.15 and 1.77, for populations from GSV and SG, respectively. The fisheries from KI and WC were relatively small, thus the survival rates were higher in these two localities with the estimates of 26.4% and 20.3%, respectively. The catch curves for SA populations from each fishery region are shown in Figure 4.32.

The historical survivorship and mortality rates of sea garfish were also estimated for the populations from GSV and SG, SA (Table 4.8). There was a gradual decline in the survival rate from 25.2% in 1954/55 to 19.9% in 1986/87, and then to 11.6% in 1998 for the GSV population. Such a decline occurred more rapidly for the SG population especially between 1954/55 and 1977/78, which reflected the dramatic increase in fishing mortality during this period. The catch curves for the historical data are shown in Figure 4.33.

Table 4.8. Estimates of the annual survivorship (s) with standard error (SE) and the instantaneous mortality rates (Z) for the historical populations of *Hyporhamphus melanochir* from Gulf St. Vincent and Spencer Gulf, South Australia. N is the sample size.

Region	Method	Year	S	SE of s	Z	Ν
Gulf St. Vincent	All	1954/55	0.252	0.006	1.38	3587
	All	1986/87	0.199	0.009	1.61	1717
	All	1998	0.116	0.008	2.15	1498
	Haul	1994	0.167	0.006	1.79	3724
	Haul	1998	0.117	0.008	2.15	1399
Spencer Gulf	All	1954/55	0.342	0.006	1.07	4475
	All	1977/78	0.193	0.002	1.64	21418
	All	1998	0.180	0.008	1.71	1904
	Haul	1994	0.209	0.004	1.57	6586
	Haul	1998	0.170	0.009	1.77	1543



Figure 4.32. The catch curves for the sea garfish populations from GSV, SG, KI, and WC of South Australia.



Figure 4.33. The historical catch curves for the sea garfish populations from CSV and SG, South Australia.

In Victoria, fish from PPB had a much higher mortality rate compared to those from CI. The estimates of survival rates were 31.2% and 8.2% in CI and PPB, respectively. Before 1995/96, the annual commercial landings of sea garfish had been the highest from PPB. However, the catch dropped about 50% in this Bay during the last 5 years, and landings from CI have become dominant in Victoria. Therefore the difference in survival rates was more likely due to the variable gear selectivity in the Bay and the Inlet. Over the last two year, ring nets have mostly replaced gar seines in CI whist the later method still produced most of the catch in PPB. It is possible that large fish  $\geq$  3 year old) were under-represented in the samples from the commercial fisheries in the PPB. The catch curves for populations from CI and PPB are presented in Figure 4.34.



Figure 4.34. The catch curves for the sea garfish populations from Corner Inlet and Port Phillip Bay, Victoria.

In WA, the survival rates of garfish were similar for fish from the south coast and the west coast with the estimates of 40.8% and 37.4%, respectively. The highest overall survival rate of 37.7% corresponded to the lowest exploitation rate of the commercial fisheries of sea garfish in WA compared to those in SA and Victoria. The catch curves for populations from the SC and WC are shown in Figure 4.35.



Figure 4.35. The catch curves for the sea garfish populations from the south and west coast of Western Australia.

# 5.4. Discussion

## Size and Age Structures

Sea garfish are closely associated with the sea grass beds throughout the southern Australian coastal waters. There were significant spatial and temporal differences in the size and age structures of the fish from the commercial fisheries in SA, Victoria, and WA. In general, the overall mean size of the fish from WA was about 3 cm bigger than those from the other two states, and the catches were dominated by 3 age classes (1, 2, and 3 year old) with a relatively more complex age compositions ranging from 0 to 10 years. In contrast, the catches from SA and Victoria consisted of 7 age-classes (0 to 6 years) with 1 and 2 year old fish dominating the catches.

Compared to the commercial fishery from Tasmania, the age structures of *Hyporhamphus melanochir* from the three mainland states were much younger with smaller mean sizes (except for the SC of WA). Samples from the commercial dab net fishery in eastern Tasmanian waters were dominated by 4 and 5 year old fish with a maximum of 9 age classes represented (Jordan *et al.* 1998). The spatial variation in size and age structures was at least partially attributed to the gear selectivity difference between the states, as evidence indicated

that dab netting tended to select larger fish than haul netting did in the SA fisheries. However, it was also possible that larger fish remained in deeper water outside the depth range of the fisheries in WA, SA and Victoria.

In SA, the commercial fisheries had undergone significant decline in the mean size and age between 1954/55 and 1998 in the GSV and SG (Figures 4.9 and 4.24). The present age composition was also much younger compared to that of a lightly fished population in Baird Bay (SA), which was dominated by 4 year old fish for both males and females with a broader age range of 0 to 10 years (Jones 1990). The temporal changes in the size and age structures were consistent with the effects expected to result from increase exploitation. There was an extensive literature on general responses of fish populations to exploitation, including those for populations from relatively shallow-water marine environments (e.g. Hempel 1978; Pauly 1979; Grosslein *et al.* 1980). The responses include the change in age structure and/or size structure with fewer old, large fish and the population dominated by new recruits; lower age at maturity and/or size at maturity; and increasing growth rate of individuals. Such responses are often observed in short-lived, fast-growing species (e.g. Pauly 1979; Grosslein *et al.* 1980), such as the sea garfish.

The age structures suggested that fish from SG started to recruit at a younger age (about 1 year) than those from GSV, KI, and WC of SA (Figure 4.18). Anecdotal evidence suggested that relatively small mesh haul nets were often used in the commercial fisheries along the northeast coast of SG (Tickera and Port Pirie). Consequently the age structure difference between the two gulfs was due more to variable gear selectivity. There was no indication of inter-annual variation in the recruitment strength in SG, as age data from TK (SG) demonstrated similar age structures between the two seasons (1997/98 and 1998/99) with 1 year old fish dominant (Figure 4.36).



Figure 4.36. Comparison of the age compositions of *Hyporhamphus melanochir* from Tickera, SA between two seasons (October-April 1997/98 and Oct-April 1998/99). N is the sample size.

In Victoria, different types of haul nets have been used in the multi-species net fisheries, including gar seines, beach seines, estuary seines ad ring nets (Knuckey *et al.* 2000). The length and mesh size of the nets differed between Bays and Inlets, and the mesh size has recently been modified (change to larger meshes) to increasingly target King George whiting (Chapter 3). Over the last two years, traditional gar seines still landed about 60% of the total catch in PPB whilst these methods has been mostly replaced by ring nets in the CI. Due to the variable gear selectivity, the size structures were relatively complex and differed between CI and PPB in Victoria.

In WA, the commercial fisheries of sea garfish were relatively small compared to the other states with most of the catches (about 85%) coming from the WC (Cockburn Sound and Quindalup). A variety of fish gear types have been recorded against this species with the main methods being beach seines, haul nets, gill nets and ring nets. The lawful mesh size of gill nets used along the SC appeared to be 16 mm larger than those operated along the WC waters. The present study found that the mean size of the fish from the SC was 3 cm bigger than those from the WC waters. Although fish from the WC of WA only included the beach seine and haul net samples, more than 50% of the SC samples (by number) were caught by gill nets. Despite the fact that the maximum age of fish caught from the SC was much older (10 years) than those from the WC (5 years), there was less than 5% of fish  $\geq 5$  year old along either coast of WA.

For our market survey, the comparison between the proportion of fish measured in each season for each region, the total weight of fish encountered at the market and the commercial catch data suggests a relatively representative sampling program in SA (Figure 4.37). Although the size and age structures were not differentiated by sex based on the market measuring survey, our study on the reproductive biology (Chapter 5) suggests that most of the commercial catches during the summer were females whilst sex ratio was more even during the winter. This applied to the fisheries throughout WA, SA, and Victoria.



Figure 4.37. The proportion of fish measured, the total weight of fish encountered at the market survey and the seasonal commercial catch of sea garfish for the Gulf St. Vincent (GSV), northern Spencer Gulf (NSG), southern Spencer Gulf (SSG), Kangaroo Island (KI), and the west coast (WC) of South Australia between February 1998 and January 1999. Spring (September-November), Summer (December-February), Autumn (March-May), Winter (June-August).

### Mortality Rates

The total mortality rates of the southern sea garfish were high in SA, Victoria and WA with the low annual survival rates of 15.7%, 21.3%, and 37.7%, respectively. Based on the study of a lightly fished population in the Baird Bay, SA, Jones (1990) provides an estimate of the natural mortality (M) of 0.55 (a survival rate of 48.2%) for both sexes combined, which allows approximate estimates of the fishing mortality (F) for each state. The F values were therefore 1.30, 1.00, and 0.43, and the relative actual fishing mortality rates ( $\mu$ ) were 59.3%, 50.7%, and 27.2% in SA, Victoria, and WA respectively. Fisheries statistics indicated that the commercial landings of sea garfish have alway been the highest in SA with approximately 60% of the national catch derived from SA waters (see Chapter 3).

In SA, the total mortality estimates increased overall between 1954/55 and 1998 at similar levels for the populations from GSV (56%) and SG (60%). However, for GSV, most of the increase occurred during the last 10 years (by 34%); whereas for SG, it mainly occurred (53%) between 1955 and 1977. In the SG, the commercial fisheries of sea garfish has remained stable since 1983/84 whilst in the GSV, the annual catch increased from 97 tonnes in 1986/87 to 158 tonnes in 1998/99 (about 63%) (Figure 4.38). Therefore the increase of Z of garfish in GSV was at least partially due to the increase in commercial exploitation, although it might also be attributed to the environmental changes which have affected the distribution of older age groups of garfish - away from the commercially fished areas.or the increase in recreational catch over this time. McGlennon and Kinloch (1997) suggested recreational garfish harvest of 42.4 tonnes for GSV (April 1994-March 1995) and 18.3 tonnes for SG (April 1995-March 1996), which consisted of 20% and 8% of the total garfish catch in GSV and SG, respectively.



Figure 4.38. The annual commercial catches of sea garfish from the Spencer Gulf and Gulf St. Vincent of South Australia.

There are only limited mortality estimates for similar species available to be compared with those reported here. Berkely and Houde (1978) suggested an annual survival rate of 14% for a heavily fished species *Hemirhamphus braziliensis* from 1 to 2 years of age; Hughes (1974) estimated survival rates for the lightly fished Pacific saury (*Cololabis saira*) from 28.7% to 10.5%; Kasim *et al.* (1996) estimated the total mortality rates for a highly exploited full beak species *Ablennes hians* between 3.23 and 3.87, which were converted to annual survival rates of 4.0-2.1%, and a natural mortality rate of 0.81, which was equivalent to a survival rate of 44.5%. Low annual survival rates appear to be typical for fish of the Order Beloniformes as they are important prey for a number of predators such as pelagic teleosts and seabirds (Ayling and Cox 1982).

Based on the age structures of the commercial fisheries, the present study provided the first estimates of mortality rates for garfish populations from SA, Victoria, and WA. Using the catch curves, we assumed that the age of fully recruited fish was consistent at 2 year old in each state. This generally agreed with the mesh selectivity study on haul nets in SA waters (Jones 1982), although fish from SG were more likely to be selected at a younger age in the fisheries due to the use of smaller mesh haul nets along the northeast coast of the gulf. An FRDC funded project (No. 1999/145) to develop a stock assessment indicator model for this species has commenced at SARDI, in which mortality, yearly recruitment, population numbers, and biomass will be further estimated for the SA populations. This likelihood estimation model is dynamic with semi-yearly time step, is length and age based, and integrates estimation of growth and mortality. The garfish fishery feature identified in the current project of sex and gear selectivity by season, reproductive seasonality, and all random stratified survey length and subsampled age data, are inputs to the FRDC 1999/145 model. Further knowledge will be required on gear selectivity for sea garfish from Victoria and WA to assess the size and age compositions of the entire populations.

# 4.5. References

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Age	Gulf St.	Vincen	t										GSV
N	Summe	r				-	Winter					-	Total
Size class (mm)	0	1	2	3	4	Total	0	1	2	3	5	Total	
170-179	1	1				2	3					3	5
180-189	2					2							2
190-199	2	1				3	1					1	4
200-209	5	6	1			12		6				6	18
210-219	2	10	2			14		3	2			5	19
220-229	4	11	4			19		9	4			13	32
230-239	1	15	12			28		6	1			7	35
240-249	2	12	13			27		5	9			14	41
250-259		13	21			34		2	14			16	50
260-269		4	24	1		29		7	16	1		24	53
270-279		9	21	3		33		1	11	2		14	47
280-289		3	13	3		19		2	9	3		14	33
290-299		1	6	5		12			10	2		12	24
300-309		2	2	1	1	6			3	1		4	10
310-319			3	2		5			2			2	7
320-329		1	3	3		7				2		2	9
330-339			1	2		3				2		2	5
340-349			2	3		5			1	1		2	7
350-359			1	1	1	3							3
360-369				1		1							1
370-379													
380-389													
390-399													
400-409													
410-419													
420-429											1	1	1
430-439													
Grand Total	19	89	129	25	2	264	4	41	82	14	1	142	406

Appendix 4.1. The seasonal age-length keys for *Hyporhamphus melanochir* from Gulf St. Vincent, South Australia.

Age	Kangaro	o Island	d									кі
N	Summer					-	Winter				Total	Total
Size class (mm)	1	2	3	4	5	Total	1	2	3	4		
170-179												
180-189												
190-199												
200-209												
210-219	1					1	2	1			3	4
220-229	2					2	5				5	7
230-239	2					2	4				4	6
240-249	3	3				6	2	2			4	10
250-259	3	8				11	4	5			9	20
260-269	3	6				9		6			6	15
270-279		9				9		7			7	16
280-289		4	2			6		12	1	1	14	20
290-299		6	3			9		2	2		4	13
300-309		1	2			3		1			1	4
310-319			2		1	3						3
320-329			1			1			1		1	2
330-339				3		3						3
340-349												
350-359												
360-369												
370-379												
380-389												
390-399												
400-409												
410-419												
420-429												
430-439												
Grand Total	14	37	10	3	1	65	17	36	4	1	58	123

Appendix 4.2. The seasonal age-length keys for *Hyporhamphus melanochir* from Kangaroo Island, South Australia.

Age	Spence	r Gulf												
N	Summe	r					-	Winter						
Size class (mm)	0	1	2	3	4	5	Total	1	2	3	4	5	6	Total
170-179														
180-189														
190-199														
200-209	3	7					10	4						4
210-219	6	23					29	16						16
220-229	8	53					61	33						33
230-239	8	55	2				65	38						38
240-249	8	71	16				95	40	7					47
250-259		64	34				98	37	13					50
260-269		44	40				84	35	26	2				63
270-279		35	46	4			85	14	31	3				48
280-289		22	39	4			65	2	29	7				38
290-299		3	35	9			47	1	14	11	1			27
300-309		1	26	12	1		40		6	7	1			14
310-319			17	10			27		5	3				8
320-329			3	9	2		14		1	6	2			ę
330-339				6			6			6	1			7
340-349				4	1		5			3	5			8
350-359					1		1			3	5	1		ç
360-369				1	4	1	6				2		2	4
370-379													1	
380-389														
390-399											1			
400-409					1		1							
410-419														
420-429														
430-439														
Grand Total	33	378	258	59	10	1	739	220	132	51	18	1	3	42

Appendix 4.3. The seasonal age-length keys for *Hyporhamphus melanochir* from Spencer Gulf, South Australia.

Age	SG	West Co	ast											wc
N	Total	Summer					Winter							Total
Size class (mm)		1	2	3	4	Total	0	1	2	3	4	5	Total	
170-179							1						1	1
180-189														
190-199														
200-209	14													
210-219	45													
220-229	94													
230-239	103		2			2		1	1				2	4
240-249	142		4			4		2	2				4	8
250-259	148		20			20		3	11				14	34
260-269	147	1	16			17		5	13				18	35
270-279	133	1	14	1		16		4	12				16	32
280-289	103		11			11		2	20				22	33
290-299	74		2	1		3		1	13	2			16	19
300-309	54		1	1		2			7	5			12	14
310-319	35			1		1			6	4	1		11	12
320-329	23			4		4			1	6			7	11
330-339	13			1		1				4	1	1	6	7
340-349	13				1	1				3	1		4	5
350-359	10									1	2		3	
360-369	10										1	1	2	2
370-379	1													
380-389														
390-399	1													
400-409	1													
410-419														
420-429														
430-439														
Grand Total	1164	2	70	9	1	82	1	18	86	25	6	2	138	220

Appendix 4.4. The seasonal age-length keys for *Hyporhamphus melanochir* from the west coast of South Australia.

Age/No.	Gulf St.	. Vince	nt				GSV	Kangaro	oo Islan	d			KI
Size class (mm)	0	1	2	3	4	5	Total	1	2	3	4	5	Total
170-179	4	1					5						
180-189	2						2						
190-199	3	1					4						
200-209	5	12	1				18						
210-219	2	13	4				19	3	1				4
220-229	4	20	8				32	7					7
230-239	1	21	13				35	6					6
240-249	2	17	22				41	5	5				10
250-259		15	35				50	7	13				20
260-269		11	40	2			53	3	12				15
270-279		10	32	5			47		16				16
280-289		5	22	6			33		16	3	1		20
290-299		1	16	7			24		8	5			13
300-309		2	5	2	1		10		2	2			4
310-319			5	2			7			2		1	3
320-329		1	3	5			9			2			2
330-339			1	4			5				3		3
340-349			3	4			7						
350-359			1	1	1		3						
360-369				1			1						
370-379													
380-389													
390-399													
400-409													
410-419													
420-429						1	1						
430-439													
Grand Total	23	130	211	39	2	1	406	31	73	14	4	1	123

Appendix 4.5. The age-length keys for *Hyporhamphus melanochir* from Gulf St. Vincent Spencer Gulf, Kangaroo Island and the west coast of South Australia.

# Appendix 4.5. Continued.

Age/No.	Spence	r Gulf						SG	West C	oast					WC	SA
Size class (mm)	0	1	2	3	4	5	6	Total	0	1	2	3	4	5	Total	Total
170-179									1						1	6
180-189																2
190-199																4
200-209	3	11						14								32
210-219	6	39						45								68
220-229	8	86						94								133
230-239	8	93	2					103		1	3				4	148
240-249	8	111	23					142		2	6				8	201
250-259		101	47					148		3	31				34	252
260-269		79	66	2				147		6	29				35	250
270-279		49	77	7				133		5	26	1			32	228
280-289		24	68	11				103		2	31				33	189
290-299		4	49	20	1			74		1	15	3			19	130
300-309		1	32	19	2			54			8	6			14	82
310-319			22	13				35			6	5	1		12	57
320-329			4	15	4			23			1	10			11	45
330-339				12	1			13				5	1	1	7	28
340-349				7	6			13				3	2		5	25
350-359				3	6	1		10				1	2		3	16
360-369				1	6	1	2	10					1	1	2	13
370-379							1	1								1
380-389																
390-399					1			1								1
400-409					1			1								1
410-419																
420-429																1
430-439																
Grand Total	33	598	390	110	28	2	3	1164	1	20	156	34	7	2	220	1913

Age	Corner I	nlet					-	-						-	СІ
N	Summe	r						Winter							Total
Size class (mm)	1	2	3	4	5	6	Total	0	1	2	3	4	6	Total	
170-179															
180-189															
190-199															
200-209									3					3	3
210-219	3						3		6					6	9
220-229	6						6		9					9	15
230-239	6						6		10					10	16
240-249	6						6		6	2				8	14
250-259	7	1					8		3	2				5	13
260-269	5	3					8		2	5				7	15
270-279	2	7					9			9				9	18
280-289		7					7			11				11	18
290-299		6					6			11	2			13	19
300-309		2	4				6			9	1			10	16
310-319			8				8			3				3	11
320-329			2				2			2	1	1		4	6
330-339		1		1			2				1	1		2	4
340-349					1		1					2		2	3
350-359					1		1					1		1	2
360-369						1	1								1
370-379												1	1	2	2
380-389															
390-399															
400-409															
410-419															
420-429															
430-439															
Grand Total	35	27	14	1	2	1	80	3	39	54	5	6	1	105	185

Appendix 4.6. The seasonal age-length keys for *Hyporhamphus melanochir* from Corner Inlet, Victoria.

Age	Port Phil	lip Bay									PPB
N	Summer					Winter					Total
Size class (mm)	1	2	3	5	Total	1	2	3	4	Total	
170-179											
180-189											
190-199											
200-209						2				2	2
210-219	1				1	1				1	2
220-229						2				2	2
230-239	1				1	1				1	2
240-249	2	4			6	3	2			5	11
250-259	1	4			5	2	2			4	9
260-269		5			5		2			2	7
270-279		6			6		1			1	7
280-289		2			2		6	2		8	10
290-299		2			2		1			1	3
300-309		1			1		2			2	3
310-319							3			3	3
320-329			1		1		1			1	2
330-339								1		1	1
340-349									1	1	1
350-359									1	1	1
360-369											
370-379				1	1						1
380-389											
390-399											
400-409											
410-419											
420-429											
430-439											
Grand Total	5	24	1	1	31	11	20	3	2	36	67

Appendix 4.7. The seasonal age-length keys for *Hyporhamphus melanochir* from Port Phillip Bay, Victoria.

Age/No.	Corne	r Inlet	t					CI	Port Pl	nillip B	Bay			PPB		Victoria
Size class (mm)	0	1	2	3	4	5	6	Total	1	2	3	4	5	Total		Total
170-179																
180-189																
190-199																0
200-209		3						3	2						2	5
210-219		9						9	2						2	11
220-229		15						15	2						2	
230-239		16						16	2						2	18
240-249		12	2					14	5	6					11	25
250-259		10	3					13	3	6					9	22
260-269		7	8					15		7					7	22
270-279		2	16					18		7					7	25
280-289			18					18		8	2				10	28
290-299			17	2				19		3					3	22
300-309			11	5				16		3					3	
310-319			3	8				11		3					3	14
320-329			2	3	1			6		1	1				2	8
330-339			1	1	2			4			1				1	5
340-349					2	1		3				1			1	4
350-359					1	1		2				1			1	3
360-369							1	1								1
370-379					1		1	2					1		1	3
380-389																
390-399																
400-409																
410-419																
420-429																
430-439																
Grand Total	3	74	81	19	7	2	2	185	16	44	4	2	1		67	252

Appendix 4.8. The age-length keys for *Hyporhamphus melanochir* from Corner Inlet and Port Phillip Bay in Victoria.

Age	South Co	oast																
N	Summer	-								Winter T								
Size class (mm)	1	2	3	4	5	6	7	10	Total		1	2	3	4	5	6	Total	
170-179	1								1									1
180-189																		
190-199	1								1									1
200-209	1								1									1
210-219	4								4									4
220-229	4	3							7									7
230-239	1								1		1						1	2
240-249	3	10	1						14		3	1					4	18
250-259	6	4	1						11		6						6	17
260-269	8	1							9		5	1					6	15
270-279	3	1	1						5		5	1					6	11
280-289	2	5	1			1			9		3	2	1				6	15
290-299	1	5							6			2	1				3	9
300-309		5	2						7			4	1	1			6	13
310-319		2	1						3		1	12	1				14	17
320-329		6	4	1					11		1	4	2				7	18
330-339		2	3	2					7			11	5	2			18	25
340-349			2	2	1	1			6			6	4	1			11	17
350-359			3	1	1				5			12	4	1			17	22
360-369			1	2					3			4	2	2			8	11
370-379			2						2			2	4		1		7	9
380-389			1	2		1			4				2	1			3	7
390-399			1						1				2				2	3
400-409						1			1				1				1	2
410-419						1			1							1	1	2
420-429																		
430-439							1	1	2									2
Grand Total	35	44	24	10	2	5	1	1	122	2	25	62	30	8	1	1	127	249

Appendix 4.9. The seasonal age-length keys for *Hyporhamphus melanochir* from the south of Western Australia.

Age	West Co	ast											wc
Ν	Summer							Winter					Total
Size class (mm)	0	1	2	3	4	5	Total	1	2	3	4	Total	
170-179	1	1					2						2
180-189	2						2						2
190-199	1						1						1
200-209													
210-219	1						1						1
220-229	1						1						1
230-239		1					1	3				3	4
240-249		1					1	1				1	2
250-259		1	1				2	1				1	3
260-269		4	3				7	4	2			6	13
270-279		1	4				5	3	2	2		7	12
280-289		1	11				12	1	3	3		7	19
290-299			5	3			8		5	4		9	17
300-309			9	2	1		12		10	5		15	27
310-319			3	3	2		8		3	1		4	12
320-329				2	1		3			4	1	5	8
330-339			2	1	1	1	5			4	2	6	11
340-349					1		1				2	2	3
350-359													
360-369													
370-379													
380-389													
390-399													
400-409													
410-419													
420-429													
430-439													
Grand Total	6	10	38	11	6	1	72	13	25	23	5	66	138

Appendix 4.10. The seasonal age-length keys for *Hyporhamphus melanochir* from the west of Western Australia.

Age/No.	South	Coas	t						SC	Wes	t Coas	st				WC	WA
Size class (mm)	1	2	3	4	5	6	7	10	Total	0	1	2	3	4	5	Total	Total
170-179	1								1	1	1					2	3
180-189										2						2	2
190-199	1								1	1						1	2
200-209	1								1								1
210-219	4								4	1						1	5
220-229	4	3							7	1						1	8
230-239	2								2		4					4	6
240-249	6	11	1						18		2					2	20
250-259	12	4	1						17		2	1				3	20
260-269	13	2							15		8	5				13	28
270-279	8	2	1						11		4	6	2			12	23
280-289	5	7	2			1			15		2	14	3			19	34
290-299	1	7	1						9			10	7			17	26
300-309		9	3	1					13			19	7	1		27	40
310-319	1	14	2						17			6	4	2		12	29
320-329	1	10	6	1					18				6	2		8	26
330-339		13	8	4					25			2	5	3	1	11	36
340-349		6	6	3	1	1			17					3		3	20
350-359		12	7	2	1				22								22
360-369		4	3	4					11								11
370-379		2	6		1				9								9
380-389			3	3		1			7								7
390-399			3						3								3
400-409			1			1			2								2
410-419						2			2								2
420-429																	
430-439							1	1	2							ļ	2
Grand Total	60	106	54	18	3	6	1	1	249	6	23	63	34	11	1	138	387

Appendix 4.11. The age-length keys for *Hyporhamphus melanochir* from the south and the west coast of Western Australia.

# CHAPTER 5. REPRODUCTIVE BIOLOGY OF SEA GARFISH

# Q. Ye, C. Noell and D. McGlennon

Objective: Investigate the relationship between habitat type, reproduction and productivity in seagrass and other inshore habitats, and determine key aspects of the early life history of garfish.

