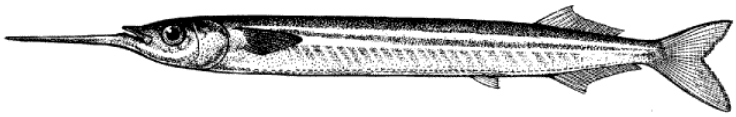
EARLY LIFE STAGES OF THE SOUTHERN SEA GARFISH, *HYPORHAMPHUS MELANOCHIR* (VALENCIENNES, 1846), AND THEIR ASSOCIATION WITH SEAGRASS BEDS

CRAIG J. NOELL



SCHOOL OF EARTH AND ENVIRONMENTAL SCIENCES
THE UNIVERSITY OF ADELAIDE
SOUTH AUSTRALIA

Submitted for the Degree of Doctor of Philosophy
on January 11, 2005

APPENDIX A. Publication from *Marine Biotechnology* that was based on results presented in Chapter 3.

Mar. Biotechnol. 3, 509–514, 2001
DOI: 10.1007/s10126-001-0059-5

MARINE BIOTECHNOLOGY

Short Communication

Molecular Discrimination of Garfish *Hyporhamphus* (Beloniformes) Larvae in Southern Australian Waters

Craig J. Noell,[1,2,*] S. Donnellan,[3] R. Foster,[3] and L. Haigh[3]

[1]*Department of Environmental Biology, Adelaide University, Australia 5005*
[2]*South Australian Research and Development Institute (Aquatic Sciences), P.O. Box 120, Henley Beach, Australia 5022*
[3]*South Australian Museum, North Terrace, Adelaide, Australia 5000*

Abstract: A multiplex polymerase chain reaction (PCR) assay developed for discrimination between garfish larvae (family Hemiramphidae, order Beloniformes) found in southern Australian waters was 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% of tests for *H. melanochir* and 94% of tests for *H. regularis*.

Key words: garfish, *Hyporhamphus*, larva, mtDNA, PCR, species identification.

INTRODUCTION

Garfishes (order Beloniformes) are targeted in major commercial and recreational fisheries in many parts of the world, including southern Australia (Collette, 1974). In this region, here defined as including southern Western Australia (W.A.), South Australia (S.A.), Victoria, and Tasmania, the garfish fishery is a multispecies fishery, with catch effort principally focused on 2 species, the southern sea garfish *Hyporhamphus melanochir* (Valenciennes 1846) and the river garfish *H. regularis* (Günther 1866).

Recent efforts to manage the commercial and recreational garfish fishery in southern Australia have focused on developing population models for predicting the strength of recruitment. Critical for such models is the identification of spawning and recruitment locations and populations. This depends on being able to identify garfish species at all life-history stages. While identifying adult garfish in southern Australia is not problematic (Collette, 1974), some uncertainty exists when allocating larvae to a particular species on the basis of traditionally used morphological characters such as pigmentation, meristic counts, and body measurements (C.J. Noell, unpublished data). This uncertainty is due to intraspecies variation, growth and development-related changes, and the occasional requirement for extrapolation between different-sized specimens. This is complicated by the fact that larvae of both species have been collected together in the same samples (C.J. Noell, unpublished data).

The identification of closely related species of organisms is a frequent problem in marine biology, especially in the egg or larval stages of the life cycle (Burton, 1996). The advent of DNA analysis based on polymerase chain reaction (PCR) has provided a quick, often cheap, and potentially automatable method to solve these problems (Silberman and Walsh, 1992; Banks et al., 1993; Medeiros-Bergen et al., 1995; Grutter et al., 2000; Rocha-Olivares et al., 2000). Here we present a method, based on PCR of the mitochondrial

Received January 17, 2001; accepted April 25, 2001.
*Corresponding author: telephone +61-8-8303-3649; fax +61-8-8303-6222; email craig.noell@student.adelaide.edu.au

control region (*CR*), that discriminates between life stages of *H. regularis* and *H. melanochir* or the eastern sea garfish *H. australis* (Steindachner 1866) in southern Australia. Initially we partially sequenced the *CR* from a selection of adult garfish samples geographically representative of each species' range to phylogenetically identify nucleotide sites that distinguish between their mitochondrial lineages. We developed a multiplex PCR assay that distinguishes the species on the basis of the presence or absence of diagnostic bands and verified the assay on an extensive sample of adults from each species.

MATERIALS AND METHODS

Specimens Examined

Adult samples for DNA analysis were collected for the 2 *Hyporhamphus* species found in southern Australian waters. A sample of *H. australis* 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 Victoria in the event the distribution of *H. australis* extended 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 1). 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.J. 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 length of tissue 2 to 4 mm 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. A fragment of approximately 443 to 462 bp from the mitochondrial *CR* was PCR amplified using primers H16498 (designed by Meyer et al., 1990) and L-M252 (Table 2). That this product was of mitochondrial origin rather than a nuclear paralogue (Zhang and Hewitt, 1996) was verified by Donnellan et al. (2001). Amplifications were carried out on a Hybaid Omn-E Thermal Cycler.

Table 1. Sample Details of Garfish Examined for Mitochondrial DNA Variation*

Location	n_s	n_{PCR}	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 Victoria	1	3	Adult
Corner Inlet Victoria	1	3	Adult
Marion Bay Tasmania (MB)	1	3	Adult
Flinders Island Transmania (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 Victoria	2	11	Adult
Hyporhamphus australis			
Broken Bay N.S.W.	1	—	Adult
Arrhamphus sclerolepis			
N.S.W.	1	—	Adult

*n_s indicates sample size for nucleotide sequencing; n_{PCR}, sample size for PCR assay. Locality codes are in parentheses.

Table 2. 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′

Reaction volumes of 50 µl contained 50 to 100 ng of template DNA, 0.2 µM of each primer, 0.2 mM each of dATP, dTTP, dGTP, and dCTP, 4 mM $MgCl_2$, 1× GeneAmp PCR Buffer II (PerkinElmer) and 1 U AmpliTaq Gold DNA polymerase (PerkinElmer). PCR cyclic conditions were 95°C for 9 minutes, 50°C for 1 minute, 72°C for 1 minute for 1 cycle;

94°C for 45 seconds, 50°C for 45 seconds, 72°C for 1 minute for 34 cycles; and 72°C for 6 minutes, 30°C for 10 seconds for 1 cycle.

PCR products were purified for sequencing with the UltraClean 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 Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems Inc.). Reaction volumes of 10 µl contained 50 to 100 ng of PCR product, 0.5 µM primer, and 3 µl BigDye. PCR cyclic conditions were 94°C for 30 seconds, 50°C for 15 seconds, 60°C for 4 minutes for 25 cycles, and 60°C for 4 minutes, 30°C for 10 seconds for 1 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 to 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 2), 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 hour 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 1). We also visually inspected for the L-M282 primer sequence in the *CR* sequences of a further 67 *H. melanochir*, sampled from across the species range, available from Donnellan et al. (2001). We subsequently PCR tested larvae that we could unequivocally assign to species on the basis of morphology from a much larger series of samples of each species (Table 1).

Results and Discussion

We initially sequenced part of the mitochondrial *CR* from 11 adult fish to survey nucleotide sequence variation in southern Australian *Hyporhamphus*. Haplotype diversity of *H. melanochir CR* was surveyed previously by Donnellan et al. (2001) 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. We chose 5 haplotypes from this study to represent the haplotype lineages identified by phylogenetic analyses of these data. Donnellan et al. (2001) also tested whether the PCR primers amplified nuclear paralogues of the *CR* in *Hyporhamphus*. These tests based on titrations of enriched mitochondrial DNA did not show any evidence that the primers we used were capable of amplifying nuclear paralogues of the *CR* in either *H. melanochir* or *H. regularis*.

The final alignment of garfish *CR* haplotypes included 423 sites. For phylogenetic analyses, alignment gaps (indels), used to optimize the sequence alignment, were treated as a fifth state. Under the MP criterion of optimality, multisite 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 1). Two major lineages, strongly supported by bootstrapping (100%), are apparent among the *Hyporhamphus* haplotypes, one including both *H. australis* and *H. melanochir* and the other including *H. regularis*. Each lineage is characterized 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 2 major lineages (21.6% to 25.6% uncorrected sequence divergence) and the small

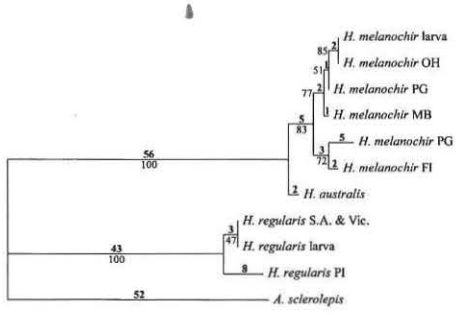


Figure 1. Phylogenetic relationships among garfish *CR* haplotypes recovered with maximum parsimony. Unbolded numerals represent bootstrap proportions from 2000 pseudoreplicates; numerals in boldface are the number of sites that change along that branch. Refer to Table 1 for locality codes.

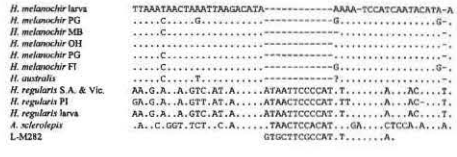


Figure 2. Part of the nucleotide sequence alignment of the mitochondrial *CR* haplotypes from adult and larval *H. melanochir* and *H. regularis, H. australis,* and the outgroup *A. sclerolepis.* This represents the section of the alignment from which the PCR primer L-M282, used to discriminate between *H. melanochir* and *H. regularis,* was designed. This section is from nucleotide sites 155 to 210 of the complete alignment. Dots (.) indicate identical nucleotides to *H. melanochir* larva; dashes (-) indicate alignment gaps; question mark (?) indicates unknown nucleotide.

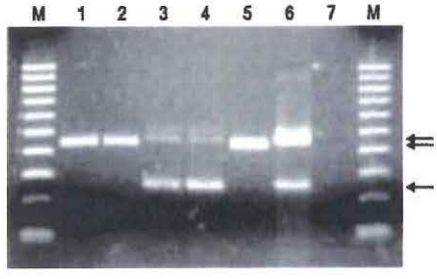


Figure 3. Electrophoretic discrimination between mtDNA *CR* multiplex PCR products from *H. melanochir* (443 bp) and *H. regularis* (462 and 264 bp). Lanes 1 and 2, *H. melanochir* larvae; lanes 3 and 4, *H. regularis* larvae; lane 5, *H. melanochir* adult; lane 6, *H. regularis* adult; lane 7, no template PCR control. M indicates 100-bp ladder for molecular weight marker. Arrows indicate the position of DNA products of 264, 443, and 462 bp.

genetic distances among conspecific haplotypes (0% to 3.2% 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 1). Although both *H. australis* and *H. melanochir* are clearly genetically distant from *H. regularis,* the *CR* haplotypes of *H. australis* and *H. melanochir* are genetically much more closely related. Collette (1974) recognized the latter pair as separate species because of 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.

Examination of the aligned *CR* sequences revealed 2 multisite 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 6 sequenced *H. regularis* specimens and the outgroup *A. sclerolepis,* while the deletion character state was present in both *H. melanochir* and *H. australis* (Figure 2). Primer L-M282, located in the vicinity of the 12-bp indel (Figure 2), was designed to amplify in combination with primer H16498 only the *H. regularis CR.* The final PCR test used was a multiplex of the 3 primers (L-M252/L-M282/H16498). While the predicted gel phenotypes for each species were the 443-bp product only for *H. melanochir* and both the 264-bp and 462-bp products for *H. regularis* (Figure 3), 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. We 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 less than 4% and 6% for *H. melanochir* and *H. regularis,*

respectively, with 95% confidence. Larvae that had been unequivocally assigned to species a priori on a morphological basis (*H. melanochir*, $n = 19$; *H. regularis*, $n = 20$) were subsequently tested, and the gel phenotypes were 100% compatible with the predicted phenotype (Figure 3).

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 for which "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 intraspecies variation and the morphologic plasticity associated with larval growth and development, among the within-species 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 nonsequencing method that is potentially automatable, permitting analysis of large numbers of specimens and thereby avoiding much of the labor-intensive identification work using morphological criteria. Furthermore, ecologists without detailed knowledge of taxonomy or molecular biology would require only a little technical training for species discrimination.

ACKNOWLEDGMENTS

We thank P. Coutin, K. Jones, D. McKeown, D. Short, G. Wright, and N.S.W. Fisheries for assistance in obtaining samples, J. Armstrong for technical assistance, and S. Cooper and A. Fowler for comments that improved the manuscript. The project was supported by Fisheries Research and Development Corporation grant 97/133.

REFERENCES

Banks, M.A., Hedgecock, D., and Waters, C. (1993). Discrimination between closely related Pacific oyster species (*Crassostrea*) via mitochondrial DNA sequences coding for large subunit rRNA. *Mol Mar Biol Biotechnol* 2:129–136.

Burton, R.S. (1996). Molecular tools in marine ecology. *J Exp Mar Biol Ecol* 200:5–101.

Collette, B.B. (1974). The garfishes (Hemiramphidae) of Australia and New Zealand. *Rec Aust Mus* 29:11–105.

Donnellan, S., Haigh, L., Elphinstone, M., McGlennon, D., and Ye, Q. (2001). Genetic discrimination between southern sea garfish (*Hyporhamphus melanochir*) stocks of Western Australia, South Australia, Victoria and Tasmania. In: *Fisheries Biology and Habitat Ecology of Southern Sea Garfish* Hyporhamphus melanochir *in Southern Australia,* Jones, G.K., Ye, Q., Ayvazian, S., and Coutin, P. (eds.). Canberra, Australia: Fisheries Research and Development Corporation. FRDC Final Draft Report 97/133.

Faber, J.E., and Stepien, C.A. (1997). The utility of mitochondrial DNA control region sequences for analyzing phylogenetic relationships among populations, species, and genera of the Percidae. In: *Molecular Systematics of Fishes,* Kocher, T.D., and Stepien, C.A. (eds.). San Diego, Calif.: Academic Press, 129–143.

Grutter, A.S., Morgan, J.A.T., and Adlard, R.D. (2000). Characterising parasitic gnathiid isopod species and matching life stages with ribosomal DNA ITS2 sequences. *Mar Biol* 136:201–205.

Medeiros-Bergen, D., Olson, R.R., Conroy, J.A., and Kocher, T.D. (1995). Distribution of holothurian larvae determined with species-specific genetic probes. *Limnol Oceanogr* 40:1225–1235.

Meyer, A., Kocher, T.D., Basaibwaki, P., and Wilson, A.C. (1990). Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature* 347:550–553.

Miller, S.A., Dykes, D.D., and Polesky, H.F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids R* 16:1215.

Rocha-Olivares, A., Moser, H.G., and Stannard, J. (2000). Molecu-

APPENDIX A. *Continued*

lar identification and description of pelagic young of the rockfishes *Sebastes constellatus* and *Sebastes ensifer*. *Fish Bull* 98:353–363.

Schwager, S.J., Federer, W.T., Mutschler, M.A., and Scully, B.T. (1993). The effect of linkage on sample size determination for multiple trait selection. *Theoret Appl Genet* 86:964–974.

Silberman, J.D., and Walsh, P.J. (1992). Species identification of spiny lobster phyllosome larvae via ribosomal DNA analysis. *Mol Mar Biol Biotechnol* 1:195–205.

