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

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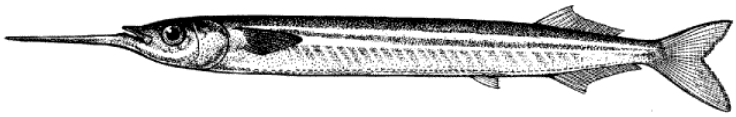

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Chapter 6 Assimilation of seagrass in the diet

6.1 INTRODUCTION

Seagrass plays a major role in supporting the processes and function of the marine environment and is an important fisheries habitat, providing nursery, feeding and spawning areas and refuge from predators (Kikuchi, 1980; Klumpp *et al.*, 1989; McArthur *et al.*, 2003). Therefore, knowledge of the dietary requirements of fish species that inhabit seagrass beds represents one of the essential components in understanding the significance of seagrass, which can help ensure that appropriate conservation measures for this habitat are implemented.

This study focuses on the importance of seagrass in the diet of *H. melanochir*, an important commercial and recreational fish species of southern Australia that is often targeted over seagrass beds. Previous studies on the diet of *H. melanochir* have been based mainly on gut content analyses, which revealed that a substantial volume indeed consisted of zosteracean seagrass (*Z. muelleri* or *Z. tasmanica*) (Ling, 1956; Thomson, 1957*a*; Robertson & Klumpp, 1983; Klumpp & Nichols, 1983; Edgar & Shaw, 1995*b*). *Hyporhamphus melanochir* appear to be one of only a few fish species that ingest seagrass in large quantities (Edgar & Shaw, 1995*b*) and they are also an important prey for commercial fishes (Western Australian salmon, Thomson, 1957*a*; mulloway, Kailola *et al.*, 1993; snook, Bertoni, 1995), and coastal water birds (large black cormorant, Mack, 1941; little penguin, Klomp & Wooller, 1988; Australasian gannet, Bunce, 2001; crested tern, pers.observ.), thus apparently representing a pathway for seagrass to higher trophic levels.

The analysis of gut contents can provide important information on prey taxonomy, however this method has several disadvantages. Gut content data only provide a snapshot of the diet, unless a frequent sampling strategy is adopted, which is often impractical. Identification of ingested material is often difficult; specifically, the gut contents of *H. melanochir* are thoroughly macerated, presumably by the action of pharyngeal teeth that are present in hemiramphids (Collette *et al.*, 1984; Greven *et al.*, 1997; Tibbetts, 1997). Furthermore, gut content analysis is also prone to biases and inaccuracies related to different digestion rates among prey species (Hyslop, 1980). For example, the importance of animals with exoskeletons and plant matter are likely to be overestimated in the diet since they are more resistant to digestion relative to soft-bodied organisms. Therefore, although *H. melanochir* undoubtedly feed on seagrass, the dependence upon seagrass as a dietary source is unresolvable by analysis of gut contents alone.

Stable isotope analysis is an alternative technique in food web studies and has been used to elucidate trophic relationships in a variety of aquatic ecosystems (e.g. freshwater, Fry & Sherr, 1984; estuarine, Peterson *et al.*, 1985; marine, Michener & Schell, 1994). The use of stable carbon and nitrogen isotopes (^{13}C and ^{12}C; ^{15}N and ^{14}N) to identify carbon sources and define trophic position for consumers is based on the following observations: (1) there is a broad range of carbon isotope compositions ($^{13}C/^{12}C$, expressed as $\delta^{13}C$) among different primary producers (Peterson & Fry, 1987); (2) the $\delta^{13}C$ signature of the consumer closely resembles that of its diet, i.e. 'you are what you eat' (DeNiro & Epstein, 1976, 1978), with only slight enrichment (<1‰) due to selective loss of ^{12}C during respiration; and (3) the $\delta^{15}N$ signature of a consumer is typically enriched by 3-4‰ relative to its diet, due to preferential loss of ^{14}N in nitrogenous waste (Minagawa & Wada, 1984). Therefore, $\delta^{13}C$ can be used to determine the carbon flow from primary producers to consumers and $\delta^{15}N$ to define the trophic level (Fry, 1991). While stable isotope analysis will augment rather than replace traditional dietary methods such as gut content analysis and direct observations of feeding, it has the advantages of providing temporally integrated information on diet (Tieszen *et al.*, 1983) and reflecting foods that are actually assimilated (not just ingested) by the consumer.

To estimate the proportions of food sources that contribute towards a diet, isotopic signatures are usually incorporated into linear mixing models (Phillips, 2001). For two food sources, isotopic signatures are only required for a single element (e.g. $\delta^{13}C$), whereas three sources require isotopic signatures of two elements (e.g. $\delta^{13}C$ and $\delta^{15}N$). A dual-isotope approach provides significantly more power to resolve trophic relationships than the use of a single stable isotope, especially where food sources are isotopically similar or when there are more than two food sources involved (Peterson *et al.*, 1985).

Stable isotope analysis on the diet of *H. melanochir* thus far is limited to the study of adult fish by Nichols *et al.* (1985), who estimated that the assimilated diet consisted of 65-80% seagrass and 20-35% benthic algae. However, only $\delta^{13}C$ signatures were utilized, no adjustment was made for enrichment in ^{13}C from diet to consumer, other potential food sources (i.e. zooplankton) were not removed of non-dietary carbonate, and it is unclear what life stage of *H. melanochir* was examined.

The present study employs stable carbon- and nitrogen-isotope ratios to investigate the diets of different life stages of *H. melanochir* from the Bay of Shoals, South Australia (S.A.). Included in the stable isotope analysis is a comparison between the isotopic signatures of *H. melanochir* with those of species that do not feed on seagrass. Specifically, the main objective was to quantify the contribution of zosteracean seagrass towards the assimilated diet of larval, juvenile and adult *H. melanochir* using two- and three-source mixing models developed by Phillips & Gregg (2001) and Phillips & Koch (2002).

6.2 MATERIALS AND METHODS

6.2.1 STUDY AREA

The Bay of Shoals (35°37′S, 137°36′E), situated on the northeast coast of Kangaroo Island, S.A., is a shallow and well-protected embayment (area *c*. 33 km^2), being exposed to very low wave energy action. It is characterised by sandy-mud tidal flats and fine sandy beaches and spits. Tides ranged from 0-1.4 m at the time of sampling. The water temperature is usually 1-2°C higher than the sea adjacent in summer, and the reverse in winter (Nichols & Hone, 1996). Extensive seagrass beds within the bay mainly consist of *Posidonia*, with patches of *Amphibolis*, *Halophila* and *Zostera*; large drifts of red algae are also common (Edyvane, 1999). A seasonal netting ban is enforced throughout the Bay of Shoals from November-April, inclusive, to minimise the mortality of undersize King George whiting *Sillaginodes punctata* taken in garfish haul nets.

6.2.2 COLLECTION OF *H. MELANOCHIR*, POTENTIAL FOOD SOURCES AND OTHER FISH

Larval, juvenile and adult *H. melanochir*, potential food sources and several other fish species were randomly collected from the Bay of Shoals in March 2000 for stable isotope analysis. Potential food sources included three species of seagrass (*Z. tasmanica*, *Halophila ovalis* and *Posidonia australis*), two species of algae (*Caulerpa remotifolia* and *Jania minuta*) and two mesh-size classes of zooplankton (160 and 500 µm). Based on the identification of zosteracean seagrass in the gut contents of *H. melanochir* from previous studies (Ling, 1956; Thomson, 1957*a*; Robertson & Klumpp, 1983; Klumpp & Nichols, 1983; Edgar & Shaw, 1995*b*), it is assumed that *Zostera* is a ‘known’ food source for juvenile and adult *H. melanochir*. The remaining plant samples of the ‘potential’ food sources were included in the stable isotope analysis because of their abundance in the Bay of Shoals and to check that the isotopic signatures of the plant samples could be differentiated from one another, thus enabling unambiguous contributions of *Zostera*, and possibly other food sources, to be predicted.

The other fish species sampled for stable isotope analysis and comparison with *H. melanochir* are listed in TABLE 6.1. Project costs limited the number of analyses for each sample (*H. melanochir*, potential food source, or other fish) to three replicates for each of the carbon and nitrogen analyses. Larval *H. melanochir* were sampled with a neuston net and immediately sorted by eye (Noell, 2003), while juvenile and adult *H. melanochir* were taken at night using a dip net and spotlight. The other fish species that co-occurred in the bay were caught by hook and line. Plankton was sampled at night by towing nets with mesh sizes of 160 and 500 µm just beneath the water surface. Approximately 30 mL of plankton was collected during each 5-min tow. Any plant or abiotic material was removed in the laboratory

TABLE 6.1 Stable isotope data used in standard mixing model and concentration-dependent model calculations (except data for fish species above the dotted line, which are for comparison only). Values for stable carbon- and nitrogen-isotope ratios are given as mean ± 1 S.D. The numbers in parentheses refer to the ranges of standard length (L_S, mm).