The reproductive biology of sea garfish, Hyporhamphus melanochir, was compared amongst populations of SA, Victoria, and WA between August 1997 and April 1998. Analyses involved determination of gonadosomatic indices, macroscopic staging of gonads, average size of largest oocytes, size/age at 50% maturity, sex ratio, and batch fecundities that were related to fish size and age. This species is a multiple batch spawner with asynchronous oocyte development and a protracted spawning season. Spawning generally occurred concurrently across southern Australia from October through March with a slightly more extended spawning season in SA and WA (September to April). For females from SA, Vic and WA, size at 50% maturity was 18.8, 20.9, and 22.8 cm SL, and age at 50% maturity was 17.5, 19.3, and 19.0 months, respectively. Sex ratios of samples from the commercial net fishery in spawning season were highly biased towards females, but were more even during non-spawning season. Garfish spawn relatively large eggs (>3 mm) and have low batch fecundity (BF), averaging 960, 758, and 1270 hydrated oocytes per batch for SA, Vic, and WA respectively, which was significantly higher (p = 0.002) in WA. BFs were linearly related to SL and ovary-free fish weight (W<sub>f</sub>) for fish from SA and Vic; whist they were best related to age linearly for those from WA. The present legal minimum size for garfish is 21, 20, and 23 cm TL in SA, Victoria, and WA, respectively, which relates to 43, 6, and 20% of mature fish.

Weekly sampling was conducted at Tickera and Port Wakefield in SA for a more detailed analysis of reproductive activity between October 1998 and April 1999. Similar to the broad scale study in the previous season, most spawning occurred between October and March with two peaks in November and February in PW, but not as distinct in TK. No weekly pattern could be determined. For females from TK and PW,  $L_{50}$  were 18.8 and 20.4 cm SL, respectively, suggesting that this parameter may vary slightly between years and areas. The mean BF were 959 and 1131 hydrated oocytes per batch for TK and PW with no significant (p = 0.1333) difference between two localities. The BF of TK fish was best described by a linearly relationship with  $W_f (R^2 = 0.74)$ ; whilst that of PW fish was more closely related to SL with a power relationship ( $R^2 = 0.73$ ).

In order to study the spatial variation and schooling behaviour of females and males during the spawning season, research sampling was conducted using dab nets and multi-panel gill nets in the inshore (<5m) and offshore (>5m) areas in SA during the third spawning season (1999/2000). Four commercial and one recreational fishery samples were also included in this study. It was concluded that females tended to form relatively large schools in the inshore shallow waters; whilst males were more widely distributed with a considerably higher proportion in the offshore deep waters. Therefore, our samples from the commercial haul net fishery, which mainly targets large schools and occurs within shallow waters (< 5m) in SA during summer months, were dominated by females throughout the reproductive season.

# **5.1. Introduction**

This chapter reports on the reproductive biology of sea garfish (*Hyporhamphus melanochir*) across southern Australian waters. It provides a detailed analysis in all three states of a) the reproductive season, using Gonadosomatic Indices (GSI's), ovarian developmental stages, and the oocyte sizes of the largest eggs, b) the size and age at first maturity, c) the sex ratios, d) the relationships between batch fecundity and size and age of garfish, and e) in SA, the spatial distribution of female and male spawning fish. The results of the latter research component provide a suggested reproductive behavioural strategy for this species.

Prior to this study on the reproductive biology was undertaken, limited information was available, mainly derived from Ling's (1958) and Thompson's (1957) studies in SA and WA, respectively. More recently, St. Hill (1996) and Jordan *et al.* (1998) investigated the reproductive biology *of H. melanochir* in eastern Tasmania, and those results are compared with the present study reported in this chapter.

Finally, this chapter reviews the information on how the minimum legal lengths set for H. melanochir in the three states relate to the reproductive parameters, such as size/age at first, 50%, and 100% maturity.

## 5.2. Methodology

# Study Area and Sample Collection

## Broad scale sampling

Broad scale fish sampling was conducted between August 1997 and April 1999 across SA, Victoria, and WA to study the geographic variation in reproductive characteristics of sea garfish. The regional breakdown and sampling sites for the three states were shown in Figure 5.1. Monthly samples of 30 fish were collected from each site and almost all of the adult fish were purchased from the local markets in each state. Whole fish samples from WA and Victoria were sent regularly in frozen condition to SARDI Aquatic Sciences for biological processing. A total of 4701 fish were collected during this period. The locality, month and year of collection and number of fish sampled are shown in Table 5.1. Juvenile samples that contributed to the analysis of growth rates in Chapter 2 were also included to investigate the size and age at first maturity.



Figure 5.1. Broad scale sampling sites and regions for the study of reproductive biology of *Hyporhamphus melanochir* across South Australia, Victoria, and Western Australia. Inset areas show sampling locations for each state in respect to Australian coastline. MB = Middle Beach, PW = Port Wakefield, KC = Kingscote, AB = Arno Bay, CP = Corny Point, PL = Port Lincoln, PP = Port Pirie, TK = Tickera, WH = Whyalla, and VB = Venus Bay.

			Year/Mor	nth																			
State	Region	Site	1997					1998												1999			
			8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4
SA	Gulf St.	Middle Beach		40		39	72		30	30	30					30							
	Vincent	Port Wakefield		40	41	40	40	90		29	30	30	29	30	30	30							
	Kangaroo Island	Kingscote			40					30	30			30									
	Spencer Gulf	Arno Bay		40		40			30	30	30	30	30	29	30	38							
		Corny Point		40	40	34	30	30	30	30	28	30			30	30							
		Port Lincoln		37					30	30	29	30	30	30	29	30							
		Tickera		40	40	40	40	30		30	30	31	30	29	31	29							
		Port Pirie		40	39	40	40	31	30	30	29	30	30	30	30	30							
		Whyalla	39	40	24					30	30			30	31	30							
	West Coast	Venus Bay	38	46					30	30	30			30									
Victoria	Corner Inlet	Corner Inlet								30	30	30	30	30	30	30	30		30	30		30	30
violona	Port Phillip Bay	Port Phillip Bay								30	30	30	30	30	30	30	30			30	30	29	30
	Western Port Bay	Western Port Bay								30	30	00	50	30	50	30	50			30	00	29	50
	Western Fort Bay	Western Fort Bay								30													
WA	South Coast	Oyster Harbour										30	30							28			
		Peaceful Bay										30											
		Princess Royal Harb	our					30															
		Wilson Inlet						82	17	12	27	30		30	30	30	30	29	30	30	30	30	25
	West Coast	Cockburn Sound							30		19	30	30	30							30		
		Eagle Bay																					30
		Peel-Harvey Inlet								30		12											

Table 5.1. Information on locality, month and year of sampling, and number of fish collected for the reproductive biology study of Hyporhamphus melanochir.
#### Weekly sampling at two sites

In order to determine greater detail in the timing of reproductive activity, during the second spawning season (1998/99), sampling was concentrated at two main ports, Tickera and Port Wakefield, in the two gulfs of SA (Figure 5.1). Weekly samples of 30-50 fish from Tickera and Port Wakefield were purchased from the SAFCOL fish market between October 1998 and April 1999. A total of 748 (652 female, 96 male) and 861 (660 female, 201 male) fish were sampled from TK and PW, respectively during this season.

#### Spatial variation and schooling behaviour between sexes

To investigate the spatial variation in sex ratio, research sampling was conducted using dab nets and gill nets at the inshore (1-5 m depth) and offshore (6-10 m depth) areas near St. Kilda and Middle Beach in the GSV of SA (Figure 5.2) during the third spawning season (1999/2000). Dab netting was conducted at night using the spotlights to locate the fish, which is also an effective commercial method for taking garfish in SA and Tasmania. The schooling behaviour of the fish sampled was recorded. Three multi-panel gill nets (each of 75 m length, 2.4 m depth and mesh sizes of 38 mm, 35 mm, and 29 mm) were also used. Two of these were floating nets whilst the third had less floats and more leads so that it sank and was set on the sea floor. A floating net was set in the inshore shallow areas and both a floating and a sinking net were set in the offshore deep waters. Gill nets were mostly deployed at night, parallel to the tidal current and hauled at dawn the following day.



Figure 5.2. Sampling sites for the study of spatial variation in sex ratio *of Hyporhamphus melanochir* from South Australia. (*Inset* area relative to Australian coastline).

Between October 1999 and February 2000, a total of 359 fish were sampled by dab netting, including 5 offshore samples and 5 inshore samples. However, the number of fish taken by gill nets was very limited (20 fish over 17 net nights), probably a result of their patchy distribution in these areas and the use of only three 75-m gill nets. Consequently, gill net samples were excluded from the quantitative analysis of the sex ratios.

During this spawning season, fish samples were also purchased from the SAFCOL Fish Market on 4 occasions in order to compare sex ratios between research and commercial samples. Additionally, a recreational rod and line sample and a research dab net sample from the Bay of Shoals in KI, and a research trawl net sample from Middle Bank of northern SG were also included in the sex ratio comparisons. The schooling behaviour of fish was recorded for each of the above samples based on observation during fishing wether fish were taken from a large school or from patchily distributed individuals. Detailed information on sampling locality, month and year, the number of fish collected, sex, and schooling behaviour are presented in Table 5.2 for all fishing methods.

Table 5.2. Information on the samples of *Hyporhamphus melanochir* for the study of spatial variation in sex ratio between October 1999 and March 2000 in SA. \* Schooling behaviour was determined based on observation during fishing if fish were sampled from large schools or patchy individuals.

Sample	Method	Year	Month	Net	Area	Location	Depth	Fish No.	Female	Male	Schooling*
Research	Dab net	1999	12		Offshore	St. Kilda	8-10 m	30	1	29	No
		2000	1		Offshore	St. Kilda	9-10 m	30	2	28	No
			1		Inshore	St. Kilda	1-2 m	30	7	23	No
			1		Offshore	St. Kilda	9-10 m	30	6	24	No
			1		Inshore	St. Kilda	2-3 m	29	17	12	No
			2		Offshore	Middle Beach	8-10 m	30	7	23	No
			2		Inshore	Middle Beach	1-2 m	ı 31	7	24	No
			2		Offshore	Middle Beach	7-8 m	29	5	24	No
			2		Inshore	Middle Beach	1 m	30	14	16	No
			3		Inshore	Bay of Shoals	1-2 m	90	83	7	Yes
	Gill net	1999	10	Float	Offshore	St. Kilda	8-10 m	1	1		No
			11	Float	Offshore	St. Kilda	8-10 m	4	3	1	No
				Sink	Offshore	St. Kilda	8-10 m	0			No
			11	Float	Offshore	St. Kilda	8 m	4	2	2	No
				Sink	Offshore	St. Kilda	8 m	0			No
			11	Float	Offshore	St. Kilda	8-10 m	3	1	2	No
				Sink	Offshore	St. Kilda	8-10 m	0			No
			12	Float	Inshore	St. Kilda	3 m	0			No
				Float	Offshore	St. Kilda	8 m	ı 3	2	1	No
				Sink	Offshore	St. Kilda	8 m	0			No
		2000	1	Float	Inshore	St. Kilda	3 m	0			No
				Float	Offshore	St. Kilda	8 m	0			No
				Sink	Offshore	St. Kilda	9 m	0			No
			1	Float	Inshore	St. Kilda	2 m	5	2	3	No
				Float	Offshore	St. Kilda	8 m	0			No
			2	Float	Offshore	Middle Beach	6 m	0			No
				Float	Offshore	Middle Beach	6 m	0			No
	Trawl	2000	2		Offshore	Middle Bank		27	14	13	Yes
Recreational	Rod & line	1999	11		Inshore	Bay of Shoals	1-3 m	40	12	28	No
Commercial	Dab net	2000	1		Inshore	Port Vincent	< 5 m	50	49	1	Yes
	Haul	2000	2		Inshore	Middle Beach	< 5 m	48	25	23	No
	Haul	2000	2		Inshore	Middle Beach	< 5 m	49	40	9	Yes
	Haul	2000	3		Inshore	Corney Point	< 5 m	30	29	1	Yes

## Laboratory Analysis

All fish were measured for the standard length (SL) (to the nearest millimetre), weighed (to the nearest gram), and dissected for the study of reproductive biology. For each fish the gonads were removed, sexed and weighed to 0.1 g. Gonadosomatic indices (GSI) were calculated as:  $GSI = [W_g/W_f]*100\%$  ( $W_g =$  gonad weight,  $W_f =$  gonad-free fish weight). Ovaries were classified macroscopically to one of eight stages of development, based on size, colour and visibility of oocytes (Ling 1958) (Table 5.3). For most aspects of reproductive

biology, macrostaging was done only for females since it was assumed that the gonad developmental stage between sexes was virtually synchronised throughout the spawning cycle. Males were staged (Table 5.4) following criteria by Ling (1958) to assess the spatial variation and schooling behaviour between sexes. However, the assignment of stages to males was relatively unclear and the results for these should be interpreted with caution.

Table 5.3.	Macroscopic	stages of	of development	of	ovaries	of	Hyporhamphus	melanochir.
(after Ling	1958).							

Stage	Characteristics
1 - Immature virgins	Ovaries small and thread-like, extending about one-third of the length of the body cavity. Sometimes only just visible: and it is almost always impossible to distinguish the sex. No ova visible.
2 - Immature virgins	Ovaries distinguishable as such. Small and thin, about 1/16 inch in diameter, occupying same space as stage I and in the body cavity. White in colour or translucent; individual ova not visible.
3 - Maturing virgins or recovering or resting mature (spent) fish	Ovaries about 1/8 inch in diameter, extending half way along length of body cavity. A blood-vessel runs along dorsolateral surface of gonads, with smaller ones ramifying over the more posterior region. Small white ova to be seen in translucent ovaries.
4 - Maturation continuing	Ovaries about same relative length as in stage III but twice as thick. Blood- vessels larger. Ova plainly visible, having a diameter of about 1 mm.
5 - Maturation still in progress	Ovaries about 1/3 inch in diameter, extending some three-quarters of the way along length of body cavity. Blood vessels ramifying over the ovaries are reduced, but the main dorsolateral ones still large. The ova appears to be clearing and are about 1.5 mm in diameter.
6 - Ripe ova, but not yet running	Ovaries lie along the entire length of the body cavity and have become much swollen to about 3/4 inch diameter. Only the large lateral blood-vessel obvious. Ripe ova 3 mm in diameter appear as a fairly turgid. There is no sign of the genital pore being open. Smaller ova constitute a second group; diameter about 1.5 mm.
7 - Running ripe	Ova shed through genital pore when slight pressure is applied to the abdomen. If some ova have been extruded the ovaries will be somewhat limp and flaccid, with the remaining large ripe ova lying free in the lumen. Blood-vessels running along the side of each ovary are still very big and clearly defined.
8 - Spent ovary.	May or may not be bloodshot, but very limp and shrunken. Tunica tough and leathery, unlike easily ruptured ovarian wall of ripe stage. Blood begins to appear at the posterior end where the ramifying vessels were obvious in the earlier stages. Only a few residual large ova remain, but many medium-sized ones of the next smallest group still visible.

Table 5.4. Macroscopic stages of development of testes of *Hyporhamphus melanochir*. (after Ling 1958).

Stage	Characteristics
1 - Immature virgins	As in stage I of the females: sex indistinguishable; the gonad a mere thread- like structure, about one-third the length of the gut space.
2 - Immature virgins.	Sex just recognizable. Testis a little thicker than in stage I, and of noticeable "brittle" structure as distinct from the somewhat elastic ovary; still extending about a third of the way from the anal end of gut cavity; coloured yellowish cream.
3 - Maturing virgins or recovering or resting spent adults.	Cream in colour and displaying a triangular cross section about 1/10 inch across. Same relative length as earlier stages.
4 - Maturing (though easily confused with spent).	Colour brownish pink. About 1/8 inch in cross section, extending half way along the body cavity. Posterior end more tubular and white.
5 - Mature, but milt not yet running.	Pale pink in colour, and swollen to about 1/4 inch across, extending half way along the body cavity. Triangular shape still obvious. Tubules visible as a tightly coiled mass. Pink colour gives way to white at posterior end, where milt is accumulating. A median blood-vessel visible in hinder region and giving off branches to each testis.
6 - Running ripe	Testes even more swollen. Very soft, and pale pink in colour with black spots on surface. Strap-like in general shape, with tubules plainly visible in the body of the organs. Genital pore open and white milt exuded by the application of slight pressure on the abdomen.
7 - Spent.	Much reduced in size and showing signs of blood, which colours the testis a dull reddish brown.

For those females with ovaries more advanced than stage 2, one ovary was split longitudinally, and the oocytes were washed from the ovary matrix in a petri dish. The diameters of the largest ten oocytes were measured using an image analysis system, which was comprised of a dissecting microscope, a video camera (Panasonic wv-GL 700), and a computer installed with VideoPro image analysis software. Counts of batch fecundity were made where gonad development was significant (hydrated oocyte diameter > 2200 µm). This was done by firstly removing a segment from the centre of the other ovary lobe, weighing to 0.001 g, and then splitting the segment before teasing out and counting of oocytes. The batch fecundity (BF) was calculated as:  $BF = [E_c /W_s] \times W_o$  ( $E_c = egg$  count,  $W_s = segment$  weight,  $W_o = total weight of two ovaries)$ .

## Statistical Analysis

The size and age at first maturity were measured for female *H. melanochir*. Those individuals with ovary  $\geq$  stage 3 during spawning season were defined as mature. Logistic curves were fitted to describe the percentage maturity at both standard length (SL cm) and age (months) using the non-linear least squares (NLIN) procedure in SAS (Anon 1989) according to the equation:

$$P_m = \frac{100}{1 + e^{-k(X-m)}}$$

where  $P_m$  is % maturity, X is the SL (cm) or age (months), k is a constant describing how rapidly fish mature, and m is the size or age at 50% maturity.

The relationships between batch fecundity (BF) and SL, ovary-free fish weight and age were estimated by linear least squares procedure (GLM) in SAS (Anon 1989) and described by the linear regression:

$$y = ax + b$$

where a and b are constants, y is the BF, and x is the SL (cm), or ovary-free fish weight (g), or age (years).

Analysis of residual plots and subsequent log transformation of data, where necessary, was done to conform to assumptions of homogeneity and normality.

The above linear function was also applied to determine the relationships between ovary weight and ovary-free fish weight for each of the ovarian developmental stages 4, 5, and 6.

The spatial variation in sex ratio was detected by a Chi-square ( $\mathbb{C}^2$ ) test using the Proc Freq procedure in SAS (Anon 1989).

# 5.3. Results

### Broad Scale Study Across Southern Australian Waters

## Seasonality of reproduction

Monthly trends in mean gonadosomatic indices (GSI) are shown in Figure 5.3 for female and male *Hyporhamphus melanochir* from South Australia, Victoria and Western Australia between August 1997 and April 1999. In general, monthly GSI's showed the same overall trend for both sexes across southern Australian waters. For fish from SA, the mean GSI's increased from a low in May to a peak in October and November for males and females, respectively; they dropped slightly and then approached a second smaller peak in February before declining through the next few months. Similar relatively high levels in GSI also occurred between September and February 1999 for Victoria and WA although there was no distinct "two peaks". The small values of the mean GSI in March 1998 for Victorian fish and between January and March 1998 for WA fish were attributed to the relatively high proportion of small immature females. However, the presence of ripe females in these samples, although comprising a small proportion, still suggests that spawning activity was occurring until at least March 1998 (Figure 5.5).

During the spawning season, ovaries reached a maximum of 5.2%, 6.0%, and 9.3%, whilst testes peaked at 1.2%, 1.7%, and 1.7% of gonad-free weight for fish from SA, Victoria, and WA, respectively. It is of note that the mean GSI of WA females was considerably higher, as a result of inclusion of more larger fish, than those from SA and Victoria. The decrease in GSI's through the later spawning period reflects the increasing proportion of recovering fish (stage 3), which completed spawning.

The two peaks in GSI's for both females and males from SA were possibly indications of two spawning peaks during the spawning season. Males were in condition slightly earlier than females, which probably ensured fertilisation. The extended period during which a wide range of GSI values are apparent indicated an asynchronous maturation of females throughout a protracted spawning season.



Figure 5.3. Monthly mean gonadosomatic indices (GSI) for male and female *Hyporhamphus melanochir* from South Australia, Victoria, and Western Australia between August 1997 and April 1999. Error bars are standard errors.

For females from SA, Victoria and WA, the relationships between ovary weight and ovaryfree fish weight were compared between fish classified at stages 4, 5, and 6 (Figure 5.4). The parameters of the linear relationships are presented in Table 5.5. From the examination of these regressions as well as the mean GSI's of fish at the three stages, it is apparent that ovaries more than doubled their weight through this hydration process (Table 5.5).



Figure 5.4. Relationships between ovary weight and ovary-free fish weight for mature females (stage 3, 4, and 5) from South Australia, Victoria, and Western Australia.

Table 5.5. The linear relationships between the ovary weight and the ovary-free fish weight and the mean gonadosomatic indices for mature females (stage 4, 5, and 6) caught between August 1997 and April 1999 from SA, Victoria, and WA. \*\* = significant at p = 0.01, ns = not significant p = 0.05.

State	Stage	N	Slope	Intercept	R-square	Р	GSI ± SE (%)
South Australia	4	268	0.033	-0.192	0.4428	< 0.0001**	3.14 ± 0.15
	5	537	0.051	-0.866	0.4941	< 0.0001**	$4.03 \pm 0.09$
	6	163	0.090	-0.538	0.4482	< 0.0001**	8.29 ± 0.24
Victoria	4	31	0.011	1.163	0.0647	0.1672 ns	2.28 ± 0.24
	5	78	0.058	0.108	0.3338	< 0.0001**	$5.85 \pm 0.25$
	6	11	0.121	-0.262	0.688	0.0016**	11.83 ± 0.86
Western Australia	4	71	0.010	1.635	0.0493	0.0628 ns	1.99 ± 0.16
	5	139	0.040	1.724	0.3067	< 0.0001**	5.20 ± 0.21
	6	50	0.078	2.172	0.3482	< 0.0001**	9.15 ± 0.46

The temporal pattern of reproductive activity is also shown in the monthly trend in ovarian developmental stages (Figure 5.5). For fish from all three states, most females were at the resting phase ( $\leq$  Stage 3) during May to August. Some fish turned ripe ( $\geq$  Stage 6) early in September, particularly those from WA (Wilson Inlet), but most became ripe, running ripe and spent during October to March, which reflected the main spawning season for garfish in Southern Australian waters. Some fish persisted in spawning condition till April in SA and WA, probably due to the warmer water temperatures in the gulfs, bays and inlets. This agreed with previous studies in the reproductive cycle of *H. melanochir*. It was found that spawning in *H. melanochir* could occur as early as September in SA (Ling 1958) and as late as April/May in WA (Thomson 1957b).

For the sea garfish from SA, two spawning peaks were also apparent in the monthly ovarian development, occurring in November/December and February. However, monthly sampling might be too coarse to detect shorter periodicity in spawning activity. Therefore, one sampling site from each gulf (Port Wakefield for GSV and Tickera for SG) was subsequently chosen for more intensive weekly sampling to further study the gonad characteristics during the second reproductive season (1998/99), the results of which are presented in the next section.



Figure 5.5. Monthly ovarian developmental stages of sea garfish from SA, Victoria, and WA between August 1997 and April 1999.

Regionally, there were similar patterns in spawning season for garfish from Gulf St. Vincent (GSV), Spencer Gulf (SG), Kangaroo Island (KI) and West Coast waters (WC) in SA (Figure 5.6) even though some paucity of monthly samples existed for KI and WC. An abnormally low percentage of ripe fish in February 1998 at GSV was attributed to a large number of smaller fish during this month taken from Middle Beach.

Based on the limited data from the WC of SA, we found that garfish may start spawning slightly earlier in this region and complete spawning 1-2 month earlier than fish from other regions of SA.



Figure 5.6. Monthly ovarian developmental stages of sea garfish from different regions of South Australian waters between August 1997 and September 1999.

In Victoria, the seasonality of reproduction was comparable for sea garfish from Port Phillip Bay (PPB) and Corner Inlet (CI) (Figure 5.7). The spawning activity started in October in both regions, and ended in February 1999 or March 1998 in PPB. The lack of samples in February 1999 from CI and the large proportion of small immature females sampled in March 1999 from both regions make it difficult to assess the full duration of the spawning period.

Nevertheless, the advanced ovarian development of fish collected in March 1998 suggests that the spawning season in Victoria is likely to extend to March with some inter-annual variability.



Figure 5.7. Monthly ovarian developmental stages of sea garfish from Port Phillip Bay and Corner Inlet, Victoria between March 1998 and April 1999.

In WA, most of the spawning fish were from the south coast, which showed a protracted spawning season between September 1998 and March 1999. Some fish persisted in spawning condition with hydrated eggs ( $\geq$  stage 5) until April along both the south and west coasts of WA (Figure 5.8). The lack of samples from the west coast precludes an assessment of the full duration of the spawning season.



Figure 5.8. Monthly ovarian developmental stages of sea garfish from the south coast and west coast of WA between January 1998 and April 1999.

## Size and age at first maturity

Among the samples from SA, Victoria, and WA, the smallest ripe females (stage 6) were 19.0, 21.3, and 22.0 cm in SL (equivalent to 21.8, 24.4, and 25.2 cm in TL) with ages of 11, 24, and 25 months, respectively. The size and age at first maturity for females were measured for those individuals that carried gonad  $\geq$  stage 3 during the spawning season. It was shown that the proportion of the fish  $\leq$  stage 3 decreased from 90% in August to 10% in November in SA (Figure 5.5). Such rapid ovarian development in the early spawning season was also evident for females from Victorian and WA waters. Therefore, it was likely that these fish would spawn during the same reproductive season. Parameters of the logistic maturity curves are provided in Table 5.6. In SA, Victoria, and WA, 50% of sea garfish were mature at standard lengths of 18.8, 20.9, 22.8 cm (equivalent to TL's of 21.5, 23.9, and 26.1 cm) (Figure 5.9) with ages of 17.5, 19.3, and 19.0 months (Figure 5.10), respectively.

State	SL-50% mature (cm)	SE	к	SE	R-square	Р	N
SA	18.8	0.16	0.5728	0.0452	0.987	< 0.0001	1975
Victoria	20.9	0.30	0.7717	0.1667	0.894	< 0.0001	226
WA	22.8	0.23	0.5262	0.0558	0.972	< 0.0001	420
	Age-50% mature (months)	SE	К	SE	R-square	Р	N
SA	17.5	1.37	0.1729	0.0373	0.920	< 0.0001	778
Victoria	19.3	2.04	0.5302	0.2663	0.961	< 0.0001	86
WA	19.0	1.03	0.2047	0.0415	0.938	< 0.0001	212

Table 5.6. Parameter estimates of the logistic curves of the size and age at the first maturity of sea garfish from SA, Victoria, and WA.