Swofford, D.L. (1999). *PAUP* Phylogenetic Analysis Using Parsimony (* and Other Methods)*. Sunderland, Massachusetts: Sinauer.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25:4876–4882.

Zhang, D-X., and Hewitt, G.M. (1996). Nuclear integrations: challenges for mitochondrial DNA markers. *Tr Ecol Evol* 11:247–251.

APPENDIX B. The complete 423 nucleotide sequence alignment of the mitochondrial *control region* haplotypes from adult and larval *Hyporhamphus melanochir* and *H. regularis*, *H. australis*, and the outgroup *A. sclerolepis*. The oligonucleotide sequence of the species-specific primer for *H. regularis*, L-M282, is included in the alignment. Boxes and arrows (➡,⬅) indicate positions and directions of primers, respectively, used in the PCR multiplex. Dots (.) indicate identical nucleotides to *H. melanochir* larva; dashes (-) indicate alignment gaps; question marks (?) indicate unknown nucleotides. R = A or G; W = A or T; S = C or G; Y = C or T; K = G or T; B = C, G or T; N = G, A, T or C. Refer to FIG. 3.1 for locality codes.

	L-M252➡									
H. melanochir larva	ATTCTAAACT	AAACTATTCT	TTGCCGGGCT	CCGCCCCAAA	-GTACATATA	TGGACATATA	CATAAATCTA	TATAGTACAT	ATATCTATGT	ATTATCCCCA
H. melanochir PG	....	....	....	....	-.........	....	....	....	....	....
H. melanochir MB	....	....	....	....	-.........	....	....	....	....	....
H. melanochir OH	....	........B.	.......S..	....	-.........	....	.......Y..	....	.........K	....
H. melanochir PG	....	....	....	....	-.........	....	....	....	..N.......	....
H. melanochir FI	....	....	....	....	-.........	....	....	....	....	....
H. australis	....	...N......	....	....	-.........	....	.......T..	..C.......	....	....
H. regularis S.A. & Vic.	.......GT.	....	....	.T........	A.........	....T....G	....T..A..	..CTA.....	.G........	....A.....
H. regularis PI	.......GT.	....	....	.T........	A.........	....T.C..G	....T..A..	..CTA.....	.G........	....A.....
H. regularis larva	.......GT.	....	.K........	.T........	A.........	....T....G	....T..A..	..CTA.....	.G........	....A.....
A. sclerolepis	??????????	??????????	??????....	........R.	-.........	.....-....	.TA.C..T..	.C........	.A..A.....	......A...

H. melanochir larva	TTCATTTATA	TTAAACATTT	ATCAATGAT-	AGAGGGTATA	TCAATGTT--	----TTAAAT	AACTAAATTA	AGACATA---	---------A	AAA-TCCATC
H. melanochir PG	....	....	.........-	....AA....	.....A..--	---------C	.....G....	.......---	---------G	...-......
H. melanochir MB	....	....	.........-	.....A....	.........--	---------C	....	.......---	----------	...-......
H. melanochir OH	....	....	.........-	....	.........--	---------.	....	.......---	---------.	...-......
H. melanochir PG	....	....	.........-	....	.........--	---------C	....	.......---	---------.	...-......
H. melanochir FI	....	....	.........-	....A.....	.....A..--	---------C	....	.......---	---------G	...-......
H. australis	....	....	.........-	.....T....	.....A..--	---------C	.....T....	.......---	---------R	...-......
H. regularis S.A. & Vic.	....	....	..G...T..-	.....AC...	...T.T..GA	TAATAA.G.A	..A.GTC.AT	.A.....ATA	ATTCCCCAT.	T..-....A.
H. regularis PI	....	....	..G.G.T..-	.....AC...	...T.T..GA	TAATGA.G.A	..A.GTT.AT	.A.....ATA	ACTCCCCAT.	TT.-....A.
H. regularis larva	....	....	..G...T..-	.....AC...	...T.T..GA	TAATAA.G.A	..A.GTC.AT	.A.....ATA	ATTCCCCAT.	T..-....A.
A. sclerolepis	..A.......	.C......AA	T.G......T	.....AC.C.	C..T.C..GA	TCAA.A..C.	GGT.TCT..C	.A.....-TA	ACTCCACAT.	..GA....CT
Primer L-M282➡									gTg cTTCgCCAT.	T.......A.

H. melanochir larva	AATACATA-A	AATGGAAGTA	TAACAGAACT	AGAATTAGTG	CCAAACGAAA	GTTTAAGACC	GATCACAACT	CTCATAAGTC	TAGTTATACC	AAGAGACACC
H. melanochir PG	.......G-.	.......A..	....	....	....	....	....	....	....	....A.....
H. melanochir MB	........-.	.......A..	....	....	....	....	....	....	....	....
H. melanochir OH	........-.	....	....	....	....	....	....	....	....	....
H. melanochir PG	........-.	.......A..	....	....	....	....	....	....	....	....
H. melanochir FI	.......G-.	.......A..	....	.........A	....	....	....	....	....	....A.....

APPENDIX B. *Continued*

```
H. australis                .........-. .......A.. .......... .......... .......... .......... .......... ......G... .......... ....A.....
H. regularis S.A. & Vic.    ..AC....T. ....A.GAA. AC.TTT..TG .T.TC.TTGC .A..CGT.T. A..A.GCC.G ATCACA.TAA .....CT... ...A...... .G..CT..A.
H. regularis PI             ..AC-...T. ....A.GAA. AC.TTT..TG .T.TC.TTGC .A..CGT.T. A..A.GCC.G ATCACA.TAA T....T.... ...A...... .G..CT..A.
H. regularis larva          ..AC....T. ....A.GAA. AC.TTT..TG .T.TC.TTGC .A..CGT.T. A..A.GCC.G ATCACA.TAA .....CT... ...A...... .G..CT..A.
A. sclerolepis              CCA.A...A. GTGA...AA. C..AGAT.GA CA.--AGTAC .AT.CGTT.. A..A.GCC.G ACCACA..AA .....C.... ...A...... .G..CT..A.

H. melanochir larva         ATCCCTAAAA TTTCAAATAT TTAATGTAGT AAGAGCCTAC CATCAGTTGA TTCCTTAATG AACACTCTTA TTGAAGGTGA GGGACAAAAA TCGTGGGGGT
H. melanochir PG            .......... C......... .......... .......... .......... ..A....... .......... ........C. ......G... ..........
H. melanochir MB            .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
H. melanochir OH            .......... .......... .......... .......... .......... .....W.... .......... .......... .......... ..........
H. melanochir PG            .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
H. melanochir FI            .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
H. australis                .......... .A........ .......... .......... .......... .......... .......... ........C. .......... ........N.
H. regularis S.A. & Vic.    ...T..G..- GA....T... .......... .....A.C.. .......... ..T....... C.T....... ....T...C. ......G..N ..........
H. regularis PI             ...T..G..- GA....T... .......... .....A.C.. .......... ..T....... C.T....... ....T...C. A.....G... ..........
H. regularis larva          ...T..G..- GA....T... .......... .....A.C.. .......... ..T....... C.T....... ....T...C. ......G... ..........
A. sclerolepis              .C.T..GG.T AA........ .......... .....A.C.. .......... ..T....... C.T....... ....T...C. ......G... ..........

                                                                                                                    ←H16498
H. melanochir larva         TTCACTTCTT GAATTATTCC TGG
H. melanochir PG            .......... .......... ...
H. melanochir MB            .......... .......... ...
H. melanochir OH            .......... .......... ...
H. melanochir PG            .......... .......... ...
H. melanochir FI            .......... .......... ...
H. australis                .......... .......... ...
H. regularis S.A. & Vic.    .......... .......... ...
H. regularis PI             .????????? ?????????? ???
H. regularis larva          .......... .......... ...
A. sclerolepis              .......... .......... ...
```

APPENDIX C. Publication from *Fishery Bulletin* that was based on results presented in Chapter 4.

Larval development of the southern sea garfish (*Hyporhamphus melanochir*) and the river garfish (*H. regularis*) (Beloniformes: Hemiramphidae) from South Australian waters

Craig J. Noell

Department of Environmental Biology
Adelaide University
South Australia 5005
Present address: SARDI Aquatic Sciences
PO Box 120
Henley Beach
South Australia 5022
E-mail address: noell.craig@saugov.sa.au

Abstract—Larval development of the southern sea garfish (*Hyporhamphus melanochir*) and the river garfish (*H. regularis*) is described from specimens from South Australian waters. Larvae of *H. melanochir* and *H. regularis* have completed notochord flexion at hatching and are characterized by an elongate body with distinct rows of melanophores along the dorsal, lateral, and ventral surfaces; a small to moderate head; a heavily pigmented and long straight gut; a persistent preanal finfold; and an extended lower jaw. Fin formation occurs in the following sequence: caudal, dorsal and anal (almost simultaneously), pectoral, and pelvic. Despite the similarities between both species and among hemiramphid larvae in general, *H. melanochir* larvae are distinguishable from *H. regularis* by 1) having 58–61 vertebrae (vs. 51–54 for *H. regularis*); 2) having 12–15 melanophore pairs in longitudinal rows along the dorsal margin between the head and origin of the dorsal fin (vs. 19–22 for *H. regularis*); and 3) the absence of a large ventral pigment blotch anteriorly on the gut and isthmus (present in *H. regularis*). Both species can be distinguished from similar larvae of southern Australia (other hemiramphids and a scomberosocid) by differences in meristic counts and pigmentation.

Manuscript accepted 25 October 2002.
Manuscript received 31 December 2002 at NMFS Scientific Publications Office.
Fish. Bull. 101:368–376 (2003).

The beloniform family Hemiramphidae (garfishes or halfbeaks) are small to medium-size surface-dwelling marine, estuarine, and freshwater fishes. The family contains 12 genera and 101 species worldwide, and more than one-third of the species belong to the genus *Hyporhamphus* (Froese and Pauly[1]). The Hemiramphidae are related to the Exocoetidae (flyingfishes) and, more distantly, to the Scomberosocidae (sauries), Belonidae (needlefishes), and Adrianichthyidae (ricefishes) (Collette et al., 1984). Six genera and 17 species of hemiramphids occur in Australian waters, where garfishes have long been considered valuable food and bait fish (Collette, 1974; Kailola et al., 1993).

Two hemiramphid species inhabit the waters of South Australia (S.A.), namely the southern sea garfish *Hyporhamphus melanochir* (Valenciennes, 1846) and the river garfish *H. regularis* (Günther, 1866). Adults of both are widely distributed along southern Australia from Western Australia (W.A.) to New South Wales, although *H. regularis* have not been recorded in Tasmania (Tas.). They support important commercial and recreational fisheries, particularly in S.A. (Kailola et al., 1993). *H. melanochir* are commonly found in sheltered coastal waters, whereas *H. regularis* are confined to estuaries (Jones et al., 1996). Juveniles and adults of both species co-occur in some estuaries of southern Australia, e.g. Port River-Barker Inlet of S.A. (34°45′S, 138°31′E) (Jones et al., 1996) and Peel-Harvey Estuary of W.A. (32°32′S, 115°43′E) (Noell, unpubl. data).

Despite their widespread distribution and economic importance, the early life history of *H. melanochir* is only partially described (i.e. reproductive biology [Ling, 1958]; egg development [Jordan et al., 1998], and there is no published information for *H. regularis*. Furthermore, although adults are easily identified with keys and descriptions provided by Collette (1974), no such information exists for the larvae. A fundamental prerequisite for any larval fish study is, undoubtedly, their accurate identification (Neira et al., 1998).

Thus far, at least some larval stages have been described for 19 hemiramphids worldwide (Sudarsan, 1966; Talwar, 1967; Hardy, 1978; Chen, 1988; Watson, 1996; Prince Jeyaseelan, 1998), eight of which belong to *Hyporhamphus*. The purposes of this paper are to describe the larval development of *H. melanochir* and *H. regularis* and to document distinguishing characters between larvae of these species.

[1] Froese, R., and D. Pauly. 2001. FishBase. World Wide Web electronic publication. Accessed 28 Nov 2001. Web site: www.fishbase.org.

Materials and methods

Most larvae were collected with a neuston net in Gulf St. Vincent (34°29′S, 138°15′E) and the Bay of Shoals (35°37′S, 137°37′E) of South Australia. The neuston net was a square-framed bongo net with a mouth area of 0.5 m^2 fitted with 500-µm mesh, to which a 30-cm diameter pneumatic float was attached to both sides of the frame. This attachment ensured that, while being towed, the top of the frame rode steadily above the water surface and that ~0.4 m^2 of the mouth area was submerged. The net was towed from the stern of the vessel inside a circular direction for 5 min at speeds of 2–4 knots. Additional larvae were collected by hand from beneath a wharf in Barker Inlet where they often school during daylight at mid-flood tide. Transforming larvae and juveniles were collected at night with a dip net and spotlight at Outer Harbor (34°46′S, 138°28′E) and Barker Inlet. The term "transforming" is used here to describe the stage between the end of the larval phase and the start of the juvenile phase, i.e. after the attainment of all fin rays and before the formation of scales. All specimens examined in this study were collected between November and March. Larvae were sorted from plankton samples immediately after collection based on reference larval specimens from the South Australian Museum fish collection that were identified to family. Larvae were fixed in 10% formalin buffered with sodium β-glycerophosphate (1 g/L) and later preserved in 70% ethanol.

A total of 47 *H. melanochir* (6.4–48.3 mm body length, BL) and 49 *H. regularis* (7.0–46.9 mm BL) larvae through juveniles were used to describe morphometrics, meristics, and pigmentation. Larvae were identified as hemiramphids based on larval and adult characters reported in the literature (Collette, 1974; Hardy, 1978; Collette et al., 1984; Chen, 1988; Watson, 1996; Trnski et al., 2000). Developmental series were assembled by using the series method (Neira et al., 1998), the accuracy of which was verified by a molecular technique (Noell et al., 2001). Terminology of early life history stages follows that of Kendall et al. (1984). Representative series for both species are deposited with the I.S.R. Munro Fish Collection (CSIRO, Hobart, Tas.). (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 Wild M3Z stereomicroscope at 6.5–40× magnifications by using various combinations of incident and transmitted light. Body measurements were taken with SigmaScan Pro® 4.01 image measurement software (SPSS Inc., 1999) and are accurate to less than 0.05 mm. This method was particularly useful for measuring cumulative distances of bent larvae. Abbreviations and definitions of routinely taken body measurements follow Leis and Carson-Ewart (2000). Lower jaw length (LJ) is defined as the horizontal distance from the tip of the lower jaw to the anterior margin of the pigmented region of the eye. Lower jaw extension (LJx) is defined as the horizontal distance from the tip of the lower jaw to the tip of the snout. Eye diameter was measured along both horizontal (EDh) and vertical midlines (EDv) of its pigmented region. Body depth was measured at two points: at the pectoral base (BDp) and at the anus (BDa). Other measurements taken were snout length (SnL), head length (HL), pre dorsal-fin length (PDL) and preanal length (PAL). All measurements are expressed as a percentage of BL. Pigment refers to melanin. Drawings were prepared with the aid of a camera lucida.

Selected specimens were cleared and stained with alcian blue and alizarin red-S, following the method of Potthoff (1984), in order to count fin rays and vertebrae. Myomeres were difficult to count reliably at either end and thus vertebral counts (which include the urostyle) of stained larvae were taken instead. For small larvae that had unformed centra, corresponding neural or haemal spines were counted to obtain the number of vertebrae.