Sample	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Trophic level	[C] (%)	[N] (%)	[C]:[N]
Fish							
Spinytail leatherjacket (*Acanthaluteres brownii*) (127)	1	-12.1	4.0	2.5	42.5	13.3	3.2
Western Australian salmon (*Arripis georgiana*) (124-133)	3	-17.5 ± 0.7	8.6 ± 0.7	3.8	44.9	14.0	3.2
Tommy ruff (*Arripis truttacea*) (151-168)	3	-14.9 ± 1.9	9.4 ± 0.7	4.1	42.8	13.4	3.2
Blue rock whiting (*Haletta semifasciata*) (151-169)	3	-14.2 ± 0.3	7.4 ± 0.5	3.5	42.8	13.3	3.2
Sixspine leatherjacket (*Meuschenia freycinetti*) (201)	1	-14.4	8.5	3.8	37.6	11.8	3.2
White trevally (*Pseudocaranx dentex*) (163-177)	3	-14.1 ± 0.7	8.6 ± 0.6	3.8	37.9	11.6	3.3
King George whiting (*Sillaginodes punctata*) (226-254)	3	-13.7 ± 1.6	6.4 ± 0.6	3.2	31.2	9.9	3.1
Snook (*Sphyraena novaehollandiae*) (392-419)	3	-16.7 ± 0.7	10.5 ± 0.6	4.4	24.7	8.0	3.1
Southern sea garfish (*Hyporhamphus melanochir*) larvae (6-9)	1*	-13.1	4.0	2.5	38.0	8.9	4.3
H. melanochir juveniles (89-148)	3	-14.8 ± 0.3	6.2 ± 0.3	3.1	40.6	12.5	3.2
H. melanochir adults (256-288)	3	-12.4 ± 0.4	3.6 ± 0.3	2.4	39.5	12.1	3.3
Seagrass							
Halophila ovalis	3	-11.6 ± 0.4	-0.9 ± 0.6	1.0	33.1	2.7	12.2
Zostera tasmanica	3	-8.6 ± 0.2	0.0 ± 0.3	1.3	38.3	2.2	17.5
Posidonia australis	3	-5.5 ± 0.1	-1.0 ± 0.5	1.0	40.8	1.3	31.7
Algae							
Caulerpa remotifolia	3	-15.7 ± 0.4	2.8 ± 0.3	2.1	39.1	2.3	17.0
Jania minuta	3	-10.5 ± 0.3	1.1 ± 0.3	1.6	19.0	0.7	28.9
Plankton							
160 µm zooplankton	3	-19.6 ± 0.2	4.1 ± 0.3	2.5	48.8	7.3	6.8
500 µm zooplankton	3	-18.5 ± 0.1	4.2 ± 0.5	2.5	46.9	7.6	6.2

*Pooled sample of 32 larvae.

from both mesh size samples under a dissecting microscope. The remaining zooplankton samples, which appeared to mainly consist of copepods, were retained for analysis. Seagrass and algae were collected by divers. Samples were frozen as soon as possible after collection and stored at -20°C until processed.

6.2.3 PROCESSING AND STABLE ISOTOPE ANALYSIS

Approximately 3-5 and 5-10 mg dry weight of animal and plant matter, respectively, was sufficient for carbon and nitrogen analysis. For juvenile and adult fish, dorsal muscle tissue was removed and skinned from each individual after measuring for standard length (L_S, mm). Samples of *H. melanochir* larvae were pooled to obtain sufficient material for stable isotope analysis and ground whole after drying. Half the zooplankton samples were acid-treated in 10% HCl and rinsed with distilled water to remove non-dietary carbonate contaminants. The acid-treated half was retained for analysis of carbon, the other half for nitrogen (acid-treating interferes with the $\delta^{15}N$ signature; Bunn *et al.*, 1995). Plants were rinsed in distilled water after leaves were scraped of epiphytes with a blunt scalpel. Samples were oven-dried at 60°C for 24 h and ground to a fine powder with a mortar and pestle for animal tissue or a ring mill (Labtechnics) for plant material. Dried material were stored in airtight containers and returned to the freezer until analysed.

All samples were oxidised and the resultant CO_2 and N_2 gases analysed with a continuous flow isotope ratio mass spectrometer (Europa Tracermass, Crewe, England) at the Centre for Catchment and Instream Research, Faculty of Environmental Sciences, Griffith University, Nathan, Queensland. Stable isotope ratios are expressed in delta (δ) notation, defined as the relative per mil (‰) difference from a standard reference material (PeeDee belemnite limestone for carbon and ambient air for nitrogen), given by:

$$\delta I = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \qquad [6.1]$$

where I is the isotope ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Positive values indicate a sample is relatively ‘enriched,’ while negative values indicate a sample is ‘depleted.’ Stable isotope ratios hereafter refer to mean values from the three replicates of each sample analysis unless stated otherwise. Analytical precision was within ±0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ based on the S.D. of 5-7 replicates each of three sucrose and ammonium sulphate standards, respectively.

6.2.4 TROPHIC LEVELS

The trophic level at which each of the consumer species occupies can indicate their likely feeding mode and, therefore, the hierarchical structure that exists among fauna of the Bay of Shoals. Trophic levels were calculated on the basis of the $\delta^{15}N$ signature of mixture M (i.e. the consumer) following the method of Hobson & Welch (1992):

$$\text{Trophic level} = \frac{\delta^{15}N_M - (-1.0)}{3.4} + 1 \quad [6.2]$$

where -1.0 is the $\delta^{15}N$ signature (‰) of the primary producer *P. australis* (i.e. the assumed baseline of the food web), 3.4 is the nitrogen isotope fractionation per trophic level (‰), and 1 refers to the trophic level above *P. australis*.

6.2.5 STANDARD LINEAR MIXING MODELS

Relative contributions (in biomass) of different food sources to the diet of *H. melanochir* were quantified with linear mixing models by a repeated/iterative method where all possible combinations of food sources were inputted. Using mixing models, the isotopic signatures of *n* elements are required to estimate the proportions of up to *n* + 1 food sources.

For the single isotope (usually $\delta^{13}C$), two-source case, a simple linear mixing model can be formulated from the following mass balance equations:

$$\delta^{13}C_M - \Delta^{13}C = f_X\delta^{13}C_X + f_Y\delta^{13}C_Y \quad [6.3]$$

$$1 = f_X + f_Y \quad [6.4]$$

and the proportions of food sources X and Y in M (i.e. the consumer, *H. melanochir*) can be calculated (Bunn & Boon, 1993) as:

$$f_X = \frac{(\delta^{13}C_M - \Delta^{13}C) - \delta^{13}C_Y}{\delta^{13}C_X - \delta^{13}C_Y} \quad [6.5]$$

$$f_Y = 1 - f_X \quad [6.6]$$

where *f* represents the fractional contribution of C from each food source to the consumer's diet, and $\Delta^{13}C$ is the isotopic fractionation (change in $\delta^{13}C$ during digestion, metabolism and assimilation) between the integrated food sources X and Y and the consumer's tissue. The isotopic fractionation is seldom known exactly for any particular animal; therefore, the commonly assumed values of 0.8‰ for $\Delta^{13}C$ and 3.4‰ for $\Delta^{15}N$ were used, based on the

seminal findings of DeNiro & Epstein (1978) and Minagawa & Wada (1984), respectively. Only the signature of the consumer is corrected in subsequent calculations since fractionation is assumed to be constant for all the food sources. For every combination where the consumer's $\delta^{13}C$ signature occurs between those of two sources (after correcting for fractionation), a valid result is produced (i.e. f_X and f_Y are both positive, and their sum equals one).

For the dual isotope (e.g. $\delta^{13}C$ and $\delta^{15}N$), three-source mixing model, the mass balance equations are:

$$\delta^{13}C_{M'} = f_X\delta^{13}C_X + f_Y\delta^{13}C_Y + f_Z\delta^{13}C_Z \quad [6.7]$$

$$\delta^{15}N_{M'} = f_X\delta^{15}N_X + f_Y\delta^{15}N_Y + f_Z\delta^{15}N_Z \quad [6.8]$$

$$1 = f_X + f_Y + f_Z \quad [6.9]$$

where X, Y, M and *f* are defined as above, and Z is the third food source. As for Eq. [6.3], the isotopic values for the consumer's tissue must be adjusted by the appropriate Δ values to account for trophic fractionation, but these terms have been omitted in Eqs [6.7] and [6.8] and in subsequent equations for simplicity of presentation, and fractionation-corrected signatures are instead denoted by the prime (′) symbol (e.g. $\delta^{13}C_{M'} = \delta^{13}C_M - \Delta^{13}C$). This linear mixing model comprises three equations with three unknowns (f_X, f_Y and f_Z), which can be solved for unique values of the unknowns. One expression of the solution (Phillips, 2001) is:

$$f_X = \frac{(\delta^{15}N_Z - \delta^{15}N_Y)(\delta^{13}C_{M'} - \delta^{13}C_Y) - (\delta^{13}C_Z - \delta^{13}C_Y)(\delta^{15}N_{M'} - \delta^{15}N_Y)}{(\delta^{15}N_Z - \delta^{15}N_Y)(\delta^{13}C_X - \delta^{13}C_Y) - (\delta^{13}C_Z - \delta^{13}C_Y)(\delta^{15}N_X - \delta^{15}N_Y)} \quad [6.10]$$

$$f_Y = \frac{(\delta^{13}C_{M'} - \delta^{13}C_Z) - (\delta^{13}C_X - \delta^{13}C_Z)\,f_X}{\delta^{13}C_Y - \delta^{13}C_Z} \quad [6.11]$$

$$f_Z = 1 - f_X - f_Y \quad [6.12]$$

In a bivariate plot of the two isotopes, the values of the consumer must fall within the triangular space enclosed by lines connecting the three food sources (once the values for these sources have been corrected for fractionation or, in the case of this study, once the value of the consumer has been corrected since fractionation among all sources is assumed to be constant) (Phillips & Koch, 2002).