Fish from SA became mature at the smallest size among the three states. At 21 cm TL, about 43% of the females were mature in SA whilst only 12% and 9% were mature in Victoria and WA, respectively (Figure 5.9). A comparison between the size at 50% maturity and the present legal minimum size limit (LMS) for each state reveals that 43, 6, and 20% of females are mature at the LMS of 21, 20, 23 cm TL in SA, Victoria, and WA, respectively (Figure 5.11). They approach 100% mature at total lengths of 33.2, 32.1, and 37.8 cm for the above three respective states.

Although sea garfish from SA approached 50% maturity earlier than those from Victoria and WA, the population from Victoria matured most rapidly (Table 5.6) (Figure 5.10).



Figure 5.9. Size at reproductive maturity of Hyporhamphus melanochir from South Australia, Victoria, and Western Australia.



Figure 5.10. Age at reproductive maturity of *Hyporhamphus melanochir* from South Australia, Victoria, and Western Australia.



Figure 5.11. Size at reproductive maturity of *Hyporhamphus melanochir* in respect to the State legal minimum size in South Australia, Victoria, and Western Australia.

# Sex ratio

The monthly percentages of male and female *H. melanochir* are shown in Figure 5.12 for samples from SA, Victorian, and WA commercial fisheries between August 1997 and April 1999. In SA, the number of females greatly exceeded that of males caught during the spawning season (October-April); whilst the sex ratios approached 1:1 for the rest of the year. Such a phenomenon was not as apparent in Victoria and WA, where sex ratio was biased toward females only during some months of the spawning season.

The seasonal pattern in sex frequency counts is unlikely to be a result of biased samples in SA, where most of the commercial catch were taken by haul nets, virtually targeting large

schools of garfish within waters of less than 5 m in depth. In contrast, a wider range of gear types were used for the garfish fisheries in Victoria and WA (see Chapter 3). The possible spatial variability in sex ratio and schooling behaviour for each sex were further investigated during the third reproductive season (1999/2000), the results of which are presented in the third section of this chapter.



FEMALE MALE

Figure 5.12. Monthly percentages of male and female sea garfish from SA, Victoria, and WA between August 1997 and April 1999.

The phenomenon of female dominant samples throughout the main spawning season was relatively consistent among the regions in SA except for WC where the lack of samples between October 1997 and January 1998 precludes a full assessment of the seasonal variability of sex ratios (Figure 5.13).



Figure 5.13. Monthly percentages of male and female sea garfish from different regions of South Australia between August 1997 and September 1999.

There was an apparent difference in the seasonality of sex ratios between the samples from PPB and CI in Victoria (Figure 5.14). In the former locality, samples were dominated by females throughout most of the months of the reproductive season; whilst in the later locality, the sex ratios seem more even or slightly biased toward males. The regional difference in sex ratio is likely a result of the use of different fishing gears in these two regions (Chapter 3). Over the last two years, ring nets have mostly replaced gar seines (similar to haul nets in SA) in CI whilst the latter method still produced most of the catch in PPB.



Figure 5.14. Monthly percentages of male and female sea garfish from Port Phillip Bay and Corner Inlet in Victoria between March 1998 and April 1999.

Along the south coast of WA, sea garfish samples were clearly dominated by females throughout the protracted spawning season (Figure 5.15). The paucity of monthly samples from the west coast makes it difficult to compare the sex ratios between the two regions.



Figure 5.15. Monthly percentages of male and female sea garfish from the south and west coasts of Western Australia between January 1998 and April 1999.

## Oocyte size

A number of distinct groups of ova at different developmental stages were typically present in ripe ovaries of *H. melanochir* (Figure 5.16). Immature ova (D in Figure 5.16) were small (<0.2mm diameter) and translucent with nucleus clearly visible. Maturing ova (0.2-1.0 mm) (C) were opaque, possessed yolk granules, and were usually oval in shape. Mature ova (B) ranged up to 1.6 mm in diameter, and were opaque, pale yellowish in colour. Ripe ova (A) were large, transparent, up to 3 mm in diameter, and filamentous (Figure 5.17). The succession of ova size classes in the mature ovaries of spawning females suggested the possibility of a multiple, intermittent spawning strategy.



Figure 5.16. Different oocyte stages in a ripe ovary of Hyporhamphus melanochir.



Figure 5.17. Hyporhamphus melanochir ripe oocytes with filaments.

The trends of monthly mean oocyte diameters, with measurements of ten oocytes chosen at random representing the largest oocyte size class for each pair of ovaries, are shown in Figure 5.18 for garfish from SA, Victoria and WA. The overall trend for each state was similar to that of the GSI's (Figure 5.3); and the seasonality was comparable between the three states.

The mean oocyte size was the smallest (600-900  $\mu$ m) during May to August while gonads were at the resting and recovering stages. They rose quickly up to 1400-3000  $\mu$ m around October and persisted throughout the main spawning season until March. The high variability in oocyte size of individual fish during the spawning season again suggested an asynchronous maturation of females. In SA, the mean oocyte size peaked in November and February corresponding to the two peaks in female GSI values.







Figure 5.18. Monthly means of the oocyte diameters of garfish from South Australia, Victoria, and Western Australia between August 1997 and April 1999.

The batch fecundities (BF) were determined for *H. melanochir* from SA, Victoria, and WA when the ova of the most advanced developed group were generally larger than 2200  $\mu$ m. Ova larger than 2200  $\mu$ m appeared to be developing to the hydration ripe stage and can be considered to present a single batch of eggs. This probably reflected the number of eggs shed at one time. The mean batch fecundities for garfish from SA, Victoria, and WA were 960, 758, and 1270 hydrated oocytes per fish, respectively (Table 5.7). There was a significant difference in BF between the three states (P = 0.001). Fish from WA had a significantly higher BF (P = 0.002) than those from SA and Victoria, which had similar fecundity estimates (P = 0.194).

The fecundities for fish from different regions of each state are also presented in Table 5.7. In general, there was no regional difference in BF for fish from SA (P = 0.853) and Victoria (P = 0.116). As BF was only estimated for WA fish from the SC, no regional comparison can be made for garfish from this state.

Table 5.7. The estimates of batch fecundity for Hyporhamphus melanochir from different
regions of South Australia, Victoria, and Western Australia between August 1997 and April
1999.

State	Region	Batch fecundity	SE	N
South Australia	Gulf St. Vincent	900	80	37
	Spencer Gulf	994	66	83
	Kangaroo Island	927	228	9
	West Coast	942	96	17
	State overall	960	46	146
Victoira	Port Phillip Bay	563	183	8
	Corner Inlet	1018	190	6
	State overall	758	142	14
Western Australia	South Coast	1270	100	52
	State overall	1270	100	52

The relationships between batch fecundity and fish size and age were compared for sea garfish from SA, Victoria and WA (Figure 6.19) with parameter estimates presented in Table 5.8. In general, larger and older fish tended to carry more ripe eggs during the spawning season. All relationships were significant except for that between BF and age for the fish from Victoria (Table 5.8), probably due to the limited sample size.

In SA, the significant relationships with SL, ovary-free fish weight ( $W_f$ ), and age explained 48.4, 42.9, and 41.5% of the total variation in numbers of hydrated oocytes respectively. The linear relationships with SL and  $W_f$  were stronger for fish from Victoria, explaining a higher percentage (> 55%) of the variation in BF. However, these relationships were much weaker for fish from WA, where BF were poorly related with SL and  $W_f$ . In fact, BF of WA fish were best related with age, which explained 67.7 % of the total variation.

There was some variability in BF of individual fish with the same length. Such variation in fish condition is possibly related to differences in food supply, population density stress, temperature and other environmental effects (Bagenal 1978; Thomson 1957b).

Table 5.8. Results from regression analyses between batch fecundity (BF) and fish size (SL in cm), ovary-free fish weight ( $W_f$  in gram), and age (year) for *Hyporhamphus melanochir* from SA, Victoria, and WA between August 1997 and April 1999. \*\* = significant at p = 0.01, ns = not significant at p = 0.05.

State	Equation	Ν	R-square	Р
South Australia	BF = 138.17 SL - 2408.9	146	0.4844	< 0.0001**
	BF = 10.41 Wf -29.91	146	0.4291	< 0.0001**
	BF = 456.67 Age + 81.67	79	0.4145	< 0.0001**
Victoria	BF = 151.75 SL - 2897.2	14	0.5939	0.0013**
	BF = 12.76 Wf - 349.09	14	0.5685	0.0018**
	BF = 383.25 Age + 356.75	6	0.1614	0.4299 ns
Western Australia	BF = 102.30 SL - 1773.3	52	0.2198	0.0005**
	BF = 4.67 Wf - 390.05	52	0.1497	0.0046**
	BF = 617.00 Age - 401.50	10	0.6768	0.0035**



Figure 5. 19. The relationships between batch fecundity and fish size (SL in cm), ovary-free fish weight ( $W_f$  in gram), and age (year) of *Hyporhamphus melanochir* from SA, Victoria, and WA between August 1997 and April 1999.

## Weekly Study at Tickera and Port Wakefield

### Seasonilty of reproduction

The temporal pattern of reproductive activity is shown in the weekly trends of mean GSI's and ovarian developmental stages of sea garfish from Tickera (TK) and Port Wakefield (PW) between October 1998 and April 1999 (Figures 5.20 and 5.21). Similar to the study throughout SA, most spawning occurred from October to March with some fish persisting in spawning condition till April.

Weekly mean GSI's showed the same overall trend for both females and males from TK and PW (Figure 5.20) although there was a limited number of males caught during the spawning season. For fish from both localities, most of the spawning occurred between October and December, with a lower level of spawning activity in the htter half of the spawning season (January-March). Mean GSI's declined with the gradual increase in proportion of spent or recovering fish. For fish from TK, the maximum weekly mean GSI was 6.3% and 1.6% of ovary-free body weight ( $W_f$ ) for females and males, respectively, which occurred in the third week of December. For fish from PW, the maximum GSI of 6.1% and 1.1% of  $W_f$  for females and males, respectively, occurred at different times of the spawning season (February for females and October for males).

With some fluctuations in spawning intensity, there were two spawning peaks in November and February for fish from PW (Figure 5.20). However, the weekly periodicity was not as distinct for fish from Tickera during the 1998/99 reproductive season (Figures 5.20 and 5.21).

For both TK and PW, the presence of different ovarian stages throughout the spawning period (Figure 5.21) and the wide variation in GSI's (Figure 5.20) indicated an asynchronous maturation and spawning of sea garfish during the protracted spawning season.



Figure 5.20. Weekly mean gonadosomatic indices (GSI) for male and female *Hyporhamphus melanochir* from Tickera and Port Wakefield, South Australia between October 1998 and April 1999. Error bars are standard errors.



Figure 5.21. Weekly ovarian developmental stages of sea garfish from Tickera and Port Wakefield, South Australia between October 1998 and April 1999.

Tickera (SG)

## Size and age at first maturity

The size and age at first maturity for females were measured for individuals that carried maturing gonads ( $\geq$  stage 3) during the spawning season (October–April) from both TK and PW (Table 5.9). It was shown that for garfish from TK and PW 50% maturity was reached at 18.8 and 20.4 cm SL (21.6 and 23.4 cm TL), respectively (Figure 5.22), and at the corresponding age of 20.7 and 20.8 months, respectively (Figure 5.23). The estimate of L<sub>50</sub> for Tickera fish in 1998/99 was the same as that for samples throughout SA (18.8 cm SL) in 1997/98.

The estimates of age at 50% maturity (age<sub>50</sub>) were not as reliable as  $L_{50}$  for these two localities. The logistic curves explained only 35.6 and 31.2% of the variation in percentage of maturity in TK and PW, respectively, even though the relationships with age were significant (p < 0.01) (Table 5.9). The poor estimates of age<sub>50</sub> were likely due to the limited and uneven sample size across the age categories throughout the 1998/99 spawning season.

Region	SL-50% mature (cm)	SE	К	SE	R-square	Р	N
Tickera	18.8	0.20	0.7872	0.1319	0.909	< 0.0001	652
Port Wakefield	20.4	0.19	0.9089	0.1373	0.962	< 0.0001	659
	Age-50% mature (month)	SE	К	SE	R-square	Р	N
Tickera	20.7	66.06	2.0547	101.3	0.356	< 0.0001	434
Port Wakefield	20.8	30.78	1.773	44.0	0.312	0.0003	125

Table 5.9. Parameter estimates of the logistic curves of the size and age at the first maturity of sea garfish from Tickera and Port Wakefield, South Australia between October 1998 and April 1999.



Figure 5.22. Size at reproductive maturity of *Hyporhamphus melanochir* from Tickera and Port Wakefield, South Australia between October 1998 and April 1999.



Figure 5.23. Age at reproductive maturity of *Hyporhamphus melanochir* from Tickera and Port Wakefield, South Australia between October 1998 and April 1999.

#### Sex ratio

The percentages of male and female *Hyporhamphus melanochir* caught from TK and PW between October 1998 and April 1999 are shown in Figure 5.24. Similarly with the results found in 1997/98 throughout SA, the number of females greatly exceeded that of males caught in both locations during the spawning season (October-March). Fish samples in this study were obtained generally from the commercial haul net fishery, which was restricted to waters of less than 5 metres in depth. In order to investigate the spatial distribution of females and males, and whether the two sexes segregate into separate spawning shoals during

their reproductive season, independent research sampling was conducted in the third spawning season (1999/2000), the results of which are presented in the next section.



Figure 5.24. Weekly percentages of male and female sea garfish from Tickera and Port Wakefield, South Australia between October 1998 and April 1999.

#### Oocyte size

A number of distinct groups of oocytes at different developmental stages were also observed during oocyte measurement for ripe ovaries of *Hyporhamphus melanochir* from Tickera and Port Wakefield. The microscopic characteristics of oocytes were as described in the broad scale study (Figures 5.16 and 5.17). The succession of oocyte size classes in the mature ovaries of spawning females again suggested the possibility of a multiple, intermittent spawning strategy.

Weekly means of the oocyte diameters for garfish from TK and PW showed the same general trend as those for overall SA (1997/98) between the months of October and April (Figure 5.25). Mean oocyte size had a trend of declining throughout the spawning season due to the increase in proportion of recovering stage fish. The oocyte diameters averaged around 2000 µms in the earlier half of the spawning season in TK, whilst decreased to the level of 1500 µms in the latter half of the season in both TK and PW. No oocyte measurements were taken for PW garfish between October and December 1998.



Figure 5.25. Weekly means of the oocyte diameters of sea garfish from Tickera and Port Wakefield, South Australia between October 1998 and April 1999.

#### Batch fecundity and age/size relationships

The batch fecundity (BF) was determined for sea garfish sampled from TK and PW between October 1998 and April 1999 (Table 5.10). The mean BF for fish from TK and PW was 959 and 1131 hydrated oocytes per fish, respectively, with no significant difference between the two localities (p = 0.1333).

Table 5.10. The estimates of batch fecundity for *Hyporhamphus melanochir* from Tickera and Port Wakefield, South Australia between October 1998 and April 1999. SE = standard error, ns = not significant at p = 0.05.

Region	Batch fecundity	± SE	Ν	Р
Tickera	959	75	60	0.1333 ns
Port Wakefield	1131	86	56	

The relationships between BF and the SL,  $W_f$ , and age of sea garfish from TK and PW are summarised in Table 5.11. All models are significant except the one between BF and age for PW, where age samples were very limited (n=5). For fish from both locations, data of BF and SL were best fitted to power relationships with 63.7 and 72.7% of variation in BF explained by SL for fish from TK, and PW, respectively (Table 5.11). Their corresponding linear relationships for log-transformed data are also showed in Figure 5.26. In general, as fish grows larger, the number of ripe eggs they carry during spawning season increases at an exponential rate.

In TK, there was a significant linear relationship between BF and  $W_{f_5}$  and age (Figure 5.26). The relationship between BF and  $W_{f_5}$  was relatively strong with a R<sup>2</sup> of 0.7433. In PW, the BF-W<sub>f\_5</sub> relationship was better described by a power function, whilst a BF-age relationship was not determined due to a lack of age samples (Table 5.11).

Table 5.11. Results from regression analyses between batch fecundity (BF) and fish size (SL) (cm), ovary-free fish weight ( $W_f$ ) (g), and age (year) for *Hyporhamphus melanochir* from Tickera and Port Wakefield, South Australia between October 1998 and April 1999. \*\* = significant at p = 0.01, ns = not significant at p = 0.05.

State	Equation	Ν	R-square	Р
Tickera	BF = 0.0023 SL**4.021	60	0.6374	< 0.0001**
	Ln BF = 4.021 LnSL - 6.07	60	0.6374	< 0.0001**
	BF = 15.84 Wf - 459.50	60	0.7433	< 0.0001**
	<u>BF = 426.27 Age + 441.02</u>	46	0.4553	< 0.0001**
Port Wakefield	BF = 0.0001 SL**4.962	56	0.7268	< 0.0001**
	Ln BF = 4.962 LnSL - 8.91	56	0.7268	< 0.0001**
	BF = 1.1877 Wf**1.490	56	0.6675	< 0.0001**
	Ln BF = 1.490 LnWf + 0.172	56	0.6675	< 0.0001**
	BF = 181.0 Age + 719.8	5	0.1534	0.5144 ns



Figure 5.26. The relationships between batch fecundity and the standard length (SL) (cm), ovary-free fish weight ( $W_t$ ) (g) and age (month) of *Hyporhamphus melanochir* from Tickera and Port Wakefield, South Australia between October 1998 and April 1999.

#### Spatial Variation and Schooling Behaviour Between Sexes

In order to analyse the spatial variation in sex ratios, between November 1999 and March 2000, all fish collected from research sampling and the commercial and recreational fisheries were categorised according to their schooling behaviour and fishing locations (depth strata). The percentages of females and males from different sampling methods are shown in Figure 5.27 and the mean sex ratios are presented in Table 5.12 for fish from the different categories. Generally, the sex ratio of garfish was biased toward females for schooling fish and samples from the shallow water during the spawning season in SA.



Figure 5.27. The percentage of females and males of schooling and non-schooling sea garfish collected from shallow inshore and deep off shore areas in the South Australian waters between November 1999 and March 2000. Symbols for the samples: CH = commercial haul net, CD = commercial dab net, R = recreational line, D = research dab netting, and T = research trawling. The dotted lines are showing the average percentages for females and males. Note: Wether fish were schooling or patchily distributed was determined by observation during fishing.

It was apparent that the shallow water samples of schooling fish (SWS) were dominated by females (92%) whilst the deep water samples of non-schooling fish (DWN) consisted mostly of males (86%) (Figure 5.27). There was a highly significant difference in sex ratio between fish from these two groups (p < 0.0001) (Table 5.13). In SA, the commercial haul net fishery has mainly been targeting large schools of garfish within the shallow waters (<5 m) in the summer. Therefore, fish samples from the commercial fisheries had a biased sex ratio toward

females, which was consistent with our findings in the previous two spawning seasons (1997/98 and 1998/99). With the same sampling method (dab netting), our research samples clearly indicate that the percentage of males were lower in shallows than in deeper waters.

In fact, all comparisons in sex ratio among different categories of fish showed significant differences except that for non-schooling fish from shallow water (SWN) and the schooling fish from deep water (DWS), both of which tended to have more even sex ratios (p = 0.2164) (Table 5.13). With overall samples pooled, the female to male ratio was 1:1, which was similar to that of fish during the non-spawning season. Consequently, the skewed sex ratios of the commercial samples in SA were likely the result of different schooling behaviour and spatial distribution between the female and male sea garfish throughout the spawning period.

Table 5.12. The overall percentage of females and males of schooling and non-schooling sea garfish collected from the shallow inshore and deep off shore areas in the South Australian waters between November 1999 and March 2000. SW = shallow inshore, DW = deep offshore, S = schooling, and N = non-schooling.

Area	Schooling	Female	Male	Total fish No.
SW	S	91.8%	8.2%	219
	Ν	39.4%	60.6%	208
SW		66.3%	33.7%	427
DW	S	51.9%	48.1%	27
	Ν	14.1%	85.9%	149
DW		19.9%	80.1%	176
	S	87.4%	12.6%	246
	Ν	28.9%	71.1%	357

Table 5.13. The results of Chi-square test of sex ratios for sea garfish among different sampling location (depth strata) and schooling behaviour of fish from the South Australian waters between November 1999 and March 2000. DW = deep inshore, SW = shallow inshore, S = schooling, and N = non-schooling, DWS = deep water schooling, DWN = deep water non-schooling, SWS = shallow water schooling, SWN = shallow water non-schooling. \*\* = significant at p = 0.01, ns = not significant at p = 0.05.

Comparision	Ν	Chi-square	Р
DW & SW	603	107.6	<0.0001**
S & N	603	200.3	<0.0001**
DWS & DWN	176	20.5	<0.0001**
SWS & SWN	427	130.8	<0.0001**
DWS & SWS	246	34.8	<0.0001**
DWN & SWN	357	27.1	<0.0001**
DWN & SWS	368	223.6	<0.0001**
DWS & SWN	235	1.53	0.2164 ns
DWS, DWN, SWS, & SWN	603	238.0	<0.0001**

The percentages of gonad developmental stages of both female and male sea garfish from different samples are shown in Figure 5.28. The ovarian stages indicated the presence of ripe females (stage = 6) in both shallow inshore and deep offshore areas during the spawning season, probably associated with the extensive distribution of seagrass beds in the gulfs of SA (i.e. both > and  $\leq 5$  m depth). On the other hand, males in spawning condition (stage 6, running ripe) were only taken from non-schooling samples, which again reflected that males were more patchily distributed probably to maximise the fertilisation rate.



Figure 5.28. The percentage of gonad developmental stages of female and male sea garfish with different schooling behaviour from the shallow inshore and deep offshore waters in South Australia between November 1999 and March 2000.

#### 5.4. Discussion

# Seasonality of Reproduction

The increase of GSI and the presence of ripe, running ripe and spent sea garfish ( $\geq$  stage 5) from spring to early autumn clearly demonstrates that spawning occurs over an extended period of at least six months throughout the South Australian, Victorian, and Western Australian waters. There was little variation in the seasonality of reproduction among the three states with slightly more extended spawning periods in SA and WA (September to April) than in Victoria (October to March), where the latitude is somewhat higher. This is in agreement with previous studies in the reproductive cycle of *Hyporhamphus melanochir*, which found that the spawning started as early as September in SA (Ling 1958), and ended as late as April/May in WA (Thomson 1957b), but only occurred between October and February/March in Tasmania (St. Hill 1996; Jordan *et al.* 1998), where water temperatures are cooler. Within each state, there was little variation in reproductive activities between regions.
The spawning cycle of sea garfish can be affected by environmental factors, such as temperature and day-length, etc. (Thomson 1957b). The timing of spawning in this species is probably linked to the timing of the summer bloom in productivity in the shelf waters across southern Australia. Seasonal variation in the production of seagrass generally suggested maximum productivity during summer, e.g. for Zostera spp.and Heteroxostra tasmanica in the Gulf St. Vincent (Silkstone 1978), and Posidonia australis in the northern Spencer Gulf (West and Larkum 1979), SA; for Heteroxostra tasmanica in Western Port and Port Phillip Bay, Victoria (Bulthuis and Woelkerling 1983), and for Amphibolis antarctica and Posidonia australis in Shark Bay, WA (Walker and McComb 1988). The extensive growth of seagrass provides abundant food for adult Hyporhamphus melanochir (Thomson 1957a; Thomson 1959; Wood 1959; Robertson & Klumpp1983) during the protracted spawning season. In addition, Ward and Mcleay (1999) and Ward et al. (2001) suggested summer/autumn blooms in zooplankton in shelf waters of central and western South Australia. The present study (Chapter 7) found that sea garfish larvae concentrated on zooplankton, which is an important food source during their early life history. As the duration of the peak productivity can vary from year to year, the extended spawning period may be a strategy to maximise the number of larvae encountering suitable feeding conditions. It is also related to the fact that Hyporhamphus melanochir are serial spawners, with asynchronous oocyte development occurring simultaneously in reproductively active ovaries. This was agreed by previous studies on sea garfish in SA (Ling 1958) and Tasmania (St. Hill 1996). Furthermore, asynchronous maturation of individuals, which was indicated by the high variability in average size of largest ten oocytes of each fish, may also influence the duration of the spawning season. Ling (1958) suggested that larger fish ripened at an earlier date than smaller individuals, which had just attained their first maturity. This was also found in our study.

In addition, our study found two spawning peaks in November/December 1997 and February 1998 for SA garfish population, which was also suggested by the study in 1954/55 (Ling 1958). However, neither distinct peaks in spawning activity were found for the Victorian and WA populations, nor were they detected for the population of sea garfish from Eastern Tasmania (Jordan *et al.* 1998). The lack of a sample of fish from November from Victoria precludes an assessment of the full picture of monthly fluctuation in spawning intensity.

Our weekly study in SA at TK and PW, SA during the 1998/99 season again indicated two spawning peaks, particularly for the PW population, although there might be some interannual variation. However, no distinct weekly pattern in reproductive activity could be determined.

## Size/Age at First Maturity

The size at first maturity for females from SA (21.5 cm TL) was considerably smaller than those from Victoria and WA. At the present LMS for each state, the percentage of mature females approached 50% in SA whilst it was less than 20% in Victoria and WA. There were 88, 79, and 78% of mature females at the mean sizes of the commercial fisheries, which were 25.5, 25.9, and 28.8 cm TL in SA, Victoria, and WA (Chapter 4), respectively.

Although there was lack of appropriate estimates in  $L_{50}$  for garfish in previous studies of the reproductive biology, the  $L_{50}$  in SA is likely to represent a decrease in the size of first mature fish from historical records. Ling (1958) reported that the smallest running ripe (stage 7) female was 22.9 cm TL, which could be exceptional as the next smallest with streaming ova was 26.5 cm TL; whilst our study found the smallest size stage 7 female to be 21.9 cm TL. The reduction in  $L_{50}$  was probably linked to the significant increase in fishing mortality in SA during the past 40 years. Lower size/age at maturity has been suggested as one of the general responses of fish populations to exploitation (Clark and Tracey 1994), particularly for short-lived, fast-growing species (e.g. Pauly 1979; Grosslein *et al.* 1980), such as the sea garfish. At present, the mortality estimate from the commercial catch was also the highest for the SA population among the three states (Chapter 4). Fisheries statistics indicated that commercial landings of garfish have always been the highest in SA with approximately 60% of the national catch taken from SA waters (see Chapter 3).