Results

Southern sea garfish (*Hyporhamphus melanochir* Valenciennes, 1846) (Fig. 1)

Description of larvae The smallest *H. melanochir* larva examined was a 6.4-mm newly hatched, laboratory-reared, postflexion-stage specimen. Some yolk remained, although yolk absorption was complete in the smallest field-collected larva (6.9 mm).

Larvae are elongate to very elongate (BDp=7–13% BL), and have a body depth slightly tapered towards the anus (BDa= 7–9% BL). Relative body depth at the pectoral base decreases slightly during larval development (Table 1). Larvae have 58–61 vertebrae (Table 2). The gut is relatively thick, long, straight, and nonstriated. PDL and PAL remain in the ranges of 70–75% and 71–76% BL, respectively (except for the 17.0-mm larva, which had a PDL and PAL of 62% BL). The first dorsal-fin ray is slightly anterior to or directly above the corresponding anal-fin ray. There is no gap between the anus and the anal fin. A long preanal finfold, initially the same length as the gut, persists through to the transformation stage before it disappears. There is no head spination. The small to moderate head (HL=16–24% BL) decreases in size in relation to BL with larval growth (Table 1). The longer lower jaw protrudes beyond the snout (LJx) by 4% BL at 11.0–11.5 mm, increasing to a maximum of 34% BL in the 29.3-mm juvenile. The mouth is oblique and reaches to the level of the center of the eye in newly hatched larvae. The maxilla subsequently moves forward in relation to the eye and by 12.1–14.4 mm it does not reach the eye. Very small villiform teeth are present on both the premaxilla and dentary in newly hatched larvae. The moderate to large eye (EDh=6–10% BL or 33–42% HL) is elongate (EDv=78–88% EDh) and decreases in size in relation to BL. A single rudimentary nasal papilla first appears as a small fleshy lump in the olfactory pit by 17.0 mm. Scales first appear between 20.4 and 29.3 mm laterally on the tail, anterior to the caudal peduncle.

Development of fins Completion of fin development in *H. melanochir* occurs in the following sequence: C → D → A → P_1, P_2 (Table 2). All principal rays of the caudal fin (7+8)

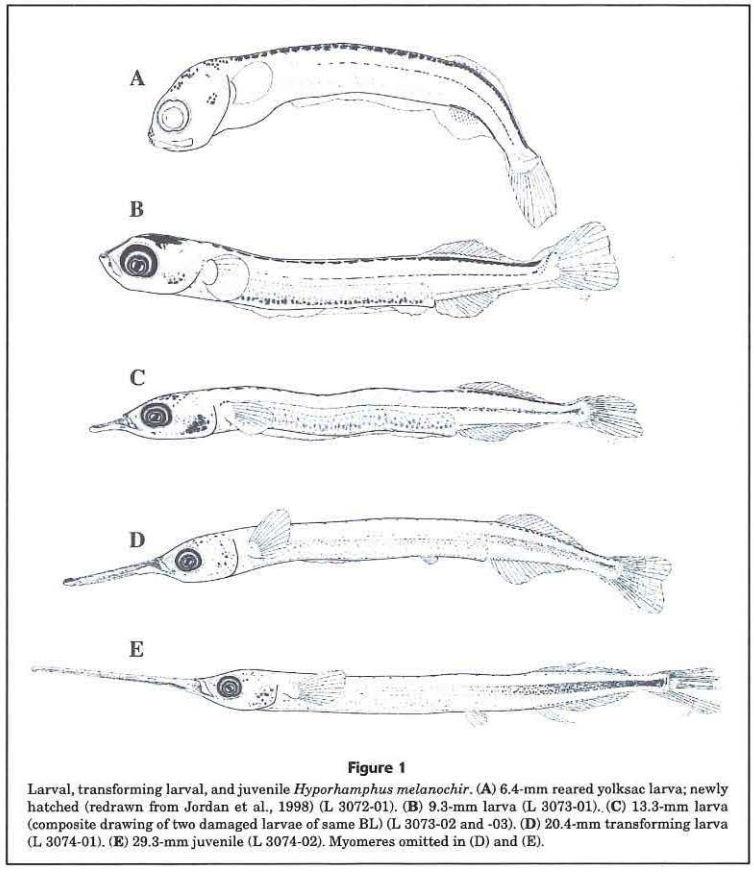


Figure 1

Larval, transforming larval, and juvenile *Hyporhamphus melanochir*. (**A**) 6.4-mm reared yolksac larva; newly hatched (redrawn from Jordan et al., 1998) (L 3072-01). (**B**) 9.3-mm larva (L 3073-01). (**C**) 13.3-mm larva (composite drawing of two damaged larvae of same BL) (L 3073-02 and -03). (**D**) 20.4-mm transforming larva (L 3074-01). (**E**) 29.3-mm juvenile (L 3074-02). Myomeres omitted in (D) and (E).

and several incipient dorsal- and anal-fin rays are present in newly hatched larvae. A full complement of 15–18 dorsal-fin and 17–20 anal-fin rays is attained at 11.4 and 12.1 mm, respectively. The pectoral base and finfold form prior to hatching, and incipient rays appear shortly after (by 7.2 mm); all 11–13 rays are formed by 19.6 mm. The pelvic fin buds appear by 13.3 mm, and all six pelvic-fin rays are formed by 19.6 mm.

Pigmentation *Hyporphamphus melanochir* larvae 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 midbrain. The extended lower jaw is heavily pigmented throughout development and melanophores extend laterally along the dentary. The eye is partially pigmented in the newly hatched larva, but fully pigmented by 6.9 mm. The gut is heavily and uniformly pigmented dorsally and laterally along the entire length, and melanophores are often coalesced, but pigmentation becomes obscured as the overlying musculature develops. Dorsal pigmentation initially consists of 12–15 large melanophore pairs in longitudinal rows between the head and origin of the dorsal fin (Fig. 2A), and a continuous band along either side of the dorsal-fin base. Dorsal pigmentation gradually decreases in intensity thereafter. Three

APPENDIX C. *Continued*

Table 1

Morphometrics of larval, transforming larval, and juvenile *Hyporhamphus melanochir* (expressed as % of BL). Mean ±SD is given when sample size n >1. Dashed lines differentiate larvae, transforming larvae, and juveniles in descending order.

BL (mm)	n	SnL	LJ	LJx	EDh	EDv	HL	PDL	PAL	BDp	BDa
6.4[1]	1	2.1	2.7	0.6	9.9	8.7	24.4	74.6	75.5	16.3[2]	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 ±0.8	3.9 ±1.1	1.1 ±0.4	9.1 ±0.3	7.3 ±0.3	22.0 ±0.8	70.8 ±0.9	72.6 ±1.0	11.9 ±0.3	8.9 ±0.3
7.5–8.0	7	3.6 ±0.9	4.6 ±1.2	1.0 ±0.4	9.1 ±0.5	7.1 ±0.3	22.8 ±1.6	71.3 ±1.3	73.0 ±0.9	11.8 ±0.6	8.9 ±0.7
8.0–8.5	9	3.6 ±0.5	4.9 ±0.8	1.3 ±0.4	8.8 ±0.3	7.1 ±0.3	21.9 ±1.0	70.9 ±0.7	72.7 ±0.9	11.7 ±0.7	8.9 ±0.7
8.5–9.0	3	3.5 ±0.8	5.0 ±1.1	1.5 ±0.3	8.6 ±0.2	7.0 ±0.3	21.1 ±0.2	71.5 ±0.2	72.5 ±0.4	11.3 ±0.5	9.1 ±0.5
9.0–9.5	4	3.4 ±0.5	5.0 ±0.7	1.6 ±0.3	8.0 ±0.3	6.5 ±0.2	20.9 ±0.8	71.7 ±0.7	72.8 ±0.6	11.4 ±0.7	8.4 ±0.3
11.0–11.5	4	3.7 ±0.7	7.2 ±1.5	3.5 ±1.2	7.5 ±0.4	6.3 ±0.2	20.3 ±1.3	71.6 ±0.6	72.3 ±0.5	10.6 ±0.7	8.5 ±0.6
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

[1] Yolksac larva.
[2] Includes yolk sac.

Table 2

Meristic counts of larval, transforming larval, and juvenile *Hyporhamphus melanochir*. Numbers in bold indicate the BL at which a full complement of rays is first attained. Dashed lines differentiate larvae, transforming larvae, and juveniles in descending order. D = dorsal; A = anal; P_1 = pectoral; P_2 = pelvic; C = caudal.

	Fin rays						
BL (mm)	D	A	P_1	P_2	C	Branchiostegal rays	Vertebrae
6.4[1]	8	9	base		0+7+8+0	3	38+21
7.2	8	8	1		0+7+8+0	3	39+20
7.3	9	10	1		0+7+8+0	3	39+19
7.6	11	11	1		0+7+8+0	3	38+20
7.9	10	11	1		0+7+8+0	3	40+20
8.3	11	11	2		0+7+8+0	4	39+20
8.4	13	14	2		1+7+8+1	5	39+21
9.4	14	16	4		1+7+8+1	5	40+21
11.4	**15**	16	6		2+7+8+1	7	38+20
12.1	16	**17**	7		2+7+8+2	7	39+20
14.4	16	19	9	bud	2+7+8+2	9	39+20
19.6	17	19	**11**	**6**	**4+7+8+4**	**12**	38+20
20.4	16	17	12	6	4+7+8+4	12	39+19
29.3	17	18	11	6	4+7+8+4	13	38+20
33.3	17	18	12	6	5+7+8+5	12	38+20
41.3	16	19	11	6	4+7+8+5	12	40+19
48.3	16	19	11	6	4+7+8+5	12	39+19

[1] Yolksac larva.

APPENDIX C. *Continued*

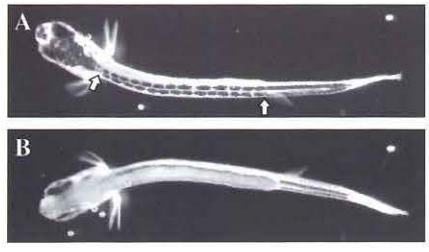


Figure 2
Pigmentation of an 8.5-mm *Hyporhamphus melanochir* larva. (**A**) Dorsal view; arrows indicate the margins of the 12–15 melanophore pairs in longitudinal rows. (**B**) Ventral view.

Table 3
Morphometrics of larval, transforming larval, and juvenile *Hyporhamphus regularis* (expressed as % of BL). Mean ±SD is given when sample size $n > 1$. Dashed lines differentiate larvae, a transforming larva, and juveniles in descending order.

BL (mm)	*n*	SnL	LJ	LJx	EDh	EDv	HL	PDL	PAL	BDp	BDa
7.0[1]	1	3.2	4.4	1.2	8.2	6.5	20.4	73.2	71.6	11.6[2]	7.4
7.5–8.0	9	2.8 ±0.3	4.4 ±0.4	1.7 ±0.3	7.6 ±0.1	6.3 ±0.1	19.9 ±0.6	73.1 ±0.6	71.8 ±0.4	11.2 ±0.2	8.2 ±1.2
8.0–8.5	12	2.8 ±0.4	4.3 ±0.5	1.5 ±0.3	7.5 ±0.3	6.2 ±0.2	19.6 ±0.9	72.8 ±0.7	71.6 ±0.6	11.0 ±0.5	7.7 ±0.3
8.5–9.0	10	2.8 ±0.2	4.2 ±0.2	1.5 ±0.2	7.2 ±0.2	5.9 ±0.1	19.2 ±0.3	72.9 ±0.9	71.8 ±0.9	10.6 ±0.2	7.4 ±0.3
9.0–9.5	5	2.8 ±0.1	4.4 ±0.4	1.6 ±0.4	7.0 ±0.1	6.0 ±0.2	19.1 ±0.4	72.3 ±0.7	71.5 ±0.6	10.6 ±0.2	7.6 ±0.3
9.5–10.0	3	2.8 ±0.1	4.4 ±0.3	1.7 ±0.2	6.9 ±0.4	5.8 ±0.2	18.7 ±0.5	72.1 ±1.6	71.2 ±1.7	10.6 ±0.4	7.3 ±0.2
10.0–10.5	3	3.0 ±0.3	4.8 ±0.9	1.8 ±0.6	6.8 ±0.2	5.8 ±0.2	18.9 ±0.6	72.2 ±0.6	71.3 ±0.6	10.2 ±0.3	7.6 ±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

[1] Yolksac larva.
[2] Includes yolk sac.

distinct lines of pigment appear along the dorsal margin in juveniles (by 29.3 mm) and remain to adult stage. A series of melanophores form a dashed, sometimes continuous, midlateral line. Melanophores appear laterally on the caudal peduncle by 14.4 mm and then proliferate anteriorly to form a broad medial stripe that remains, forming a silver stripe from the caudal peduncle to the operculum of adults. Ventral pigmentation consists of continuous bands of melanophores either side of the anal-fin base (Fig. 2B). Fins are unpigmented, except the caudal fin, which has small melanophores on the ray bases.

River garfish (*Hyporhamphus regularis* Günther, 1886) (Fig. 3)

Description of larvae The smallest *H. regularis* larva examined (7.0 mm) had completed notochord flexion and had a yolk sac. Yolk absorption was complete by 7.6 mm.

Larval *H. regularis* closely resemble larval *H. melanochir* morphologically (see Tables 1 and 3), but differ somewhat in relative length of the lower jaw, relative positions of the dorsal- and anal-fin origins, and in number of vertebrae. The longer lower jaw protrudes beyond the snout (LJx) by

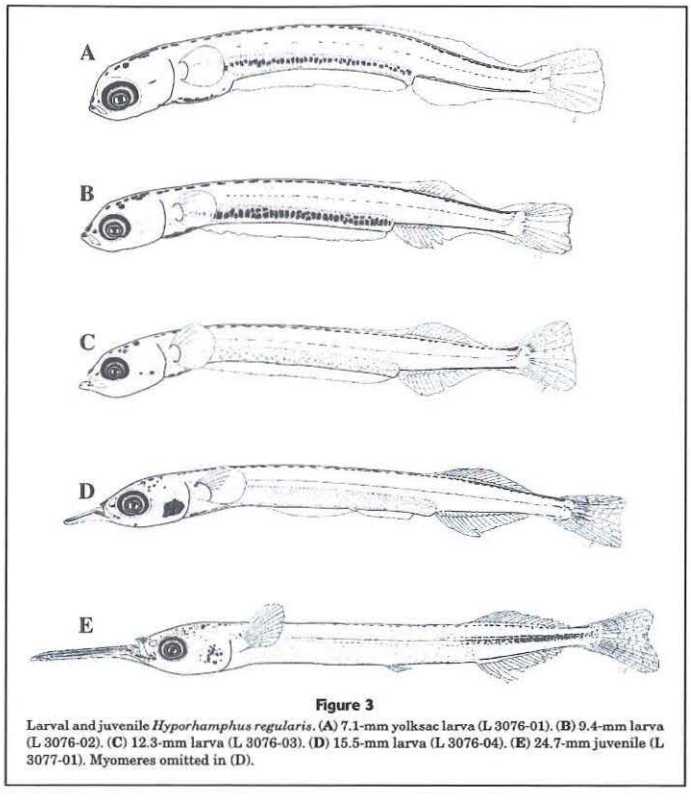


Figure 3

Larval and juvenile *Hyporhamphus regularis*. (**A**) 7.1-mm yolksac larva (L 3076-01). (**B**) 9.4-mm larva (L 3076-02). (**C**) 12.3-mm larva (L 3076-03). (**D**) 15.5-mm larva (L 3076-04). (**E**) 24.7-mm juvenile (L 3077-01). Myomeres omitted in (D).