6.2.6 UNCERTAINTY IN SOURCE PROPORTION ESTIMATES

For both two- and three-source models, the variances, standard errors and confidence intervals for source proportion estimates are presented following calculations of Phillips &

Gregg (2001) to account for the observed variability in the isotopic signatures of the sources as well as the mixture.

6.2.7 CONCENTRATION-WEIGHTED MIXING MODEL

In the three-source mixing model, there is an implicit assumption that the proportion of C that the consumer derives from source X is the same as the proportion of N that it derives from source X (i.e. f_X is the same in Eqs [6.7] and [6.8], and similarly for sources Y and Z. This assumption appears reasonable if the food sources have similar C and N concentrations (e.g. where sources belong to the same class of food). However, since it is likely that *H. melanochir* is omnivorous, and concentrations are expected to differ substantially among plant and animal food sources, a concentration-weighted mixing model (Phillips & Koch, 2002) was used to incorporate these differences and adjust source proportion estimates.

Calculations for uncertainty in source proportions and the concentration-weighted mixing model were carried out using spreadsheets developed by the respective authors (see Phillips & Gregg, 2001 & Phillips & Koch, 2002 for website addresses).

6.3 RESULTS

6.3.1 CARBON ISOTOPE VALUES

Stable carbon-isotope ratios of biota collected in the Bay of Shoals ranged from -19.6 to -5.5‰ (TABLE 6.1; FIG. 6.1). In general, plants were ^{13}C-enriched (-15.7 to -5.5‰) and zooplankton ^{13}C-depleted (-19.6 to -18.5‰) relative to fish (-17.5 to -12.1‰). All potential food sources for *H. melanochir*, i.e. seagrass, algae and zooplankton, were discernible by their δ^{13}C signatures. The δ^{13}C signatures for larval, juvenile and adult *H. melanochir* of -13.1, -14.8 and -12.4‰, respectively, were within the range obtained for all other fish species examined (-17.5 to -12.1‰).

6.3.2 NITROGEN ISOTOPE VALUES AND TROPHIC LEVELS

Stable nitrogen-isotope ratios ranged from -1.0 to 10.5‰ (TABLE 6.1; FIG. 6.1). As expected, δ^{15}N signatures of plants spanned a narrow range (-1.0 to 2.8‰) and were depleted compared to consumers. Of the consumers, fish were generally ^{15}N-enriched relative to zooplankton, except for larval and adult *H. melanochir* and spinytail leatherjacket (*Acanthaluteres brownii*), which shared similar δ^{15}N signatures (*c.* 4‰). Juvenile *H. melanochir* were enriched in ^{15}N (6.2‰) relative to larvae yet still at the lower range for all fish species.

On the basis of their δ^{15}N signatures, larval and adult *H. melanochir*, *A. brownii*, and zooplankton (both 160- or 500-um size fractions) occupied the third trophic level, and

juvenile *H. melanochir* occupied the fourth. The highest order consumer was snook (*Sphyraena novaehollandiae*), which occupied the fifth trophic level.

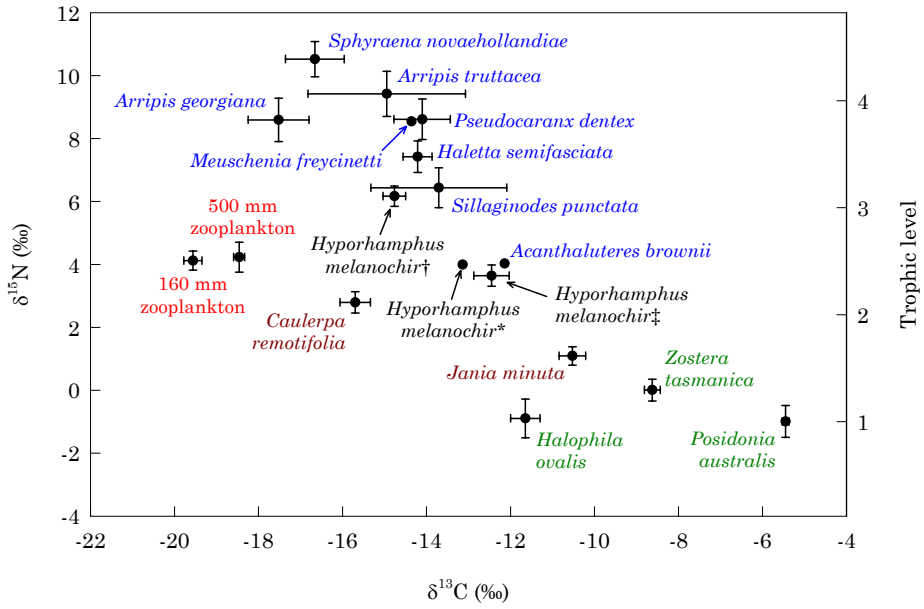

FIG. 6.1 Bivariate plot of the stable carbon- and nitrogen-isotope ratios (mean ± 1 S.D.) of fish (blue text, except *Hyporhamphus melanochir*, which is black), seagrass (green), algae (brown) and plankton (red) in the Bay of Shoals. *Larval *H. melanochir*; †juvenile *H. melanochir*; ‡adult *H. melanochir*. Classification of trophic level was based on a 3.4‰ increase in $\delta^{15}N$ from one level to the next.

6.3.3 RELATIVE CONTRIBUTIONS OF FOOD SOURCES TO THE DIET OF *H. MELANOCHIR*

Plant and zooplankton samples considered to be potential food sources for *H. melanochir* appeared abundant in the Bay of Shoals at the time of their collection, all of which were isotopically distinct from one another either by $\delta^{13}C$ alone or by $\delta^{13}C$ and $\delta^{15}N$ combined. Based on the standard mixing models and the concentration-dependent model, several combinations of these sources explain the isotopic signatures for *H. melanochir* (for example, see FIG. 6.2).

6.3.3.1. Two-source linear mixing model

Based on the two-source model, the same combinations of potential food sources were calculated for the diets of *H. melanochir* larvae, juveniles and adults since no food source had a $\delta^{13}C$ signature between these life stages; however, the relative contributions of the sources within the combinations varied (TABLE 6.2). Out of 21 possible combinations of two food sources, 12 could be solved for each life stage by the linear mixing model; these combinations were therefore considered to be the actual assimilated diet of *H. melanochir*.

For larvae, the diet consisted either of *Caulerpa* (57-83%) and another plant (*Halophila*, 43%; *Zostera*, 25%; *Jania*, 34%; or *Posidonia*, 17%), or zooplankton (160- or 500-µm size

fraction, 29-65%) and a plant (*Halophila*, 66-71%; *Zostera*, 46-51%; *Jania*, 57-62%; or *Posidonia*, 35-40%).

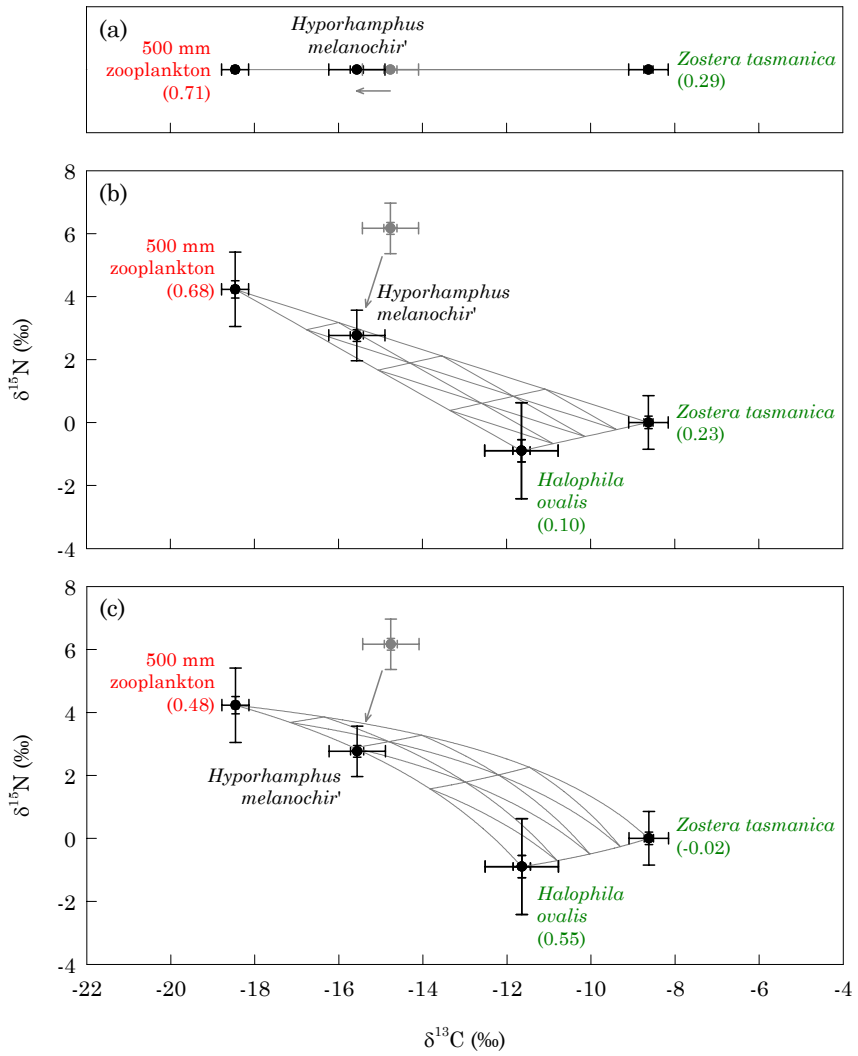

FIG. 6.2 Examples for estimating proportions (in parentheses) of different food sources in the diet of juvenile *Hyporhamphus melanochir* using (a) two- and (b) three-source standard mixing models and (c) the concentration-dependent model. Stable isotope ratios are shown for *H. melanochir* and food 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.