Furthermore, with the broad distribution of sea garfish across the regions in each state, there might be spatial variation in age and size at maturity, which are determined both by gene pool and by environment (Stearns and Crandall 1984). Due to limited sample size and/or small number of immature individuals, estimation and comparison of regional  $L_{50}$  was not done within each state. The weekly study in SA during the second spawning season (1998/99) suggested the same  $L_{50}$  (18.8 cm SL) for the TK population as for the whole SA population in 1997/98. Nevertheless, the somewhat higher  $L_{50}$  (20.4 cm SL) for PW population likely suggested both inter-annual and spatial variation.

## **Fecundity**

The fecundity of *Hyporhamphus melanochir* has been researched previously (Ling 1958; Thomson 1957b; St. Hill, 1996). However, the estimates varied, possibly due to different

criterion in obtaining egg counts. In our study, batch fecundity was estimated as the number of oocytes that became hydrated and were larger than 2200 µms on the day that a fish was caught, and here ranged from 93 to 3,884 depending on fish size and/or age. The overall mean BF was the highest for fish from WA, and this was probably associated with larger fish caught in Wilson Inlet. The BF-SL and BF-W<sub>f</sub> relationships for SA and Victorian fish demonstrated little variation in the rate of egg production with fish size and weight. However, with the inclusion of greater numbers of larger fish, the relationship for WA fish may suggest that as garfish grow larger, a higher proportion of energy is allocated to egg production, as found for other fish species (DeMartini and Foutain 1981; Hunter and Macewicz 1985). Higher level of energy allocation toward reproduction for larger fish was also reflected in the relatively higher GSI for both female and male sea garfish from WA (present study) and Tasmania (Jordan et al. 1998). In fact, during the second spawning season, the power relationships between BF and SL for fish from TK and PW in South Australia suggested an increasing rate of egg production with fish size. Furthermore, there was great variability in BF for individuals with the same size or age. Thomson (1957b) and Bagenal (1978) suggested that fecundity of an individual fish can be affected by feeding or other factors, such as temperature.

The "post-ovulatory follicle" method was suggested by Hunter and Macewics (1985) for estimating spawning fraction and frequency for serial spawners. Attempts at obtaining such estimates were unsuccessful due to problems encountered in a pilot histological study. The conventional histological preparations from ovaries of sea garfish with hydrated oocytes resulted in sections of poor quality, with the oocytes, post-ovulatory follicles and ovary matrix being disrupted, and therefore uninterpretable. Further study is needed to develop the histological method to study ovarian histology for sea garfish in order to establish spawning frequency and total fecundity.

## Sex Ratio

There were biased sex ratios toward females for the samples from commercial net fisheries (determined by the haul net fishery in SA) during both spawning seasons 1997/98 and 1998/99. Similar results were found by Ling (1958) and St. Hill (1996). Our study in the third spawning season (1999/2000) in SA suggested significant differences in spatial distribution and schooling behaviour between sexes. Females tended to form large schools in the shallow inshore waters (<5 m), which were targeted by the commercial haul net fishery in SA. In contrast, males were relatively widely dispersed and more patchy in distribution with a significantly higher proportion in deeper offshore waters (>5 m). We suggest this to be a

strategy to maximise the probability of ripe females encountering males in spawning condition.

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# CHAPTER 6. EARLY LIFE HISTORY AND HABITAT ECOLOGY OF SEA GARFISH

# Noell, C.J.

Objective: Investigate the reproductive biology, productivity and habitat utilisation of sea garfish in representative shallow water habitats and improve understanding of early life history and recruitment.

Abstract: A multiplex polymerase chain reaction (PCR) assay was developed for discrimination between garfish larvae (family Hemiramphidae, order Beloniformes) found in southern Australian waters based on species-specific amplification of part of the mitochondrial control region. The species were easily discerned by the number and distinct sizes of PCR products (*Hyporhamphus melanochir*, 443 bp; H. regularis, 462 and 264 bp). Although based on a single gene, the method will correctly identify the species of individuals in at least 96% and 94% of tests for *H. melanochir* and *H.* regularis, respectively. Once verified by the molecular technique, larval development of H. melanochir and H. regularis was illustrated and described for specimens collected from regions of Gulf St. Vincent, South Australia. Larvae of both species have completed flexion at hatch and are characterised by their elongate body with distinct rows of pigmentation on dorsal, lateral and ventral sides; small to moderate head; heavily pigmented long straight gut; persistent preanal finfold; and extended lower jaw. Fin development proceeds in sequence: caudal; dorsal and anal; pectoral; and pelvic. Despite similarities, *H. melanochir* larvae are distinguishable from *H. regularis* by: (i) absence of the large ventral pigment blotch present in H. regularis; (ii) 12-15 paired melanophores in longitudinal rows along the dorsal margin between the head and origin of dorsal fin (vs. 19-22 for H. regularis); and (iii) 58-61 myomeres (vs. 51-54 for H. regularis). A logistic regression analysis of body measurements revealed a significant difference between combined measurements of eye diameter and pre-anal fin length. Development of the caudal complex of both species appears identical and consists of well-fused hyperal elements 1 and 2, late-fusing hyperal elements 3 and 4, a fifth hypural, and a parahypural element. Abundances of *H. melanochir* larvae from 57 plankton stations in Gulf St. Vincent in Dec 1998 and Dec 2000 averaged 4.8 and 12.3 larvae.1000 m<sup>-2</sup> of surface water, respectively. The distribution of these larvae indicated positive spatial autocorrelation, i.e. a non-randomness or clustering of similar abundance values. Most larvae were found in the upper regions of the gulf. The fact that these regions are almost entirely occupied by seagrass habitat supports the notion that the demersal eggs of *H. melanochir* become attached to seagrass and/or algae following spawning. A gyre in the upper gulf, influenced by prevailing southerly winds, the Coriolis effect, and land boundaries, may explain retention of larvae. The importance of seagrass beds to H. melanochir spawning is also supported by anecdotal evidence and literature on eggs of other Beloniformes, which are also demersal and attach to marine plants.

## 6.1. Introduction

A complex picture is emerging of the links between fish and seagrass. Seagrass beds have been found to support, in general, a greater diversity and abundance of fish than unvegetated habitats, including species of commercial and recreational value (Bell and Pollard, 1989; Connolly, 1994; Edgar and Shaw, 1995). Seagrass habitats are believed to act as: a source of enhanced food production; a refuge from predators; or a "sink" in inshore waters where larvae are transported by prevailing currents.

The southern sea garfish, *Hyporhamphus melanochir*, occur in close association with shallow seagrass beds around the coastline of South Australia, particularly in sheltered bays and estuaries of Gulf St. Vincent and Spencer Gulf where they are targeted by commercial haulnetters and dabnetters. Few fish species actually spawn over seagrasses, and although no previous studies of the reproductive ecology of *H. melanochir* in South Australia have been undertaken, there exists a small amount of anecdotal evidence that suggests that their eggs may be deposited on or become attached to seagrass blades or algae.

Ling (1958), who investigated the gonad reproductive cycle of this species, described the ripe garfish ovum as a large, clear, structure "covered by adhesive filaments..." He postulated that such ornamentation enabled the eggs to become attached, similarly with other hemiramphids, to "weed" (seagrass) at the bottom of sheltered bays such as in the two gulfs of South Australia. Ling (1958) further suggests that the development of eggs of *H. melanochir* is *in situ* and supports this with the statement "vast shoals of tiny garfish are obtainable at these same localities a few months after spawning takes place."

More recently, Jordan *et al.* (1998) described and illustrated *H. melanochir* eggs from coastal waters of eastern Tasmania. These were found entangled, by their filaments, among drift algae described as a red filamentous type (Jordan, personal commun.). Indeed, most species of the order Beloniformes produce large, demersal eggs with attaching filaments, and are often found associated with some form of vegetation (Collette *et al.*, 1984; Leis and Trnski, 1989; Parenti, 1993; Watson, 1996). It is therefore apparent from these observations, and the available literature on spawning behaviour of closely related species (Table 6.1), that more information on spawning areas of *H. melanochir* could be gained from the collection of eggs or larvae of this species.

Before this is reported in this chapter, it is necessary to ensure that, as the river garfish *H. regularis* (Günther, 1866) also occurs in regions of Gulf St. Vincent (Glover, 1985), there is no confusion in accurately identifying *H. melanochir* eggs and larvae. Therefore, the first two sections of this chapter concentrate on developing a method to differentiate the larvae of the two species. The identification, to genus and/or species, of developing fish larvae through to transformation to juveniles often rely on morphological characters different to those used to identify their adult counterparts (Neira *et al.*, 1998). This is certainly apparent for other hemiramphids found overseas (e.g. Sudarsan, 1966; Hardy and Johnson, 1974; Chen, 1988; Sokolovsky and Sokolovskaya, 1999). The advent of polymerase chain reaction (PCR)-based DNA analysis has provided a quick, often cheap, and potentially automatable method to identify closely related species of organisms, especially in the egg or larval stages of the life cycle (Silberman and Walsh, 1992; Banks *et al.*, 1993; Medeiros-Bergen *et al.*, 1995;

Species	Family	Authors	Location	Attachment	Collection method	Notes
Belone belone	Belonidae	Fonds et al. (1974)	Wadden Sea, Netherlands	Submerged algae or Zostera	NC	
Cheilopogon furcatus	Exocoetidae	Shiganova and	Central part of North	Fragment of halyard (from a	Pleiston net	
		Kovalevskaya (1991)	Atlantic Ocean	capron flag)		
Cololabis saira	Scomberosocidae	Ahlstrom and Stevens	Puget Sound, USA, to	Cables or ropes of gear	Neuston net	
		(1976)	southern Baja	suspended in water; large		
		<b>F</b> 1 10 11 (1000)	California, Mexico	invertebrates, e.g. salps	0	
		Tanaka and Oozeki (1996)	Sanriku Coast, Japan	Floating Sargassum	? Din not	Found in outs of invanile Schootes aphageli that more appointed
		Nagasawa and Domon (1997)	Sea of Japan	Drifting seaweed	Dip net	Found in guts of juvenile Sebastes schlegeli that were associated with seaweed
Cypsilurus spp.	Exocoetidae	Delsman (1924)	Coromandel Coast, India	Bundles of palm leaves	?	Leaves attached to a rope set as a fish attraction device (FAD)
Exocoetid spp.	Exocoetidae	Hunte <i>et al.</i> (1995) and references therein	Various locations	Floating <i>Sargassum</i> , seagrass, driftwood, straw, feathers, coconuts, empty bottles, nets, coconut branches, banana leaves, sugar cane trash, pleuston organisms, submerged substrata	Various methods	Some objects were set as FADs
Hemiramphus brasiliensis	Hemiramphidae	Berkeley and Houde (1978)	Southeast Florida, USA	Floating blades of the seagrass,	Surface plankton	
-	•	• · · ·		Syringodium filiforme	tows	
Hemirhamphus intermedius	Hemiramphidae	Graham (1939)	Otago Harbour, New Zealand	Weed	Seine	Found in stomachs of mullet caught in same haul as parent garfish
Hemirhamphus marginatus	Hemiramphidae	Talwar (1967)	Pāmban Island, India	Sargassum	Hand	Washed up on Dhanushkodi beach
Hirundichthys affinis	Exocoetidae	Hunte et al. (1995)	Eastern Caribbean	Coconut fronds	Hand	Set as a FAD
Hyporhamphus melanochir	Hemiramphidae	Ling (1958)	Encounter Bay, South Australia	?	?	Only a single egg found, probably belonging to <i>H. melanochir</i>
		Jones (1990)	Baird Bay, South Australia	Gillnet	Gillnet	Eggs coated the meshes of the gillnets as spawning <i>H. melanochir</i> were being hauled
		Jordan et al. (1998)	Great Oyster Bay, Tasmania, Australia	Drifting red filamentous algae	Beam trawl	None found among Heterozostera beds in Norfolk Bay, Tasmania
		Noell (unpub. data)	Bay of Shoals, South Australia	Small tufts of <i>Jania minuta</i> on <i>Posidonia</i>	Dab net	Incidentally taken while dab netting for adult <i>H. melanochir</i>
Hyporhamphus quoyi	Hemiramphidae	Sudarsan (1966)	Beach at Mandapam, India	Seaweeds	Hand	Washed ashore
Hyporhamphus sajori	Hemiramphidae	Sokolovsky and Sokolovskaya (1999)	Peter the Great Bay, Russia	Floating and attached Sargassum miyabei	IKS-80 egg net	
		Nagasawa and Domon (1997)	Sea of Japan	Drifting seaweed	Dip net	Found in guts of juvenile <i>Sebastes schlegeli</i> that were associated with drifting seaweed
Hyporhamphus unifasciatus	Hemiramphidae	Olney and Boehlert (1988)	Chesapeake Bay, USA	Floating blades of Zostera	Pushnet	
Strongylura marina	Belonidae	Zeckua Ramos and Martinez Perez (1993)	Tecolutla Estuary, Mexico	Seagrass	Hand	

Table 6.1. Review of the literature on Beloniform fish eggs with filaments used for attachment to fixed or floating objects. NC = not collected; ? = information not available.

Burton, 1996; Grutter *et al.*, 2000; Rocha-Olivares *et al.*, 2000). It also provides independent verification of morphological characters used to differentiate larvae.

Therefore, the objectives of this study were to: (i) develop a molecular technique to discriminate *H. melanochir* and *H. regularis* larvae found in the Gulf St. Vincent (described in Noell *et al.*, in press); (ii) describe and draw the larval development of these two species; (iii) predict the spawning areas of *H. melanochir* within Gulf St. Vincent from the distribution and abundance of eggs and larvae; and (iv) assess the reliance upon seagrass for spawning by *H. melanochir* with the aid of recently completed, comprehensive, benthic habitat maps for the whole gulf region (Edyvane, 1999).

## 6.2. Materials and methods

## Molecular discrimination of Hyporhamphus larvae

## Specimens examined

Adult samples for DNA analysis were collected for the two *Hyporhamphus* species found in southern Australian waters. A sample of the eastern sea garfish *H. australis* (Steindachner, 1866) found in New South Wales (N.S.W.), was included to ensure that our test could successfully discriminate this species from *H. regularis* of eastern Vic., just in case the distribution of *H. australis* extended to there. Our analysis will also provide a preliminary assessment of discrimination of *H. australis* and *H. melanochir* whose distributions overlap in southern N.S.W. A snub-nosed garfish *Arrhamphus sclerolepis* (Günther, 1866) was used for the outgroup (Table 6.2). Adults were identified using the keys and descriptions in Collette (1974). A sample of larval *H. melanochir* and *H. regularis*, identified *a priori* by C. Noell, was included to establish that this life stage could be successfully genotyped.

## DNA extraction, PCR amplification and nucleotide sequencing

DNA was extracted from either larvae preserved in 70% ethanol or frozen livers of adult fish using a salt extraction method (Miller *et al.*, 1988). A 2-4 mm length of tissue taken from the tail end of all larvae (n = 39; body length range 5.8-26.3 mm) was sufficient to obtain enough DNA for PCR analysis. An approximately 443-462 bp fragment from the mitochondrial control region (CR) was PCR amplified using primers H16498 (designed by Meyer et al., 1990) and L-M252 (Table 6.3). Verification that this product was of mitochondrial origin rather than a nuclear paralogue (Zhang and Hewitt, 1996) was done in Chapter 1. Amplifications were carried out on a Hybaid Omn-E Thermal Cycler. Reactions volumes of 50 µL contained 50-100 ng template DNA, 0.2 µM of each primer, 0.2

mM each of dATP, dTTP, dGTP and dCTP, 4 mM MgCl<sub>2</sub>, 1× GeneAmp® PCR Buffer II (Perkin Elmer) and 1 U AmpliTaq Gold<sup>™</sup> DNA polymerase (Perkin Elmer). PCR cyclic conditions were: 95°C 9 min, 50°C 1 min, 72°C 1 min for one cycle, 94°C 45 s, 50°C 45 s, 72°C 1 min for 34 cycles and 72°C 6 min, 30°C 10 s for one cycle.

Table 6.2. Sample details of garfish examined for mitochondrial DNA variation.  $n_{\rm S}$  = sample size for nucleotide sequencing;  $n_{\rm PCR}$  = sample size for PCR assay. Locality codes in parentheses.

Location	ns	<i>n</i> <sub>PCR</sub>	Life stage
Hyporhamphus melanochir			
Cockburn Sound W.A.	1	3	adult
Oyster Harbour W.A. (OH)	1	3	adult
Thevenard S.A.	1	3	adult
Tickera S.A.	1	3	adult
Arno Bay S.A.	1	3	adult
Port Gawler S.A. (PG)	2	6	adult
Western Port Vic.	1	3	adult
Corner Inlet Vic.	1	3	adult
Marion Bay Tas. (MB)	1	3	adult
Flinders Island Tas. (FI)	1	3	adult
Bay of Shoals, Kangaroo Island S.A.	1	19	larval
Hyporhamphus regularis			
Port Adelaide S.A.	1	20	larval
Angas Inlet S.A.	1	18	adult
Onkaparinga River S.A.	2	11	adult
Peel Inlet, Mandurah W.A. (PI)	1	8	adult
Broken Bay N.S.W.	-	1	adult
Gippsland Lakes Vic.	2	11	adult
Hyporhamphus australis			
Broken Bay N.S.W.	1	-	adult
Arrhamphus sclerolepis			
N.S.W.	1	-	adult

Table 6.3. Oligonucleotide sequences of primers used to discriminate garfish *Hyporhamphus* species found in southern Australian waters.

Primer	Sequence
L-M252	5'-ACCATCAGCACCCAAAGCTAGG-3'
L-M282	5'-GTGCTTCGCCATATAATCCAAC-3'
H16498 (Meyer et al., 1990)	5'-CCTGAAGTAGGAACCAGATG-3'

PCR products were purified for sequencing with the UltraClean<sup>TM</sup> PCR Clean-Up DNA Purification Kit (Mo Bio Laboratories, Inc.). Both strands of the purified PCR product were cycle sequenced with the same primers used for PCR with the BigDye<sup>TM</sup> Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems Inc.). Reaction volumes of 10  $\mu$ L contained 50-100 ng PCR product, 0.5  $\mu$ M primer and 3  $\mu$ L BigDye<sup>TM</sup>. PCR cyclic conditions were: 94°C 30 s, 50°C 15 s, 60°C 4 min for 25 cycles and 60°C 4 min, 30°C 10 s for one cycle. Products were run on an ABI 373A automated DNA sequencer.

### Phylogenetic analysis

The sequence alignment, done initially with CLUSTAL X (Thompson *et al.*, 1997), was improved manually. Individual sequences of the alignment are deposited with GenBank under accession numbers AF368258-AF368268. Phylogenetic relationships among garfish haplotypes were reconstructed with the maximum parsimony (MP) criterion of optimality with branch and bound searches. Phylogenetic trees were tested for robustness with bootstrapping (2000 pseudoreplicates done with branch and bound searches). All phylogenetic analyses were performed with PAUP\* 4.0b4a (Swofford, 1999).

## PCR test for species identification

A species-specific primer for *H. regularis*, L-M282 (Table 6.3) was designed from the aligned garfish *CR* sequences once apomorphic sites had been identified from the phylogenetic analysis. This internal primer was used in conjunction with the external primers L-M252 and H16498 in a multiplex PCR with reaction volumes and cyclic conditions the same as those already described. Because of the presence of the external primer pair unsuccessful amplifications could be detected for any of the species, i.e. the external primer pair acts as an amplification control. Amplified DNA fragments were electrophoresed for 1 h at 100 V in a 1.5% agarose gel, stained with ethidium bromide and visualised by UV transillumination.

A random sample of 30 individuals is sufficient to detect at least one copy of a haplotype (i.e. a gel phenotype) that occurs at 10% frequency with 95% confidence (Schwager *et al.*, 1993). So, the PCR test was validated on 49 adult *H. regularis* (6 were also sequenced) and 33 *H. melanochir* samples (11 were also sequenced) (Table 6.2). The *CR* sequences of a further 67 *H. melanochir* (available in Chapter 1) sampled from across the species range were also visually inspected for the L-M282 primer sequence. Larvae that could be unequivocally assigned to species based on morphology from a much larger series of samples of each species (Table 6.2) were subsequently tested by the multiplex PCR.

## Larval development of H. melanochir and H. regularis

#### Collection of larvae

Hemiramphid larvae are most commonly found at the water surface (Collette, 1984; Leis and Trnski, 1989; Watson, 1996), and were therefore collected by sampling the neuston. Plankton net tows were conducted throughout the Gulf St. Vincent aboard RV *Ngerin* in December 1998 and in the Bay of Shoals, Kangaroo Island, aboard RV *Pagrus* in November 1999 and March 2000 (Figure 6.1). A

square-framed (each square 0.5 m wide) bongo plankton net 3 m long with 500 µm mesh was equipped with a 30 cm diameter pneumatic float either side of the frame to ensure the top of the frame rode steadily above the water surface (Figure 6.2). Larvae were also hand-collected in January and November 1999 with an aquarium net from beneath a wharf in Barker Inlet (Figure 6.1) where they have been observed to school during daylight at mid-flood tide (Bruce<sup>1</sup>). Transforming (or metamorphosing) larvae and juveniles were rarely encountered using the described methods, probably as a result of accumulated mortality and increased avoidance capacity (Sandknop *et al.*, 1984). Instead, these larger specimens were attracted using a spotlight at night and collected by dip netting at Outer Harbour and Barker Inlet in January 2000, some of which were in close association with floating *Zostera*.



Figure 6.1. Map of Gulf St. Vincent showing land wind stations (%) and the locations for sampling *H. melanochir* eggs ( $\alpha$ ) and larvae( $\alpha$ ).

Hemiramphid larvae were easily sorted by eye from plankton samples immediately after collection, assisted by the prior examination of reference larval specimens from the South Australian Museum fish collection (identified to family level by B. Bruce). Larvae were fixed in 10% formalin buffered with sodium  $\beta$ -glycerophosphate (1 g.L<sup>-1</sup>) and later preserved in 70% alcohol.

<sup>&</sup>lt;sup>1</sup> Bruce, B. D., CSIRO Marine Research, GPO Box 1538, Hobart, Tasmania, Australia 7001. Personal commun., 1998.



Figure 6.2. The hauling (A) and operation (B) of the neuston plankton net used to collect *H. melanochir* larvae.

## Larval development

Totals of 47 *H. melanochir* (6.4-48.3 mm body length, BL) and 49 *H. regularis* (7.0-46.9 mm BL) larvae, transforming larvae and juveniles were used to describe body morphology, pigmentation and meristics. Developmental size series for each species was assembled using the series method (Neira *et al.*, 1998) after the initial identification to Hemiramphidae was subsequently checked microscopically with larval and adult characters reported in the literature (Chen, 1988; Collette, 1974; Collette *et al.*, 1984; Leis and Trnski, 1989; Watson, 1996). Terminology of early life history stages follows Kendall *et al.* (1984). Representative series for both species are deposited with the I.S.R. Munro Fish Collection (CSIRO, Hobart, Tasmania). {Registration numbers: *H. melanochir* (*n* = 13), CSIRO L 3072-01, 3073-01 to -08, 3074-01 to -02, 3075-01 to -02; *H. regularis* (*n* = 12), CSIRO L 3076-01 to -07, 3077-01 to -02, 3078-01 to -03}.

Larvae were examined with a bright/dark field Wild M3Z stereomicroscope (6.5-40× magnification) using various combinations of incident and transmitted light. Body measurements were taken using SigmaScan Pro<sup>®</sup> 4.01 image measurement software. This method was particularly useful for measuring cumulative distances of bent larvae that adopt a curvilinear form. The image capture and resolution capabilities of the industrial CCD camera (Panasonic GP-KR222) and monitor enabled measurements to the nearest 2-12.3  $\mu$ m (= 1 screen pixel) at 6.5-40× magnifications. Abbreviations and definitions of routinely taken body measurements follow Leis and Trnski (1989) (Figure 6.3). All hemiramphid larvae examined were post-flexion, so BL is defined as the distance from the tip of the snout to the posterior margin of the hypural bones (i.e. standard length). Snout length (SnL) and lower jaw length (LJ) are defined as the distance from the tip of the upper and lower jaw, respectively, to the anterior margin of the eye. The lower jaw extension (LJx) is simply the difference between LJ and SnL. The eye diameter was measured along both the horizontal (EDh) and vertical (EDv) axes due to its oval shape. Body depth was measured at two points, body depth at pectoral (BDp) and body depth at anus (BDa), as defined by Bruce (1995). No attempt was made to adjust body measurements of

hemiramphid larvae from preserved to live lengths as no significant difference was found for the morphologically similar saury larvae when preserved in alcohol (Oozeki *et al.*, 1991). All descriptions of pigmentation refer to melanin only.



Figure 6.3. Body measurements of Hyporhamphus larvae.

Selected specimens were cleared and stained with alcian blue and alizarin red-S following the method of Potthoff (1984). These were used to count fin rays and vertebrae, and to describe the fin development sequence and the development of the caudal complex. Abbreviations and terminology of caudal skeleton follows Fujita and Oozeki (1994). The term 'ossified' refers solely to structures stained positively for bone. Myomeres were difficult to reliably count at the extremes and were therefore determined directly from the number of vertebrae (which includes the urostyle), assuming a near one-to-one correspondence between myomeres and vertebrae. Corresponding neural and haemal spines accounted for vertebrae of small larvae that have unformed centra.

Illustrations were prepared with the aid of a camera lucida. To depict adequate detail when drawing the elongate bodies of hemiramphid larvae, larvae were examined at higher magnifications, drawn in sections and then aligned using reference points. Some body measurements were taken similarly by capturing adjoining images at higher magnifications to increase resolution and therefore improve measurement accuracy. A planachromatic objective was ideal for both methods to avoid distortion at the periphery of the field of view.

# Logistic regression analysis of body measurements

Logistic regression analysis was used to determine if differences in body measurements could distinguish between *H. melanochir* and *H. regularis* larvae. The analysis sample used to develop the logistic regression model consisted of the 41 *H. melanochir* (6.4-17.0 mm BL) and 44 *H. regularis* (7.0-13.1 mm BL) larvae used to describe larval development (excluding transforming larvae and juveniles). Body measurements were firstly transformed, however, to reduce size effects caused by allometric growth following an equation taken from Thorpe (1975):

$$\hat{Y}_i = \log_{10} Y_i - b(\log_{10} X_i - \log_{10} \overline{\overline{X}})$$

where  $\hat{Y}_i$  is the adjusted value of the *i*th specimen;  $Y_i$  is the raw value of the *i*th specimen; *b* is the pooled regression coefficient of  $\log_{10} Y$  against  $\log_{10} X$ ;  $X_i$  is the body length of the *i*th specimen; and  $\overline{X}$  is the grand mean of body lengths. Following transformation, each body measurement variable was regressed against BL. The regression coefficient of each transformed variable on BL was close to zero and insignificant, which suggest negligible effects of allometry.