4% BL at 13.1 mm and increases to a maximum of 24% BL in the 31.5 mm juvenile. The first dorsal-fin ray is slightly posterior to or directly above the corresponding anal-fin ray. Larvae have 51–54 vertebrae. Scales first appear between 18.1 and 24.7 mm laterally on the tail, anterior to the caudal peduncle.

Development of fins Completion of fin development in *H. regularis* occurs in the following sequence: C → D → A → P_1, P_2 (Table 4). Development of the caudal fin is incomplete at birth; 6 + 7 principal rays are present in the 7.0-mm yolksac larva, and the full complement (7+8) shortly after, by 7.7 mm. Distinct anal-fin bases are visible at 7.0 mm. A full complement of 14–17 dorsal and 15–19 anal-fin rays is attained at 10.1 and 10.5 mm, respectively. The pectoral base and finfold are present at birth, and incipient rays first appear by 8.1 mm; all 11–12 rays are formed by 18.1 mm. The pelvic fin buds appear by 13.1 mm, and all six pelvic-fin rays are formed by 18.1 mm.

Pigmentation Pigmentation of *H. regularis* larvae is similar to that of *H. melanochir* larvae except along the dorsal and ventral margins. Dorsal pigmentation consists of 19–22 melanophore pairs in longitudinal rows between the head and dorsal fin origin (Fig. 4A). A large pigment blotch is present ventrally on the isthmus and anteriorly on the gut.

Discussion

This study provides the first descriptions of larval development of hemiramphids endemic to marine (*H. melanochir*) and estuarine (*H. regularis*) waters of Australia.

Table 4

Meristic counts of larval, transforming larval, and juvenile *Hyporhamphus regularis*. Numbers in bold indicate the BL at which a full complement of rays is first attained. Dashed lines differentiate larvae, a transforming larva, and juveniles in descending order. D = dorsal; A = anal; P_1 = pectoral; P_2 = pelvic; C = caudal.

	Fin rays						
BL (mm)	D	A	P_1	P_2	C	Branchiostegal rays	Vertebrae
7.0[1]	anlage	bases	base		0+6+7+0	2	35+19
7.7	4	6	base		0+**7**+8+0	3	34+19
7.8	6	7	base		0+7+8+0	4	34+18
8.1	5	7	1		0+7+8+0	4	34+19
8.3	8	9	2		0+7+8+0	4	33+20
8.6	9	11	2		0+7+8+0	5	34+19
8.9	11	11	2		0+7+8+1	5	34+20
9.3	11	12	3		1+7+8+1	5	33+18
9.6	10	11	3		0+7+8+1	5	35+19
10.1	**14**	14	4		1+7+8+1	6	33+20
10.5	13	**15**	5		1+7+8+1	6	35+19
13.1	14	16	7	bud	2+7+8+2	8	35+19
18.1	14	17	**11**	**6**	4+7+8+4	**12**	35+18
24.7	16	17	12	6	4+7+8+4	11	34+19
31.5	15	17	12	6	4+7+8+4	11	34+18
33.8	15	18	11	6	4+7+8+4	11	33+19
46.9	16	17	11	6	4+7+8+4	11	35+18

[1] Yolksac larva.

Both *H. melanochir* and *H. regularis* share characters common to other described hemiramphid larvae. They are generally characterized by their lack of head or fin spines; elongate body; long straight gut; extended lower jaw; a main pigmentation pattern consisting of rows of melanophores on the dorsal, lateral, and ventral surfaces of the body; and advanced state of development at hatching (Collette et al., 1984; Watson, 1996; Trnski et al., 2000). Although the size at which fins develop varies slightly between *H. melanochir* and *H. regularis*, the sequence of development for both species is the same as that for most hemiramphids, i.e. C → D, A → P_1 → P_2 (Collette et al., 1984).

Hyporhamphus melanochir larvae are distinguishable from *H. regularis* by 1) having 58–61 vertebrae (vs. 51–54 for *H. regularis*); 2) having 12–15 melanophore pairs in longitudinal rows along the dorsal margin between the head and origin of the dorsal fin (vs. 19–22 for *H. regularis*); and 3) the absence of a large ventral pigment blotch anteriorly on the gut and isthmus which is present in *H. regularis*. Despite the difficulty in counting myomeres, either the number of vertebrae in cleared and stained specimens or the number of myomeres between the pectoral-fin base and anus (usually three less than the number of precaudal vertebrae; see Tables 2 and 4) revealed a consistent difference between both species.

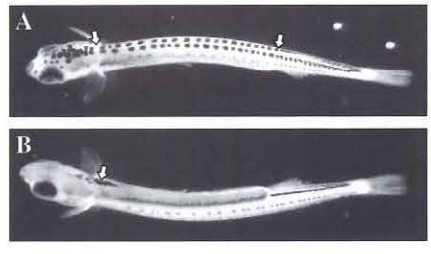


Figure 4

Pigmentation of an 8.7-mm *Hyporhamphus regularis* larva. (**A**) Dorsal view; arrows indicate the margins of the 19–22 melanophore pairs in longitudinal rows. (**B**) Ventral view; arrow indicates the ventral pigment blotch.

The geographic distributions of larval *H. melanochir* and *H. regularis* were separate in most samples; only three *H. melanochir* were found among *H. regularis* from Barker Inlet, whereas no *H. regularis* were among *H. melanochir* from the Bay of Shoals or Gulf St. Vincent. Larvae of other

hemiramphid species may overlap in distribution with those of *H. melanochir* and *H. regularis* outside South Australian waters. Meristic characters (summarized in Table 5) can often distinguish *H. melanochir* and *H. regularis* from the other species, except the eastern sea garfish (*H. australis*), which has overlapping meristic counts and currently undescribed larvae. The storm garfish (*Hemiramphus robustus*) has fewer anal-fin rays (11–14) and develops both a dark blotch below the dorsal fin and a pigmented pelvic fin in the juvenile stage (Collette, 1974; Collette et al., 1984). The long-finned garfish (*Euleptorhamphus viridis*), an oceanic species that rarely frequents nearshore waters, is strikingly different from other hemiramphids, being much more elongate and slender, and having divergent meristic counts, including more dorsal- and anal-fin rays (21–25 and 20–24, respectively), more vertebrae (69–73), fewer pectoral-fin rays (7–9), and fewer gill rakers (25–33) (Collette, 1974; Hardy, 1978; Chen, 1988; Trnski et al., 2000).

Larvae of the saury (*Scomberosox saurus*) (family Scomberosocidae) also occur in southern Australia and are the only other species that could be confused with hemiramphids. These are distinguishable from hemiramphid larvae by their higher myomere count (62–70), greater number of principal caudal-fin rays (16–17), presence of dorsal and anal finlets, and much heavier pigmentation (Bruce and Sutton, 1998; Trnski et al., 2000).

Acknowledgments

I am grateful to D. Short, L. Triantafillos, and the crew of the RV *Ngerin* for assisting in the field collection of specimens. The South Australian Museum allowed access to catalogued hemiramphid specimens, and A. Jordan donated a newly hatched *H. melanochir* larva and assisted with the examination of distinguishing characters. B. Bruce and F. J. Neira provided tips on larval drawing techniques. I also thank B. Bruce, S. Donnellan, A. Fowler, A. Jordan, F. J. Neira, T. Trnski, and T. Ward for kindly reviewing the manuscript. This project was supported by a Fisheries Research and Development Corporation grant 97/133 and was undertaken while receiving an Australian Postgraduate Award (Industry) at Adelaide University.

Table 5

Meristic counts of adult 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 *H. melanochir* and *H. regularis* in this study are also included. Vertebrae are given as precaudal + caudal; gill rakers are given as upper + lower. ? = no information available. D = dorsal; A = anal; P_1 = pectoral; P_2 = pelvic; C = caudal.

	Fin rays							
Species	D	A	P_1	P_2	C	Branchiostegal rays	Vertebrae	Gill rakers
Euleptorhamphus viridis	21–25	21–24 20–24[2]	8–9 7–9[2]	6[2]	?+7+8+?[4]	?	69 – 73 (44 – 46) + (26 – 29) = 70 – 75[4]	(5 – 9) + (18 – 23) = 25 – 33
Hemiramphus robustus	13–15	11–14	12–13	6[5]	4+7+8+5[5]	13[5]	(35 – 37) + (17 – 19) = 52–55 (33 –34) + (16 – 17) = 49-50[1]	(27 – 33) + (20 – 25) = ?
Hyporhamphus australis	15–17	17–20	11–13 10–13[1]	6[5]	4+7+8+4[5]	12–13[5]	(37 – 39) + (18 – 20) = 56–58 (38 – 40) + ?[1]	(31 – 39) + (23 – 33) = ?
Hyporhamphus melanochir	15–18	17–20	11–13	6[3]	4–5+7+8+4–5[5,6]	12–13[5,6]	(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[3]	4+7+8+4[5,6]	10–12[5,6]	(33 – 38) + (18 – 20) = 51 – 58 (33 – 35) + (18 – 20) = 51 – 54[6]	(30 – 36) + (21 – 27) = 52 – 61

[1] Parin et al. (1980).
[2] Chen (1988).
[3] Gomon et al. (1994).
[4] Trnski et al. (2000).
[5] Noell (unpubl. data).
[6] This study.

Literature cited

Bruce, B. D., and C. A. Sutton.
1998. Scomberosocidae: sauries. *In* Larvae of temperate Australian fishes: laboratory guide for larval fish identification (F. J. Neira, A. G. Miskiewicz, and T. Trnski, eds.), p. 98–101. Univ. Western Australia Press, Perth, Western Australia.

Chen, C. H.
1988. Hemiramphidae. *In* An atlas of the early stage fishes in Japan (M. Okiyama, ed.), p. 265–275. Tokai Univ. Press, Tokyo. [In Japanese.]

Collette, B. B.
1974. The garfishes (Hemiramphidae) of Australia and New Zealand. Rec. Aust. Mus. 29:11–105.

Collette, B. B., G. E. McGowen, N. V. Parin, and S. Mito.
1984. Beloniformes: development and relationships. *In* Ontogeny and systematics of fishes (H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall Jr., and S. L. Richardson, eds.), p. 335–354. Am. Soc. Ichthyol. Herpetol., Spec. Publ. 1.

Gomon, M. F., C. J. M. Glover, and R. H. Kuiter.
1994. The fishes of Australia's south coast, 992 p. State Print, Adelaide, Australia.

Hardy, Jr., J. D.
1978. Development of fishes of the mid-Atlantic Bight: an atlas of egg, larval and juvenile stages. Vol. II. Anguillidae through Syngnathidae. U.S. Fish Wildl. Serv. FWS/OBS-78/12, 458 p.

Jones, G. K., J. L. Baker, K. Edyvane, and G. J. Wright.
1996. Nearshore fish community of the Port River-Barker Inlet Estuary, South Australia. I. Effect of thermal effluent on the fish community structure, and distribution and growth of economically important fish species. Mar. Freshwater Res. 47:785–800.

Jordan, A. R., D. M. Mills, G. Ewing, and J. M. Lyle.
1998. Assessment of inshore habitats around Tasmania for life-history stages of commercial finfish species. Fishing Research and Development Corporation, Final Report Project 94/037, 176 p.

Kailola, P. J., M. J. Williams, P. C. Stewart, R. E. Reichelt, A. McNee, and C. Grieve.
1993. Australian fisheries resources, 422 p. Bureau of Resource Sciences and the Fisheries Research and Development Corporation, Canberra, Australia.

Kendall, A. W., Jr., E. H. Ahlstrom, and H. G. Moser.
1984. Early life history stages of fishes and their characters. *In* Ontogeny and systematics of fishes (H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall Jr., and S. L. Richardson, eds.), p. 11–22. Am. Soc. Ichthyol. Herpetol., Spec. Publ. 1.

Leis, J. M., and B. M. Carson-Ewart.
2000. The larvae of Indo-Pacific coastal fishes: an identification guide to marine fish larvae. Fauna Malesiana Handbooks, 2, 850 p. Brill, Leiden.

Ling, J. K.
1958. The sea garfish, *Reporhamphus melanochir* (Cuvier and Valenciennes) (Hemiramphidae), in South Australia: breeding, age determination, and growth rate. Aust. J. Mar. Freshwater Res. 9:60–110.

Neira, F. J., A. G. Miskiewicz, and T. Trnski.
1998. Larvae of temperate Australian fishes: laboratory guide for larval fish identification, 474 p. Univ. Western Australia Press, Perth, Western Australia.

Noell, C. J., S. Donnellan, R. Foster, and L. Haigh.
2001. Molecular discrimination of garfish *Hyporhamphus* (Beloniformes) larvae in southern Australian waters. Mar. Biotechnol. 3:509–514.

Parin, N. V., B. B. Collette, and Y. N. Shcherbachev.
1980. Preliminary review of the marine halfbeaks (Hemiramphidae, Beloniformes) of the tropical Indo-West-Pacific. Trudy Inst. Okeanol. Akad. NAUK SSSR 97:7–173. [In Russian.]

Potthoff, T.
1984. Clearing and staining techniques. *In* Ontogeny and systematics of fishes (H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall Jr., and S. L. Richardson, eds.), p. 35–37. Am. Soc. Ichthyol. Herpetol., Spec. Publ. 1.

Prince Jeyaseelan, M. J.
1998. Manual of fish eggs and larvae from Asian mangrove waters, 193 p. UNESCO, Paris.

SPSS Inc.
1999. SigmaScan® Pro 5.0. Chicago, Il.