The $\delta^{13}C$ signature of juveniles was very similar to that of *Caulerpa* (after being adjusted for fractionation), which explains the almost exclusive contribution of this source calculated in the juvenile diet (97-99%). In other combinations, contributions of other plants were

comparatively less (and therefore zooplankton was greater) than for the corresponding larval diet (*Halophila*, 43-51%; *Zostera*, 29-37%; *Jania*, 37-44%; or *Posidonia*, 22-28%).

TABLE 6.2 Relative contributions (*f*, in %) of food sources (X and Y) in the diets of larval, juvenile and adult *Hyporhamphus melanochir* estimated by the two-source mixing model. Values for *f* are given as **mean** ± 1 S.E. The 95% confidence interval (C.I.) was not calculated for larval food sources since only one pooled sample of larvae was analysed. Combinations are only given where $0 \leq f \leq 100\%$ for all sources; these are listed in alphanumeric order. Boxes indicate the combinations where *Zostera* is the main plant food source.

Source X	f_X	C.I.$_X$	Source Y	f_Y	C.I.$_Y$
Larval *H. melanochir*					
Caulerpa	**57** ± 8		*Halophila*	**43** ± 8	
Caulerpa	**75** ± 4		*Zostera*	**25** ± 4	
Caulerpa	**66** ± 6		*Jania*	**34** ± 6	
Caulerpa	**83** ± 3		*Posidonia*	**17** ± 3	
Halophila	**71** ± 4		160 µm zooplankton	**29** ± 4	
Halophila	**66** ± 4		500 µm zooplankton	**34** ± 4	
Zostera	**51** ± 3		160 µm zooplankton	**49** ± 3	
Zostera	**46** ± 3		500 µm zooplankton	**54** ± 3	
Jania	**62** ± 3		160 µm zooplankton	**38** ± 3	
Jania	**57** ± 4		500 µm zooplankton	**43** ± 4	
Posidonia	**40** ± 2		160 µm zooplankton	**60** ± 2	
Posidonia	**35** ± 2		500 µm zooplankton	**65** ± 2	
Juvenile *H. melanochir*					
Caulerpa	**97** ± 6	77-100	*Halophila*	**3** ± 6	0-23
Caulerpa	**98** ± 4	87-100	*Zostera*	**2** ± 4	0-13
Caulerpa	**97** ± 5	82-100	*Jania*	**3** ± 5	0-18
Caulerpa	**99** ± 3	91-100	*Posidonia*	**1** ± 3	0-9
Halophila	**51** ± 2	44-57	160 µm zooplankton	**49** ± 2	43-56
Halophila	**43** ± 3	34-51	500 µm zooplankton	**57** ± 3	49-66
Zostera	**37** ± 2	31-42	160 µm zooplankton	**63** ± 2	58-69
Zostera	**29** ± 2	22-37	500 µm zooplankton	**71** ± 2	63-78
Jania	**44** ± 2	38-51	160 µm zooplankton	**56** ± 2	49-62
Jania	**37** ± 2	29-44	500 µm zooplankton	**63** ± 2	56-71
Posidonia	**28** ± 1	24-32	160 µm zooplankton	**72** ± 1	68-76
Posidonia	**22** ± 1	17-28	500 µm zooplankton	**78** ± 1	72-83
Adult *H. melanochir*					
Caulerpa	**40** ± 7	17-62	*Halophila*	**60** ± 7	38-83
Caulerpa	**65** ± 4	53-78	*Zostera*	**35** ± 4	22-47
Caulerpa	**53** ± 5	35-70	*Jania*	**47** ± 5	30-65
Caulerpa	**76** ± 3	67-85	*Posidonia*	**24** ± 3	15-33
Halophila	**80** ± 4	68-92	160 µm zooplankton	**20** ± 4	8-32
Halophila	**77** ± 4	63-90	500 µm zooplankton	**23** ± 4	10-37
Zostera	**58** ± 2	48-68	160 µm zooplankton	**42** ± 2	32-52
Zostera	**53** ± 3	42-64	500 µm zooplankton	**47** ± 3	36-58
Jania	**70** ± 3	60-80	160 µm zooplankton	**30** ± 3	20-40
Jania	**66** ± 3	51-80	500 µm zooplankton	**34** ± 3	20-49
Posidonia	**45** ± 2	37-52	160 µm zooplankton	**55** ± 2	48-63
Posidonia	**40** ± 2	32-48	500 µm zooplankton	**60** ± 2	52-68

For the adult diet, the potential combinations and proportions of food sources were similar to those of larvae, but with a slightly greater proportion of seagrass since the $\delta^{13}C$ signature of adults is closer to the signatures of seagrass species. The adult diet consisted

either of *Caulerpa* (40-76%) and another plant (*Halophila*, 60%; *Zostera*, 35%; *Jania*, 47%; or *Posidonia*, 24%), or zooplankton (160- or 500-µm size fraction, 20-60%) and a plant (*Halophila*, 77-80%; *Zostera*, 53-58%; *Jania*, 66-70%; or *Posidonia*, 40-45%).

6.3.3.2. Three-source linear mixing model

Out of 35 possible combinations of three food sources in the assimilated diet of *H. melanochir*, 5 could be solved for larvae, 12 could be solved for juveniles, while none were possible for adults. Based on three-source model calculations, the larval diet consisted of *Halophila* (69-70%), 160 µm zooplankton (25-30%), and one of the other sources (1-4%) (TABLE 6.3). In the juvenile diet, zooplankton (160 and/or 500 µm) was part of every valid combination. In most of these combinations, zooplankton was the predominant food source (56-73%) in combination with one or two plants (*Halophila*, 1-13%; *Zostera*, 3-34%; *Jania*, 25-44%; or *Posidonia*, 3-27%). Other combinations included *Caulerpa* (41-92%), zooplankton (3-32%), and another plant (*Zostera*, 5%; *Jania*, 4-27%; or *Posidonia*, 1%).

6.3.3.3. Concentration-dependent mixing model

Out of 35 possible combinations of three food sources in the assimilated diet of *H. melanochir*, 15 could be solved for juveniles only. No valid combinations were calculated for larvae or adults using the concentration-dependent mixing model. For the juvenile diet, most combinations consisted almost exclusively of *Caulerpa* (95-99%) (TABLE 6.4), which is consistent with the two-source mixing model. Other combinations included *Halophila* (27-52%), zooplankton (24-48%) and another plant (*Caulerpa*, 49%; *Zostera*, 17%; *Jania*, 30%; or *Posidonia*, 9%).

6.4 DISCUSSION

Stable isotope analysis was used to examine the importance of seagrass, particularly *Zostera*, in the diets of larval, juvenile and adult *H. melanochir*, the contributions of which (along with other potential food sources) were estimated using mixing models developed by Phillips & Gregg (2001) and Phillips & Koch (2002). The $\delta^{13}C$ signature of *Zostera* and other seagrasses collected in this study were typical of those in temperate regions (McMillan, 1980; Hemminga & Mateo, 1996). The $\delta^{13}C$ signature of adult *H. melanochir* from the Bay of Shoals (-12.4‰) was slightly different from adults caught at Corner Inlet, Vic. (-12.1‰, Nichols *et al.*, 1985), but different to those caught at One Tree Point, Tas. (22.8‰, Fenton, 1996), probably due to spatial (Jennings *et al.*, 1997), temporal (Goering *et al.*, 1990), and ontogenetic (Lindsay *et al.*, 1998) variations that occur within a species, or possibly due to spatial differences in diet composition. In a study of food chains in seagrass

TABLE 6.3 Relative contributions (*f*, in %) of food sources (X, Y, and Z) in the diets of larval and juvenile *Hyporhamphus melanochir* estimated by the three-source mixing model. Values for *f* are given as **mean** ± 1 S.E. The 95% confidence interval (C.I.) was not calculated for larval food sources since only one pooled sample of larvae was analysed. Combinations are only given where $0 \leq f \leq 100\%$ for all sources; these are listed in alphanumeric order. Boxes indicate the combinations where *Zostera* is the main plant food source.