Logistic regression analysis is used to determine the probability of a dichotomous outcome (dependent variable, i.e. *H. melanochir* or *H. regularis*) predicted by a set of independent variables (body measurements) in the form:

$$P = e^{y}/(1 + e^{y})$$

where P is the probability that the larva is H. melanochir, and y is the linear regression:

$$y = B_0 + B_1 X_1 + B_2 X_2 + \ldots + B_n X_n$$

where  $B_0$  is the regression constant, and  $B_1, ..., B_n$  are the coefficients associated with each morphometric predictor variable  $X_1, ..., X_n$ . A probability of 0.5 is the cutting score for the prediction of species (i.e. *H. regularis* < 0.5 ≤ *H. melanochir*). The forward stepwise logistic regression procedure in SPSS<sup>®</sup> 10.0 was used to estimate the model by maximum likelihood. Independent variables were entered into the model at the 0.05 significance level and removed at the 0.10 level, with the greatest reduction in the log-likelihood value (-2LL) used to guide variable entry. The Wald statistic was used to assess the significance of the coefficients for the variables included in the model, while the overall model fit was assessed by the change in the -2LL value, estimates of  $R^2_{logit}$ , accuracy of classification tables, and the Hosmer and Lemeshow Test (Hair *et al.*, 1998). Finally, the estimated logistic regression model was cross-validated with a holdout sample of 20 *H. melanochir* (6.0-13.4 mm BL) and 22 *H. regularis* (7.1-15.5 mm BL) larvae.

## Links between the distribution of eggs and larvae and spawning of H. melanochir

## Collection of eggs

Attempts to find and sample eggs of *H. melanochir* were conducted in Gulf St. Vincent at Middle Beach and Port Wakefield (Figure 6.1). These locations were chosen for the following reasons:

evidence that spawning has occurred in these areas from the discovery of eggs (Ling, 1958); the presence of seagrass meadows presumably required for attachment of eggs (Edyvane, 1999); reproductive activity of *H. melanochir* is synchronistic throughout South Australian commercial fishing areas (Ling, 1958; Chapter 5 of this report); and accessibility by boat.

Seven sites at Middle Beach and 12 sites at Port Wakefield were equally spaced by  $\approx 3.04$  km (2 min longitude) along three to four transects, which were roughly perpendicular to the coast and  $\approx 5.54$  km apart (3 min latitude). Final site selection within these locations was determined after depth-stratified ground truthing by SCUBA, swimming for 50 m parallel to the shoreline. Sites with extensive seagrass (and algal) cover with depths of 1-10 m were marked with GPS and subsequently revisited each month for a spawning season (Oct 1998-Apr 1999). Given the adhesive and filamentous properties of the eggs of *H. melanochir* and, therefore, the unlikelihood of dislodgment by a pump or suction device, the most appropriate sampling method was initially believed to be the systematic removal of plant material by SCUBA. Samples were collected approximately monthly at alternating locations. Because sampling from the boat required calm weather conditions, it was often difficult to link sampling date with any environmental variable (e.g. moon phase, tidal rhythm). The sampling procedure for each location included spot dives at each of 3-5 randomly chosen sites (suitably covered with vegetation) within that location, with  $3 \times 1 \text{ m}^2$  of vegetation harvested per site using shears and a catch bag. The number of sites sampled was mainly dictated by SARDI dive policy restrictions and weather conditions. For each location, a total of 9-15 m<sup>2</sup> of collected plant material was placed in hessian bags, refrigerated overnight at the laboratory, and sorted for eggs the following day.

Sampling by SCUBA proved to be relatively cost-ineffective and was replaced with a beam trawl (Figure 6.4) for the following spawning season (Oct 1999-Apr 2000) after Jordan *et al.* (1998) successfully sampled eggs of *H. melanochir* in Tasmanian coastal waters using this method. A 5 m long net with 18 mm panel mesh and 12 mm codend mesh was attached to an aluminium beam trawl with a  $1.2 \times 0.75$  m opening. The beam trawl was towed off the stern (at the same sites as described above) for 20 s at a mean speed of  $0.83 \text{ m.s}^{-1}$ , effectively covering a swept area of 20 m<sup>2</sup> of substratum per tow. A digital video camera was mounted at the top of the frame so that the benthic habitat could be recorded in the event that eggs were found. Sampling frequency and treatment of collected plant material was the same as for the first spawning season. The beam trawl was also operated in Bay of Shoals, Kangaroo Island, in Mar 2000 following the discovery, by a fish processor, of *H. melanochir* eggs 12 months earlier. It is unlikely that these eggs belonged to the estuarine-dependent *H. regularis* since they were taken by a dab netter amongst a haul of adult *H. melanochir* in spawning condition and their appearance matched the descriptions of *H. melanochir* eggs by Jordan *et al.* (1998). In

addition to the regular field collections described, floating plant material was also collected opportunistically from the boat (although not quantitatively).



Figure 6.4. Side view  $(\mathbf{A})$  and front view  $(\mathbf{B})$  of beam trawl used to sample benchic habitat. Note: digital video camera mounted on top of frame.

## Distribution and abundance of H. melanochir larvae

The methodology for collection of hemiramphid larvae was described in the section on larval development. *H. melanochir* larvae collected in Dec 1998 (cruise 1) and Dec 2000 (cruise 2) were used to examine distribution and abundances of larvae in Gulf St. Vincent (Figure 6.1). Stations were positioned at every 7 min latitude (= 12.93 km) and longitude ( $\approx$  10.65 km) within the coordinates 34°18'S, 137°44'E to 35°42'S, 138°26'E. The neuston net was towed for 5 min at a vessel speed of 2-4 knots and inside a circular arc to avoid interference by propeller wash. A calibrated flowmeter (General Oceanics, model 2030R) was mounted at the centre of each net mouth, and the average of the two readings used to estimate the area of surface water swept according to the manufacturer's calculations. Since *H. melanochir* larvae are entirely neustonic, abundances of larvae were standardised to 1000 m<sup>2</sup> of surface water rather than the volume of water filtered.

## Spatial analysis of larval abundances

The correlation among neighbouring abundances of larvae in the irregular lattice of Gulf St. Vincent stations was measured using Moran's I spatial autocorrelation statistic (Moran, 1950), with a lag distance specified as the minimum nearest neighbour (10.65 km). Moran's I is defined by:

$$I = \frac{n \sum_{i} \sum_{j} W_{ij} (x_i - \overline{x}) (x_j - \overline{x})}{S_0 \sum_{i} (x_i - \overline{x})}$$

where n = number of samples,  $x_i$  = the variate value at sample i,  $\overline{x}$  = mean of x over all locations,  $W_{ij}$ = proximity of observations i and j, and  $S_0 = \sum_i \sum_j W_{ij}$  ( $i \neq j$ ). Abundances were firstly log

transformed  $(\log(x+1))$  to standardise and normalise the distribution of abundances. Values of *I* that exceed the expected value of -1/(n-1) indicate positive spatial autocorrelation, in which similar values, either high values or low values are spatially clustered. The significance of *I* was tested by the standard *z*-statistic at the  $\alpha = 0.05$  level (Zar, 1999) under the assumption of randomisation. Correlograms of *I* vs distance classes of 10.65 km were calculated for both cruises (i.e. those samples lying within 0 to 10.65 km of each other, 10.65 to 21.3 km of each other, etc.) to allow inferences on spatial structure and patch sizes to be made. Spatial analysis was carried out using an add-in program for Excel, Rook's Case v0.9.5a.

#### Wind data

Real-time wind data for seven land-based stations situated around Gulf St. Vincent (Figure 6.1) were obtained from the Bureau of Meteorology, and consisted of three-hourly readings of surface wind speed (m.s<sup>-1</sup>) and direction for 1 Oct-17 Dec, 1998 and 1 Oct-7 Dec, 2000. These were compared with distribution and abundances of larvae to predict the influence that prevailing winds and currents may have on larval movements.

## 6.3. Results

### Molecular discrimination of Hyporhamphus larvae

The final alignment of garfish *CR* haplotypes included 423 sites. For phylogenetic analyses, alignment gaps (indels), used to optimise the sequence alignment, were treated as a fifth state. Under the MP criterion of optimality, multi-site gaps were treated as a single "mutation". Under these conditions, 142 nucleotide sites were variable and 100 were parsimony informative. The MP analysis recovered a single tree of 185 steps (Figure 6.5). Two major lineages, strongly supported by bootstrapping (100%), are apparent among the *Hyporhamphus* haplotypes, one including both *H. australis* and *H. melanochir* and the second including *H. regularis*. Each lineage is characterised by a long basal branch (40 or more characters changing along these branches) and short branches among haplotypes reflecting the substantial nucleotide divergence between the two major lineages (21.6 to 25.6% uncorrected sequence divergence). For both *H. melanochir* and *H. regularis*, sequences derived from larvae were identical in each case to a haplotype found among the adults (Figure 6.5).



Figure 6.5. Phylogenetic relationships among garfish *CR* haplotypes recovered with maximum parsimony. Unbolded numerals represent bootstrap proportions from 2000 pseudoreplicates; numerals in boldface are the number of sites that change along that branch. Refer to Table 6.2 for locality codes.

Examination of the aligned CR sequences revealed two multi-site indels of 6 and 12 bp starting at nucleotide positions 149 and 178 respectively of the alignment. The insertion character state for both indels is present in the six sequenced *H. regularis* specimens and the outgroup *A. sclerolepis*, while the deletion character state was present in both H. melanochir and H. australis (Figure 6.6). Primer L-M282, located in the vicinity of the 12 bp indel (Figure 6.6), was designed to amplify in combination with primer H16498 only the *H. regularis CR*. The final PCR test used was a multiplex of the three primers (L-252/L-M282/H16498). While the predicted gel phenotypes for each species were the 443 bp product only for *H. melanochir* and both the 264 and 462 bp products for *H. regularis* (Figure 6.7), a third outcome, the 264 bp product only, was observed in a minor proportion of *H. regularis* samples. The results of the PCR multiplex were 100% compatible with the *a priori* species identification of the 33 adult H. melanochir and 49 adult H. regularis tested. Inspection of a further 67 H. melanochir partial CR sequences along with the 11 that were also subjected to the multiplex PCR revealed that the 12 bp sequence required for annealing of the 5' end of L-M282 in *H. regularis* was deleted. It was therefore inferred that the 264 bp product would not be amplified from the samples that had been sequenced only. These sample sizes for adults of known species identity (*H. melanochir*, n = 100; *H. regularis*, n = 49) represent the ability to detect a copy of the other species' gel phenotype if it were present at a frequency of <4% and 6% for *H. melanochir* and *H. regularis*, respectively, with 95% confidence. Larvae that had been unequivocally a priori morphologically assigned to species (H. *melanochir*, n = 19; *H. regularis*, n = 20) were subsequently tested and the gel phenotypes were 100% compatible with the predicted phenotype (Figure 6.7).

H. melanochir larva	ТТАААТААСТАААТТААGACATAАААА-ТССАТСААТАСАТА-А
H. melanochir PG	GGG
H. melanochir MB	C
H. melanochir OH	
H. melanochir PG	C
H. melanochir FI	G
H. australis	CT
H. regularis S.A. & Vic.	AA.G.AA.GTC.AT.AATAATTCCCCAT.TAACT.
H. regularis PI	GA.G.AA.GTT.AT.AATAACTCCCCAT.TTAACT.
H. regularis larva	AA.G.AA.GTC.AT.AATAATTCCCCAT.TAACT.
A. sclerolepis	.AC.GGT.TCTC.ATAACTCCACATGACTCCA.AA.
Primer L-M282	GTGCTTCGCCAT.TA.

Figure 6.6. Part of the nucleotide sequence alignment of the mitochondrial *CR* haplotypes from adult and larval *H. melanochir* and *H. regularis*, *H. australis* and the outgroup *A. sclerolepis*. This represents the section of the alignment from which the PCR primer, L-M282, used to discriminate between *H. melanochir* and *H. regularis* was designed. This section is from nucleotide sites 155 to 210 of the complete alignment. Dots (.) indicate identical nucleotides as *H. melanochir* larva; dashes (-) indicate alignment gaps; question mark (?) indicates unknown nucleotide.



Figure 6.7. Electrophoretic discrimination between mtDNA *CR* multiplex PCR products from *H. melanochir* (443 bp) and *H. regularis* (462 and 264 bp). Lanes 1 and 2, *H. melanochir* larvae; lanes 3 and 4, *H. regularis* larvae; lane 5, *H. melanochir* adult; lane 6, *H. regularis* adult; lane 7, no template PCR control. M indicates 100 bp ladder for molecular weight marker. Arrows indicate the position of DNA products of 264, 443 and 462 bp.

## Larval development descriptions

## Southern sea garfish (Hyporhamphus melanochir Valenciennes, 1847) (Figure 6.8)

# Morphology

The smallest *H. melanochir* larva examined was a 6.4 mm newly hatched specimen (donated by A. Jordan from egg rearing experiments). This larva was well developed having already undergone flexion during the embryonic stage. Immediately after hatching, the mouth and gut are fully functional, the eyes are partially pigmented and there is a minimal yolk reserve. Yolk reserves are exhausted within 24 h after hatching (Jordan *et al.*, 1998). Larvae were collected during the daytime and consequently, the gas bladder was probably deflated and therefore inconspicuous in all specimens examined.



Figure 6.8. Development of *Hyporhamphus melanochir*. (**A**) 6.4 mm newly hatched larva (illustration modified from Jordan *et al.*, 1998); (**B**) 9.3 mm larva; (**C**) 13.3 mm larva (composite illustration of two similarly sized but damaged specimens); (**D**) 20.4 mm transforming larva; (**E**) 29.3 mm juvenile. Myomeres were difficult to see in specimens (**D**) and (**E**) and consequently omitted.

Body measurements of larval and juvenile *H. melanochir* are summarised in Table 6.4. Body measurements of the 17 mm larva are not consistent with the sample and therefore not considered for describing morphology. Larvae are elongate to very elongate (BDp = 8-13% of BL), with body depth slightly tapered toward the anus (BDa = 7-9% of BL), and have 58-61 myomeres. BDp decreases slightly throughout larval development. The gut is relatively thick, long, and remains straight and non-striated. The numbers of abdominal and caudal myomeres remain constant, which suggests no ontogenetic shift occurs in the position of the anus. Both PDL and PAL are relatively constant with ranges of 70-75 and 71-76% of BL, respectively. The position of the first dorsal fin ray was slightly

BL (mm)	n	Snout length	Lower jaw length	Lower jaw extension	Horizontal eye diameter	Vertical eye diameter	Head length	Pre-dorsal fin length	Pre-anal length	Body depth at pectoral	Body depth at anus
6.4 <sup>1</sup>	1	2.1	2.7	0.6	9.9	8.7	24.4	74.6	75.5	16.3 <sup>2</sup>	8.0
6.9	1	3.0	4.0	1.0	9.2	7.5	23.5	69.7	71.9	12.7	8.6
7.0-7.5	9	$2.8 \pm 0.8$	$3.9 \pm 1.1$	$1.1 \pm 0.4$	$9.1 \pm 0.3$	$7.3 \pm 0.3$	$22.0\pm0.8$	$70.8\pm0.9$	$72.6\pm1.0$	$11.9\pm0.3$	$8.9 \pm 0.3$
7.5-8.0	7	$3.6 \pm 0.9$	$4.6 \pm 1.2$	$1.0 \pm 0.4$	$9.1 \pm 0.5$	$7.1 \pm 0.3$	$22.8 \pm 1.6$	$71.3 \pm 1.3$	$73.0\pm0.9$	$11.8\pm0.6$	$8.9\pm0.7$
8.0-8.5	9	$3.6 \pm 0.5$	$4.9\pm0.8$	$1.3 \pm 0.4$	$8.8 \pm 0.3$	$7.1 \pm 0.3$	$21.9 \pm 1.0$	$70.9\pm0.7$	$72.7\pm0.9$	$11.7 \pm 0.7$	$8.9 \pm 0.7$
8.5-9.0	3	$3.5 \pm 0.8$	$5.0 \pm 1.1$	$1.5 \pm 0.3$	$8.6 \pm 0.2$	$7.0 \pm 0.3$	$21.1 \pm 0.2$	$71.5\pm0.2$	$72.5\pm0.4$	$11.3\pm0.5$	$9.1 \pm 0.5$
9.0-9.5	4	$3.4 \pm 0.5$	$5.0 \pm 0.7$	$1.6 \pm 0.3$	$8.0 \pm 0.3$	$6.5 \pm 0.2$	$20.9\pm0.8$	$71.7 \pm 0.7$	$72.8\pm0.6$	$11.4 \pm 0.7$	$8.4 \pm 0.3$
11.0	1	4.4	9.4	5.0	8.0	6.6	21.8	71.1	72.6	11.5	9.2
11.0-11.5	3	$3.4 \pm 0.6$	$6.4 \pm 0.5$	$3.0 \pm 0.9$	$7.3 \pm 0.2$	$6.2 \pm 0.1$	$19.8\pm0.9$	$71.8\pm0.6$	$72.2\pm0.6$	$10.3 \pm 0.4$	$8.3 \pm 0.5$
12.1	1	4.1	9.0	4.9	7.6	6.4	19.7	72.6	72.6	10.2	8.6
14.4	1	3.7	12.3	8.6	6.9	5.8	19.2	70.2	71.4	9.4	7.8
17.0	1	2.9	10.3	7.4	5.6	4.7	15.9	61.7	61.7	7.3	6.6
19.6	1	4.9	28.7	23.8	6.0	4.9	18.6	70.4	71.2	8.2	7.4
20.4	1	4.0	24.2	20.2	5.7	5.0	17.5	72.5	71.6	8.9	7.6
29.3	1	4.4	38.4	34.0	5.3	4.8	17.0	69.9	71.0	8.2	7.2
33.3	1	4.9	38.3	33.4	5.3	4.6	17.9	71.7	72.7	8.5	7.6
41.3	1	5.2	36.2	31.1	5.5	5.1	18.6	74.1	74.1	9.2	7.8
48.3	1	5.6	36.9	31.3	5.2	4.8	17.8	74.0	74.0	9.7	8.1

Table 6.4. Body measurements of larval, transforming and juvenile *Hyporhamphus melanochir* (expressed as a percentage of body length, BL). Means  $\pm$  standard deviations are given when sample size, n > 1. Dotted lines differentiate larvae, transforming larvae and juveniles.

<sup>1</sup> Yolk-sac larva <sup>2</sup> Includes yolk sac.

anterior, if not equal, to that of the corresponding anal fin ray. There is virtually no gap because the anal fin is situated immediately posterior to the anus. A long preanal finfold, initially the same length as the gut, persists throughout the larval stage, but gradually deteriorates until 20.4 mm when there is only small residual material. There is no head spination. The small to moderate head (HL = 18-24% of BL) is initially oval and becomes increasingly elongate although decreasing in size relative to BL. SnL increases gradually (2-5% of BL) as the apex becomes more pointed. The longer lower jaw protrudes beyond the snout (LJx) by 5% of BL at 11.0-12.1 mm and increases to a maximum of 34% of BL in the 29.3 mm juvenile. The mouth is oblique and reaches to the centre of the eye in the newly hatched larva. It subsequently moves forward relative to the eye so, by 12.1-14.4 mm, the maxilla does not reach the eye. Very small pointed teeth are just visible on both the premaxilla and dentary in newly hatched larvae. The ovoid eye (EDv = 78-88% of EDh) is moderate to large (EDh = 6-10% of BL or 33-42% of HL) and decreases with development. A single rudimentary nasal papilla first appears as a small fleshy lump in the olfactory pit by 17.0 mm. Scale formation first occurs between 20.4 and 29.3 mm laterally anterior to the caudal peduncle.

#### Fin development

The development of fins in larval and juvenile *H. melanochir* is summarised in Table 6.5. Completion of fin development occurs in the following sequence: caudal; dorsal and anal (almost simultaneously); pectoral; and pelvic. The caudal fin is developed at hatch with all principal rays (7+8) present in the newly hatched larva. Ossification of the principal caudal fin rays is medial to distal, commences by 14.4 mm and is complete by 29.3 mm. Several dorsal and anal fin rays are incipient at hatch. A full complement of 15-18 dorsal and 17-20 anal fin rays is reached at 11.4 and 12.1 mm, respectively. Ossification of dorsal and anal fin rays is anterior to posterior, commences by 29.3 mm and is complete by 41.3 mm. The pectoral base and transparent fin form prior to hatch. Incipient rays appear soon after at 7.2 mm. Pectoral fin development is slow, however; the complete fin ray count of 11-13 is not reached until 19.6 mm. Almost immediately after their formation, pectoral fin rays commence ossifying dorsal to ventral by 20.4 mm and are ossified by 41.3 mm. The onset of pelvic fin development is delayed until a bud appears at 14.4 mm. All six pelvic fin rays are present at 19.6 mm, begin ossifying distal to proximal by 29.3 mm and are ossified by 48.3 mm.

## Pigmentation

Although there is some variability in the size, number and distribution of melanophores, certain pigmentations persist throughout the *H. melanochir* larval developmental series. *H. melanochir* larvae are post-flexion and are moderately to heavily pigmented. Head pigmentation consists of melanophores on the tip of the lower jaw, snout, olfactory pit and opercula, and a patch of several

large melanophores on the occiput. The extended lower jaw is heavily pigmented throughout its development and melanophores extend to laterally along the dentary. The eve is partially pigmented in the newly hatched larva, but intensely pigmented soon after by 6.9 mm. The gut is heavily and uniformly pigmented dorsally and laterally along the entire length, the many melanophores often coalesced, but pigmentation appears less intense with increasing overlying musculature. Dorsal pigmentation is prominent with paired longitudinal rows of usually 12-15 large melanophores along the dorsal margin between the head and origin of the dorsal fin. These rows continue with crowded, and often fused, melanophores along the base of the dorsal fin (Figure 6.9A). The numbered melanophores become interspersed with smaller melanophores in transforming larvae by 19.6 mm; the dorsal larval pigmentations gradually diminish thereafter. Three lines appear along the dorsal margin in juveniles by 29.3 mm and remain through to adults. A series of melanophores form a dashed, sometimes continuous, midlateral line. Melanophores appear laterally on the caudal peduncle by 14.4 mm, and then accumulate posterior to anterior to form a broad medial stripe that remains through to adults where it appears as a silver stripe running from the caudal peduncle to the opercula. Ventral pigmentation consists of fused melanophores either side along the base of the anal fin (Figure 6.9B). Fins are devoid of pigmentation except the caudal fin, which has small melanophores on the bases of the fin rays.

Table 6.5. Meristic counts for cleared and stained larval, transforming and juvenile *Hyporhamphus melanochir*. Blanks indicate character is absent. Caudal fin rays are given as upper procurrent, upper principal, lower principal and lower procurrent; vertebrae are given as abdominal and caudal centra. Numbers in bold indicate the body length (BL) at which a full complement of rays is first attained; single and double underlined numbers indicate ossifying and completely ossified, respectively.

DL (mm)	C4			Fin rays			Branc	<b>X</b> 7 ( 1
BL (mm)	Stage	Dorsal	Anal	Pectoral	Pelvic	Caudal	ostegal rays	Vertebrae
6.4	larval <sup>1</sup>	9	8	base		<b>7</b> + <b>8</b>	3	39+20
7.2	larval	8	8	1		7+8	3	39+20
7.3	larval	9	10	1		7+8	3	39+19
7.6	larval	11	11	1		7+8	3	38+20
7.9	larval	10	11	1		7+8	3	40 + 20
8.3	larval	11	11	2		7+8	4	39+20
8.4	larval	13	14	2		1 + 7 + 8 + 1	5	39+21
9.4	larval	14	16	4		1 + 7 + 8 + 1	5	40+21
11.4	larval	15	16	6		2+7+8+1	7	38+20
12.1	larval	16	17	7		2+7+8+2	7	39+20
14.4	larval	16	19	9	bud	2+ <u>7</u> + <u>8</u> +2	9	<u>39+20</u>
19.6	transforming	17	19	11	6	<u>4</u> +7+8+ <u>4</u>	12	38+20
20.4	transforming	16	17	<u>11</u>	6	4+7+8+4	<u>12</u>	<u>39</u> + <u>19</u>
29.3	juvenile	<u>17</u>	<u>19</u>	11	<u>6</u>	5+ <u>7</u> + <u>8</u> +5	13	38+20
33.3	juvenile	17	18	12	6	5+7+8+5	<u>12</u>	38+20
41.3	juvenile	<u>16</u>	<u>19</u>	<u>11</u>	6	4+7+8+5	12	40+19
48.3	juvenile	16	19	11	<u>6</u>	<u>4</u> +7+8+ <u>5</u>	12	39+19

<sup>1</sup>Yolk-sac larva.



Figure 6.9. Dorsal (**A**) and ventral (**B**) pigmentation of an 8.5 mm *Hyporhamphus melanochir* larva. Arrows indicate the margins of the paired row of 12-15 melanophores.

## River garfish (Hyporhamphus regularis Günther, 1886) (Figure 6.10)

## Morphology

The smallest *H. regularis* larva examined was a 7.0 mm specimen. This larva was well developed having already undergone flexion during the embryonic stage. At this size, the mouth and gut are fully functional, the eyes are pigmented, and there is a medium yolk sac. Larvae were collected during the daytime and consequently, the gas bladder was probably deflated and therefore inconspicuous in all specimens examined.