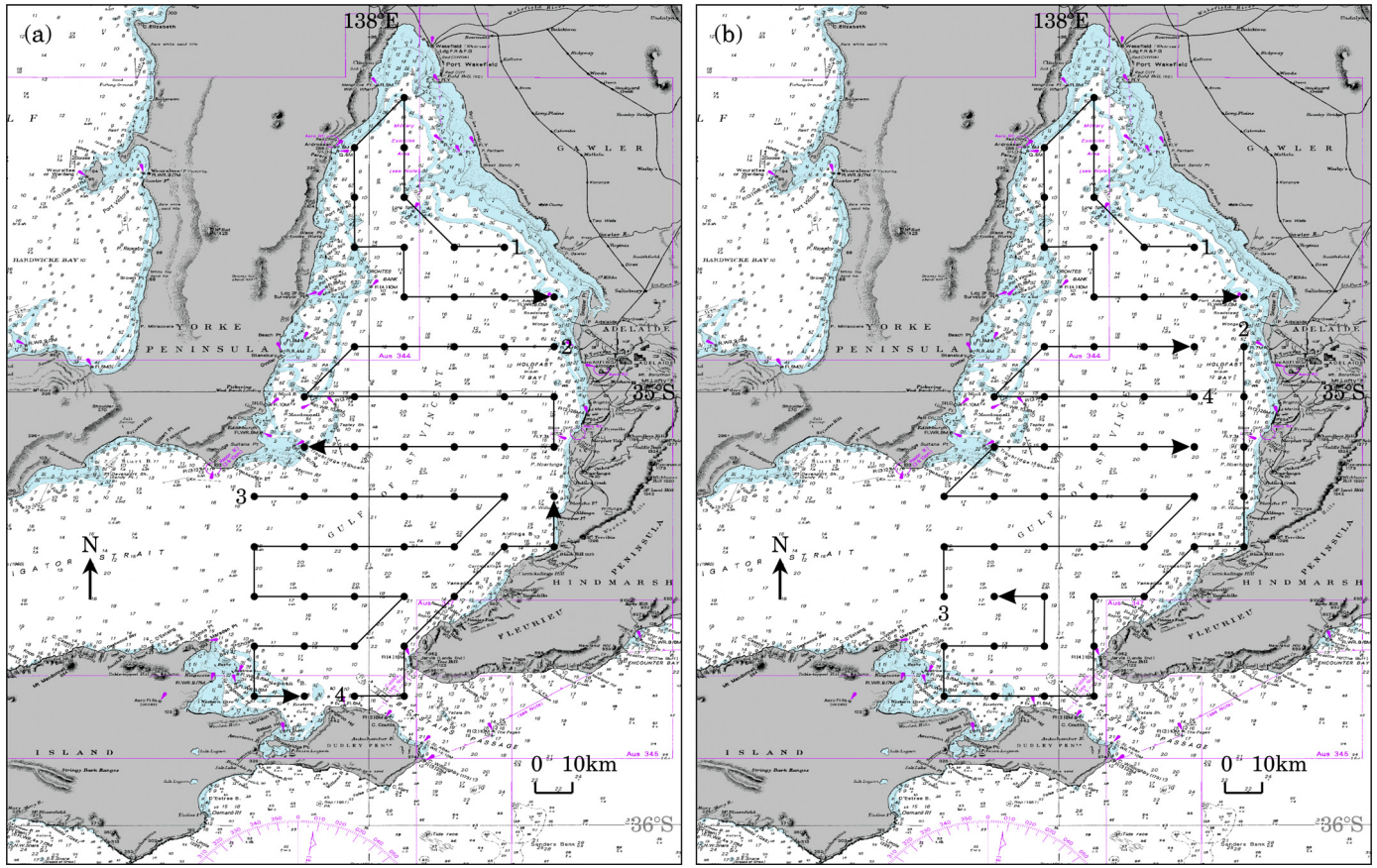
Sudarsan, D.
1966. Eggs and larvae of a hemirhamphid fish from Mandapam. J. Mar. Biol. Assoc. India 8:342–346.

Talwar, P. K.
1967. Studies on the biology of *Hemiramphus marginatus* (Forsskål) (Hemirhamphidae-Pisces). J. Mar. Biol. Assoc. India 9:61–69.

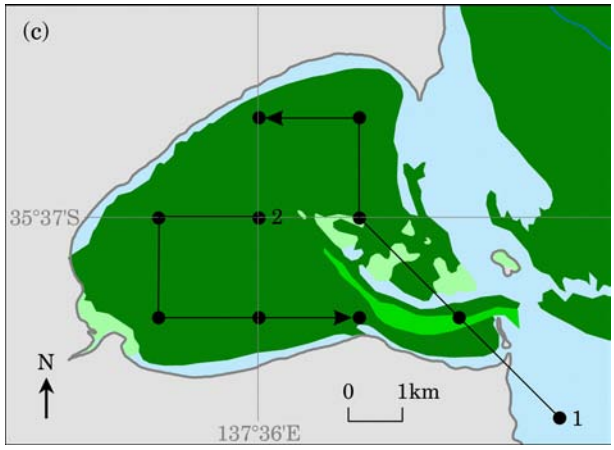
Trnski, T., J. M. Leis, and B. M. Carson-Ewart.
2000. Hemiramphidae. *In* The larvae of Indo-Pacific coastal fishes: an identification guide to marine fish larvae (J. M. Leis, and B. M. Carson-Ewart, eds.), p. 154–158. Fauna Malesiana Handbooks, 2. Brill, Leiden.

Watson, W.
1996. Hemiramphidae: halfbeaks. *In* The early stages of fishes in the California Current region (H. G. Moser, ed.), p. 634–641. Calif. Coop. Oceanic Fish. Invest. Atlas 33.

APPENDIX D. Cruise paths for RV *Ngerin* in Gulf St Vincent during (a) Dec 14-17, 1998 (cruise 1) and (b) Dec 4-7, 2000 (cruise 3), and for RV *Pagrus* in the Bay of Shoals during (c) March 3-5, 2000 for determining the distribution and abundance of *Hyporhamphus melanochir* larvae.



APPENDIX D. *Continued*



References

Ahlstrom, E. H. & Stevens, E. (1976). Report of neuston (surface) collections made on an extended CalCOFI cruise during May 1972. *CalCOFI Report* **18,** 167-180.

Anderson, R. O. & Gutreuter, S. J. (1983). Length, weight, and associated structural relationships. In *Fisheries Techniques* (Nielsen, L. A. & Johnson, D. L., eds), pp. 283-300. Bethesda, Maryland: American Fisheries Society.

Aoki, Y. (1988). Spawning season of Clupeidae, Belonidae, Hemiramphidae and Exocoetidae in Ogasawara (Bonnin) Islands. *Suisanzoshoku* **36,** 79-86. [In Japanese.]

Bailey, K. M. & Houde, E. D. (1989). Predation on eggs and larvae of marine fishes and the recruitment problem. *Advances in Marine Biology* **25,** 1-83.

Banks, M. A., Hedgecock, D. & Waters, C. (1993). Discrimination between closely related Pacific oyster species (Crassostrea) via mitochondrial DNA sequences coding for large subunit rRNA. *Molecular Marine Biology and Biotechnology* **2,** 129-136.

Barber, P. M., Fletcher, J. M. & Newman, H. M. (1993). Demonstration of the glycocalyces associated with three oral gram-negative bacterial species using a modern acrylic resin technique. *Journal of Structural Biology* **110,** 180-187.

Bartlett, S. E. & Davidson, W. S. (1992). FINS (Forensically Informative Nucleotide Sequencing): a procedure for identifying the animal origin of biological specimens. *BioTechniques* **12,** 408-411.

Bell, J. D. & Pollard, D. A. (1989). Ecology of fish assemblages and fisheries associated with seagrasses. In *Biology of the Seagrasses: A Treatise on the Biology of Seagrasses with Special Reference to the Australian Region* (Larkum, A. W. D., McComb, A. J. and Shepherd, S. A. eds), pp. 565-609. Amsterdam: Elsevier.

Berkeley, S. A. & Houde, E. D. (1978). Biology of two exploited species of halfbeaks, *Hemiramphus brasiliensis* and *H. balao* from southeast Florida. *Bulletin of Marine Science* **28,** 624-644.

Bertoni, M. (1995). The reproductive biology and feeding habits of the snook (*Sphyraena novaehollandiae*). *Southern Fisheries* **3**(2), 34-35.

Blaxter, J. H. S. (1988). Pattern and variety in development. In *Fish Physiology. Vol. XI: The Physiology of Developing Fish. Part A. Eggs and Larvae* (Hoar, W. S. & Randall, D. J., eds), pp. 1-58. London: Academic Press, Inc.

Borowitzka, M. A. & Lethbridge, R. C. (1989). Seagrass epiphytes. In *Biology of the Seagrasses: A Treatise on the Biology of Seagrasses with Special Reference to the Australian Region* (Larkum, A. W. D., McComb, A. J. and Shepherd, S. A. eds), pp. 458-499. Amsterdam: Elsevier.

Bradford, M. J. (1992). Precision of recruitment predictions from early life stages of marine fishes. *Fishery Bulletin* **90,** 439–453.

Bradford, M. J. & Cabana, G. (1997). Interannual variability in stage-specific survival rates and the causes of recruitment variation. In *Early Life History and Recruitment in Fish Populations* (Chambers, C. & Trippel, E. A., eds), pp. 469–494. London: Chapman and Hall.

Bruce, B. (1989). Studying larval fish ecology. *Safish* **13(4),** 4-9.

Bruce, B. D. & Sutton, C. A. (1998). Scomberosocidae: sauries. In *Larvae of Temperate Australian Fishes: Laboratory Guide for Larval Fish Identification* (Neira, F. J., Miskiewicz, A. G. & Trnski, T., eds), pp. 98-101. Nedlands: University of Western Australia Press.

Bunce, A. (2001). Prey consumption of Australasian gannets (*Morus serrator*) breeding in Port Phillip Bay, southeastern Australia, and potential overlap with commercial fisheries. *ICES Journal of Marine Science* **58,** 904-915.

Bunn, S. E. & Boon, P. I. (1993). What sources of organic carbon drive food webs in billabongs? A study based on stable isotope analysis. *Oecologia* **96,** 85-94.

Bunn, S. E., Loneragan, N. R. & Kempster, M. A. (1995). Effects of acid washing samples on stable isotope ratios of C and N in penaeid shrimp and seagrass: implications for food web studies using multiple stable isotopes. *Limnology and Oceanography* 40, 622-625.

Burton, R. S. (1996). Molecular tools in marine ecology. *Journal of Experimental Marine Biology and Ecology* **200,** 5-101.

Busson-Mabillot, S. (1977). Un type particulier de sécrétion exocrine: celui de l'appareil adhésif de l'oeuf d'un poisson téléostéen. *Biol. Cellulaire* **30,** 233-244.

Bye, J. A. T. (1976). Physical oceanography of Gulf St Vincent and Investigator Strait. In *Natural History of the Adelaide Region* (Twidale, C. R., Tyler, M. J. & Webb, B. P. eds), pp. 143-160. Adelaide: Royal Society of South Australia.

Bye, V. J. (1984). The role of environmental factors in the timing of reproductive cycles. In *Fish Reproduction: Strategies and Tactics* (Potts, G. W. & Wootton, R. J., eds), pp. 187-205. London: Academic Press.

Campana, S. E. & Jones, C. M. (1992). Analysis of otolith microstructure data. In *Otolith Microstructure Examination and Analysis* (Stevenson, D. K. & Campana, S. E., eds), pp 73-100. Canadian Special Publication of Fisheries and Aquatic Sciences 117

Campana, S. E. & Neilson, J. D. (1985). Microstructure of fish otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* **42,** 1014-1032.

Carlander, D. K. (1987). A history of scale age and growth studies of North American freshwater fish. In *Age and Growth of Fish* (Summerfelt, C. R. & Gordon, H. E., eds), pp. 3-14. Ames, Iowa: Iowa State University.

Carr, W. E. S. & Adams, C. A. (1973). Food habits of juvenile marine fishes occupying seagrass beds in the estuarine zone near Crystal River, Florida. *Transactions of the American Fisheries Society* **102,** 511-540.

Chen, C. H. (1988). Hemiramphidae. In *An Atlas of the Early Stage Fishes in Japan* (Okiyama, M., ed.), pp. 265-275. Tokyo: Tokai University Press. [In Japanese.]

Chidambaram, K. & Menon, M. D. (1948). Notes on the development of *Megalops cyprinoides* and *Hemirhamphus georgii*. *Proceedings of the Zoological Society, London* **117,** 756-763.

Coates, D. & Van Zwieten, P. A. M. (1992). Biology of the freshwater halfbeak *Zenarchopterus kampeni* (Teleostei: Hemiramphidae) from the Sepik and Ramu River basin, northern Papua New Guinea. *Ichthyological Exploration of Freshwater* **3,** 25-36.

Coetzee, D. J. (1981). Analysis of the gut contents of the needlefish, *Hyporhamphus knysnaensis* (Smith), from Rondevlei, southern Cape. *South African Journal of Zoology* **16,** 14-20.

Coffroth, M. A. & Mulawka, J. M., III. (1995). Identification of marine invertebrate larvae by means of PCR-RAPD species-specific markers. *Limnology and Oceanography* **40,** 181-189.

Collette, B. B. (1974). The garfishes (Hemiramphidae) of Australia and New Zealand. *Records of the Australian Museum* **29,** 11-105.

Collette, B. B., McGowen, G. E., Parin, N. V. & Mito, S. (1984). Beloniformes: development and relationships. In *Ontogeny and Systematics of Fishes* (Moser, H. G., Richards, W. J., Cohen, D. M., Fahay, M. P., Kendall, A. W., Jr. & Richardson, S. L., eds), pp. 335-354. American Society of Ichthyology and Herpetology, Special Publication No. 1.

Connolly, R. M. (1994). A comparison of fish assemblages from seagrass and unvegetated areas of a southern Australian estuary. *Australian Journal of Marine and Freshwater Research* **45,** 1033-1044.

Crossland, S., Coates, D., Grahame, J. & Mill, P. J. (1993). Use of random amplified polymorphic DNAs (RAPDs) in separating two sibling species of *Littorina*. *Marine Ecology Progress Series* **96,** 301-305.

Cushing, D. H. (1969). The regularity of the spawning season of some fishes. *Journal du Conseil International pour l'Exploration de la Mer* **33,** 81–97.

Cushing, D. H. (1990). Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. *Advances in Marine Biology* **26,** 249–293.

Delsman, H. C. (1924). Fish eggs and larvae from the Java Sea. 3. A pelagic scomberosocid egg. *Treubia* **5,** 408-418.

DeNiro, M. & Epstein, S. (1976). You are what you eat (plus a few ‰): the carbon isotope cycle in food chains. *Geological Society of America Abstracts with Program* **8,** 834-835.

DeNiro, M. & Epstein, S. (1978). Influence of the diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* **42,** 495-506.

deVlaming, V. (1983). Oocyte development patterns and hormonal involvements among teleosts. In *Control Processes in Fish Physiology* (Rankin, J. C., Pitcher, T. J. & Duggan, R. T., eds), pp. 176-199. London: Croom Helm Ltd.

Donato, A., Giannetto, S., Contini, A. & Mauceri, A. (1978). Some aspects of oogenesis in a teleost, *Belone belone* L. *Rivista di Biologia Normale e Patologica* **4,** 41-62 (in Italian with English summary).

Donnellan, S., Haigh, L., Elphinstone, M., McGlennon, D. & Ye, Q. (2002). Genetic discrimination between southern sea garfish (*Hyporhamphus melanochir*) stocks of Western Australia, South Australia, Victoria and Tasmania. In Fisheries Biology and Habitat Ecology of Southern Sea Garfish (*Hyporhamphus melanochir*) in Southern Australia (Jones, G. K., Ye, Q., Ayvazian, S. & Coutin, P., eds), pp. 9-34. FRDC Project 97/133. Canberra, Australia: Fisheries Research and Development Corporation.

Duarte, C. M. & Alcaraz, M. (1989). To produce many small or few large eggs: a size-independent reproductive tactic of fish. *Oecologia* **80,** 401-404.

Dulčić, J. (1998). Larval growth of sprat, *Sprattus sprattus phalericus*, larvae in the Northern Adriatic. *Fisheries Research* **36,** 117-126.

Dumont, J. N. & Brummet, A. R. (1980). The vitelline envelope, chorion, and micropyle of *Fundulus heteroclitus* eggs. *Gamete Research* **3,** 25-44.

Durai, A. A., Ramanathan, N. & Balasubrahmanyan, K. (1988). Breeding biology of *Hyporhamphus unifasciatus* (Ranzani) from coastal waters of Parangipettai, east coast of India. In *The First Indian Fisheries Forum, Proceedings. Dec 4-8, 1987, Mangalore, Karnataka* (Joseph, M. M. ed.), pp. 199-202. Asian Fisheries Society, Indian Branch.

