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

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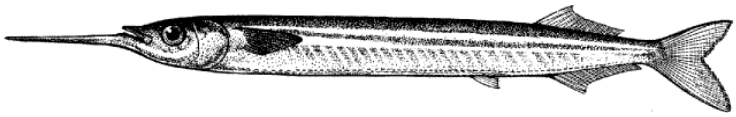

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Chapter 5 Distribution, age, growth and back-calculated spawning dates of larvae

5.1 INTRODUCTION

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

Hyporhamphus melanochir occurs in close association with shallow seagrass beds around the coastline of S.A., particularly in sheltered bays and estuaries of Gulf St Vincent and Spencer Gulf where they are targeted by commercial haul netters and dab netters. Few fish species are known to spawn over seagrass (Bell & Pollard, 1989), although there exists a small amount of anecdotal evidence that suggests that *H. melanochir* may spawn there and their eggs may be deposited on or become attached to seagrass blades or algae.

Ling (1958) investigated the gonad reproductive cycle of this species and described the ripe garfish oocyte as a large, clear structure 'covered by adhesive filaments...' He postulated that such ornamentation allowed the eggs to become attached, as reported for other hemiramphids (Collette *et al.*, 1984), to 'weed' (seagrass) at the bottom of sheltered bays such as in the two gulfs of South Australia. Ling (1958) further suggested that the development of eggs of *H. melanochir* is *in situ* on the seagrass blades after finding that 'vast shoals of tiny garfish are obtainable at these same localities a few months after spawning takes place.'

More recently, Jordan *et al.* (1998) described and illustrated the development of *H. melanochir* eggs from the coastal waters of eastern Tasmania. These were found entangled, by their filaments, among drift algae described as a red filamentous type (Jordan[††], personal communication). Indeed, most beloniforms produce large, demersal eggs with attaching filaments that are often found associated with some form of vegetation (Collette *et al.*, 1984; Parenti, 1993; Watson, 1996; Trnski *et al.*, 2000). The available literature on spawning behaviour of closely related species (TABLE 5.1) suggests a close association of *H. melanochir* eggs/larvae and seagrass. Information on spawning areas of adult *H. melanochir* could be

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TABLE 5.1 Review of the literature on Beloniform eggs that have filaments used for attachment to fixed or floating objects. ? Information not available.

Species	Family	Authors	Location	Attachment	Collection method	Comments
Belone belone	Belonidae	Fonds *et al.* (1974)	Wadden Sea, Netherlands	Submerged algae or *Zostera*	Observed only	
Cheilopogon furcatus	Exocoetidae	Shiganova & Kovalevskaya (1991)	Central part of North Atlantic Ocean	Fragment of halyard	Pleiston net	
Cololabis saira	Scomberosocidae	Ahlstrom & Stevens (1976)	Puget Sound, U.S.A., to southern Baja California, Mexico	Cables or ropes of gear suspended in water; large invertebrates, e.g. salps	Neuston net	
		Tanaka & Oozeki (1996)	Sanriku Coast, Japan	Floating *Sargassum*	?	
		Nagasawa & Domon (1997)	Sea of Japan	Drifting seaweed	Dab net	Found in guts of juvenile *Sebastes schlegeli* that were associated with seaweed
Cypsilurus spp.	Exocoetidae	Delsman (1924)	Coromandel Coast, India	Bundles of palm leaves	?	Leaves attached to a rope set as a fish attraction device (FAD)
Exocoetid spp.	Exocoetidae	Hunte *et al.* (1995) and references therein	Various locations	Floating *Sargassum*, seagrass, driftwood, straw, feathers, coconuts, empty bottles, nets, coconut branches, banana leaves, sugar cane trash, pleuston organisms, submerged substrata	Various methods	Some objects were set as FADs
Hemiramphus brasiliensis	Hemiramphidae	Berkeley & Houde (1978)	Southeast Florida, U.S.A.	Floating blades of the seagrass *Syringodium filiforme*	Surface plankton tows	
Hemirhamphus georgii (=*Rhynchorhamphus georgii*)	Hemiramphidae	Chidambaram & Menon (1948)	West coast of India	Coconut husk washed ashore, floating seaweed	Hand	

