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

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FIG. 6.2 Examples for estimating proportions (in parentheses) of different food sources in the diet of juvenile Hyporhamphus melanochir using (a) two- and (b) three-source standard mixing models and (c) the concentration-dependent model. Stable isotope ratios are shown for H. melanochir and food sources. The prime symbol (‘) indicates values are corrected for fractionation. Error bars show ± 1 S.E. (inner bars) and 95% C.I. (outer bars). Note: in the bivariate plots of the two isotopes, the mean composition of the consumer falls within the mixing triangle in (b) but not in (c). The vertices of each triangle represent the position of the consumer if its diet consisted exclusively of a particular food source. Lines within the triangle are ‘iso-diet’ lines, along which the proportion of a food source is invariant. Iso-diet lines are shown at 25% intervals, from 100% at the vertex to 0% along the side of the triangle opposite the vertex.
FIG. 6.3 Photomicrographs of zooplankton found in the gut contents of *Hyporhamphus melanochir* larvae after the larvae were cleared and stained for fin and vertebrae meristics. Both photomicrographs are at the same scale.
Abstract

This study investigates early life stages of the southern sea garfish (*Hyporhamphus melanochir*) and their association with seagrass in Gulf St Vincent, South Australia. The overall aims were to identify and describe the early life stages of *H. melanochir* and to explore the possible relationship(s) between these life stages and seagrass habitat with the emphasis on seagrass as a requirement for spawning or as a food source.

The reproductive biology of female *H. melanochir* from the commercial fishery was assessed by microscopic examination of ovaries, oocyte size distributions, gonadosomatic indices, and macroscopic ovarian stages. Five stages of oocyte development were identified and described: perinucleolar, yolk vesicle, yolk globule, migratory nucleus and hydrated. A coherence between histological and whole oocyte descriptions is demonstrated. *Hyporhamphus melanochir* are characterised as multiple spawners with group-synchronous oocyte development and indeterminate fecundity. A protracted spawning season from October to March was indicated by the occurrence of ripe ovaries and increases in gonadosomatic index. Females reach sexual maturity at 193 mm standard length, and batch fecundity ranged from 201-3044 oocytes depending on fish size. Spawning shoals are segregated by sex, as indicated by commercial samples, with a biased female-to-male ratio of 4.5:1 during the spawning season (1.2:1 during the non-spawning season). In addition, features of the oocyte surface were closely examined, which revealed that the filaments on the chorion of the hydrated oocyte are adhesive. These adhesive filaments presumably allow the fertilised egg to become attached to vegetative substrate by adhesion and/or entanglement.

*H. melanochir* larvae were discriminated from another hemiramphid species, river garfish (*H. regularis*), which is also known to occur in the study area, based on species-specific amplification of part of the mitochondrial control region using a multiplex polymerase chain reaction (PCR) assay. The species were easily discerned by the number and distinct sizes of PCR products [*H. melanochir*, 443 bp; river garfish (*H. regularis*), 462 and 264 bp]. Although based on a single gene, this molecular method will correctly identify the species of individuals in at least 96% and 94% of tests for *H. melanochir* and *H. regularis*, respectively.

Subsequent to verifying the identification of species by molecular discrimination, the larval development of *H. melanochir* and *H. regularis* were described. Larvae of *H. melanochir* and *H. regularis* had completed notochord flexion at hatching and are characterized by their elongate body with distinct rows of melanophores along the dorsal, lateral and ventral surfaces; small to moderate head; heavily pigmented, long straight gut; persistent preanal finfold; and extended lower jaw. Fin formation occurs in the sequence: caudal, dorsal and anal (almost simultaneously), pectoral, pelvic. Despite the similarities between both species and among hemiramphid larvae in general, *H. melanochir* larvae are distinguishable from *H. regularis* by: having 58-61 vertebrae (v. 51-54 for *H. regularis*); having 12-15 melanophore pairs in longitudinal rows along the dorsal margin between the head and origin of the dorsal fin (v. 19-22 for *H. regularis*); and the absence of a large ventral pigment blotch anteriorly on the gut and isthmus (present in *H. regularis*). A logistic regression analysis of body measurements also revealed interspecies differences in the combined measurements of eye diameter
and pre-anal fin length. Both species can be distinguished from morphologically similar larvae found in southern Australia (other hemiramphids and a scomberosocid) by differences in meristic counts and pigmentation.

_Hyporhamphus melanochir_ larvae were successfully collected throughout Gulf St Vincent using a neuston net; however, attempts to sample eggs were unsuccessful. Abundances of larvae in the gulf averaged 4.8 and 12.3 larvae 1000 m$^{-2}$ of surface water in December 1998 and December 2000, respectively. Larvae exhibit fast growth, as indicated by otolith growth increments, with back-calculated spawning dates falling within the October-March spawning season. Spatial analysis of larval distributions revealed a positive spatial autocorrelation, i.e. non-randomness or clustering of similar abundance values. Most larvae were found in the upper region of the gulf, and the prevalence of seagrass habitat throughout this region supports the view that the demersal eggs of _H. melanochir_ become attached to seagrass and/or algae following spawning. A gyre in waters of the upper gulf, influenced by prevailing southerly winds, the Coriolis effect, and land boundaries, may explain retention of larvae. The importance of seagrass beds to _H. melanochir_ spawning is also supported by anecdotal evidence and available literature on eggs of other Beloniformes, which are also demersal and attach to marine plants.

Dual stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) of larval, juvenile and adult _H. melanochir_ and several potential food sources from the Bay of Shoals was carried out to estimate the importance of zosteracean seagrass towards the assimilated diet of _H. melanochir_. Although the diet of _H. melanochir_ larvae is probably planktonivorous, their isotopic signatures partly reflect the parental diet due to the influence of pre-existing tissue in addition to growth. According to mixing model calculations, the signatures of juveniles can be explained by a diet consisting of 23-37% _Zostera_, 0-10% _Halophila_ and the remainder zooplankton, whilst the diet of adults consists of 53-58% _Zostera_ and the remainder zooplankton. These findings indicate an increasing dependence upon _Zostera_ with growth of _H. melanochir_.

The results of this study enhance the completeness of our understanding of the fisheries biology and ecology of _H. melanochir_. Significant contributions are provided in reproductive biology and larval biology, seagrass beds (in combination with mixed algae) are demonstrated to be an important habitat for spawning, and _Zostera_ seagrass is shown to be a necessary food source in the diet of juveniles and adults.
Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

[Signature]
January 11, 2005
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