Edgar, G. J. & Shaw, C. (1995*a*). The production and trophic ecology of shallow-water fish assemblages in southern Australia I. Species richness, size-structure and production of fishes in Western Port, Victoria. *Journal of Experimental Marine Biology and Ecology* **194,** 53-81.

Edgar, G. J. & Shaw, C. (1995*b*). The production and trophic ecology of shallow-water fish assemblages in southern Australia II. Diets of fishes and trophic relationships between fishes and benthos at Western Port, Victoria. *Journal of Experimental Marine Biology and Ecology* **194,** 83-106.

Edgar, G. J. & Shaw, C. (1995*c*). The production and trophic ecology of shallow-water fish assemblages in southern Australia III. General relationships between sediments, seagrasses, invertebrates and fishes. *Journal of Experimental Marine Biology and Ecology* **194,** 107-131.

Edgar, G.J., Watson, G., Hammond, L. S. & Shaw, C. (1994). Comparisons of species richness, size-structure and production of benthos in vegetated and unvegetated habitats in Western Port, Victoria. *Journal of Experimental Marine Biology and Ecology* **176,** 201-226.

Edyvane, K. S. (1999). Conserving Marine Biodiversity in South Australia Part 2 – Identification of Areas of High Conservation Value in South Australia. Urrbrae: South Australian Research and Development Institute.

Ekau, W. (1998). Comparative growth analysis of *Engraulis anchoita* larvae from southern Brazil. *Archive of Fishery and Marine Research* **46,** 1-17.

Elgar, M. A. (1990). Evolutionary compromise between a few large and many small eggs: comparative evidence in teleost fish. *Oikos* **59,** 283-287.

Faber, J. E. & Stepien, C. A. (1997). The utility of mitochondrial DNA control region sequences for analyzing phylogenetic relationships among populations, species, and genera of the Percidae. In *Molecular Systematics of Fishes* (Kocher, T. D. & Stepien, C. A., eds), 129-143. San Diego, California: Academic Press.

Fassel, T. A., Mozdziak, P. E., Sanger, J. R. & Edmiston, C. E. (1998). Superior preservation of the staphylococcal glycocalyx with aldehyde-ruthenium red and select lysine salts using extended fixation times. *Microscopy Research & Technique* **41,** 291-297.

Fell, J. W. (1993). Rapid identification of yeast species using three primers in a polymerase chain reaction. *Marine Biology and Biotechnology* **2,** 174-180.

Fenton, G. E. (1996). Diet and predators of *Tenagomysis tasmaniae* Fenton, *Anisomysis mixta australis* (Zimmer) and *Paramesopodopsis rufa* Fenton from south-eastern Tasmania (Crustacea: Mysidacea). *Hydrobiologia* **323,** 31-44.

Fletcher, M. & Floodgate, G. D. (1973). An electron-microscopic demonstration of an acidic polysaccharide involved in the adhesion of a marine bacterium to solid surfaces. *Journal of General Microbiology* **74,** 325-334.

Fogarty, M. J., Sissenwine, M. P. & Cohen, E. B. (1991). Recruitment variability and the dynamics of exploited marine populations. *Trends in Ecology and Evolution* **6,** 241–246.

Fonds, M., Rosenthal, H. & Alderdice, D. F. (1974). Influence of temperature and salinity on embryonic development, larval growth and number of vertebrae of the garfish, *Belone belone*. In *The Early Life History of Fish* (Blaxter, J. H. S., ed.), pp. 509-525. Berlin: Springer-Verlag.

Fowler, A. J., McLeay, L. & Short, D. A. (1999). Reproductive mode and spawning information based on gonad analysis for the King George whiting (Percoidei: Sillaginidae) from South Australia. *Marine and Freshwater Research* **50,** 1-14.

Fry, B. (1991). Stable isotope diagrams of freshwater food webs. *Ecology* **72,** 2293-2297.

Fry, B. & Arnold, C. (1982). Rapid $^{13}C/^{12}C$ turnover during growth of brown shrimp (*Penaeus aztecus*). *Oecologia* **54,** 200-204.

Fry, B. & Sherr, E. B. (1984). $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contributions in Marine Science* **27,** 13-47.

Fuiman, L. A. (2002). Special considerations of fish eggs and larvae. In *Fishery Science. The Unique Contributions of Early Life Stages* (Fuiman, L. A. & Werner, R. G., eds), pp. 1-32. Oxford, U.K.: Blackwell Science.

Gaughan, D. J., Fletcher, W. J. & White, K. V. (2001). Growth rate of larval *Sardinops sagax* from ecosystems with different levels of productivity. *Marine Biology* **139,** 831-837.

Geller, J. B., Carlton, J. T. & Powers, D. A. (1993). Interspecific and intrapopulation variation in mitochondrial ribosomal DNA sequences of *Mytilus* spp. (Bivalvia: Mollusca). *Molecular Marine Biology and Biotechnology* **2,** 44-50.

Goering, J., Alexander, V. & Haubenstock, N. (1990). Seasonal variability of stable carbon and nitrogen isotope ratios of organisms in a North Pacific bay. *Estuarine, Coastal and Shelf Science* **30,** 239-260.

Gomon, M. F., Glover, C. J. M. & Kuiter, R. H. (1994). *The Fishes of Australia's South Coast*. Adelaide: State Print.

Graham, D. H. (1939). Breeding habits of the fishes of Otago Harbour and adjacent seas. *Transactions of the Royal Society of New Zealand* **69,** 361-372.

Greven, H., Wanninger, A. & Clemen, G. (1997). Dentigerous bones and dentition in the hemiramphid fish *Dermogenys pusillus* (Atheriniformes, Teleostei). *Annals of Anatomy* **179,** 21-32.

Grutter, A. S., Morgan, J. A. T. & Adlard, R. D. (2000). Characterising parasitic gnathiid isopod species and matching life stages with ribosomal DNA ITS2 sequences. *Marine Biology* **136,** 201-205.

Guandalini, E., Mantovani, A., Fazzi, P., Gelosi, E., Colombari, P. T., Mazzini, M. & Fausto, A. M. (1994). Histological study on the oocyte filaments of the silverside *Odonthestes bonariensis*. *Journal of Fish Biology* **44,** 673-682.

Gunn, J. S., Bruce, B. D., Furlani, D. M., Thresher, R. E. & Blaber, S. J. M. (1989). Timing and location of spawning of blue grenadier, *Macruronus novaezelandiae* (Teleostei: Merlucciidae), in Australian coastal waters. *Australian Journal of Marine and Freshwater Research* **40,** 97-112.

Hair, J. F., Anderson, R. E., Tatham, R. L. & Black, W. C. (1998). *Multivariate Data Analysis*. Upper Saddle River, New Jersey: Prentice-Hall, Inc.

Hardy, J. D., Jr. (1978). Development of Fishes of the Mid-Atlantic Bight: An Atlas of Egg, Larval and Juvenile Stages. Vol. II. Anguillidae through Syngnathidae. U.S. Fisheries and Wildlife Service, FWS/OBS-78/12.

Hart, N. H., Pietri, R. & Donovan, M. (1984). The structure of the chorion and associated surface filaments in *Oryzias* – evidence for the presence of extracellular tubules. *Journal of Experimental Zoology* **230,** 273-296.

Hederstrom, H. (1959). Observations on the age of fishes. *Report of the Institute of Freshwater Research, Drottningholm* **40,** 161-164.

Hemminga, M. A. & Mateo, M. A. (1996). Stable carbon isotopes in seagrasses: variability in ratios and use in ecological studies. *Marine Ecology Progress Series* **140,** 285-298.

Henry, G. W. & Lyle, J. M. (2003). The national recreational and indigenous fishing survey. FRDC Project 99/158. Canberra, Australia: Fisheries Research and Development Corporation.

Herald, P. J. & Zottola, E. A. (1988). The use of transmission electron microscopy to study the composition of *Pseudomonas fragi* attachment material. *Food Microstructure* **7,** 53-57.

Herzka, S. Z. & Holt, G. J. (2000). Changes in isotopic composition of red drum (*Sciaenops ocellatus*) larvae in response to dietary shifts: potential applications to settlement studies. *Canadian Journal of Fisheries and Aquatic Sciences* **57,** 137-147.

Hesslein, R. H., Hallard, K. A. & Ramlal, P. (1993). Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$. *Canadian Journal of Fisheries and Aquatic Sciences* **50,** 2071-2076.

Hill, R. S., Allen, L. D. & Bucklin, A. (2001). Multiplexed species-specific PCR protocol to discriminate four N. Atlantic *Calanus* species, with an mtCOI gene tree for ten *Calanus* species. *Marine Biology* **139,** 279-287.

Hobson, K. A. & Clark, R. G. (1992). Assessing avian diets using stable isotopes. I: Turnover of ^{13}C in tissues. *Condor* **94,** 181-188.

Hobson, K. A. & Welch, H. E. (1992). Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. *Marine Ecology Progress Series* **84,** 9-18.

Houde, E. D. (1987). Fish early life dynamics and recruitment variability. *American Fisheries Society Symposia* **2,** 17–29.

Houde, E. D. (2002). Mortality. In *Fishery Science. The Unique Contributions of Early Life Stages* (Fuiman, L. A. & Werner, R. G., eds), pp. 64-87. Oxford, U.K.: Blackwell Science.

Hu, Y., Lutz, R. A. & Vrijenhoek, R. C. (1992). Electrophoretic identification and genetic analysis of bivalve larvae. *Marine Biology* **113,** 227-230.

Hunte, W., Oxenford, H. A. & Mahon, R. (1995). Distribution and relative abundance of flyingfish (Exocoetidae) in the eastern Caribbean. II. Spawning substrata, eggs and larvae. *Marine Ecology Progress Series* **117,** 25-37.

Hunter, J. R., Lo, N. C. H. & Leong, R. J. H. (1985). Batch fecundity in multiple spawning fishes. In *An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: Application to the Northern Anchovy,* Engraulis mordax (Lasker, R. ed.), pp 67-77. U.S. Department of Commerce, NOAA Technical Report NMFS 36.

Hunter, J. R. & Macewicz, B. J. (1985). Measurement of spawning frequency in multiple spawning fishes. In *An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: Application to the Northern Anchovy,* Engraulis mordax (Lasker, R. ed.), pp 79-94. U.S. Department of Commerce, NOAA Technical Report NMFS 36.

Hyslop, E. J. (1980). Stomach content analysis – a review of methods and their application. *Journal of Fish Biology* **17,** 411-429.

Jenkins, G. P. (1987). Age and growth of co-occurring larvae of two flounder species, *Rhombosolea tapirina* and *Ammotretis rostratus*. *Marine Biology* **95,** 157-166.

Jenkins, G. P., May, H. M. A., Wheatley, M. J. & Holloway, M. G. (1997). Comparison of fish assemblages associated with seagrass and adjacent unvegetated habitats of Port Phillip Bay and Corner Inlet, Victoria, Australia, with emphasis on commercial species. *Estuarine, Coastal and Shelf Science* **44,** 569-588.

Jennings, S., Reñones, O., Morales-Nin, B., Polunin, N. V. C., Moranta, J. & Coll, J. (1997). Spatial variation in the ^{15}N and ^{13}C stable isotope composition of plants, invertebrates and fishes on Mediterranean reefs: implications for the study of trophic pathways. *Marine Ecology Progress Series* **146,** 109-116.

Johnson, J. E. (1981). The seasonality of the algal epiphytes of *Posidonia sinuosa* in upper Spencer Gulf. M.Sc. Thesis, University of Adelaide.

Jones, C. (1986). Determining age of larval fish with the otolith increment techniques. *Fishery Bulletin* **84,** 91-103.

Jones, C. M. (1992). Development and application of the otolith increment techniques. In *Otolith Microstructure Examination and Analysis* (Stevenson, D. K. & Campana, S. E., eds), pp 1-11. Canadian Special Publication of Fisheries and Aquatic Sciences 117.

Jones, G. K. (1990). Growth and mortality in a lightly fished population of garfish (*Hyporhamphus melanochir*), in Baird Bay, South Australia. *Transactions of the Royal Society of South Australia* **114,** 37-45.

Jones, G. K., Baker, J. L., Edyvane, K. & Wright, G. J. (1996). Nearshore fish community of the Port River-Barker Inlet Estuary, South Australia. I. Effect of thermal effluent on the fish community structure, and distribution and growth of economically important fish species. *Marine and Freshwater Research* **47,** 785-800.

Jones, G.K., Hall, D. A., Hill, K. L. & Staniford, A. J. (1990). The South Australian Marine Scalefish Fishery. Stock assessment, economics, management. South Australian Department of Fisheries, Green Paper.

Jones, G. K. & Kangas, M. (1987). A review of the catch and effort in the commercial marine scale fishery in South Australian waters, 1976–1986. South Australian Department of Fisheries, Fisheries Research Paper 18.

Jones, G. K., Ye, Q., Ayvazian, S. & Coutin, P. (2002). Fisheries biology and habitat ecology of southern sea garfish (*Hyporhamphus melanochir*) in southern Australian waters. FRDC Project 97/133. Canberra, Australia: Fisheries Research and Development Corporation.

Jordan, A. R. (1994). Age, growth and back-calculated birthdate distributions of larval jack mackerel, *Trachurus declivis* (Pisces: Carangidae), from eastern Tasmanian coastal waters. *Australian Journal of Marine and Freshwater Research* **45,** 19-33.

Jordan, A. R., Mills, D. M., Ewing, G. & Lyle, J. M. (1998). Assessment of inshore habitats around Tasmania for life-history stages of commercial finfish species. FRDC Project 94/037. Canberra, Australia: Fisheries Research and Development Corporation.

Kailola, P. J., Williams, M. J., Stewart, P. C., Reichelt, R. E., McNee, A. & Grieve, C. (1993). *Australian Fisheries Resources*. Canberra, Australia: Bureau of Resource Sciences and the Fisheries Research and Development Corporation.

Kawamura, G., Takimoto, M. & Sobajima, N. (1990). Larval growth and age- and size-related variation in development of sense organs in the halfbeak, *Hemiramphus sajori*. In *The Second Asian Fisheries Forum* (Hirano, R. & Hanyu, I., eds), pp. 407-410. Manila, Philippines: Asian Fisheries Society.

Kemp, W. M. (2000). Seagrass ecology and management: an introduction. In *Seagrasses: Monitoring, Ecology, Physiology, and Management* (Bortone, S. A., ed.), pp. 1-6. Fort Myers, Florida, U.S.A.: CRC Press.

Kendall, A. W., Jr., Ahlstrom, E. H. & Moser, H. G. (1984). Early life history stages of fishes and their characters. In *Ontogeny and Systematics of Fishes* (Moser, H. G., Richards, W. J., Cohen, D. M., Fahay, M. P., Kendall, A. W., Jr. & Richardson, S. L., eds), pp. 11-22. American Society of Ichthyology and Herpetology, Special Publication No. 1.

Kikawa, S. (1964). Estimated number of eggs spawned out of the ovaries of an indomaguro (*Thunnus thynnus maccoyii?*). *Report of the Nankai Regional Fisheries Research Laboratory* **20,** 27-34. [In Japanese.]