Source X	f_X	C.I.$_X$	Source Y	f_Y	C.I.$_Y$	Source Z	f_Z	C.I.$_Z$
Larval *H. melanochir*								
Caulerpa	**3** ± 52		*Halophila*	**70** ± 22		160 µm zooplankton	**27** ± 30	
Halophila	**69** ± 25		*Zostera*	**1** ± 21		160 µm zooplankton	**29** ± 7	
Halophila	**70** ± 21		*Jania*	**1** ± 22		160 µm zooplankton	**29** ± 5	
Halophila	**69** ± 24		*Posidonia*	**1** ± 15		160 µm zooplankton	**30** ± 8	
Halophila	**70** ± 8		160 µm zooplankton	**25** ± 66		500 µm zooplankton	**4** ± 73	
Juvenile *H. melanochir*								
Caulerpa	**88** ± 133	0-100	*Zostera*	**5** ± 39	0-90	500 µm zooplankton	**7** ± 93	0-100
Caulerpa	**41** ± 857	0-100	*Jania*	**27** ± 368	0-100	160 µm zooplankton	**32** ± 489	0-100
Caulerpa	**92** ± 96	0-100	*Jania*	**4** ± 36	0-82	500 µm zooplankton	**3** ± 60	0-100
Caulerpa	**92** ± 101	0-100	*Posidonia*	**3** ± 23	0-52	500 µm zooplankton	**5** ± 78	0-100
Halophila	**10** ± 14	0-43	*Zostera*	**23** ± 11	0-47	500 µm zooplankton	**68** ± 4	57-78
Halophila	**1** ± 11	0-28	*Jania*	**44** ± 11	19-68	160 µm zooplankton	**56** ± 3	50-61
Halophila	**13** ± 12	0-42	*Jania*	**25** ± 12	0-52	500 µm zooplankton	**62** ± 3	56-68
Halophila	**13** ± 13	0-45	*Posidonia*	**16** ± 8	0-33	500 µm zooplankton	**72** ± 6	56-87
Zostera	**3** ± 58	0-100	*Jania*	**41** ± 72	0-100	160 µm zooplankton	**56** ± 14	26-87
Zostera	**34** ± 5	21-46	160 µm zooplankton	**38** ± 48	0-100	500 µm zooplankton	**29** ± 52	0-100
Jania	**39** ± 109	0-100	*Posidonia*	**3** ± 69	0-100	160 µm zooplankton	**58** ± 40	0-100
Posidonia	**27** ± 4	17-36	160 µm zooplankton	**54** ± 50	0-100	500 µm zooplankton	**19** ± 54	0-100

TABLE 6.4 Relative contributions (f, in %) of three food sources (X, Y, and Z) in the diet of juvenile *Hyporhamphus melanochir* estimated by the concentration-dependent model. As defined by Phillips & Koch (2002), $f_{X,B}$, $f_{Y,B}$, and $f_{Z,B}$ represent the fractions of assimilated biomass of sources in the mixture (equivalent to f_X, f_Y, and f_Z in the standard mixing model, indicated in boldface). Similarly, $f_{X,C}$, $f_{Y,C}$, $f_{Z,C}$, $f_{X,N}$, $f_{Y,N}$, and $f_{Z,N}$ represent the fractions of assimilated C or N of the individual sources in the mixture. Combinations are only given where $0 \leq f \leq 100\%$ for all sources after rounding; these are listed in alphanumeric order.

Source X	$f_{X,B}$	$f_{X,C}$	$f_{X,N}$	Source Y	$f_{Y,B}$	$f_{Y,C}$	$f_{Y,N}$	Source Z	$f_{Z,B}$	$f_{Z,C}$	$f_{Z,N}$
Caulerpa	95	97	99	*Halophila*	0	0	0	*Jania*	5	3	2
Caulerpa	99	99	99	*Halophila*	0	0	0	*Posidonia*	1	1	1
Caulerpa	49	49	31	*Halophila*	27	23	20	500 µm zooplankton	24	29	49
Caulerpa	95	97	99	*Zostera*	0	0	0	*Jania*	5	3	2
Caulerpa	99	99	99	*Zostera*	0	0	0	*Posidonia*	1	1	1
Caulerpa	96	96	94	*Zostera*	3	3	3	160 µm zooplankton	1	1	3
Caulerpa	97	97	96	*Zostera*	2	2	2	500 µm zooplankton	1	1	2
Caulerpa	95	98	99	*Jania*	5	3	2	160 µm zooplankton	0	0	0
Caulerpa	95	97	99	*Jania*	5	3	2	500 µm zooplankton	0	0	0
Caulerpa	99	99	99	*Posidonia*	1	1	1	160 µm zooplankton	0	0	0
Caulerpa	99	99	99	*Posidonia*	1	1	1	500 µm zooplankton	0	0	0
Halophila	36	29	21	*Zostera*	17	16	8	160 µm zooplankton	47	55	72
Halophila	33	32	24	*Jania*	30	17	5	160 µm zooplankton	37	52	71
Halophila	43	34	25	*Posidonia*	9	9	3	160 µm zooplankton	48	57	73
Halophila	52	44	28	160 µm zooplankton	6	7	8	500 µm zooplankton	42	50	64

communities, Nichols et al. (1985) examined stable isotope signatures of representative fauna associated with seagrass, and concluded that the signature for *H. melanochir* (presumably adult stage) could be explained by a diet that consisted of 65-80% *Zostera* and the remainder benthic algae.

Based on $\delta^{15}N$ signatures, the trophic positions occupied by adults and larvae (third level) suggest these two life stages be classified as herbivores, while juvenile *H. melanochir* (fourth) are classified as omnivores. In comparison, most other species are higher consumers, of which the highest is snook (*S. novaehollandiae* (fifth trophic level), a species known todemonstrate carnivory or piscivory (Bertoni, 1995). *Acanthaluteres brownii* was the only other species that occupied the same trophic level as *H. melanochir* adults/larvae, which is consistent with their diet including algae (Bell & Pollard, 1989). The $\delta^{15}N$ signatures of *H. melanochir* larvae surprisingly indicate herbivory. The low $\delta^{15}N$ is likely to be the result of inheritance of parental isotopic composition. The parental influence is expected to have a significant effect on early-stage larvae, which develop mostly by yolk assimilation, an effect that will be diluted as exogenous feeding progresses (Vander Zanden *et al.*, 1998; Leite *et al.*, 2002). Therefore, the isotopic composition of recently-hatched *H. melanochir* larvae probably does not reflect the larval diet as much as the energy source of adults that supports larval production.

Larvae analysed in this study were *c.* 8 mm body length (L_B). Assuming isometric growth, these larvae would be approximately double the weight of newly hatched larvae (6.4 mm L_B; Noell, 2003). Since the tissue synthesized during growth of larvae will reflect the isotopic composition of the diet from the time at which exogenous feeding first occurred, a larva that doubles its weight after hatching should have an isotopic composition that is determined equally by growth and pre-existing muscle tissue (Wainright *et al.*, 1996). In addition to tissue growth, isotopic composition is also affected by metabolic turnover. Previously determined turnover rates for various consumers, including postlarval brown shrimp (Fry & Arnold, 1982), gerbils (Tieszen *et al.*, 1983), Japanese quail (Hobson & Clark, 1992), broad whitefish (Hesslein *et al.*, 1993), and larval red drum (Herzka & Holt, 2000), suggest that turnover rates are specific to the taxon and tissue being analysed. Since the larvae collected in this study were estimated to be only 5 days old post hatch (Chapter 5), which is less than the turnover half-life for most organisms, it is assumed that metabolic turnover had a relatively minor effect on their isotopic signature.

The linear mixing models adopted for this study only apply if all the important food sources have been measured, and if the number of sources being considered is limited to that required in the calculations. The method works best when the stable isotope values of the food sources differ substantially and show low variance (Phillips & Gregg, 2001). Although no information exists for isotopic fractionation specific to *H. melanochir*, the re-calculation of

results using values between 0.1 and 1.5‰ (the 95% C.I. for all organisms in the study by DeNiro & Epstein, 1978) indicate that the two-source model is quite robust to this error source, with relative contributions affected by only ±7%.

Across all of the models used in this study, most of the combinations of food sources that explain the isotopic signatures of *H. melanochir* were considered doubtful, either because *Zostera* was not included as one of the sources or an improbable contribution of another food source was predicted. For example, based on the substantial evidence by traditional dietary methods that zosteracean seagrass is ingested in large quantities by *H. melanochir* (Ling, 1956; Thomson, 1957*a*; Robertson & Klumpp, 1983; Klumpp & Nichols, 1983; Edgar & Shaw, 1995*b*), and assuming similar rates of decomposition in the gut for different plants, the predominance of another plant over *Zostera* is unlikely. This presumption was supported somewhat by the lack of *Posidonia*, for example, in the gut contents of some fish that were examined despite being the predominant seagrass species in the sampling area (pers. observ.). Furthermore, even though *Zostera* was considerably less abundant than *Posidonia*, fragments of *Zostera* leaves were still identifiable among the thoroughly macerated gut contents.