TABLE 5.1 *Continued*

Species	Family	Authors	Location	Attachment	Collection method	Comments
Hemirhamphus intermedius	Hemiramphidae	Graham (1939)	Otago Harbour, New Zealand	Weed	Seine	Found in stomachs of mullet caught in same haul as parent garfish
Hemirhamphus marginatus	Hemiramphidae	Talwar (1967)	Pāmban Island, India	*Sargassum*	Hand	Washed up on Dhanushkodi beach
Hirundichthys affinis	Exocoetidae	Hunte *et al.* (1995)	Eastern Caribbean	Coconut fronds	Hand	Set as a FAD
Hyporhamphus melanochir	Hemiramphidae	Ling (1958)	Encounter Bay, South Australia	?	?	Only a single egg found, probably belonging to *H. melanochir*
		Jones (1990)	Baird Bay, South Australia	Gillnet	Gillnet	Eggs coated the meshes of the gillnets as spawning *H. melanochir* were being hauled
		Jordan *et al.* (1998)	Great Oyster Bay, Tasmania	Drifting red filamentous algae	Beam trawl	None found among *Z. tasmanica* beds in Norfolk Bay, Tasmania
		Noell (unpubl. data)	Bay of Shoals, South Australia	Small tufts of *Jania minuta* on *Posidonia*	Dab net	Incidentally taken whilst dab netting for adult *H. melanochir*
Hyporhamphus quoyi	Hemiramphidae	Sudarsan (1966)	Beach at Mandapam, India	Seaweeds	Hand	Washed ashore
Hyporhamphus sajori	Hemiramphidae	Sokolovsky & Sokolovskaya (1999)	Peter the Great Bay, Russia	Floating and attached *Sargassum miyabei*	IKS-80 egg net	
		Nagasawa & Domon (1997)	Sea of Japan	Drifting seaweed	Dab net	Found in guts of juvenile *Sebastes schlegeli* that were associated with drifting seaweed
Hyporhamphus unifasciatus	Hemiramphidae	Olney & Boehlert (1988)	Chesapeake Bay, U.S.A.	Floating blades of *Zostera*	Pushnet	
Strongylura marina	Belonidae	Zeckua Ramos & Martinez Perez (1993)	Tecolutla Estuary, Mexico	Seagrass	Hand	

inferred from the distribution of eggs or larvae of this species. This chapter investigates the spatial distribution of *H. melanochir* eggs and larvae.

This study also examines the growth of larvae and the temporal distribution of hatching dates of *H. melanochir*. Few estimates of growth of early life stages of hemiramphids are available in the literature, and all are derived from length frequency data (Li *et al.*, 1997; Sokolovsky & Sokolovskaya, 1999). The discovery of growth increments in the microstructure of otoliths (Panella, 1971) has led to a more accurate estimation of the age and growth of larval fish. Numerous studies have demonstrated that such increments form daily (see reviews by Campana & Neilsen, 1985, and Jones, 1986). Ageing by counting otolith growth increments provides a means for constructing growth functions and back-calculating hatching dates (Gunn *et al.*, 1989; Campana & Jones, 1992; Jordan, 1994), and thereby indicating the duration that larvae have spent adrift since hatching.

The main objectives of this chapter are to: (a) determine broad spawning area patterns in Gulf St Vincent from the distribution and abundance of *H. melanochir* eggs and larvae; (b) confirm that *H. melanochir* larvae are concentrated in the neuston; (c) back-calculate hatching dates through the determination of the age and growth of larvae; and (d) assess the reliance upon seagrass for spawning by *H. melanochir* with the aid of comprehensive benthic habitat maps of the gulf (Edyvane, 1999).

5.2 MATERIALS AND METHODS

5.2.1 COLLECTION OF EGGS

Attempts to find and collect eggs of *H. melanochir* were conducted in Gulf St Vincent at Middle Beach and Port Wakefield, S.A., during the spawning season from October 1998-March 1999 (FIG. 5.1). These locations were chosen for the following reasons: evidence that spawning had occurred from the discovery of eggs there (Ling, 1958); the presence of seagrass meadows on which eggs could be attached (Edyvane, 1999); and boat accessibility.

Seven sites at Middle Beach and 12 sites at Port Wakefield were equally spaced *c.* 3.0 km (2’ longitude) apart along three to four transects, which were roughly perpendicular to the coast and *c.* 5.5 km apart (3’ latitude). Final site selection within these locations was determined after depth-stratified ground truthing by SCUBA divers, swimming for 50 m parallel to the shoreline. Sites with extensive seagrass (and algal) cover at depths of 1-10 m were marked with a handheld global positioning system (GPS). Samples were collected approximately monthly over the sampling period, alternating between locations. Because sampling from the boat required calm weather conditions, it was difficult to obtain samples with regular periodicity and thus link sampling date with any environmental variable (e.g. moon phase, tidal rhythm). The adhesive and filamentous properties of the eggs of *H.*

melanochir make them difficult to collect. It is unlikely that they will be dislodged with a pump and the most appropriate sampling method was believed to be small-scale removal of benthic and drifting plant material by divers. The sampling procedure for each location included spot dives at three to five randomly chosen sites that were covered with vegetation, with 3 × 1 m^2 of plant material collected from each site using shears and a catch bag. The number of sites sampled was dependent on SARDI dive policy restrictions and weather conditions. For each location, the total volume of plant material collected in an area of 9-15 m^2 was placed in hessian bags, refrigerated overnight in the laboratory, and searched for eggs the following day by one person.