Kikuchi, T. (1980). Faunal relationships in temperate seagrass beds. In *Handbook of Seagrass Biology: An Ecosystem Perspective* (Phillips, R. C. & McRoy, C. P., eds), pp. 153-172. New York: Garland STPM Press.

Kirkman & Walker (1989). Regional studies – Western Australian seagrass. In *Biology of Seagrasses: A Treatise on the Biology of Seagrasses with Special Reference to the Australian Region* (Larkum, A. W. D., McComb, A. J. & shepherd, S. A., eds), pp. 157-181. Amsterdam: Elsevier.

Klomp, N. I. & Wooller, R. D. (1988). Diet of little penguins, *Eudyptula minor*, from Penguin Island, Western Australia. *Australian Journal of Marine and Freshwater Research* **39,** 633-639.

Klumpp, D. W., Howard, R. K. & Pollard, D. A. (1989). Trophodynamics and nutritional ecology of seagrass communities. In *Biology of Seagrasses: A Treatise on the Biology of Seagrasses with Special Reference to the Australian Region* (Larkum, A. W. D., McComb, A. J. & Shepherd, S. A., eds), pp. 394-457. Amsterdam: Elsevier.

Klumpp, D. W. & Nichols, P. D. (1983). Nutrition of the southern sea garfish *Hyporhamphus melanochir*: gut passage rate and daily consumption of two food types and assimilation of seagrass components. *Marine Ecology Progress Series* **12,** 207-216.

Kovalevskaya, (1982). Superfluous reproduction and development of flying fishes of the family Exocoetidae. *Journal of Ichthyology* **22,** 48-54.

Kryzhanovskii, S. G. (1949). Eco-morphological principles and patterns of development among minnows, loaches and catfishes (Cyprinoidei and Siluroidei). Part II. Ecological groups of fishes and patterns of their distribution. Academy of Sciences of the USSR. Trudy of the Institute of Animal Morphology, Vol. 1, pp. 237-331. *Fisheries Research Board of Canada Translation* Series No. 2945. 1974.

Lam, T. J. (1983). Environmental influences on gonadal activity in fish. In *Fish Physiology* (Hoar, W. S. & Randall, D. J., eds), pp. 65-116. London: Academic Press.

Lambert, T. C. & Ware, D. M. (1984). Reproductive strategies of demersal and pelagic spawning fish. *Canadian Journal of Fisheries and Aquatic Sciences* **41,** 1565-1569.

Lasker, R. (1978). The relationship between oceanographic conditions and larval anchovy food in the California current: identification of factors contributing to recruitment failure. *Rapp. P.-v. Réun. Cons. Int. Explor. Mer*. **173,** 212–230.

Legendre, P. & Fortin, M. -J. (1989). Spatial pattern and ecological analysis. *Vegetatio* **80,** 107-138.

Leis, J. M. & Carson-Ewart, B. M. (2000). *The Larvae of Indo-Pacific Coastal Fishes: An Identification Guide to Marine Fish Larvae*. Fauna Malesiana Handbooks, 2. Brill, Leiden.

Leite, R. G., Araújo-Lima, C. A. R. M., Victoria, R. L. & Martinelli, L. A. (2002). Stable isotope analysis of energy sources for larvae of eight fish species from the Amazon floodplain. *Ecology of Freshwater Fish* **11,** 56-63.

Les, D. H., Moody, M. L., Jacobs, S. W. L. & Bayer, R. J. (2002). Systematics of seagrasses (Zosteraceae) in Australia and New Zealand. *Systematic Botany* **27,** 468-484.

Li, H., Liu, Q. & Shen, Z. (1997). Investigation of an iterative method for fitting von Bertalanffy growth equation using data on the halfbeak fish *Hemirhamphus sajori* (T et S). *Fisheries Research* **29,** 289-294.

Lindsay, D. J., Minagawa, M., Mitani, I. & Kawaguchi, K. (1998). Trophic shift in the Japanese anchovy *Engraulis japonicus* in its early life history stages as detected by stable isotope ratios in Sagami Bay, central Japan. *Fisheries Science* **64,** 403-410.

Ling, J. K. (1956). The life history of the sea garfish *Reporhamphus melanochir* (Cuv. & Val.) (Hemirhamphidae) in South Australia. M.Sc. Thesis, University of Adelaide.

Ling, J. K. (1958). The sea garfish, *Reporhamphus melanochir* (Cuvier and Valenciennes) (Hemiramphidae), in South Australia: breeding, age determination, and growth rate. *Australian Journal of Marine and Freshwater Research* **9,** 60-110.

Litvaitis, M. K., Nunn, G., Thomas, W. K. & Kocher, T. D. (1994). A molecular approach for the identification of meiofaunal turbellarians (Platyhelminthes, Turbellaria). *Marine Biology* **120,** 437-442.

Lockley, A. K. & Bardsley, R. G. (2000). Novel method for the discrimination of tuna (*Thunnus thynnus*) and bonito (*Sarda sarda*) DNA. *Journal of Agriculture and Food Chemistry* **48,** 4463-4468.

Lowerre-Barbieri, S. K. & Barbieri, L. R. (1993). A new method of oocyte separation and preservation for fish reproduction studies. *Fishery Bulletin* **91,** 165-170.

Mack, G. (1941). Cormorants and the Gippsland Lakes fishery. *Memoirs of the National Museum of Victoria* **12,** 95-117.

McArthur, L. C., Boland, J. W., Brown, B. & Jones, G. K. (2003). Investigation of the influence of seagrass on fisheries catch level using isotonic regression. *Environmental Modeling and Assessment* **8,** 285-290.

MacArthur, R. H. & Wilson, E. O. (1967). *The Theory of Island Biogeography*. Princeton, New Jersey, U.S.A.: Princeton University Press.

McBride, R. S. & Thurman, P. E. (2003). Reproductive biology of *Hemiramphus brasiliensis* and *H. balao* (Hemiramphidae): maturation, spawning frequency, and fecundity. *Biological Bulletin* **204,** 57-67.

McGarvey, R. & Feenstra, J. E. (2004). Stock assessment models with graphical user interfaces for key South Australian marine finfish stocks. FRDC Project 1999/145. Canberra, Australia: Fisheries Research and Development Corporation.

McGlennon, D. & Kinloch, M. A. (1997). Resource allocation in the South Australian marine scalefish fishery. FRDC Project 93/249. Canberra, Australia: Fisheries Research and Development Corporation.

McMillan, C. (1980). $^{13}C/^{12}C$ ratios in seagrasses. *Aquatic Botany* **9,** 237-249.

Medeiros-Bergen, D., Olson, R. R., Conroy, J. A. & Kocher, T. D. (1995). Distribution of holothurian larvae determined with species-specific genetic probes. *Limnology and Oceanography* **40,** 1225-1235.

Mertz, G. & Myers, R. A. (1994). Match/mismatch predictions of spawning duration versus recruitment variability. *Fisheries Oceanography* **3,** 236–245.

Mertz, G. & Myers, R. A. (1996). Influence of fecundity on recruitment variability of marine fish. *Canadian Journal of Fisheries and Aquatic Sciences* **53,** 1618–1625.

Meyer, A., Kocher, T. D., Basaibwaki, P. & Wilson, A. C. (1990). Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature* **347,** 550-553.

Michener, R. H. & Schell, D. M. (1994). Stable isotope ratios as tracers in marine aquatic food webs. In *Stable Isotopes in Ecology and Environmental Science* (Lajtha, K. & Michener, R. H., eds), pp. 138-157. Oxford: Blackwell Scientific Publications.

Miller, S. A., Dykes, D. D. & Polesky, H. F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* **16,** 1215.

Miller, T. J., Crowder, L. B., Rice, J. A. & Marschall, E. A. (1988). Larval size and recruitment mechanisms in fishes: toward a conceptual framework. *Canadian Journal of Fisheries and Aquatic Sciences* **45,** 1657–1670.

Minagawa, M. & Wada, E. (1984). Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. *Geochimica et Cosmochimica Acta* **48,** 1135-1140.

Mooi, R. D. (1990). Egg surface morphology of Pseudochromoids (Perciformes, Percoidei), with comments on its phylogenetic implications. *Copeia* **1990,** 455-475.

Moran, P. A. P. (1950). Notes on continuous stochastic phenomena. *Biometrika* **37,** 17-23.

Morgan, M. J. & Trippel, E. A. (1996). Skewed sex ratios in spawning shoals of Atlantic cod (*Gadus morhua*). *ICES Journal of Marine Science* **53,** 820-826.

Morioka, S. & Machinandiarena, L. (2001). Comparison of daily increment formation pattern between sagittae and lapilli of ling (*Genypterus blacodes*) larvae and juveniles collected off Argentina. *New Zealand Journal of Marine and Freshwater Research* **35,** 111-119.

Mowry, R. W. (1956). Alcian blue techniques for the histochemical study of acidic carbohydrates. *Journal of Histochemistry and Cytochemistry* **4,** 407.

Myers, R. A. (1998). When do environmental-recruit correlations work? *Reviews in Fish Biology and Fisheries* **8,** 285–305.

Nagasawa, T. & Domon, K. (1997). The early life history of kurosoi, *Sebastes schlegeli* (Scorpaenidae), in the Sea of Japan. *Ichthyological Research* **44,** 237-248.

Neira, F. J., Miskiewicz, A. G. & Trnski, T. (1998). *Larvae of Temperate Australian Fishes: Laboratory Guide for Larval Fish Identification*. Nedlands: University of Western Australia Press.

Nichols, J. W. & Hone, P. W. (1996). Marine biogeography of Kangaroo Island. South Australian Research and Development Institute (Aquatic Sciences, West Beach), Internal Report No.2.

Nichols, P. D., Klumpp, D. W. & Johns, R. B. (1985). A study of food chains in seagrass communities III. Stable carbon isotope ratios. *Australian Journal of Marine and Freshwater Research* **36,** 683-690.

Noell, C. J. (2003). Larval development of *Hyporhamphus melanochir* and *H. regularis* (Beloniformes: Hemiramphidae) from South Australian waters. *Fishery Bulletin* **101,** 368-376.

Noell, C. J., Donnellan, S., Foster, R. & Haigh, L. (2001). Molecular discrimination of garfish *Hyporhamphus* (Beloniformes) larvae in southern Australian waters. *Marine Biotechnology* **3,** 509-514.

Olney, J. E. & Boehlert, G. W. (1988). Nearshore ichthyoplankton associated with seagrass beds in the lower Chesapeake Bay. *Marine Ecology Progress Series* **45,** 33-43.

Oozeki, Y., Watanabe, Y., Kuji, Y. & Takahashi, S. (1991). Effects of various preservatives on the body length of saury larvae. *Bulletin of the Tohoku National Fisheries Research Institute* **53,** 15-21. [In Japanese.]

Panella, G. (1971). Fish otoliths: daily growth layers and periodical patterns. *Science* **173,** 1124-1127.

Parenti, L. R. (1993). Relationships of Atherinomorph fishes (Teleostei). *Bulletin of Marine Science* **52,** 170-196.

Parin, N. V., Collette, B. B. & Shcherbachev, Y. N. (1980). Preliminary review of the marine halfbeaks (Hemiramphidae, Beloniformes) of the tropical Indo-West-Pacific. *Trudy Instituta Okeanologii, Akademiia NAUK SSSR* **97,** 7-173. [In Russian.]

Parry, G. D., Campbell, S. J. & Hobday, D. K. (1990) Marine resources off east Gippsland, southeastern Australia. Marine Science Laboratories, Technical Report 72.

Patzner, R. A. (1984). The reproduction of *Blennius pavo* (Teleostei, Blenniidae). II. Surface structures of the ripe egg. *Zoologischer Anzeiger (Jena)* **213,** 44-50.

Pauly, D. & Pullin, R. S. V. (1988). Hatching time in spherical, pelagic, marine fish eggs in response to temperature and egg size. *Environmental Biology of Fishes* **22,** 261-271.

Paxton, J. R., Hoese, D. F., Allen, G. R. & Hanley, J. E. (1989). *Zoological Catalogue of Australia. Vol.7 Pisces Petromyzontidae to Carangidae*. Canberra: Australian Biological Resources Survey.

Pepin, P. (1990). Biological correlates of recruitment variability in North Sea fish stocks. *Journal du Conseil International pour l'Exploration de la Mer* **47,** 89–98.

Pepin, P. (2002). Population analysis. In *Fishery Science. The Unique Contributions of Early Life Stages* (Fuiman, L. A. & Werner, R. G., eds), pp. 112-142. Oxford, U.K.: Blackwell Science.

Pepin, P. & Myers, R. A. (1991). Significance of egg and larval size to recruitment variability of temperate marine fish. *Canadian Journal of Fisheries and Aquatic Sciences* **48,** 1820–1828.

Peters, J. S. & Schmidt, D. J. (1997). Daily age and growth of larval and early juvenile Spanish mackerel, *Scomberomorus maculates*, from the South Atlantic Bight. *Fishery Bulletin* **95,** 530-539.

Peterson, B. J. & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* **18,** 293-320.

Peterson, B. J., Howarth, R. W. & Garritt, R. H. (1985). Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. *Science* **227,** 1361-1363.

Phillips, D. L. (2001). Mixing models in analyses of diet using multiple stable isotopes: a critique. *Oecologia* **127,** 166-170.

Phillips, D. L. & Gregg, J. W. (2001). Uncertainty in source partitioning using stable isotopes. *Oecologia* **127,** 171-179. [http://www.epa.gov/wed/pages/models/isotopes/isoerror1_04.htm]

Phillips, D. L. & Koch, P. L. (2002). Incorporating concentration dependence in stable isotope mixing models. *Oecologia* **130,** 114-125. [http://www.epa.gov/wed/pages/models/isoconc/isoconc1_01.htm]

Potthoff, T. (1984). Clearing and staining techniques. In *Ontogeny and Systematics of Fishes* (Moser, H. G., Richards, W. J., Cohen, D. M., Fahay, M. P., Kendall, A. W., Jr. & Richardson, S. L., eds), pp. 35-37. American Society of Ichthyology and Herpetology, Special Publication No. 1.

Potts, G. W. (1984). Parental behaviour in temperate marine teleosts with special reference to the development of nest structures. In *Fish Reproduction: Strategies and Tactics* (Potts, G. W. & Wootton, R. J., eds), pp. 223-244. London: Academic Press.

Prager, M. H. & Fabrizio, M. C. (1990). Comparison of logistic regression and discriminant analysis for stock identification of anadromous fish, with application to striped bass (*Morone saxatilis*) and American shad (*Alosa sapidissima*). *Canadian Journal of Fisheries and Aquatic Sciences* **47,** 1570-1577.

Prince Jeyaseelan, M. J. (1998). Manual of Fish Eggs and Larvae from Asian Mangrove Waters. Paris: UNESCO.

Quiñonez-Velázquez, C. (1999). Age validation and growth of larval and juvenile haddock, *Melanogrammus aeglefinus*, and pollock, *Pollachius virens*, on the Scotian Shelf. *Fishery Bulletin* **97,** 306-319.

Quinteiro, J., Sotelo, C. G., Rehbein, H., Pryde, S. E., Medina, I., Pérez-Martín, R. I., Rey-Méndez, M. & Mackie, I. M. (1998). Use of mtDNA direct polymerase chain reaction (PCR) sequencing and PCR-restriction fragment length polymorphism methodologies in species identification of canned tuna. *Journal of Agriculture and Food Chemistry* **46,** 1662-1669.