Of the six combinations where *Zostera* was predicted to be the main plant source, two were predicted for larvae (from the two-source model), four for juveniles (two from each of the two- and three-source models), and two for adults (from the two-source model). The results from the two-source model indicate that the $\delta^{13}C$ signatures of different life stages of *H. melanochir* can be explained by a diet consisting of 46-51% *Zostera* (and the remainder zooplankton) for larvae, 29-37% *Zostera* for juveniles, and 53-58% *Zostera* for adults. If it is assumed that the isotopic signature of larvae is derived from the parental (adult) diet, it is proposed that *H. melanochir* undergo an ontogenetic shift throughout its life history from planktonivory in larvae to ominivory in juveniles and adults by gradually introducing increasing proportions of *Zostera* into the diet. Results of this study are consistent with this proposed feeding cycle for *H. melanochir*.

An increasing dependence upon *Zostera* with growth of *H. melanochir* is not surprising, particularly when it is considered that the pharyngeal mill required for effective maceration (and assimilation) of seagrass is not sufficiently developed until the early juvenile stage in hemiramphids (Tibbetts, 1991). Certainly, it was noted that the gut contents of *H. melanochir* larvae that had been cleared and stained for obtaining meristic counts (Chapter 4) showed no signs of *Zostera* fragments, but instead consisted almost entirely of zooplankton (FIG. 6.3), a finding consistent with larvae of another hemiramphid, *Hemiramphus sajori* (Wada & Kuwahara, 1994). Of the identifiable zooplankton observed in the gut contents of *H. melanochir* larvae (TABLE 6.5), the dominant food items belonged to the subclass Copepoda (orders Calanoida and Cyclopoida). Although not quantitatively assessed, the two

zooplankton size fractions yielded similar taxonomic groups (yet differed in size composition) that were consistent with observations of gut contents in cleared and stained larvae.

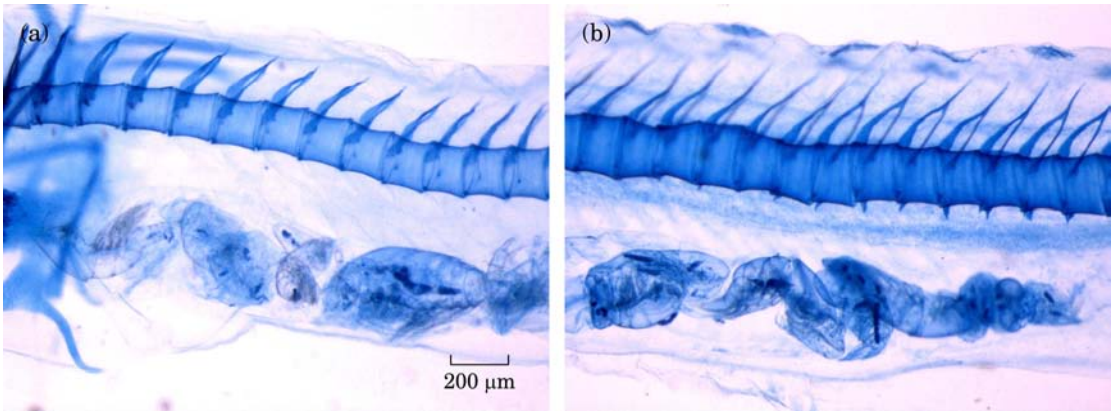

FIG. 6.3 Photomicrographs of zooplankton found in the gut contents of *Hyporhamphus melanochir* larvae after the larvae were cleared and stained for fin and vertebrae meristics. Both photomicrographs are at the same scale.

TABLE 6.5 Taxonomy of zooplankton positively identified among the gut contents of *Hyporhamphus melanochir* larvae. These food items were incidentally found after larvae were cleared and stained for the examination of fin and vertebrae meristics (Chapter 4). Taxa are listed in alphabetical order.

Class	Subclass	Order	Notes
Crustacea			eggs
	Copepoda	Calanoida	
		Cyclopoda	
	Malacostraca	Cumacea	
		Decapoda	
		Isopoda	
			zoeae
		Mysidacea	
	Ostracoda		
Scyphozoa			

The results from the three-source model indicate that the diet of juvenile *H. melanochir* consists of either: 23% *Zostera*, 10% *Halophila* and 68% 500-µm zooplankton; or 34% *Zostera*, 38% 160-µm zooplankton and 29% 500-µm zooplankton. The latter combination of food sources and their relative contributions is basically the same as the results of the two-source model for juveniles (if both zooplankton size fractions are combined as one source). The combination that includes *Halophila* appears implausible since, as mentioned above, no plant material other than *Zostera* was visible when the gut contents of selected fish were examined. No reasonable combinations were calculated for larvae or adults by the three-

source model or for any life stage by the concentration-dependent model, which may indicate their unsuitability for assessing diet in these instances.

Failure to fall within the mixing triangle of the three-source model indicates that either: (1) a source that is neither important nor part of the diet has been included in the model; (2) an important food source has been missed; (3) an incorrect trophic fractionation value has been used; or (4) an assumption of the mixing model has been violated (Phillips & Koch, 2002). It should also be noted that the mixture falling within the mixing triangle is no guarantee that none of these violations have occurred, since multiple errors could possibly compensate for each other, or individual errors might shift the mixture but still within the triangle. On the other hand, a mixture that includes a food source that falls just outside the mixing triangle may be considered a reasonable combination if some part of that food's associated error or confidence interval is inside the triangle. The contribution of this food source is negative and close to zero; therefore, the results almost effectively revert to those of the two-source model. In any case, model results should be viewed as a first approximation until trophic fractionation is determined specifically for *H. melanochir* of different life stages, and errors are reduced through larger sample sizes.

The occurrence of seagrass as a major food item in an omnivorous adult diet and/or an increasing dependence upon seagrass with successive life stages following an early planktonivorous stage are common observations among the Hemiramphidae (e.g. *H. brasiliensis*, Randall, 1967; Berkeley & Houde, 1978; *H. unifasciatus*, Carr & Adams, 1973; *H. knysnaensis*, Coetzee, 1981). The conclusion by Talwar (1962*a*) that '*H. quoyi* and *He. far* feed exclusively on seagrass and green algae,' based on commercial catches probably caught during daylight, is possibly biased towards these food items, particularly if the feeding strategy of this species is similar to that of *H. melanochir*, which feed on seagrass during the day and switch to zooplankton that emerge into the water column over seagrass beds at night (Robertson & Klumpp, 1983).

Seagrass meadows are widely distributed throughout Gulf St Vincent, except off southern Fleurieu Peninsula where the bottom habitat is predominantly calcareous reef and algae (Edyvane, 1999). Although most of the commercially-caught *H. melanochir* are taken by haul nets that are operated almost exclusively over *Zostera* (or 'garweed,' as it is commonly known among fishers), some commercial fishers use a dab net and spotlight to catch *H. melanochir* at night over variable habitats. It is difficult to speculate what *H. melanochir* might feed upon in coastal waters where seagrass is sparse, such as off southern Fleurieu Peninsula. *Hyporhamphus melanochir* are targeted by dab netters in these waters only during summer months, which coincides with the spawning period, and in a study of the breeding habits of *H. melanochir*, Ling (1958) noted that:

> *"Spawning fish ceased to feed after about September. Feeding was resumed early in the following year, or after spawning had been completed in the case of late spawners."*

Hyporhamphus melanochir have also been known to ingest algae (Ling, 1956; Thomson, 1957*a*); however, there were no signs of algae in the gut contents of the small sample examined in this study. Further work is required to clarify the composition of the diet of *H. melanochir* taken from areas where seagrass, particularly *Zostera*, does not occur.

One of the main advantages of stable isotope analysis is that it provides temporally integrated information on diet and is therefore not subject to a distinctive diel feeding behaviour. Although zooplankton provides a richer source of energy and protein than *Zostera*, and is possibly the preferred food of *H. melanochir* (Klumpp & Nichols, 1983), it nevertheless appears that a large quantity of *Zostera* must be ingested by *H. melanochir* juveniles and adults to assimilate the required nutrients when zooplankton availability is limited.

Chapter 7 General Discussion

7.1 OVERVIEW

This study represents the outcome for some of the recommendations presented at a National Garfish Workshop held in 1995 at SARDI Aquatic Sciences, which identified at that time, many gaps in our understanding on the fishery biology, habitat association and economic status of *H. melanochir* in southern Australian waters (W.A., S.A., Vic. and Tas.). This study focused on the *H. melanochir* population of Gulf St Vincent, South Australia. The overall aims of this study 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 an assimilated direct food source. In this chapter, I highlight the main findings discussed in each chapter (including their relevance in the context of the garfish-seagrass relationship), and discuss directions for further research and implication towards management.

7.2 EXPERIMENTAL FINDINGS

7.2.1 REPRODUCTIVE BIOLOGY

As an initial step in addressing the association with seagrass, the reproductive biology of *H. melanochir* from the commercial fishery was described using a holistic approach by microscopic examination of ovaries, oocyte size distributions, gonadosomatic indices, and macroscopic ovarian stages (Chapter 2). Although much of the macroscopic gonad data presented here repeat and update the work of Ling (1958), some revision was deemed necessary to complement the addition, in this study, of important histological information and thus demonstrate coherence between histological and whole oocyte descriptions.