Body measurements of larval and juvenile *H. regularis* are summarised in Table 6.6. Larvae are elongate to very elongate (BDp = 9-12% of BL), with body depth slightly tapered toward the anus (BDa = 7-8% of BL), and have 51-54 myomeres. BDp decreases slightly throughout larval development. The gut is relatively thick, long, and remains straight and non-striated. The numbers of abdominal and caudal myomeres remain constant. Both PDL and PAL are relatively constant with ranges of 72-74 and 71-73% of BL, respectively. The position of the first dorsal fin ray was slightly posterior, if not equal, to that of the corresponding anal fin ray. There is virtually no gap because the anal fin is situated immediately posterior to the anus. A long preanal finfold, initially the same length as the gut, persists throughout the larval stage, but gradually deteriorates. Some residual finfold is still apparent at 18.1 mm. The head is small to moderate (HL = 19-20% of BL), elongate and lacks spination. SnL increases gradually (3-5% of BL) as the apex becomes more pointed. The longer lower jaw protrudes beyond the snout (LJx) by 4% of BL at 13.1 mm and increases to a maximum of 24% of BL in the 31.5 mm juvenile. The mouth is oblique and reaches to the anterior margin of the eye at 7.0 mm but proceeds to move forward relative to the eye. Very small pointed teeth are just visible on both the premaxilla and dentary in the smallest specimen. The ovoid eye (EDv = 79-86% of EDh) is moderate to large (EDh = 6-10% of BL or 33-42% of HL) and decreases with development. A single

rudimentary nasal papilla first appears as a small fleshy lump in the olfactory pit by 18.1 mm. Scale formation first occurs between 18.1 and 24.7 mm laterally anterior to the caudal peduncle.



Figure 6.10. Development of *Hyporhamphus regularis*. (A) 7.1 mm larva; (B) 9.4 mm larva; (C) 12.3 mm larva; (D) 15.5 mm larva; (E) 24.7 mm juvenile. Myomeres were difficult to see in specimen (D) and consequently omitted.

# Fin development

The development of fins in larval and juvenile *H. regularis* is summarised in Table 6.7. Completion of fin development occurs in the following sequence: caudal; anal and dorsal (almost simultaneously); pectoral; and pelvic. Development of the caudal fin is incomplete at hatch, with 6+7 principal rays

BL (mm)	n	Snout length	Lower jaw length	Lower jaw extension	Horizontal eye diameter	Vertical eye diameter	Head length	Pre-dorsal fin length	Pre-anal length	Body depth at pectoral	Body depth at anus
$7.0^{1}$	1	3.2	4.4	1.2	8.2	6.5	20.4	73.2	71.6	11.6 <sup>2</sup>	7.4
7.5-8.0	9	$2.8 \pm 0.3$	$4.4 \pm 0.4$	$1.7 \pm 0.3$	$7.6\pm0.1$	$6.3 \pm 0.1$	$19.9\pm0.6$	$73.1\pm0.6$	$71.8\pm0.4$	$11.2 \pm 0.2$	$8.2 \pm 1.2$
8.0-8.5	12	$2.8\pm0.4$	$4.3 \pm 0.5$	$1.5 \pm 0.3$	$7.5\pm0.3$	$6.2 \pm 0.2$	$19.6\pm0.9$	$72.8\pm0.7$	$71.6\pm0.6$	$11.0\pm0.5$	$7.7 \pm 0.3$
8.5-9.0	10	$2.8\pm0.2$	$4.2 \pm 0.2$	$1.5 \pm 0.2$	$7.2\pm0.2$	$5.9 \pm 0.1$	$19.2\pm0.3$	$72.9\pm0.9$	$71.8\pm0.9$	$10.6\pm0.2$	$7.4 \pm 0.3$
9.0-9.5	5	$2.8\pm0.1$	$4.4 \pm 0.4$	$1.6 \pm 0.4$	$7.0 \pm 0.1$	$6.0 \pm 0.2$	$19.1 \pm 0.4$	$72.3\pm0.7$	$71.5\pm0.6$	$10.6\pm0.2$	$7.6 \pm 0.3$
9.5-10.0	3	$2.8\pm0.1$	$4.4 \pm 0.3$	$1.7 \pm 0.2$	$6.9\pm0.4$	$5.8 \pm 0.2$	$18.7\pm0.5$	$72.1 \pm 1.6$	$71.2 \pm 1.7$	$10.6\pm0.4$	$7.3 \pm 0.2$
10.0-10.5	3	$3.0 \pm 0.3$	$4.8\pm0.9$	$1.8\pm0.6$	$6.8\pm0.2$	$5.8 \pm 0.2$	$18.9\pm0.6$	$72.2\pm0.6$	$71.3\pm0.6$	$10.2\pm0.3$	$7.6 \pm 0.3$
13.1	1	4.0	7.6	3.7	6.9	5.4	19.6	73.2	71.9	9.1	7.8
18.1	1	4.5	18.6	14.1	6.3	5.4	19.9	73.8	72.6	9.5	8.1
24.7	1	5.6	27.7	22.1	6.3	5.5	20.8	72.9	73.6	9.7	8.1
31.5	1	6.2	30.0	23.9	6.0	5.5	21.0	74.4	75.5	10.7	8.5
33.8	1	6.4	27.7	21.3	6.1	5.6	21.6	74.3	74.3	10.9	8.9
46.9	1	7.3	damaged	damaged	5.6	4.7	21.8	75.4	75.4	11.2	9.8

Table 6.6. Body measurements of larval, transforming and juvenile *Hyporhamphus regularis* (expressed as a percentage of body length, BL). Means  $\pm$  standard deviations are given when sample size, n > 1. Dotted lines differentiate larvae, transforming larvae and juveniles.

<sup>1</sup> Yolk-sac larva <sup>2</sup> Includes yolk sac.

present in the 7.0 mm yolk-sac larva, but the full complement (7+8) is reached soon after by 7.7 mm. Ossification of the principal caudal fin rays is medial to distal, commences by 18.1 mm and is complete by 24.7 mm. A subdivided dorsal anlage and distinct anal fin bases are present at 7.0 mm. A full complement of 14-17 dorsal and 15-19 anal fin rays is reached at 13.1 and 10.5 mm, respectively. Ossification of dorsal and anal fin rays is anterior to posterior, commences by 24.7 mm and is complete by 33.8 mm. The pectoral base and transparent fin form by 7.0 mm with incipient rays first appearing by 8.1 mm. Pectoral fin development is slow, however; the complete fin ray count of 11-12 is not reached until 18.1 mm. Pectoral fin rays also commence ossifying dorsal to ventral at this size and are ossified by 33.8 mm. The onset of pelvic fin development is delayed until a bud appears at 13.1 mm. All six pelvic fin rays are present at 18.1 mm, begin ossifying distal to proximal by 31.5 mm and are ossified by 46.9 mm.

Table 6.7. Meristic counts for cleared and stained larval, transforming and juvenile *Hyporhamphus regularis*. Blanks indicate character is absent. Caudal fin rays are given as upper procurrent, upper principal, lower principal and lower procurrent; vertebrae are given as abdominal and caudal centra. Numbers in bold indicate the body length (BL) at which a full complement of rays is first attained; single and double underlined numbers indicate ossifying and completely ossified, respectively.

BL (mm) Stage				Branc	Vertebrae			
BL (IIIII)	Stage	Dorsal	Anal	Pectoral	Pelvic	Caudal	ostegal rays	vertebrae
7.0	larval <sup>1</sup>	anlage	bases	base		6+7	2	35+19
7.7	larval	4	6	base		<b>7</b> + <b>8</b>	3	34+19
7.8	larval	6	7	base		7+8	4	34+18
8.1	larval	5	7	1		7+8	4	34+19
8.3	larval	8	9	2		7+8	4	33+20
8.6	larval	9	11	2		7+8	5	34+19
8.9	larval	11	11	2		0+7+8+1	5	34+20
9.3	larval	11	12	3		1 + 7 + 8 + 1	5	33+18
9.6	larval	10	11	3		0+7+8+1	5	35+19
10.1	larval	14	14	4		1+7+8+1	6	33+20
10.5	larval	13	15	5		1+7+8+1	6	35+19
13.1	larval	14	16	7	bud	2+7+8+2	8	35+19
18.1	transforming	14	17	<u>11</u>	6	<b>4</b> + <u>7</u> + <u>8</u> + <b>4</b>	12	<u>35+18</u>
24.7	juvenile	<u>16</u>	<u>17</u>	12	6	<u>5+7+8</u> +5	<u>11</u>	<u>34+19</u>
31.5	juvenile	15	17	12	<u>6</u>	4+7+8+4	11	34+18
33.8	juvenile	<u>15</u>	<u>18</u>	<u>11</u>	6	4+7+8+4	11	33+19
46.9	juvenile	16	17	11	<u>6</u>	4 + 7 + 8 + 4	11	35+18

<sup>1</sup>Yolk-sac larva

#### Pigmentation

Although there is some variability in the size, number and distribution of melanophores, certain pigmentations persist throughout the *H. regularis* larval developmental series. *H. regularis* larvae are post-flexion and are moderately to heavily pigmented. Head pigmentation consists of melanophores on the tip of the lower jaw, snout, olfactory pit, and a patch of several large melanophores on the occiput. Small melanophores first appear on the opercula by 8.9 mm and increase in size and number thereafter. The extended lower jaw is heavily pigmented throughout its development and

melanophores extend to laterally along the dentary. The eye is intensely pigmented. The gut is heavily and uniformly pigmented dorsally and laterally along the entire length, the many melanophores sometimes coalesced, but pigmentation appears less intense with increasing overlying musculature. Dorsal pigmentation is prominent with paired longitudinal rows of usually 19-22 large melanophores along the dorsal margin between the head and origin of the dorsal fin. These rows continue with crowded, and often fused, melanophores along the base of the dorsal fin (Figure 6.11A). The numbered melanophores become interspersed with smaller melanophores in juveniles by 24.7 mm; the dorsal larval pigmentations gradually diminish thereafter. Three lines appear along the dorsal margin by 31.5 mm and remain through to adults. A series of melanophores form a dashed, sometimes continuous, midlateral line. Melanophores appear laterally at the caudal peduncle by 15.5 mm, and then accumulate posterior to anterior to form a broad medial stripe that remains through to adults where it appears as a silver stripe running from the caudal peduncle to the opercula. Ventral pigmentation consists of a large pigment blotch along the isthmus between the head and abdomen, and fused melanophores either side along the base of the anal fin (Figure 6.11B). Fins are devoid of pigmentation except the caudal fin, which has small melanophores on the bases of the fin rays.



Figure 6.11. Dorsal (**A**) and ventral (**B**) pigmentation of an 8.7 mm *Hyporhamphus regularis* larva. Arrows indicate: (**A**) the margins of the paired row of 19-22 melanophores; and (**B**) the pigment blotch along the isthmus between the head and abdomen.

## Logistic regression analysis of body measurements

Of the ten adjusted body measurements considered for logistic regression analysis, only the EDh and PDL were selected as the dependent variables, the estimated coefficients of which were statistically significant at the 0.02 level or less (Table 6.8). Therefore, body measurements EDh and PDL are significant and should be interpreted. Results of the logistic regression analysis emphasises the differences in certain body measurements that exists between *H. melanochir* and *H. australis* that may otherwise appear not so obvious. For example, the relationship between EDh and BL was subsequently examined and differences between species clearly demonstrated (Figure 6.12).

Table 6.8. Summary of statistics for independent variables included in the logistic regression model. Regression coefficient (*B*) is given as maximum likelihood estimate  $\pm$  standard error.

Variable	В	Wald	Р
EDh	$135.646 \pm 41.125$	10.879	0.001
PDL	$-316.740 \pm 135.066$	5.499	0.019
Constant	$818.587 \pm 453.099$	3.264	0.071



Figure 6.12. Relationship between the unadjusted horizontal eye diameter to body length ratio (EDh/BL) (%) and body length (mm) for larval *Hyporhamphus melanochir* (solid circles) and *H. regularis* (open circles). Also included are data for transforming larval and juvenile *H. melanochir* (solid triangles) and *H. regularis* (open triangles).

Various measures were used to evaluate the final model fit, and all of these in combination provide support for acceptance of the two-variable model as a significant logistic regression model that is suitable for further examination. A chi-square test indicated that the reduction in the -2LL value from the base model was highly significant ( $\chi^2 = 105.745$ , df = 2, *P* << 0.001). The high  $R^2_{\text{logit}}$  of 0.898 is further indicative of a good model fit. Finally, the classification matrix show extremely high hit ratios of correctly classified cases for the two-variable model, with both analysis and holdout samples each having an overall hit ratio of 97.6% (Table 6.9). Only three cases were misclassified for both analysis and holdout samples combined (Figure 6.13). As expected, the Hosmer and Lemeshow Test revealed a non-significant difference between the observed and predicted classifications of species ( $\chi^2 = 0.478$ , df = 7, *P* = 1.000).

Although a marginal improvement in model fit was obtained with the entry of EDv (as indicated by the -2LL and  $R^2_{\text{logit}}$  values), the Wald statistic revealed a non-significant variable coefficient and so, Edv was subsequently omitted. Even so, the two-variable model was clearly preferred because of its better classification accuracy of the holdout sample.

Table 6.9. Classification matrix for the estimated logistic regression model. For each cell, the number on the left denotes the analysis sample and the number on the right denotes the holdout sample. Hit ratio is the percent correctly classified.

Astual spacing		Predicted	$\mathbf{U}$ it notio (0/)		
Actual species	n -	H. melanochir	H. regularis	- Hit ratio (%)	
H. melanochir	41/20	40/20	1/0	97.6/100.0	
H. regularis	44/22	1/1	43/21	97.7/95.5	
Total	85/42			97.6/97.6	



Figure 6.13. Adjusted horizontal eye diameter (EDh) and pre-dorsal fin length (PDL) data for larval *Hyporhamphus melanochir* (solid symbols) and *H. regularis* (open symbols) superimposed on a 3D mesh plot of the two-variable logistic regression model. Dataset comprises analysis (circles) and holdout (triangles) samples. The line bisecting the mesh plot indicates the 0.5 probability cutoff score for species prediction.

## Development of the caudal complex of Hyporhamphus species

The caudal complexes of *H. melanochir* and *H. regularis* are anatomically identical during larval development and so a single description for the *Hyporhamphus* genus is given (Figure 6.14). However, slight variations may occur between species in their size at which some structures form. For such variations the BL is given for *H. melanochir*, followed by *H. regularis* (in parentheses), unless specified.

The parahypural (PH) and five hypural elements ( $HY_1$ ,  $HY_2$ ,  $HY_3$ ,  $HY_4$ ,  $HY_5$ ) are all present at 7.0 mm and are formed ventral to the urostyle (US). The uppermost  $HY_5$  was not visible in the 6.4 mm *H*. *melanochir* larva. The first and second hypural elements are fused to form a plate ( $HY_{1-2}$ ) leaving a small foramen that remains in juveniles. The third and fourth hypural elements ( $HY_3$ ,  $HY_4$ ) are fused

at 33.8 mm, leaving a small slit-like foramen. At this size, both the upper and lower hypural plates  $(HY_{1-2} \text{ and } HY_{3-4})$  are large, triangular, and of equal size and opposite symmetry. One principal caudal fin ray is attached to HY<sub>5</sub>, six to HY<sub>3</sub> and HY<sub>4</sub>, six to HY<sub>1-2</sub>, one to the PH and one to the haemal spine of the preural centrum 2 (HPU<sub>2</sub>). All 7+8 principal rays had formed in all cleared and stained specimens except the 7.0 mm *H. regularis* larva (Tables 6.5 and 6.7). The hypurapophysis (PP) of the PH is attached basally to the ural centrum (UC) and is well developed at 33.8 mm. Each of the elements supporting the principal rays is ossifying, except HY<sub>5</sub>, at 14.4 mm (18.1 mm).



Figure 6.14. Development of the caudal complex in *Hyporhampus* species. (A) 7.0 mm *Hyporhamphus regularis* larva; (B) 7.8 mm *H. regularis* larva; (C) 10.5 mm *H. regularis* larva; (D) 14.4 mm *Hyporhamphus melanochir* larva; (E) 19.6 mm *H. melanochir* transforming larva; (F) 33.8 mm *H. regularis* juvenile. Abbreviations: EP, epural; HPU, haemal spine of preural centrum; HY, hypural; NO, notochord; NPU, neural spine of preural centrum; PH, parahypural; PP, hypurapophysis; PU, preural centrum; RC, radial cartilage; UN, uroneural; US, urostyle. Heavy stippling indicates cartilage; light stippling indicates one (except notochord). Scale bar indicates 0.5 mm.

Two epural elements ( $EP_2$  and  $EP_3$ ) appear dorsal to the US at 7.6-7.8 mm and a third ( $EP_1$ ) at 9.4 mm (10.5 mm). Both upper and lower procurrent rays begin forming by 8.4 mm (9.3 mm), increasing to four or five each by 19.6 mm (18.1 mm) (Tables 6.5 and 6.7). The epural elements appear to support the upper procurrent rays whilst haemal spines of the preural centra and a radial cartilage (RC) support the lower procurrent rays. A uroneural (UN) appears dorsal to the UC at 7.6 mm (9.3 mm), is

ossifying at 14.4 mm (18.1 mm), and is enlarged and partially fused to  $HY_5$  in the 33.8 mm juvenile. Neural and haemal spines of the preural centra appear to ossify simultaneously at 14.4 mm (18.1 mm).  $HPU_2$  is enlarged and blade-like at 33.8 mm.

### Links between the distribution of eggs and larvae and spawning of H. melanochir

## Collection of eggs

An area of 9-15 m<sup>2</sup> of vegetation was collected from harvesting by SCUBA and, although a much greater volume could be sampled using a beam trawl with equal effort, the volume of material collected in both sampling methods was restricted by the time and labour required to sort for eggs. A maximum 70 L of vegetation could be thoroughly sorted per person in a day. With an estimated towing time of 45 s required to collect this amount of vegetation at a speed of 0.83 m.s<sup>-1</sup>, only 45 m<sup>2</sup> of benthic habitat was sampled. The plant material collected by both methods invariably consisted mainly of the seagrasses *Zostera muelleri* ("eelgrass"), *Heterozostera tasmanica* ("garweed"), *Posidonia* spp. ("tapeweed"), *Amphibolis* sp. ("wireweed"), or drifting or attached macroalgae, but no eggs of *H. melanochir* were found among these samples.

## Distribution and abundance of H. melanochir larvae

Of the 57 stations, totals of 108 and 320 *H. melanochir* larvae were collected from cruise 1 and cruise 2, respectively. These occurred at mean abundances of 4.8 and 12.3 larvae.1000 m<sup>-2</sup> of surface water (Figures 6.15 and 6.16), and 49 and 79% frequency of occurrence of at all stations, respectively. Larval abundance reached a maximum of 40 larvae.1000 m<sup>-2</sup> for cruise 1 and 84 larvae.1000 m<sup>-2</sup> for cruise 2. In general, the greatest abundances of larvae were concentrated in the northern part of the gulf where extensive dense seagrass beds also occur (Figures 6.15 and 6.16). The size composition of the samples was clearly dominated by larvae 5.3-10.4 mm body length, accounting for 84% of the total sample from cruise 1, with larvae >15 mm rarely taken (Figure 6.17).

### Spatial analysis of larval abundances

Moran's *I* statistic for log transformed abundances of larvae for cruise 1 (I = 0.316; z = 2.984; P < 0.005) and cruise 2 (I = 0.253; z = 2.415; P < 0.02) indicate positive spatial autocorrelations within Gulf St. Vincent that were significantly different from a random spatial distribution of larvae. It is apparent, from correlograms of Moran's *I* vs distance among stations, that a similar spatial structure of larval abundance existed between cruise 1 and 2, with significant positive spatial autocorrelation for patch sizes of 10.65-31.95 km (Figure 6.18).



Figure 6.15. Distribution and abundance of *H. melanochir* larvae collected 14-17 Dec 1998 (cruise 1) aboard RV *Ngerin* superimposed on seagrass habitat map (Edyvane, 1999).



Figure 6.16. Distribution and abundance of *H. melanochir* larvae collected 4-7 Dec 2000 (cruise 2) aboard RV *Ngerin* superimposed on seagrass habitat map (Edyvane, 1999).



Figure 6.17. Length frequency distribution of H. melanochir larvae collected in Dec 1998 from Gulf St. Vincent.



Figure 6.18. Correlogram of Moran's *I* statistic vs distance class for abundances of *H. melanochir* larvae collected in Dec 1998 and Dec 2000. Autocorrelation values significant at  $\alpha = 0.5$  level are indicated with solid circles. Histogram shows the number of neighbour pairs at each distance class. The width of each distance class is 10.65 km.

### Wind data

The prevailing winds between October and the end of each cruise in December for Gulf St. Vincent appear to be predominantly from a SE to SW direction, based on wind data from land stations situated around the gulf (Figures 6.19 and 6.20). In particular, these directions account for 65-71% of all wind readings for November and December (Tables 6.10 and 6.11).



Figure 6.19. Three-hourly incident wind vectors (m.s<sup>-1</sup>, degrees True) from land stations situated around Gulf St. Vincent for 1 Oct-17 Dec, 1998.


Figure 6.20. Three-hourly incident wind vectors (m.s<sup>-1</sup>, degrees True) from land stations situated around Gulf St. Vincent for 1 Oct-7 Dec, 2000.

Table 6.10. Frequency of occurrence (%) of wind direction and wind speed for seven land stations situated around Gulf St. Vincent for 1 Oct-17 Dec, 1998. Zeros indicate frequencies <0.5%.

			Octobe	r				lovemb			December						
		Sp	eed (m.	.s <sup>-1</sup> )			Speed (m.s <sup>-1</sup> )			Speed (m.s <sup>-1</sup> )						Grand	
Direction	0-5	5-10	10-15	15-20	Total	0-5	5-10	10-15	15-20	Total	0-5	5-10			20-25	Total	total
N	4	3	1	0	8	2	1			3	3	2	1		0	6	6
NE	7	4	0		11	5	4	0		9	5	4				9	10
Е	5	1			6	8	7	1		16	5	5	0			10	11
SE	6	3	0		9	11	17	3		31	10	14	5	0		29	22
S	6	9	1		16	6	9	4	0	19	6	15	5			26	19
SW	8	13	3	0	23	4	9	2		15	5	7	1	0		14	18
W	6	8	1	0	15	2	1	0		3	1	1				2	8
NW	5	5	1	0	11	2	1	0		3	2	2	1	0		4	7
Total	46	45	8	1		40	50	10	0		37	50	12	0	0		
Readings			1466					1435					82				3723

Table 6.11. Frequency of occurrence (%) of wind direction and wind speed for seven land stations situated around Gulf St. Vincent for 1 Oct-7 Dec, 2000. Zeros indicate frequencies <0.5%.

				ober				N	lovemb	er		December					
			Speed	(m.s <sup>-1</sup> )				Sp	eed (m.			Speed (m.s <sup>-1</sup> )				Grand	
Direction	0-5	5-10	10-15	15-20	20-25	Total	0-5	5-10	10-15	15-20	Total	0-5	5-10	10-15	15-20	Total	total
N	5	3	1	0		9	3	1	0	0	4	3	1	0		4	6
NE	9	6	1			16	4	3	1		7	4	4			7	11
Е	5	3				8	6	4	0		10	5	5			9	9
SE	5	3				8	11	17	4	0	32	10	18	0		28	21
S	6	9	2	0		16	10	14	3		27	11	16	3	0	30	22
SW	7	9	3	0	0	19	5	4	0		10	6	7	0		13	14
W	6	8	1			15	3	2	0		6	2	1			4	10
NW	5	2	1	0		8	2	2	0		4	2	2			4	6
Total	49	42	7	1	0		44	46	10	0		42	53	4	0		
Readings				64					1612					374			3650

### 6.4. Discussion

#### Molecular discrimination of Hyporhamphus larvae

Initially, part of the mitochondrial *CR* from 11 adult fish was sequenced to survey nucleotide sequence variation in southern Australian *Hyporhamphus*. *H. melanochir CR* haplotype diversity was surveyed previously in Chapter 2 with a denaturing gradient gel/nucleotide sequencing approach in which 39 haplotypes were identified among 273 fishes sampled from across the species range in southern Australia. Five haplotypes were chosen from this study to represent the haplotype lineages identified by phylogenetic analyses of these data. It was also tested in Chapter 1 whether the PCR primers amplified nuclear paralogues of the *CR* in *Hyporhamphus*. These tests based on titrations of enriched mtDNA did not show any evidence that the primers used in this study were capable of amplifying nuclear paralogues of the *CR* in either *H. melanochir* or *H. regularis*.

Although both *H. australis* and *H. melanochir* are clearly genetically distant to *H. regularis*, the *CR* haplotypes of *H. australis* and *H. melanochir* are genetically much more closely related. Collette (1974) recognised the latter pair as separate species based on the lack of morphological intermediates in the region where their distributions overlap in southern N.S.W. A more thorough survey of *CR* 

haplotype diversity in these species in this region would be required before *CR* sequences could be used to discriminate between these taxa.

The results of this study demonstrate the impact that PCR technology using the mitochondrial *CR* has on resolving the discrimination of larvae of hemiramphid species from across southern Australia. The mitochondrial *CR* can have high haplotype diversity but low nucleotide diversity within fish taxa, such as is the case for garfish in this study, as well as for species of perches of the family Percidae (Faber and Stepien, 1997) in contrast with high nucleotide divergence between related taxa. Divergence often includes indels making the *CR* ideal for species-level discrimination tests. However, unlike some other mitochondrial genes where "universal" PCR primers are available, e.g. *cytb* and *16S rRNA*, initial PCR amplification of the *CR* can be problematic because of the limitations on the taxonomic scope of the homology of available *CR* primers.

Morphological criteria that could be used to discriminate between southern Australian garfish species throughout their early life histories now can be independently verified by molecular techniques. The molecular method described allows partitioning of morphological variation, due to intra-species variation and the morphological plasticity associated with larval growth and development, among the within- and between-species components. A possible outcome of this analysis is that the morphological characters may still be unable to adequately discriminate between the larvae of these species, in which case the molecular approach could replace the morphological one entirely. Also, regardless of whether larval identification by morphology alone is achievable, morphological identification may require more work per specimen, making it relatively more efficient to use the molecular approach.

This study demonstrates a non-sequencing based method that is potentially automatable, permitting analysis of large numbers of specimens and thereby avoiding much of the labour-intensive identification work using morphological criteria. Furthermore, ecologists without detailed knowledge of taxonomy or molecular biology would require only a little molecular technical training for species discrimination.

#### Larval development of H. melanochir and H. regularis

This study provides the first descriptions of larval development of hemiramphids endemic to either Australian marine (*H. melanochir*) or estuarine (*H. regularis*) waters. Both species share characters common to other described hemiramphid larvae. Hemiramphid larvae are generally characterized by their lack of head or fin spines; elongate body; long straight gut; extended lower jaw; relatively small mouth; a main pigmentation pattern consisting of rows of melanophores on the dorsal, lateral and

ventral sides of the body; and advanced state of development at hatching (Collette *et al.*, 1984; Leis and Trnski, 1989; Watson, 1996). Although the formation of fins is comparatively slightly advanced for *H. melanochir* larvae than for *H. regularis*, both species exhibit the same developmental sequence as most Hemiramphidae: caudal; dorsal and anal; pectoral; and pelvic (Collette *et al.*, 1984).