Randall, J. E. (1967). Food habits of reef fishes of the West Indies. *Studies in Tropical Oceanography* **5,** 665-847.

Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology* **17,** 208-212.

Ricker, W. E. (1975). Computation and interpretation of biological statistics of fish populations. *Bulletin of the Fisheries Research Board of Canada* **191,** 1-382 .

Ricker, W. R. (1979). Growth rates and models. In *Fish Physiology* (Hoar, W. S., Randall, D. J. & Brett, J. R., eds), pp. 677-743. New York, NY: Academic Press.

Riehl, R. (1984). The oocytes of the goby (*Pomatoschistus minutus*. IV. Electron microscopic autoradiography of ^{3}H-proline incorporation. *Cytologia* **49,** 127-142.

Riehl, R. & Appelbaum, S. (1991). A unique adhesion apparatus on the eggs of the catfish *Clarias gariepinus* (Teleostei, Clariidae). *Japanese Journal of Ichthyology* **38,** 191-197.

Robertson, A. I. & Howard, R. K. (1978). Diel trophic interactions between vertically-migrating zooplankton and their fish predators in an eelgrass community. *Marine Biology* **48,** 207-213.

Robertson, A. I. & Klumpp, D. W. (1983). Feeding habits of the southern Australian garfish *Hyporhamphus melanochir*: a diurnal herbivore and nocturnal carnivore. *Marine Ecology Progress Series* **10,** 197-201.

Rocha-Olivares, A., Moser, H. G. & Stannard, J. (2000). Molecular identification and description of pelagic young of the rockfishes *Sebastes constellatus* and *Sebastes ensifer*. *Fishery Bulletin* **98,** 353-363.

Rollefsen, G. (1953). The selectivity of different fishing gear used in Lofoten. *Journal du Conseil International pour l'Exploration de la Mer* **19,** 191-194.

Rosen, D. E. & Parenti, L. R. (1981). Relationships of *Oryzias*, and the groups of atherinomorph fishes. *American Museum Novitates* **2719,** 1-25.

Rosenthal, H. & Fonds, M. (1973). Biological observations during rearing experiments with the garfish *Belone belone*. *Marine Biology* **21,** 203-218.

Rothschild, B. J. & DiNardo, G. T. (1987). Comparison of recruitment variability and life history data among marine and anadromous fish. *American Fisheries Society Symposia* **1,** 531–546.

Sadovy, Y. & Shapiro, D. Y. (1987). Criteria for the diagnosis of hermaphroditism in fishes. *Copeia* **1987,** 136-156.

Saidapur, S. K. (1982). Structure and function of postovulatory follicles (corpora lutea) in the ovaries of nonmammalian vertebrates. *International Review of Cytology* **75,** 243-285.

St Hill, J. L. (1996). Aspects of the biology of southern sea garfish, *Hyporhamphus melanochir*, in Tasmanian waters. B.Sc. Hons Thesis, University of Tasmania.

Sandknop, E. M., Sumida, B. Y. & Moser, H. G. (1984). Early life history descriptions. In *Ontogeny and Systematics of Fishes* (Moser, H. G., Richards, W. J., Cohen, D. M., Fahay, M. P., Kendall, A. W., Jr. & Richardson, S. L., eds), pp. 23-24. American Society of Ichthyology and Herpetology, Special Publication No. 1.

Saunders, A. J. & Montgomery, J. C. (1985). Field and laboratory studies of the feeding behaviour of the piper *Hyporamphus ihi* with reference to the lateral line in feeding. *Proceedings of the Royal Society of London* **B224**, 209-222.

Sawada, M. (1999). ROOKCASE: an Excel 97/2000 Visual Basic (VB) add-in for exploring global and local spatial autocorrelation. *Bulletin of the Ecological Society of America* **80,** 231-234. [http: //www.uottawa.ca/academic/arts/geographie/lpcweb/newlook/data_and_downloads/download/ sawsoft/rooks.htm]

Schwager, S. J., Federer, W. T., Mutschler, M. A. & Scully, B. T. (1993). The effect of linkage on sample size determination for multiple trait selection. *Theoretical and Applied Genetics* **86,** 964-974.

Scott, L. C., Boland, J. W., Edyvane, K. S. & Jones, G. K. (2000). Development of a seagrass-fish habitat model – I: A Seagrass Residency Index for economically important species. *Environmetrics* **11,** 541-552.

Seber, G. A. F. (1982). *The Estimation of Animal Abundance*. Griffin, London.

Secor, D. H., Dean, J. M. & Laban, E. H. (1992). Otolith removal and preparation for microstructural examination. In *Otolith Microstructure Examination and Analysis* (Stevenson, D. K. & Campana, S. E., eds), pp 19-57. Canadian Special Publication of Fisheries and Aquatic Sciences 117.

Seddon, S., Connolly, R. M. & Edyvane, K. S. (2000). Large-scale seagrass dieback in northern Spencer Gulf, South Australia. *Aquatic Botany* **66,** 297-310.

Seddon, S. (2001). Causes and ecological consequences of the Spencer Gulf seagrass dieback. Ph.D. Thesis, University of Adelaide.

Shepherd, S. A., McComb, A. J., Bulthuis, D. A., Neverauskas, V., Steffensen, D. A. & West, R. (1989). Decline of seagrasses. In *Biology of Seagrasses: A Treatise on the Biology of Seagrasses with Special Reference to the Australian Region* (Larkum, A. W. D., McComb, A. J. & shepherd, S. A., eds), pp. 346-393. Amsterdam: Elsevier.

Shigonova, T. A. & Kovalevskaya, N. V. (1991). Larvae and juveniles of Beloniformes from the Northeastern Atlantic. *Journal of Ichthyology* **31,** 92-100.

Silberman, J. D. & Walsh, P. J. (1992). Species identification of spiny lobster phyllosome larvae via ribosomal DNA analysis. *Molecular Marine Biology and Biotechnology* **1,** 195-205

Silva, E. I. L. & Davies, R. W. (1988). Notes on the biology of *Hemiramphus limbatus* (Hemiramphidae: Pisces) in Sri Lanka. *Tropical Freshwater Biology* **1,** 42-49.

Snyder, D. E. (1983). Fish eggs and larvae. In *Fisheries Techniques* (Nielsen, L. A. & Johnson, D. L., eds), pp. 165-197. Bethesda, Maryland: American Fisheries Society.

Sokolovsky, A. S. & Sokolovskaya, T. G. (1999). Some aspects of biology of the Japanese halfbeak *Hyporhamphus sajori* from Peter the Great Bay, Sea of Japan. *Russian Journal of Marine Biology* **25,** 426-430.

SPSS Inc. (1999*a*). *SigmaScan® Pro 5.0 User's Guide*. Chicago, Il.

SPSS Inc (1999*b*). *SPSS® Regression Models 10.0*. Chicago, Il.

SPSS Inc. (2002). *SigmaPlot® 8.0 Programming Guide*. Chicago, Il.

Steinberg, D. & Colla, P. (1999). Logistic regression. In *SYSTAT® 9 Statistics I* (SPSS, Inc., eds), pp. 547-614. Upper Saddle River, New Jersey: Prentice Hall.

Sudarsan, D. (1966). Eggs and larvae of a hemirhamphid fish from Mandapam. *Journal of the Marine Biological Association of India* **8,** 342-346.

Suyehiro, Y. (1942). A study on the digestive system and feeding habits of fish. *Japanese Journal of Zoology* **10,** 1-303.

Swofford, D. L. (1999). PAUP* Phylogenetic Analysis using Parsimony (* and other Methods). Sunderland, Massachusetts: Sinauer.

Takahashi, S. (1978). The adherent filaments of eggs of isaza (*Chaenogobius isaza* Tanaka, Pisces). *Zoological Magazine* **87,** 216-220.

Talwar, P. K. (1962*a*). Studies on the food and feeding relationships of the halfbeak fishes (Hemirhamphidae) from the Gulf of Mannar and Palk Bay. *Indian Journal of Fisheries* **9,** 1-9.

Talwar, P. K. (1962*b*). A contribution to the biology of the halfbeak, *Hyporhamphus georgii* (Cuv. & Val.) (Hemirhamphidae). *Indian Journal of Fisheries* **9A,** 168-196.

Talwar, P. K. (1967). Studies on the biology of *Hemiramphus marginatus* (Forsskål) (Hemirhamphidae-Pisces). *Journal of the Marine Biological Association of India* **9,** 61-69.

Tanaka, Y. & Oozeki, Y. (1996). Where are the eggs of the Pacific saury, *Cololabis saira*? *Ichthyological Research* **43,** 329-333.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25,** 4876-4882.

Thomson, J. M. (1957*a*). The food of Western Australian estuarine fish. Western Australian Department of Fisheries, Fisheries Bulletin No. 7.

Thomson, J. M. (1957*b*). The size at maturity and spawning times of some Western Australian estuarine fishes. Western Australian Department of Fisheries, Fisheries Bulletin No. 8.

Thomson, J. M. (1959). Some aspects of the ecology of Lake Macquarie, N.S.W., with regard to an alleged depletion of fish. IX The fishes and their food. *Australian Journal of Marine and Freshwater Research* **10,** 365-374.

Thorpe, R. S. (1975). Quantitative handling of characters useful in snake systematics with particular reference to intraspecific variation in the Ringed Snake *Natrix natrix* (L.). *Biological Journal of the Linnean Society* **7,** 27-43.

Tibbetts, I. R. (1991). The trophic ecology, functional morphology and phylogeny of the hemiramphidae (beloniformes). Ph.D. Thesis, University of Queensland.

Tibbetts, I. R. (1997). The distribution and function of mucous cells and their secretions in the alimentary tract of *Arrhamphus sclerolepis krefftii*. *Journal of Fish Biology* **50,** 809-820.

Tieszen, L. L., Boutton, T. W., Tesdhal, K. G. & Slade, N. A. (1983). Fractionation and turnover of stable isotopes in animal tissues: implications for $\delta^{13}C$ analyses of diet. *Oecologica* **57,** 32-37.

Trnski, T., Leis, J. M. & Carson-Ewart, B. M. (2000). Hemiramphidae. In *The Larvae of Indo-Pacific Coastal Fishes: An Identification Guide to Marine Fish Larvae* (Leis, J. M. & Carson-Ewart, B. M., eds), pp. 154-158. Fauna Malesiana Handbooks, 2. Brill, Leiden.

Tyler, C. R. & Sumpter, J. P. (1996). Oocyte growth and development in teleosts. *Reviews in Fish Biology and Fisheries* **6,** 287-318.

Vander Zanden, M. J., Hulshof, M., Ridgway, M. S. & Rasmussen, J. B. (1998). Application of stable isotope techniques to trophic studies of age-0 smallmouth bass. *Transactions of the American Fisheries Society* **127,** 729-739.

Wada, Y. & Kuwahara, A. (1994). Feeding habits of the halfbeak *Hemiramphus sajori* larvae in Kumihama Bay and the adjacent waters, Japan Sea. *Bulletin of the Kyoto Institute of Oceanic and Fishery Science* **17,** 59-65. [In Japanese.]

Wainright, S. C., Fuller, C. M., Michener, R. H. & Richards, R. A. (1996). Spatial variation of trophic position and growth rate of juvenile striped bass (*Morone saxatilis*) in the Delaware River. *Canadian Journal of Fisheries and Aquatic Sciences* **53,** 685-692.

Wallace, R. A. & Selman, K. (1981). Cellular and dynamic aspects of oocyte growth in teleosts. *American Zoologist* **21,** 325-343.

Wallman, J. F. & Donnellan, S. C. (2001). The utility of mitochondrial DNA sequences for the identification of forensically important blowflies (Diptera: Calliphoridae) in southeastern Australia. *Forensic Science International* **120,** 60-67.

Watanabe, Y., Butler, J. L. & Mori, T. (1988). Growth of Pacific saury, *Cololabis saira*, in the northeastern and northwestern Pacific Ocean. *Fishery Bulletin* **86,** 489-498.

Ward, T. & McLeay, L. (1999). Spawning biomass of pilchards (*Sardinops sagax)* in shelf waters of central and western South Australia. Report to the Pilchard Working Group. 38 pp.

Ward, T., McLeay, L., Dimmlich, W. F., Rogers, P. J., Matthews, R., Kaempf, J. & Schmarr, D. (2001). Why do juvenile southern bluefin tuna, *Thunnus maccoyii*, aggregate and grow quickly in the Great Australian Bight during summer-autumn? *Fishery Bulletin*. [in review].

Watson, W. (1996). Hemiramphidae: halfbeaks. In *The Early Stages of Fishes in the California Current Region* (Moser, H. G., ed.). *CalCOFI Atlas* **33,** 634-641.

Wear, R. G. (1974). Incubation in British decapod crustacean, and the effects of temperature on the rate and success of embryonic development. *Journal of the Marine Biological Association of the United Kingdom* **54,** 745-762.

West, G. (1990). Methods of assessing ovarian development in fishes: a review. *Australian Journal of Marine and Freshwater Research* **41,** 199-222.

Winstanley, R. H., Potter, M. A. & Caton, A. E. (1983). Australian cephalopod resources. *Memoirs of the National Museum of Victoria* **44,** 243–253.

Ye, Q., Noell, C. & McGlennon, D. (2002*a*). Reproductive biology of sea garfish. In Fisheries biology and habitat ecology of southern sea garfish (*Hyporhamphus melanochir*) in southern Australian waters (Jones, G. K., Ye, Q., Ayvazian, S. & Coutin, P., eds), pp. 209-253. FRDC Project 97/133. Canberra, Australia: Fisheries Research and Development Corporation.

Ye, Q., Short, D. A., Green, C. & Coutin, P. (2002*b*). Age and growth rate determination. In Fisheries biology and habitat ecology of southern sea garfish (*Hyporhamphus melanochir*) in southern Australian waters (Jones, G. K., Ye, Q., Ayvazian, S. & Coutin, P., eds), pp. 35-99. FRDC Project 97/133. Canberra, Australia: Fisheries Research and Development Corporation.

Zar, J. H. (1999). *Biostatistical Analysis*. Upper Saddle River, New Jersey: Prentice Hall.

Zeckua Ramos, M. C. & Martinez Perez, J. A. (1993). Study of the ontogenetic development of the needlefish *Strongylura marina* in the estuarine system of Tecolutla, Veracruz, Mexico. *Revista de Zoologia (Iztacala)* **4,** 7-22. [In Spanish.]

Zhang, D-X. & Hewitt, G. M. (1996). Nuclear integrations: challenges for mitochondrial DNA markers. *Trends in Ecology and Evolution* **11,** 247-251.

Electronic references

AODC (2002). *Sea Surface Temperatures.* Australian Oceanographic Data Centre. [http://www.aodc.gov.au/products/data/aussst.html]

CSIRO Marine Research. (2004). *The Facts: CSIRO Marine Research – Understanding Our Oceans. Southern Bluefin Tuna.* [http://www.marine.csiro.au/LeafletsFolder/31sbt/31sbt.html]

Froese, R. & Pauly, D. (2001). *112 Species in Family Hemiramphidae (Halfbeaks).* FishBase. [http://www.fishbase.org]

Geoscience Australia (2002). *Compute Sunrise, Sunset & Twilight Times.* Geoscience Australia. [http://www.auslig.gov.au/geodesy/astro/sunrise.htm]