In S.A., *H. melanochir* reproduce over a protracted spawning season extending at least from October to March as indicated by the occurrence of ripe ovaries and increases in gonadosomatic index. The size at which 50% of fish are sexually mature in S.A. is 193 mm L_S, which is smaller than in other states (Ye *et al.*, 2002*a*). Sex ratios of fish caught in the S.A. fishery during the spawning season were highly biased towards females, which were found, from other independent surveys, to form large schools in relatively shallow waters (<5 m) where the haul net fishery takes place (Ye *et al.*, 2002*a*). In contrast, mature males are more widely dispersed, with higher proportions in deeper waters. Ye *et al.* (2002*a*) suggested that this segregation of schools by sex may be a strategy that maximises the probability of a ripe male encountering and fertilising with a ripe female inshore, where vegetation is

abundant for the attachment of eggs (Edyvane, 1999; Chapter 5). However, given that seagrass beds in Gulf St Vincent can be continuous up to 30 km offshore, and that females form schools in depths <5 m, it appears more probable that spawning behaviour is related to depth rather than seagrass. This was the conclusion of Jordan *et al.* (1998), who found that there was no evidence of spawning over seagrass in Great Oyster Bay, Tas., but instead was concentrated in depths <5 m over habitat that only comprised drift algal beds.

Hyporhamphus melanochir are characterised as gonochoristic multiple spawners with group-synchronous oocyte development and indeterminate fecundity. Five stages of oocyte development were identified and described: perinucleolar, yolk vesicle, yolk globule, migratory nucleus and hydrated, all of which appear as distinct cohorts in oocyte size distributions and thereby closely correspond to whole oocyte classifications given by Ling (1958). The presences of postovulatory follicles indicate, as its name suggests, that ovulation of the oocyte has occurred and that spawning is imminent or has also occurred, depending on the stage of its deterioration or resorption. Postovulatory follicles were occasionally observed while examining whole hydrated oocytes in disrupted ovary segments under a light microscope; however, none were found in any ovarian sections, and so could not be characterised histologically. Therefore, it was not possible to estimate spawning frequency (and annual fecundity) since it requires either the known duration of the hydrated oocyte stage or the classification of postovulatory follicle deterioration into distinct histological stages (each with an assigned age since spawning) (Hunter & Macewicz, 1985). In order to obtain enough females in spawning condition and determine spawning frequency, a large number of fish would need to be sampled over a 24-h period to account for the lack of spawning synchrony in *H. melanochir*, indicated by the significant variation in proportions of gonad stages and gonadosomatic index within the same month during the spawning season.

Examination of the oocyte morphology using electron microscopic techniques revealed, significantly, that the filaments of the hydrated oocyte are adhesive, which presumably allow the fertilised egg to become attached to benthic marine plants by adhesion and/or entanglement. To my knowledge, no other study has demonstrated the presence of an adhesive substance on the chorionic filaments of fish eggs. The proposed functional mechanism by which *H. melanochir* eggs attach to substrate is supported by the numerous observations of similarly ornamented eggs of other marine beloniform species attached to various marine and anthropogenic objects.

Along the continuum of the traditional paradigm of *r*- and *K*-selection (proposed by MacArthur & Wilson, 1967), *H. melanochir* displays characteristics that are consistent with those of a *K*-strategist. *Hyporhamphus melanochir* produces relatively few (F_B = 201-3044) large eggs (*c.* 3 mm in diameter) that mature slowly (up to 30 d, Jordan *et al.*, 1998), and well-developed larvae, all of which are life history traits typical of a *K*-strategist, as opposed

to an *r*-strategist, e.g. southern bluefin tuna (*Thunnus maccoyii*), which produces many small eggs (14-15 million eggs, 0.5-1.0 mm in diameter, Kikawa, 1964)) that mature early (2-3 d, CSIRO Marine Research, 2004). Iteroparous spawning (i.e. multiple spawnings in a reproductive season) over a protracted spawning period may be a 'bet-hedging' strategy to optimise survival of larvae by reducing the risk of starvation or guarding against mass predation in the event of adverse environmental conditions (Lambert & Ware, 1984). In general, *K*-strategists also invest a high degree of parental care, however, the attachment of eggs to benthic plants in *H. melanochir* may be an adaptation that avoids the need for parental care since they are kept off the bottom and unexposed to predators (Potts, 1984).

7.2.2 EARLY LIFE STAGES

SCUBA and beam trawling surveys of demersal eggs and neuston surveys for larvae were undertaken to determine the association of these early life stages with the shallow water seagrass habitat of Gulf St Vincent. However, before this was undertaken, and as *H. regularis* are known to potentially co-occur in this region, genetic and morphometric discrimination methods were developed to confidently distinguish between larvae of both species in neuston samples. The accurate identification and description of early life stages is a fundamental step for the research of population dynamics for any species since the misidentification of a species can have serious/major implications.

In the absence of tank rearing experiments, another method was sought to verify that aprioristically identified larvae from neuston tow collections are in fact *H. melanochir*. A multiplex PCR assay was developed for discrimination between larvae of different hemiramphid species found in southern Australian waters based on species-specific amplification of part of the mitochondrial control region (Chapter 3). Both *H. melanochir* and *H. regularis* were easily discerned by the number and distinct sizes of the PCR products [*H. melanochir*, 443 bp; river garfish (*H. regularis*), 462 and 264 bp]. Whilst this assay requires only a minute amount of tissue and is expedient, inexpensive and potentially automatable, it most importantly provides a method that is very accurate in resolving the discrimination of larvae.

In addition to larvae, this assay can be used to verify the identity of eggs of both species before examining for any distinguishing morphological characters. The development of *H. melanochir* eggs has already been described by Jordan *et al.* (1998) based on artificial fertilisation and rearing experiments; however, *H. regularis* eggs are yet to be described. The molecular method could possibly also benefit another project in progress[⌗⌗] by providing a

[⌗⌗] Principal Investigators: Dr Charles Gray, Dr John Stewart & Dr Ron West. Life history, reproductive biology, habitat use and fishery status of eastern sea garfish (*Hyporhamphus australis*) and river garfish (*H. regularis ardelio*) in N.S.W. waters. FRDC Project No. 2001/080.

means for discriminating between hemiramphid larvae of the eastern coast of Australia, especially if there are any difficulties encountered in assembling a developmental series for each of the constituent species based on morphology. Similar to the South Australian fishery for *H. melanochir*, at least two species of hemiramphids co-occur in the region that the N.S.W. commercial garfish fishery operates, namely *H. australis*, *H. regularis ardelio* and possibly *A. sclerolepis*.

Once the identity of *H. melanochir* and *H. regularis* larvae were verified using molecular tools, morphological descriptions of both species were provided to facilitate accurate identification of field-collected specimens (Chapter 4). Larvae of *H. melanochir* and *H. regularis* had completed notochord flexion at hatching and are characterised 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: (1) having 58-61 vertebrae (*v.* 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 (*v.* 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*). Furthermore, logistic regression analysis of body measurements revealed a significant difference between larvae of both species in the combined measurements of eye diameter and pre-anal fin length. Both species can be distinguished from similar larvae of southern Australia (other hemiramphids and a scomberosocid) by differences in meristic counts and pigmentation.

7.2.3 DISTRIBUTION OF LARVAE

Attempts to regularly sample *H. melanochir* eggs among seagrass to demonstrate the importance of this habitat for spawning proved to be unsuccessful and cost-ineffective. Two methods were used: firstly, seagrass and drift algae were collected by divers and searched in the laboratory for entangled eggs, and secondly, a beam trawl was towed in areas of dense seagrass and algae after Jordan *et al.* (1998) were successful in collecting *H. melanochir* eggs among drift algae in Tasmanian coastal waters using this method. Eventually, the search for eggs was aborted and the relationship between *H. melanochir* and seagrass had to be investigated using alternative methods.

The importance of seagrass for spawning in *H. melanochir* was inferred by a conceptual model that links between the abundance and distribution of larvae in Gulf St Vincent and regions of extensive seagrass cover, prevailing winds in the gulf, and the time between hatching and the date of capture of larvae (Chapter 5). Previously, it was unknown where *H.*

melanochir larvae are concentrated in the water column, but now we know that they are only found at the water surface to 20 cm in depth. In contrast to the eggs of *H. melanochir*, larvae were easily collected in the neuston from many regions of Gulf St Vincent. The distribution of larvae was found to be non-random or spatially autocorrelated, i.e. there was a tendency for similar abundance values to be spatially clustered. Most larvae were found in the upper region of the gulf, which is almost entirely occupied by seagrass, as shown in comprehensive benthic habitat maps (Edyvane, 1999). These results support the view that the demersal eggs of *H. melanochir* were spawned in these areas and subsequently became attached to seagrass and/or algae. 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.