The distributions of H. melanochir and H. regularis larvae were geographically separate on most occasions in this study; only three H. melanochir larvae were found amongst H. regularis at Barker Inlet, whilst no H. regularis were amongst H. melanochir collected from plankton stations at Kangaroo Island and throughout the Gulf of St. Vincent. Although this provided a useful starting point, species identity was more rigorously established using the series method, the accuracy of which has previously been verified by a molecular technique (Noell et al., in press). H. melanochir larvae can be distinguished from *H. regularis* by the following criteria: (i) the absence of a large ventral pigment blotch which is present in *H. regularis* (Jordan<sup>2</sup>); (ii) only 12-15 paired melanophores in longitudinal rows along the dorsal margin between the head and origin of the dorsal fin (vs. 19-22 for H. regularis); and (iii) 58-61 myomeres (vs. 51-54 for H. regularis). Despite the difficulty in counting all myomeres, either the number of vertebrae in cleared and stained specimens or the number of myomeres more apparent between the pectoral fin base and anus (usually three less than the number of abdominal vertebrae) revealed a consistent difference between species. The ranges for the vertebral counts for both species usually do not overlap (Collette, 1974), as was the case in this study (Table 6.12). Further examination of body measurements by logistic regression analysis revealed a significant difference exists in the combined measurements of EDh and PDL between H. melanochir and *H. regularis* larvae. This difference appeared to be mainly attributed to the consistently larger EDh of *H. melanochir* than *H. regularis* at a given body size throughout larval development. No other hemiramphid, or beloniform, species were present in larval samples.

Larvae of other hemiramphid species found in southern Australia may have overlapping geographic distributions with *H. melanochir* and *H. regularis* larvae, though unlikely within S.A. One or a set of meristic characters can primarily distinguish both *H. melanochir* and *H. regularis* from these other hemiramphids, which are summarised in Table 6.12. Larvae of the eastern sea garfish *Hyporhamphus australis* have yet to be described but have more gill rakers than *H. melanochir*. Otherwise, *H. australis* larvae are expected to be morphologically similar to *H. melanochir*, based on similarities in remaining meristic characters and adult morphology, and so could possibly be distinguished from *H. regularis* using the same criteria aforementioned. Given the close proximity of *H. melanochir* and *H. australis* populations at Eden N.S.W. (Collette, 1974), and without evidence that larvae of both species are allopatric, *H. australis* larvae may need to be reared from artificial fertilisation in order to

Table 6.12. Adult meristic characters of hemiramphids found in southern Australia. Data collated from Collette (1974) except where footnoted. A second range from another source is given if not in total agreement with Collette (1974). The distinguishing vertebral counts for *Hyporhamphus melanochir* and *Hyporhamphus regularis* in this study are also included. Caudal fin rays are given as upper procurrent, upper principal, lower principal and lower procurrent; vertebrae are given as abdominal and caudal centra; gill rakers are given as first and second arch. ? = no information available.

Species		Fin rays					Vertebrae	Gill rakers	
species	Dorsal	Anal	Pectoral	Pelvic	Caudal	ostegal rays	venebrae	Gill fakers	
Hyporhamphus melanochir	15-18	17-20	11-13	6 <sup>4</sup>	4-5+7+8+4-56	12-13 <sup>6</sup>	(36-41) + (18-21) = 55-61 $(38-40) + (19-21) = 58-61^{6}$	(27-35) + (21-29) = ?	
Hyporhamphus regularis	14-17	15-19	11-12	6 <sup>4</sup>	4-5+7+8+4-56	11-126	(33-38) + (18-20) = 51-58 $(33-35) + (18-20) = 51-54^{6}$	(30-36) + (21-27) = 52-61	
Hyporhamphus australis	15-17	17-20	11-13 10-13 <sup>1</sup>	6 <sup>5</sup>	4+7+8+4 <sup>5</sup>	12-13 <sup>5</sup>	(37-39) + (18-20) = 56-58 (38-40) + ?1	(31-39) + (23-33) = ?	
Hemiramphus robustus	13-15	11-14	12-13	6 <sup>5</sup>	4+7+8+5 <sup>5</sup>	135	(35-37) + (17-19) = 52-55 $(33-34) + (16-17) = 49-50^{10}$	(27-33) + (20-25) = ?	
Euleptorhamphus viridis	21-25	21-24 20-24 <sup>2</sup>	8-9 7-9 <sup>2</sup>	6 <sup>2</sup>	?+7+8+? <sup>3</sup>	?	$69-73$ $(44-46) + (26-29) = 70-75^3$	(5-9) + (18-23) = 25-33	

<sup>1</sup> Parin *et al.*, 1980.

<sup>2</sup> Chen, 1988.

<sup>3</sup>Leis and Trnski, 1989.

<sup>4</sup> Gomon *et al.*, 1994.

<sup>5</sup>Noell, C. J. Dep. Environmental Biology, Adelaide Univ., South Australia 5005. Unpub. data, 2000.

<sup>6</sup>This study.

morphologically distinguish them from *H. melanochir* with confidence. Storm garfish *Hemiramphus robustus* have fewer anal rays, and develop both a dark blotch below the dorsal fin and pigmented pelvic fin as juveniles (Collette, 1974; Collette *et al.*, 1984). The long-finned garfish *Euleptorhamphus viridis* is an oceanic species that is rarely seen in near-shore waters. Nevertheless, this species is strikingly different from other hemiramphids, being much more elongate and slender, and having divergent meristic counts for a suite of characters: more dorsal and anal fin rays; more vertebrae; fewer pectoral fin rays; and fewer gill rakers.

Larvae of the saury *Scomberosox saurus* (family Scomberosocidae) also occur in southern Australia and are the only other known species in this area that could be confused with hemiramphids. However, these can be distinguished from hemiramphids by their higher myomere count (62-70), more principal caudal fin rays (16-17), presence of dorsal and anal finlets, heavier pigmentation, and more laterally compressed body (Leis and Trnski, 1989; Bruce and Sutton, 1998).

The enlarged or well-developed uroneural, hypurapophysis, haemal spines and hypural plates of the caudal complex are all conditions common to exocoetoidei, a suborder of beloniformes (Rosen, 1964). This complex, along with specialized musculature, form a forked caudal fin with a larger lower lobe (apparent in juveniles and adults), which is presumably associated with the beloniform habit of skipping, skittering or gliding over the water surface (Gosline, 1971).

Finally, the occurrence of *H. regularis* larvae in Barker Inlet in November and January, as well as the collection of transforming larvae and juveniles of both *H. melanochir* and *H. regularis* on the same night, indicate that *H. regularis* spawn in the estuary in late spring and during summer, which at least partly coincides with the *H. melanochir* spawning period.

#### Links between the collection of eggs and larvae and spawning of H. melanochir

In this study, sampling surveys were conducted for eggs and larvae of *H. melanochir* in Gulf St. Vincent of South Australia to predict spawning areas and to assess the importance of seagrass for spawning. No eggs of *H. melanochir* were found in vegetation samples collected either by SCUBA or beam trawl sampling. However, this does not discount seagrass beds as a critical habitat for spawning. If the 15-45 m<sup>2</sup> of plant material sortable per person in a day is compared to the estimated 2436 km<sup>2</sup> (= 24.4 x 10<sup>8</sup> m<sup>2</sup>) that seagrass occupies in the Gulf St. Vincent (Edyvane, 1999), it is not surprising the difficulty encountered during this study in finding eggs of *H. melanochir*. Nor were there any eggs found among floating objects (usually drift algae or detached seagrass). Although floating material provides a structure for the demersal and adhesive eggs of *H. melanochir* to attach to by their filaments, it is not considered as important as the extensive seagrass beds and drifting algae below the

surface. This is because the amount of floating material necessary to support the estimated abundance of *H. melanochir* eggs on the surface water layer appeared to be insufficient to explain the observed abundance of larvae.

Perhaps the most important direct evidence of *H. melanochir* spawning over seagrass in South Australia is the discovery of eggs of *H. melanochir*, albeit by a fish processor, attached to seagrass and algae taken whilst parent fish were dabnetted from the Bay of Shoals at Kangaroo Island. Also, eggs have been found along the east coast of Tasmania attached to filamentous drift algae (Jordan *et al.*, 1998). The fact that both of these samples of eggs were heavily entangled and attached to filamentous algae, or fine epiphytic algae on the fronds of *Posidonia* sp., suggest that spawning is not so much as dependent on seagrass *per se* as the relatively large surface area that seagrass and algae effectively provide for attachment of eggs. Jordan *et al.* (1998) also found that eggs attached to artificial substrate in rearing experiments fully developed through to the larval stage whilst unattached eggs perished, which also supports the structural requirement. Also, Jones (1990) found *H. melanochir* eggs adhering to set gill nets in Baird Bay, South Australia.

H. melanochir larvae were sampled using a neuston plankton net, in contrast to the unsuccessful attempts to find eggs, and the effectiveness of this technique presents a standard methodology suitable for annual monitoring of year classes. Distributions and abundances of larvae throughout Gulf St. Vincent indicate a non-random spatial structure, where similar abundance values are spatially clustered. Furthermore, despite the greater number of larvae collected in cruise 2 than cruise 1, a similar spatial pattern was apparent for both cruises. Most larvae were collected in the northern part of the gulf, which is almost entirely occupied by seagrass habitat. During the peak spawning period of November to December, when it is assumed most of the collected larvae were hatched, prevailing winds for the whole gulf region were generally from a southerly direction. Local wind direction and speed is probably the most important factor that influences the general circulation of water in Gulf St. Vincent (Bye, 1976). It is therefore likely that these southerlies, combined with the Coriolis effect and land boundaries, influence the clockwise gyre in the upper gulf which, in turn, may explain retention of larvae following spawning over the extensive seagrass beds in this region. A notable exception to the concentration of larvae in the upper gulf were small numbers found at the entrance to the gulf between Yorke Peninsula, Kangaroo Island, and Fleurieu Peninsula. With southerly winds for the whole gulf, and easterlies predominantly registered at Kingscote, it is likely that these larvae originated from the northeast coast of Kangaroo Island, where dense seagrass beds also occur.

The vast majority of larvae collected during cruise 1 were 5.3-10.4 mm body length. The sagittal otolith from a 7.8 mm *H. melanochir* larva (median body length for the dominant size mode) typically has 13-15 microincrements (Noell, unpub. data), which presumably corresponds to the number of

days spent adrift since hatch. Therefore, it is predicted that most of the larvae collected are unlikely to have been transported far from the origin of spawning (assuming eggs were not subjected to drifting). Obviously, the age structure of the sampled larvae coupled with knowledge of influential larval transport processes, i.e. wind data and swimming behaviour, would enable more precise spawning locations within Gulf St. Vincent to be predicted. For example, larvae of a related species, *Belone belone*, can easily maintain a swimming speed of one body length per second (Rosenthal and Fonds, 1973), and *H. melanochir* larvae are also known to be competent swimmers soon after hatch (Jordan *et al.*, 1998). Age data will be analysed in the near future and integrated with the distribution and abundance of larvae presented in this study.

It is apparent, from the broad-scale distribution and abundances of larvae, that spawning of *H. melanochir* does take place over or adjacent to extensive seagrass areas. This is largely supported by anecdotal evidence and the literature presented on the eggs of most Beloniformes, which are demersal, have filaments, and are reliant upon seagrass beds and/or macroalgae as suitable structures for their attachment. The notable absence of eggs of *H. melanochir* in plankton collections held at SARDI Aquatic Sciences (taken by traditional surface and midwater tows) further suggests that these eggs are no different to those of other Beloniformes.

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# CHAPTER 7. AN ECONOMIC ANALYSIS OF THE SOUTHERN SEA GARFISH FISHERY IN SOUTH AUSTRALIA

# J.B. Morison and J. Presser

*Objective:* Investigate the potential for higher economic yield in the South Australian fishery by the improvement of harvest, post-harvest and marketing strategies.

An economic model of the fishery was developed for the analysis. A base case economic position of the fishery was derived using current or recent catch, effort, cost and price data. The model was designed to demonstrate the change in economic rent and the change in net returns per kilogram of garfish from changes in harvesting strategies, i.e. changes in harvesting times (seasonal closures) and target size (increased legal minimum size).

The analysis showed that the potential economic gains from increasing the minimum legal size are likely to be limited unless there is a significant increase in the sustainable catch in the fishery associated with the increased minimum legal size. A seasonal closure was shown to have higher potential returns, although the impact on price and CPUE resulting from a closure are uncertain. A significant improvement in the analysis would be the development of an integrated bio-economic model in which the biological and economic interactions could be better specified and taken account of for both biological and economic analyses of the fishery.

Some investigations into the marketing of garfish outside South Australia were undertaken because of the potential for obtaining higher prices in those markets. Opportunities appear limited in other Australian markets (Sydney and Melbourne) but premiums available for similar species in export markets, particularly Japan, suggest that investment in packaging and trial shipments may return net benefits to the fishery.

### 7.1 Introduction

This component of the research is to provide an economic analysis of a range of different strategies to improve the economic performance of the South Australian commercial garfish fishery without increasing overall catches. The study was undertaken to determine seasonal and size related consumer demand and market prices in combination with an analysis of the costs associated with different harvesting strategies, to provide a cost-benefit analysis of management options for the fishery. In addition, opportunities for export market development for garfish products were investigated.

### 7.2 Background

The southern sea garfish supports valuable commercial and recreational fisheries across its distribution in Western Australia, South Australia, Tasmania and Victoria. The most significant commercial fishery exists in South Australia with catches of up to 513 tonnes with a landed value of \$2.3 million (1996/97), compared with 37 tonnes in Western Australia and 100 tonnes in Victoria.

Most of the commercial catch in South Australia is taken by the haul net method of fishing, with 87% being taken by this method in 1998/99. Dab net fishing accounts for the remainder of commercial catches in South Australia (see Chapter 3).

The fishery in South Australia, however, is characterised by a large disparity in wholesale prices. This disparity is driven predominantly by the size of fish (with larger fish commanding a higher price) and by the seasonality of supply.

An analysis of prices at the Adelaide Central Fish Market during 1998/99 revealed a variation of prices of between \$6.00 per kilogram to \$12.00 per kilogram for the larger fish (> 24 cm TL), and from \$2.00 per kilogram to \$6.50 per kilogram for the small fish (21 - 24 cm TL). In South Australia, the minimum legal length of southern sea garfish is 21 cm. Garfish sampled at the fish market between February 1998 to January 1999 revealed a mean size of 25.6 cm, with 71% ranging between 23 and 28 cm, and 9% of the fish being more than 30 cm (Ye, 1999).

The length frequency distribution of market samples of garfish during the summer months is generally skewed to the right, having a mode of 23 cm, whilst the distribution in the winter months being more normal, having a mode of 26 cm (Ye, 1999). This is influenced by the catches from the haul net sector, which tends to capture large quantities of smaller fish during the summer months, whereas fish captured by the dab net method are considerably bigger although not in the same quantity.

As a consequence of the increase in supply of garfish from January to April in most years, and the generally higher proportion of smaller fish, prices for garfish of all size categories are depressed. Anecdotal information from fishers and processors suggests that if the smaller fish were removed from the market (and not caught by fishers), the prices received for larger fish would be more consistent throughout the year.

In South Australia, there is a high demand for garfish in the fresh fish market, restaurant trade and take-away food outlets. A spot check on retail prices at the Adelaide Central Market in June 2000 showed a marked difference in the price of garfish fillets according to size category. Small fillets ranged in price from \$12.90 to \$14.95 per kilogram, whereas large fillets were \$21.00 - \$21.95 per kilogram (Ye, pers. comm.). Not only is there a lower consumer demand for the small fillets, but it costs more in time and labour for the small fish to be filleted.

There appears to be some potential for the value of the fishery to increase with improved harvesting strategies and product development by implementing management strategies that encourage fishers to catch larger fish and/or reducing fishing effort and catches during periods of low economic return.

The economic study investigated three options for change to the management arrangements and analysed the effect these could have on the economic performance of the commercial fishery. These options included:

- increasing the minimum legal size from 21 cm to 24 cm;
- introducing a 2-month closure of the fishery during the months that fishers would normally get the lowest returns; and
- introduce a 2-month closure and increase the legal minimum length from 21 cm to 24 cm.

# 7.3 Method

The approach adopted for the analysis can be described in a number of steps.

- 1. Collect data on the physical and financial performance of operators in the garfish fishery and the performance of the fishery overall.
- 2. Construct a model of the current financial performance of the garfish fishery with the current management arrangements in place (i.e. a base case analysis).
- 3. Develop a set of possible changes to the management of the fishery and describe these changes in terms of how they would affect the financial performance of the fishery.
- 4. Impose these changes on the model of the fishery to derive a range of financial outcomes for each alternative management arrangement.
- 5. Compare the financial performance of the fishery under the base case analysis with the estimated performance under each of the alternative management systems.

# 7.4 Data collection

The data used for the analysis were obtained from three general sources:

- (i) a survey of licence holders;
- (ii) SARDI catch and effort data; and
- (iii) financial performance data for the marine scalefish fishery.

#### Survey of licence holders

In May 2000, a stratified sample survey of 30 licence holders in the South Australian marine scalefish fishery was undertaken to collect data relating to the commercial garfish fishery. The sample was selected from fishers who targeted garfish, and stratified according to fishing method (haul net and dab net) and annual catch quantity. A postal survey collected information on targeted fishing effort for garfish, fishing costs, prices received, investments and fishing activity. Personal interviews with garfish fishers were also undertaken to verify and clarify information being collected and to explore with the fishers the different options for management that were being considered.

#### SARDI catch and effort and price data

For both the haul net and dab net sectors, monthly data for the period July 1998 to June 1999 were provided on catch and targeted effort and used to derive estimates of CPUE (catch per unit effort). Price data over a similar period (12 August 1998 to 31 July 1999) enabled estimates of gross income per month and gross income per day fished to be derived.

#### Financial performance data in the Marine Scalefish Fishery

It is a legislative requirement that all the major fisheries in South Australia operate in accordance with fishery management plans that determine the primary management objectives of the fishery. Economic performance indicators are a feature of these plans and annual reports for the marine scalefish fishery have been prepared for 1997/98 and 1998/99 (EconSearch 1999, 2000).

The results provided in these reports and the underlying data used to generate the results have, together with the survey of garfish fishers and SARDI data, provided the basis for deriving a cost and return structure for different types of fishing enterprises operating in South Australia's garfish fishery.

# 7.5 Garfish Industry Model of Financial Performance

#### Classification of garfish fishers

Data available indicated that the licence holders operating in the fishery vary considerably in the time they spend targeting garfish. To generate a model of fishery performance, it was necessary to stratify the population of garfish fishers according to the costs, catch, income and nature of the garfish fishing operation.

The dab net and haul net sectors of the garfish fishery can be classified into three groups according to the quantity of catch per fisher in the fishery. The frequency distribution of licence holders of garfish catch is summarised in Table 7.1.

Dal	b Net Sector	Hau	Net Sector
Catch (kg) No. of licences		Catch (kg)	No. of licences
0-500	30	0-1,000	25
501 - 3,000	17	1,001 - 10,000	52
3,001 - 7,500	5	10,001 - 20,000	10
Total	52	Total	87

# Table 7.1: Number of licence holders by garfish catch, 1998/99 <sup>a</sup>

<sup>a</sup> These summary data are derived from frequency distributions with intervals of 500kg for the dab net sector and 1,000kg for the haul net sector.

# **Representative operators**

For the purpose of aggregating from representative operators to the whole fishery, the licence holders in the lowest catch category were excluded from the analysis. It was estimated that these licence holders, who made up around 40 per cent of the number of licence holders with recorded garfish catch in 1998/99, contributed less than 5 per cent of total catch in that year.

For the middle and high catch categories in each sector, a "representative" operator was derived from the available survey responses and the base data used in the Economic Indicators reports (EconSearch 1999, 2000).

The summary features for each of the representative operators are provided in Table 7.2. These representative operators provide a reasonable basis for aggregating to the total fishery. For example, on the basis of the average catch per operator and the number of operators in each category given in Table 7.2, the total catch for the garfish fishery is estimated to be 406.6 tonnes, just over 96 per cent of the actual catch recorded for 1998/99.

# Table 7.2: Base case data for garfish fishers, 1998/99

	Dab Net O	perators	Haul Net C	Operators
	High catch	Low catch	High catch	Low catch
No. of operators <sup>a</sup>	5	17	10	52
Time targeting garfish <sup>b</sup>	50%	20%	50%	20%
Garfish Catch				
Av no. of trips/operator <sup>c</sup>	65	23	127	33
Av catch/trip (kg) <sup>d</sup>	78	65	114	123
Av catch/operator (kg) <sup>e</sup>	5,070	1,495	14,478	4,059
Size Category of Catch <sup>f</sup>				
Small	15%	15%	25%	25%
Medium	40%	40%	60%	60%
Large	45%	45%	15%	15%
Income/Operator				
Garfish income <sup>g</sup>	\$33,457	\$9,866	\$73,123	\$20,500
Other income <sup>h</sup>	\$13,876	\$22,202	\$13,876	\$22,202
Total income	\$47,333	32,067	\$86,999	\$42,702
Costs & Returns/Operator				
Total cash costs <sup>i</sup>	\$28,343	\$28,815	\$47,099	\$36,729
Depreciation <sup>j</sup>	\$10,625	\$10,625	\$15,625	\$15,625
EBIT <sup>k</sup>	\$9,437	-\$6,301	\$26,061	-\$7,865

<sup>a</sup> Derived from Table 7.1. These operators account for over 95 per cent of the State's total garfish catch.

- <sup>b</sup> Based on the number of target garfish effort (boat days) data that corresponds to frequency distribution of catch data (Table 7.1) and average days fished by licence holders in the marine scalefish fishery.
- <sup>c</sup> Target garfish data (boat days) data provided by SARDI.
- <sup>d</sup> Weighted average catch per trip calculated over a 12 month period, July 1998 to June 1999 SARDI data.
- <sup>e</sup> Calculated as the number of trips per operator times the average garfish catch per trip.
- <sup>f</sup> Derived from the operator survey.
- <sup>g</sup> Calculated on the basis of monthly catch and monthly prices.
- <sup>h</sup> Derived from EconSearch (2000).
- <sup>i</sup> Calculated on the basis of survey responses and EconSearch (2000).
- <sup>j</sup> Derived from EconSearch (2000).
- <sup>k</sup> Earnings Before Interest and Tax.

# Economic performance

The base case data (Table 7.2) were used to derive a measure of the economic performance of the garfish fishery. For this analysis, economic rent was used as the indicator of the fishery's economic performance.

The analysis is somewhat complicated by the fact that each of the operators targeting garfish spend a good deal of their time targeting other species as well. While the direct costs such as fuel and labour can be attributed to garfish or other species in a relatively straightforward way, allocating overheads between the different targeted species is more difficult.

As a result, performance indicators were estimated for the total fishing operations of the licence holders who target garfish rather than just the garfish fishery. As the objective of the analysis is to estimate the economic consequences of changes in the management of the garfish fishery, these should be fully reflected in the changes in the estimated economic rent.

In general terms, economic rent can be defined as the difference between the price of a good produced using a natural resource and the unit costs of turning that natural resource into the good. In this case, the natural resource is that part of the marine scalefish fishery that includes garfish and other species targeted by garfish fishers. The good produced from this resource is the landed garfish and other targeted species.

The estimated economic rent generated by garfish fishers is shown in Table 7.3. The aggregate income of around \$3.8 million is made up of sales of garfish (\$2.1 million) and other species (\$1.7 million). The costs incurred in generating that income includes labour (hired labour and imputed return to owner operators - \$1.2 million), materials and services (such as fuel, bait, overheads such as administration and licences - \$1.7 million), depreciation (\$1.1 million) and the opportunity cost of the capital applied to the fishery (\$0.9 million). The opportunity cost is equivalent to what the fishers' investment could have earned in the next best alternative use.

Determining the opportunity cost of capital involves an assessment of the degree of financial risk involved in the activity. For a risk-free operation, an appropriate opportunity cost of capital might be the long-term real rate of return on government bonds. The greater the risks involved, the greater is the necessary return on capital to justify the investment in that particular activity. For this analysis the long-term (10 year) real rate of return on government (treasury) bonds of 5 per cent has been used and a risk premium of 5 per cent has been applied given the relatively high risk nature of the industry.

What remains after the value of these inputs (labour, capital, materials, services) has been deducted is the value of the natural resource itself. It was estimated that there was no economic rent generated by garfish fishers in 1998/99, with a calculated value of -\$1.1 million. However, it is clear from the data provided in Table 2, that the garfish fishery itself is generating positive returns where those with a high level of dependence on garfish (high catch dab and haul net operators) generating positive returns while those with a high level of dependence on other species (low catch operators) yielding negative returns.

	1998/99
	( <b>\$m</b> )
Gross Income	3.87
Less Labour	1.19
Less Materials & /Services	1.70
Less Depreciation	1.15
Less Opportunity Cost of Capital (@10%)	0.92
Economic Rent	-1.07

# Table 7.3: Estimated economic rent generated by garfish fishers – base case, 1998/99

# 7.6 Assessment of Management Strategies

The objective of the analysis was to make an assessment of changes to the current management arrangements for the garfish fishery. The management changes that were assessed are:

- Increase the legal size from 21 cm to 24 cm;
- Introduce a 2 month closure of the fishery
- Introduce a 2 month closure and increase the legal size from 21 cm to 24 cm

For each assessment, a number of assumptions were made to enable the model to estimate the likely economic performance of the fishery.

### Increase the legal size from 21 cm to 24 cm

- It was assumed that the size distribution of garfish for dab net fishers would change from small 15%, medium 40% and large 45% to small 0%, medium 55% and large 45%.
- The size distribution for haul net fishers was assumed to change from small 25%, medium 60% and large 15% to small 0%, medium 85% and large 15%.
- The cost of increased search and sorting times were included as a range of values in the analysis. An increase in search and sorting times impacts on the cost of fuel, provisions, repairs and maintenance and labour. For dab net operators these costs were adjusted under the following scenarios: zero cost impact (0%), low cost impact (5%) and high cost impact (10%). For haul net operators the corresponding values were 0%, 10% and 20%. These values were higher than for dab

net operators reflecting the additional sorting time that would be expected for the haul net operators.

- Because of the uncertainty of the possible costs associated with new or modified nets, these costs were also included as a range of values in the analysis for the haul net operators. Net costs were adjusted under the following scenarios: zero cost impact (\$0), low cost impact (\$2,500 per operator) and high cost impact (\$5,000 per operator).
- Catch levels were assumed to remain the same although it is recognised that there would be an adjustment period of possibly a couple of years before that would be case.
- No long-term improvement in total catch was incorporated into the analysis.
- Effort (number of days fished) in targeting garfish and other species was assumed to remain unchanged for both dab net and haul net fishers.

# Introduce a 2-month closure of the fishery

- It was assumed the fishery would be closed for 2 months of the year. For modelling purposes, the actual months for closure were assumed to be those with the lowest gross income per day (which in turn were determined by monthly prices and monthly CPUE). For both the haul net and dab net sectors, the months with the lowest gross income per day were January and February.
- It was further assumed that the effort would be transferred to the months with the highest gross income per day. For the dab net fishery these are the months of November and December. For the haul net fishery these are the months of June and July.
- The size distribution of the garfish catch for both dab and haul net fishers was assumed to remain unchanged with the closure, although it could be expected that the size distribution for the haul net sector in particular would increase if net fishing does not occur during the months when smaller fish are more abundant.
- Effort (number of days fished) was assumed to remain the same, simply transferred between months. Catch levels change as a result, as more effort is occurring in the high catch rate month.
- It was recognised that CPUE is likely to change in some months as a result of the closure and shift in the timing of effort. In the months with increased effort CPUE was adjusted under the following scenarios: zero CPUE impact (0% decline), low CPUE impact (10% decline) and high CPUE impact (20% decline). CPUE was assumed to remain unchanged from the base case for all other months.
- Similarly, it was recognised that the price received for garfish is likely to change in some months, particularly in the months to which effort has been transferred. In the months with increased effort,

price was adjusted under the following scenarios: zero price impact (0% decline), low price impact (10% decline) and high price impact (20% decline). Price was assumed to remain unchanged from the base case for all other months.

It is worth reiterating that it is uncertain what the impact of management changes will be in terms of CPUE, size and age distribution, fishing time and market prices, among other things. The analyses were based on a simple economic model of the fishery and detailed consideration of all the biological effects and interactions was beyond the scope of the study and a number of assumptions, as specified above, have been made. A significant improvement would be the development of an integrated bio-economic model in which the biological and economic interactions of the fishery could be better specified.

#### 7.7. Results

The results are presented in a way that show the change in economic performance of the fishery from the base case. As indicated in Table 7.3, the economic rent generated by garfish fishers in 1998/99, the base case result, was estimated to be -\$1.07 million.

#### Increase the legal size from 21 cm to 24 cm

The financial implications for the garfish fishers of an increase in legal size to 24 cm was estimated under a range of assumptions regarding the impact of the changes on operating and capital costs (zero, low and high).

The values in column (1) of Table 7.4 are based on the assumption that the change in legal size would have zero impact on the cost of fishing operations. The financial outcomes under this set of assumptions were measured in three ways. First, the total annual economic rent generated by garfish fishers was estimated to be -\$890,000. Second, although the estimated economic rent is negative, it does represent an increase of \$180,000 over the base case. Third, it was estimated that the change would result in an increase in average profit of \$0.45 per kilogram of garfish<sup>1</sup>.

Under the low cost scenario (column 2), the financial results are less attractive than under the zero cost scenario. The improvement in economic rent over the base case is \$100,000 and the increase in average profit per kilogram is 28 cents. Under the high cost assumptions (column 3), the improvement over the base case is smaller again but still positive.

<sup>&</sup>lt;sup>1</sup> Improvements in profit per kilogram were measured in terms of earnings before interest and tax (EBIT). This can give a different result to improvements in economic rent per kilogram as EBIT does not account for the opportunity cost of capital.

	Cost scenario	s of an increase in l	egal size to 24cm
Assumptions	Zero cost impact (1)	Low cost impact (2)	High cost impact (3)
Increase in search & sorting time – dab netters	0%	5%	10%
Increase in search & sorting time – haul netters	0%	10%	20%
Increase in capital costs (\$/fisher) – dab netters	\$0	\$0	\$0
Increase in capital costs (\$/fisher) – haul netters	\$0	\$2,500	\$5,000
Results:			
Total Annual Economic Rent	-\$0.89m	-\$0.98m	-\$1.06m
Improvement in Annual Economic Rent over Base Case	\$0.18m	\$0.10m	\$0.01m
Improvement in garfish profit per kg (EBIT/kg)	\$0.45/kg	\$0.28/kg	\$0.10/kg

## Table 7.4: Financial implications of an increase in legal size to 24 cm

The analysis was extended to investigate under what set of cost increases would the change to an increase in legal size to 24cm be no longer worthwhile. The results for three sets of costs assumptions are shown in Table 7.5.

The first scenario indicates that with no change in the search and sorting time costs but an increase in the cost of nets of \$13,100 per haul net fisher, the potential gains from an increase in legal size to 24cm would be completely nullified.

The second breakeven scenario indicates that the gains from an increase in legal size would be fully offset with an increase in the search and sorting time costs of 18% for dab net fishers and 36% for haul net fisher (no change in the cost of nets or other equipment).

The third scenario, involving an increase in the search and sorting time costs of 10% for dab net fishers and 20% for haul net fishers plus a \$5,800 increase in the cost of nets for haul net fishers, would also bring about a breakeven result.

	Cost scenarios to generate a breakeven outcome					
Assumptions:	(1)	(2)	(3)			
Increase in search & sorting time –dab netters	0%	18%	10%			
Increase in search & sorting time -haul netters	0%	36%	20%			
Increase in capital costs (\$/fisher) – dab netters	\$0	\$0	\$0			
Increase in capital costs (\$/fisher) – haul netters	\$13,100	\$0	\$5,800			
Results:						
Improvement in Annual Economic Rent over Base Case	\$0.0m	\$0.0m	\$0.0m			

## Table 7.5: Increase in legal size to 24 cm – breakeven assumptions

# Introduce a 2-month closure of the fishery

The financial implications for the garfish fishers of a 2-month closure of the fishery were estimated under a range of assumptions regarding the impact of the changes on CPUE and garfish prices (zero, low and high).

The values in column (1) of Table 7.6 are based on the assumption that a 2month closure of the fishery and a transfer of effort to the high catch months would have zero impact on price and CPUE in the high catch months. The financial outcomes under this set of assumptions were measured in three ways. First, the total annual economic rent generated by garfish fishers was estimated to be -\$230,000. Second, although the estimated economic rent is negative, it does represent an increase of \$850,000 over the base case. Third, it was estimated that the closure would result in an increase in average profit of \$1.57 per kilogram of garfish.

Under the low price and low CPUE impact scenario (column 2), the financial results are less attractive than under the zero impact scenario. The improvement in economic rent over the base case is \$530,000 and the increase in average profit per kilogram is \$1.04. Under the high price and CPUE impact assumptions (column 3), the improvement over the base case is \$270,000 for the fishery as a whole and EBIT is \$0.66/kg higher.

	CPUE & Pri	ce Scenarios: 2-n	nonth closure
Assumptions:	Zero price & CPUE impact (1)	Low price & CPUE impact (2)	High price & CPUE impact (3)
Fall in CPUE in high value months-dab and haul netters	0%	10.0%	20.0%
Fall in price in high value months-dab and haul netters	0%	10.0%	20.0%
Results:			
Total Annual Economic Rent	-\$0.23m	-\$0.55m	-\$0.81m
Improvement in Annual Economic Rent over Base Case	\$0.85m	\$0.53m	\$0.27m
Improvement in garfish profit per kg (EBIT/kg)	\$1.57/kg	\$1.04/kg	\$0.66/kg

# Table 7.6: Financial implications of a 2-month closure and transfer of effort

The analysis was extended to investigate under what price and CPUE impacts would the 2-month closure be no longer worthwhile. The results for three sets of assumptions are shown in Table 7.7.

The first scenario indicates that a 30% decline in both CPUE and price in the 2-months to which effort is transferred would completely nullify the potential gains from a 2month closure. The second breakeven scenario indicates that the gains from a 2-month closure would be fully offset with a 52% decline in CPUE in the 2-months to which effort is transferred (no change in garfish prices from the base case). The third scenario, involving a 49% decline in prices in the 2-months to which effort is transferred but no change in CPUE from the base case, would also bring about a breakeven result.

	Impact scen	Impact scenarios to generate a breakeven outcome				
	(1)	(2)	(3)			
Assumptions:						
Fall in CPUE in high value months-dab and haul netters	30%	52%	0%			
Fall in price in high value months-dab and haul netters	30%	0%	49%			
Results:						
Improvement in Annual Economic Rent over Base Case	\$0.0m	\$0.0m	\$0.0m			

Table 7.7: 2-month closure and transfer of effort – breakeven assumptions

#### Introduce a 2-month closure and increase the legal size to 24 cm

A final set of analyses was conducted to consider the financial implications for the garfish fishers of a 2-month closure of the fishery and an increase in the legal size to 24 cm (Table 7.8). Because no direct interaction between the management changes was modelled, the results are approximately the sum of those provided in Tables 7.4 and 7.6. The interpretation is identical to that provided for the earlier sets of results.

	Cost, CPUE & Price Scenarios: Increase Minimum Size to 24cm plus 2-month closure				
	Zero cost impact	Low cost impact	High cost impact		
Assumptions:					
Increase in search & sorting time –dab netters	0%	5%	10%		
Increase in search & sorting time -haul netters	0%	10%	20%		
Increase in capital costs (\$/fisher) – dab netters	\$0	\$0	\$0		
Increase in capital costs (\$/fisher) – haul netters	\$0	\$2,500	\$5,000		
Fall in CPUE in high value months-dab and haul netters	0%	10.0%	20.0%		
Fall in price in high value months-dab and haul netters	0%	10.0%	20.0%		
Results:					
Total Annual Economic Rent	\$0.03m	-\$0.40m	-\$0.80m		
Improvement in Annual Economic Rent over Base Case	\$1.10m	\$0.67m	\$0.28m		
Improvement in garfish profit per kg (EBIT/kg)	\$2.04/kg	\$1.35/kg	\$0.65/kg		

# 7.8 Conclusions

The aim of this economic analysis has been to show the potential financial impacts for garfish fishers if changes in the way the fishery is managed are implemented. There is considerable conjecture among fishers and fisheries managers about the merits, or otherwise, of the management changes that have been analysed. The objective here has not been to advocate any particular change but simply to present a set of analyses and results that may provide some insights to assist future management decisions.

In general the results of the two options signify positive but not substantial gains to the fishery. The increase in minimum legal size would appear to bring only a marginal benefit to the fishery, particularly if there are additional fishing costs associated with extra searching and sorting time and/or additional gear costs for haul net fishers. It is likely that the benefits from this change would be greater, on a per kilogram basis, for dab net fishers than haul net fishers, as the additional costs associated with the change would impact more heavily on the haul net fishers.

An important proviso to the results needs to be reiterated. That is, the analysis has not taken into account any improvement in the sustainable catch of the fishery associated with the management changes. If, for example, the increase in minimum legal size from 21 cm to 24 cm resulted in an increase in the sustainable catch of the fishery, then the results presented here would underestimate the actual benefit. A preliminary yie ld per recruit model, based on biological parameters from the SA garfish fishery during the 1980's, concluded that very slight gains in yield would occur if the minimum legal size was raised, at relatively high levels of fishing effort (Jones et al, 1990). A more detailed integrated age structured biological model, incorporating updated catch and effort data and detailed growth and reproduction information is currently being developed (McGarvey, FRDC grant  $99/145^2$ ).

The results indicate that the gains from a 2 month seasonal closure, where effort is shifted to more profitable times of the year, are potentially higher than for an increase in minimum legal size. This would suggest, indirectly, that there are likely to be benefits to the fishery from the introduction of a quota. By capping the quantity of fish that can be taken, individual fishers will target the fishery at times when net returns are greatest. This would have the added advantage of avoiding situations of oversupply in the market at times when there is an abundance of fish, which would otherwise result in depressed market prices.

Of course, there are significant issues associated with the introduction of quota that would need to be considered, not least of which is a closer examination of the expected economic benefits. These issues would also include method of quota allocation, adequacy of base data to set a TAC that reflects the sustainable harvest for the resource, and the cost and effectiveness of stock assessment, monitoring, surveillance and compliance.

One of the difficulties in undertaking the analysis was being able to accurately specify the biological implications of the various management changes. It is uncertain what the impact of management changes will be in terms of CPUE, size and age distribution, fishing time and market prices, among

other things. The analyses were based on a simple economic model of the fishery. Assumptions regarding the biological performance of the fishery resulting from various management changes were imposed on the model to generate estimates of financial and economic outcomes. A significant improvement would be the development of an integrated bio-economic model in which the biological and economic interactions could be better specified.

### 7.9 Garfish Marketing

A study of the nature of demand for garfish in South Australia, undertaken over 20 years ago (Gleeson 1979), indicated two fundamental market characteristics that are unlikely to have changed over that time. The first was that the price elasticity of demand is relatively low (i.e. price inelastic). This means that a relatively large fall in the price of garfish will result in only a slight increase in the quantity demanded and total revenue will fall. The second finding was that the income elasticity of demand is negative and large. This means that a relatively small increase in household income will result in a relatively large decrease in the quantity of garfish demanded. In other words consumers view garfish as an "inferior" good and will substitute other fish species (e.g. whiting) or other sources of protein for garfish as their incomes increase.

These characteristics of the garfish market in South Australia would indicate that it is almost imperative that fishers and processors look to other markets if they are to obtain positive net returns over time. The economic analysis in the previous sections was undertaken to demonstrate the potential benefits from changes in harvesting methods that would result in either an increase in average marketed size (increase legal minimum size) or a change in the time at which the product is brought to the market (seasonal closure). The potential benefits from such changes could be enhanced and sustained if markets offering consistent price premiums over the local market could be accessed.

Consequently, some investigations into the marketing of garfish outside South Australia were undertaken because of the potential for obtaining higher prices in those markets. The investigations were of a very preliminary nature due to the lack of published data on garfish in other markets. Most of the information was of an anecdotal nature provided by fishers and processors who have experience in interstate and overseas markets and others. A number of general points about the potential marketing of South Australian garfish can be made.

1. Interstate markets - prices in Sydney and Melbourne markets are very similar to those in South Australia, and so there would seem to be little advantage in incurring the additional marketing

<sup>&</sup>lt;sup>2</sup> McGarvey, R. (1999) Stock assessment models with graphical user interfaces for key South Australian marine fin fish stocks. FRDC grant 99/145.

costs to place product on those markets. With the decline in Victorian landings in the last few years, and assuming the demand is still there, there might be a niche that can be filled by the other main producer states of South Australia, Tasmania and Western Australia.

- 2. Japan 1999 Japanese auction prices for halfbeaks (similar to southern sea garfish) range from \$14/kg for imported fish, to \$27/kg for locally caught fish. Given the movements in the exchange rate since that time the price for halfbeaks would be equivalent to approximately \$15/kg at current exchange rates. If allowance were made for import duties, freight and packaging, the net return to the fisher would be around \$5/kg. The freight cost is based on utilising an AV container. During the rock lobster season (November to May) there would be opportunities to share space in an AV container with a shipment of rock lobster. Similar opportunities may exist with the export of tuna and other seafood at other times of the year. The freight cost could be around \$2/kg higher if the product was sent as loose freight. Previous experience in the Japanese market would suggest that price does not vary with size as it does in South Australia, which would provide even greater premiums for small and medium sized garfish.
- 3. Other foreign markets In general, there does not appear to be the same market for marine scale fish in other east and south east Asian markets as there is in Japan and therefore there are not the price premiums available to justify the packaging and freight costs involved. There is a need to investigate other possible overseas markets, in particular the European market Greece, Italy, France, Spain, Portugal where species taxonomically affiliated to southern sea garfish (e.g. *Belone belone*) are traditionally popular fish and are taken in fisheries that are likely to be under significant pressure from over harvesting and environmental degradation.
- 4. There is a market for garfish as bait for recreational fishers, both in Australia and in other high recreational fishing areas (e.g. Florida; McBride *et al*, 1996). With the decline in the pilchard fishery in WA, which had been a traditional market for bait for recreational fishers throughout Australia, there might be a small niche for garfish here. Garfish is a traditional bait species for catching mulloway, billfish and dolphin fish.
- 5. For dab net fishers there may be some opportunities from differentiating the quality of garfish caught by hauling nets and dab nets. Fish caught in hauling nets can be "scaled", which may result in discounting in some segments of the market. Depending on the relative perceptions by processors / consumers of fish quality, dab netted fish may be able to attract a premium in upmarket areas (eg sashimi in the Sydney market).

6. The analysis by Gleeson (1979) suggested garfish is considered an inferior good among South Australian consumers (i.e they will substitute other goods for garfish as their income levels increase). Changing this perception in the market is another area that could lift returns to the fishery. One avenue for doing this would be to highlight the fact that garfish has good eating qualities in terms of human health. According to Nichols *et al* (1999), levels of oils and omega-3 fatty acids for sea garfish provide a high quality fish diet for human consumption. With the species being acceptable to a vast socio-economic range of traditional markets, takeaway, restaurant and fresh fish, the species could be marketed stressing its health food attributes.

#### Acknowledgments

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## DIRECT BENEFITS AND BENEFICIARIES

This study has provided evidence, through validation techniques, that sectioned otoliths are an acceptable method for age determination of sea garfish, and hence the estimation of age composition of catches. It has also confirmed the need for calibration of the age determination when it is undertaken by more than one laboratory.

Using catch and effort data for different gear types in the SA commercial fishery, there was little evidence of interaction between hauling and dab nets, mainly because of temporal/spatial differences in peak fishing effort by the two methods. However, catch per unit effort data used on it's own for assessment purposes, is of concern if temporal changes in fishing techniques are not taken into account. This study showed that if future comparisons of the relative abundances of the same species between states are to be undertaken using catch and effort data, standardisation of fishing methods and gear types between the states is essential.

This study has successfully highlighted that time series of age composition data of the fished component of stocks should be included in future stock assessments of this species. The SA garfish fishery has these sets of data and will now be used, along with the catch and effort, and other biological parameters in developing the age structure model of the SA garfish fishery (FRDC project 99/145).

Although the egg surveys over seagrass beds were unsuccessful, additional evidence that sea garfish spawn in waters associated with shallow seagrass areas was provided in this study. Also, the entrainment of the highest densities of garfish larvae in the northern GSV waters through wind driven surface currents, where highest concentrations of seagrasses occurred, further inferred the importance of seagrasses in the early life history of this species. The neuston net used to sample the garfish larvae was also shown to be a cost-effective device for monitoring abundance of larvae over extensive areas. However, long term monitoring of larval abundances linked with the age compositions in the fishery are necessary if they are to be used as a method for monitoring pre-recruit indices.

The economic study of the SA garfish fishery, provided good evidence that those fishers who displayed high dependence on garfish as part of their multi-species marine scalefish fishing operations, generated positive returns. The preliminary economic model also showed that economic benefits would accrue if changes in management strategies (i.e. altering size limits and/or fishing seasons) took place; however, improvements to the analysis would occur if an integrated bio-economic model was developed.

Finally the results of this project have considerable benefits to the SA Marine Scalefish Fishery Management Committee and the South Australian Recreational Fishers Advisory Committee, with the current consideration to changes in the management of the sea garfish fishery, including minimum legal lengths, and recreational bag and boat limits in that state.

# CONCLUSIONS

This report is the outcome of the recommendations from the 1995 garfish workshop which identified at the time, many gaps in our understanding on the fishery biology, habitat association and economic status of sea garfish, *Hyporhamphus melanochir* in southern Australian waters (WA, SA, Victoria and Tasmania). In most of the southern states, sea garfish comprises a significant part of the inshore multi-species commercial net and recreational line fisheries, as seen through increasing development through higher harvesting rates. The project underpinning this report has been a collaborative one between SARDI, WA Fisheries, MAFRI, the South Australian Museum (SAM) and EconSearch Pty Ltd.

Fundamental to the future management of this species is knowledge on whether there are genetically distinct stocks present within its broad geographical range. The mitochondrial DNA based stock discrimination study on adult fish (Chapter 2), concluded that the species showed significant regional differentiation, except for those occurring within the SA gulfs and Victorian waters. Therefore, the species should comprise four management units: a) WA, b) west coast of SA, c) SA gulfs and Victoria, and d) Tasmania.

Before an assessment of the size and age structure of sea garfish could be made, the best method for determining the growth rates and age was successfully investigated through the age validation of otoliths (marginal increments and tetracycline marking) as well as calibration between two research laboratories (Chapter 3).

Sea garfish were found to be a finfish species which exhibited medium growth rates and life span. Maximum ages for fish from SA, Vic and WA were 6, 6 and 10 years, respectively. In all states, growth rates of male garfish were significantly more rapid than for females, however, the mean maximum lengths for female fish was higher than those for males. Fish reached the minimum legal lengths in each state (21, 20 and 23 cm TL, resp.) at similar ages (13 - 15 months).

The effect of the fishery on the stocks was determined through a detailed temporal analysis of the catch, fishing effort and catch rates (Chapter 4) as well as, in the case of the SA fishery, the changes in size and age composition of the commercially fished component of the stock over the history of the

fishery (Chapter 5). The commercial fishery for sea garfish is part of a multispecies net fishery in all three states, with hauling nets being the main method of capture. However, because of different regulations in the net dimensions and the method of recording fishing effort, it has not been possible to use catch rate data to compare the relative abundances of sea garfish between the three states. In SA, the state with the highest catch and effort in the commercial fishery, trends in catch per unit effort (CPUE) between 1983/84 and 99/00 were either stable or increasing in all regions. In contrast, garfish catches in the Victorian fishery declined over the same period, mainly due to a reduction in fishing effort and CPUEs for haul seine and ring nets in Port Phillip Bay and Western Port Bay. In WA, the smallest commercial fishery of the three states, catches have risen slightly over time, however, meaningful interpretation of CPUEs in the fishery could not be made. In the SA commercial fishery, comparison of temporal trends in CPUEs between hauling and dab nets found no evidence of any interactions between these two gear types. The information on recreational catches in all three states during the 1990s showed that they comprised up to 15% of the total state catch; however, there were insufficient temporal data for any investigation to be made of interactions with the commercial gear.

The size and age structures of commercial catches of sea garfish were determined from samples collected during measuring programs at local markets in each state between February 1998 and June 1999 (Chapter 5). The average size and age of fish caught in SA and Victoria were similar (25.5 and 25.9 cm TL; 1.6 and 1.7 yrs of age, resp.), and total mortality rates (Z) estimated from catch curves were also similar (1.9 and 1.6, resp.). However, for WA, the average size and age of fish caught were significantly higher (28. 8 cm TL, 2.2 yrs of age), with a wider range of ages (0 - 10 yrs) represented, resulting in a lower total mortality rate (0.98) than the other two states. The inter-state differences in mortality rates were probably a function of the differences in the relative size (total catches) of their respective fisheries. In the South Australian fishery, there was considerable spatial and temporal variation in size and age of fish. Additionally, fish caught by hauling nets, on average were smaller than those taken by dab netting.

Previous measuring programs undertaken in the SA fishery, dating back to 1954/55, were used to detect increasing total mortality rates of the fished stocks in the two main fishing areas, Spencer Gulf and Gulf St. Vincent. The increase in Z is suggested to be a function of higher catches in these areas, as Z only rose in those years, when respective catches also increased.

In 1997/98, sea garfish reproduced over a protracted spawning season concurrently across southern Australia, with the season in WA and SA extending from September to April, and in Victoria and eastern Tasmania from October to March (Chapter 6). In SA, there were two distinct spawning peaks in Nov/Dec and February, whereas in the other states, no distinct peaks were detected. Sea garfish is

a serial batch spawner producing relatively small numbers of large eggs. Batch fecundity ranged between 93 and 3884, depending on the size. Sex ratios of fish caught in the SA fishery were found to be highly biased towards females during the spawning season, apparently due to the tendency for female fish to form larger schools in relatively shallow (< 5 m.) waters, where the hauling net fishery took place. In contrast, mature males were more widely dispersed, with higher proportions in deeper waters.

In SA, the size at 50% maturity of 21.5 cm TL was the lowest of all states (23.9 and 26.1 cm TL for Victoria and WA resp.). There is also a suggestion that the size at first maturity has decreased in SA during the past 40 years, as a general response of fish populations to fishing.

SCUBA and beam trawling surveys of demersal and adhesive eggs and neuston surveys for larvae were undertaken to determine the association of these early life history stages with the shallow water seagrass habitat of Gulf St. Vincent (Chapter 7). However, before this was undertaken, and as two species of garfish were known to potentially co-occur in this region, genetic and morphometric discrimination methods were developed to confidently identify sea garfish larvae in neuston samples from the other species (river garfish, *H. regularis*). No eggs were found in either the SCUBA or beam trawling surveys, however, some eggs were discovered, through a fish processor, attached to seagrass and filamentous algae, whilst commercial dab net fishing was being undertaken in the Bay of Shoals, KI. The association between sea garfish spawning areas and sea grass was further confirmed from the neuston surveys of larvae, where most larvae collected during 1998 and 2000 spawning seasons were found in the northern region of the GSV, which is almost entirely occupied by seagrass habitat. Wind speed and direction information during October, November and December from key sites situated around the gulf also provided an explanation of the ability of the larvae to be retained in these northern regions. Small numbers of larvae found in the southern entrance to the gulf were explained again from the wind data and the presence of dense seagrass areas adjacent to the north coast of KI. The neuston sampling method was concluded to be a cost-effective technique for determining interannual variation in larval densities, however, longterm monitoring is required to link this variability to variation in age structure of the fished component of the stock.

An economic study of the SA commercial garfish fishery was undertaken to investigate the potential for higher economic yields through improving harvest and post-harvest and marketing strategies (Chapter 8). Using data collected on the financial performance of hauling and dab net fishers, it was concluded that those fishers with a high dependence on garfish, within their other multi-species operations, generated positive returns. An economic model was then designed to examine the effects of changes in economic rent and net returns per kg from changes in management strategies (ie increase in minimum legal size and harvesting period – seasonal closure). The model showed that

although some economic gains from a rise in the min. size could occur only if there was a significant increase in the catch, greater benefits to fishers would occur with a seasonal closure during summer months. Significant improvements to the analysis would be made if an integrated bio-economic model was developed.

Some investigations were made on the inter-state and international marketing potential for garfish. Opportunities appear to be limited in other Australian markets, however, export markets, particularly to Japan may return benefits to the fishery.

The results of this project will be communicated to the garfish fishing industries and Fisheries Managers through summarising written reports as well as oral presentations at Fisheries Management Committees in each state.

# **APPENDIX 1: INTELLECTUAL PROPERTY**

Not applicable.

# **APPENDIX 2: STAFF**

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