Hatching and spawning dates of collected larvae were back-calculated from age and growth data for larvae and empirical estimates of egg incubation period. Most larvae collected were *c.* 10 d old, which corresponds to the time that larvae have spent adrift since hatching. If the eggs of *H. melanochir* become attached to fixed substrate, then the hatched larvae will emerge at the same location that spawning had occurred, but on the other hand, if eggs become attached to drifting or floating vegetation, then the distance that larvae are transported from the origin of spawning could be significantly influenced by oceanographic forces acting on drifting eggs during the estimated long incubation period of 12-16 d. Despite the potential for eggs and larvae to be transported far from their origin of spawning, larvae of different ages remained concentrated in the upper region of the gulf. Retention of larvae in this region can be explained by entrainment of larvae through a gyre in the waters of the upper gulf, which is influenced by a combination of surface currents driven by prevailing southerly winds during the summer, the Coriolis effect and land boundaries (Bye, 1976). Smaller concentrations of larvae at the southern entrance to the gulf were explained again from wind data and the presence of dense seagrass areas adjacent to the north east coast of Kangaroo Island.

Although the distribution and abundance of *H. melanochir* larvae infer the importance of seagrass habitat for spawning, there is no conclusive evidence that seagrass *per se* is critical. In other words, *H. melanochir* may not necessarily seek out species of seagrass for the attachment of their eggs. Instead, the importance of seagrass may lay with the entire physical structure and area that seagrass and algae collectively provide as suitable habitat for the attachment of eggs. There are several explanations that are compatible with this possibility: (a) Jordan *et al.* (1998) could not find eggs among *Zostera* beds after successfully finding them among drift algae in the coastal waters of Tas.; (b) exhaustive searches for eggs in areas of dense seagrass during the spawning season were unsuccessful; (c) the incidental finding by a fish processor of eggs entangled among *Posidonia* revealed that the eggs were actually

attached to fine epiphytic algae that were growing on the *Posidonia* fronds; (d) eggs will not survive on bare substrate (Jordan *et al.*, 1998); (e) the length of the filaments indicates that eggs are more likely to become entangled with filamentous algae than with the relatively wide fronds of seagrass itself; (f) and despite the filaments on the eggs being coated with an adhesive substance, the strength of this adhesiveness is unlikely to be sufficient to attach to a clean seagrass frond (free of epiphytic growth) and withstand dislodgment from external forces. The abundance of algal epiphytes on *Posidonia* may also provide a visual cue for the garfish to spawn since the epiphyte standing stock can be highest during the summer months (Johnson, 1981; Borowitzka & Lethbridge, 1989), which coincides with the spawning season of *H. melanochir*, thus providing suitable habitat for attachment of its eggs. It is expected that a greater abundance of algal epiphytes would increase the chances of survival of developing eggs by concealing them from predators.

7.2.4 DIET

In addition to the association between seagrass and *H. melanochir* for spawning, the importance of seagrass to the assimilated diet of different life stages of *H. melanochir* was also examined. A number of studies have shown that *H. melanochir* feed upon zosteracean seagrass in large quantities based on their gut contents (Ling, 1956; Thomson, 1957*a*; Klumpp & Nichols, 1983; Robertson & Klumpp, 1983; Edgar & Shaw, 1995*b*). Although gut content data can provide direct evidence of ingestion and important information on taxonomy of prey items, it cannot provide a measure of the amount of seagrass that is actually assimilated in the diet of *H. melanochir*, merely what is ingested.

As an alternative technique, stable isotope analysis was undertaken in this study to complement observations of gut contents and also circumvent some of the disadvantages of gut content analysis (as discussed in Chapter 6). Stable carbon- and nitrogen-isotope signatures of potential food sources of *H. melanochir* and of the muscle tissue of *H. melanochir* itself were used in two- and three-source mixing models to assess the assimilated contributions of constituent food items of the diet. *Hyporhamphus melanochir* larvae appear to have an exclusively planktonivorous diet (based on observations of specimens that were cleared and stained in Chapter 4). However, it is presumed that the stable isotope signatures of young larvae in this study partly reflect the parental diet since their signatures are equally influenced by pre-existing tissue and 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. It was found, therefore, that with growth of *H. melanochir*, there is an increasing dependence on *Zostera* seagrass in its diet. Omnivory in the juvenile stages after a planktonivorous larval stage is similarly found among other

hemiramphids (Randall, 1967; Carr & Adams, 1973; Berkeley & Houde, 1978; Coetzee, 1981). It appears that hemiramphids have suitably adapted to feeding on plants by the function of a pharyngeal mill, which thoroughly macerates the seagrass after ingestion to obtain the required nutrients (Tibbetts, 1991). The insufficient development of this pharyngeal mill in larvae, along with the limitation in mouth gape, would explain why larvae are incapable of feeding on seagrass until they have reached the juvenile stage.

7.3 DIRECTIONS FOR FURTHER RESEARCH

This study on early life stages of *H. melanochir* and their association with seagrass coincided with a larger study on the fisheries biology of *H. melanochir* in southern Australian waters (Jones *et al.*, 2002), which provided information on the stock structure, age and growth, status of the fishery, reproductive biology and economic analysis of the fishery. Despite the recent growth of research on this species, there is scope for further research, particularly from an ecological perspective.

The main limitation of this study was the inability to sample eggs on a regular basis and thus provide direct evidence for an association between spawning of *H. melanochir* and habitat. Instead, this remains a difficult issue. The methods used in this study to collect *H. melanochir* eggs were cost-ineffective and provided insufficient data. Unfortunately, other objectives and limited resources allocated towards this part of the project prevented the exploration of alternative methods. Therefore, the development of a suitable sampling method is still required in order to derive any quantitative relationship between egg production and some measurable attribute of seagrass or another type of habitat.

Furthermore, assuming the importance of seagrass to *H. melanochir* for spawning, there is potential for further research into the effects of commercial fishing gear on benthic habitat. The main type of gear used in the fishery for *H. melanochir* is power haul nets, where fish are targeted usually over a large area of seagrass habitat, then encircled with the net. However, whilst the net is being hauled in, thus restricting the encircled area, the lead rope of the gear is being dragged over seagrass, and it is not known whether this could have a detrimental effect on the survival of attached eggs or not.

7.4 IMPLICATIONS TOWARDS MANAGEMENT

Results of this study are derived from specimens that were collected from Gulf St Vincent, South Australia. Although research focused upon a specific population, it is expected that, in addition to reproductive biology and the identification and description of larvae, the importance of seagrass to *H. melanochir* demonstrated here is applicable to all populations of

H. melanochir across southern Australia. As a consequence to this garfish-seagrass relationship, a number of management issues are identified.

The neuston sampling method used to collect *H. melanochir* larvae in this study was shown to be a cost-effective device for monitoring abundance of larvae over extensive areas, thus providing the basis for further investigation of the relationships between the spatial distribution of *H. melanochir* larvae and the processes that influence their distribution patterns, i.e. spawning location and habitat requirements, and wind-driven surface currents. A reliable sampling method also has important implications towards determining the relative success of recruitment of year classes into the fished stock. Given the difficulty in sampling the demersal eggs of *H. melanochir*, an alternative approach that may contribute to our understanding and the ability to determine recruitment success may be the larval abundance index or larval production method, where the objective is to derive a measure of offspring production that closely reflects fluctuation in spawning-stock biomass (Pepin, 2002). In accordance with this objective, further sampling of *H. melanochir* larvae should be conducted in Spencer Gulf in addition to Gulf St Vincent since, it was recently demonstrated by genetic discrimination of *H. melanochir* stocks that, populations from both gulfs can be considered a single management unit (Donnellan *et al.*, 2002). When linked with age compositions in the fishery, long-term monitoring of larval abundances can ultimately be used to validate stock assessment models and develop pre-recruit indices that can facilitate effective and timely decisions for management of the fishery (McGarvey & Feenstra, 2004).

If *H. melanochir* eggs are vulnerable to fishing gear, as discussed above in potential research, then entire year classes can be heavily impacted upon, and restrictions on haul netting during peak spawning may need to be considered. Certainly, fishers already reduce effort during the summer months in targeting *H. melanochir* over seagrass beds due to strong winds and poor weather conditions at that time of year as well as a preference for targeting other species, e.g. *A. georgianus*. My experience with haul net fishers targeting garfish is that they have a great regard for the environment, yet they are probably not fully aware of the importance of the habitat that seagrass beds provide for spawning of *H. melanochir* and the attachment of eggs. Therefore, there may eventually be a need for the fishing industry and fisheries scientists to collaborate and to agree on how a realistic view of the impact of haul netting on seagrass beds should be dealt with, and provide recommendations to management accordingly.

A global trend is evident of regional declines in seagrass abundance, and most of these declines appear to be related to human-induced disturbances, many of which are related to reductions in light available for plant photosynthesis (Kemp, 2000). In the face of widespread decline in seagrass habitat across southern Australia, it is imperative that available resources are allocated towards controlling the processes that destroy seagrass or reduce seagrass beds

(Bell & Pollard, 1989), thereby increasing the likelihood of *H. melanochir* encountering suitable habitat for spawning and feeding. On the other hand, if seagrass loss is allowed to continue, this obviously would have a detrimental impact on any species that is dependent on seagrass, such as *H. melanochir*, as well as the socioeconomy of local fishing communities that relies on the sustainability of that species.

This research, in conjunction with a closely-related project on fisheries biology of adults (Jones *et al.*, 2002), significantly enhances the completeness of our understanding of the fisheries biology and ecology of *H. melanochir* with a description of the early life stages and their association with seagrass regarding spawning and diet. It is envisaged that this new information may be incorporated into the development of future management plans for the fishery, which will ultimately ensure the concomitant sustainability of both resources in the long term.