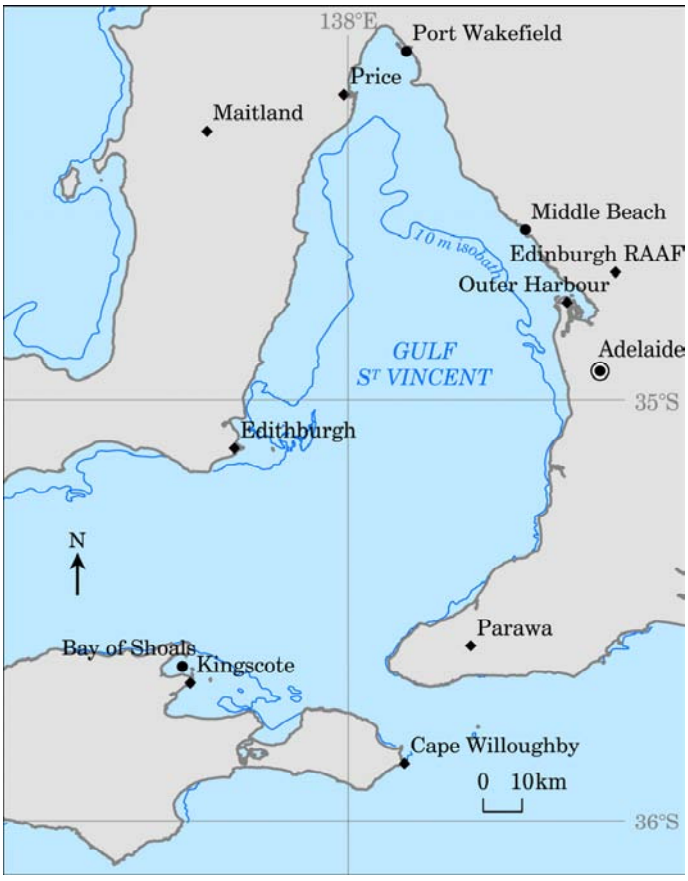

FIG. 5.1 Map of Gulf St Vincent showing land-based wind measurement stations (⬥) and the locations that were searched for *Hyporhamphus melanochir* eggs (●).

Collecting plant material using divers proved to be relatively cost-ineffective and was replaced with collection by a beam trawl for the following spawning season (October 1999-March 2000). Jordan *et al.* (1998) found *H. melanochir* eggs in the coastal waters of Tas. using this method. A 5-m long net with 18-mm panel mesh and 12-mm codend mesh was attached to an aluminium beam trawl frame with a 1.2 × 0.75 m mouth (FIG. 5.2). The beam trawl was towed from astern (at the same sites described above) for 20 s at a mean speed of

0.83 m s^{-1}, with a swept bottom area of *c*. 20 m^2 per tow. A digital video camera was mounted at the top of the frame so that eggs could be linked to benthic habitat type in the event that they were found. Sampling frequency and treatment of collected plant material was the same as for the previous spawning season. The beam trawl was also operated in the Bay of Shoals (FIG. 5.1) in March 2000 following the discovery, by a fish processor, of *H. melanochir* eggs 12 months earlier. They were incidentally collected by a dab netter in the same haul as adult *H. melanochir* in spawning condition, and their morphology was identical to that described for *H. melanochir* eggs by Jordan *et al.* (1998). In addition to the regular sampling procedure described, floating plant material was collected opportunistically from the boat.

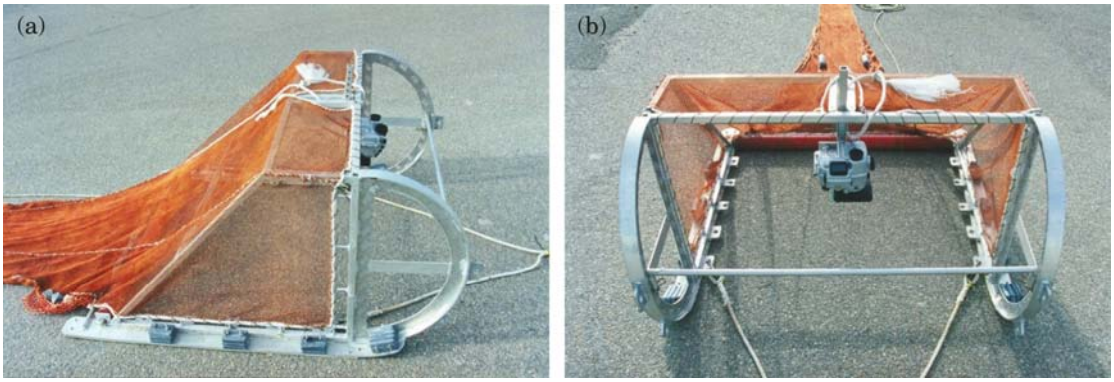

FIG. 5.2 (a) Side and (b) front views of the beam trawl used to sample benthic habitat for the collection of *Hyporhamphus melanochir* eggs. Note: the digital video camera mounted on top of the trawl frame.

5.2.2 COLLECTION OF LARVAE

Hemiramphid larvae are most commonly found at the water surface (Collette *et al.*, 1984; Watson, 1996; Trnski *et al.*, 2000), and were therefore collected in this study by sampling the neuston. The neuston net configuration consisted of 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 (FIG. 5.3). This attachment ensured that, while being towed, the top of the frame rode steadily above the water surface and that *c*. 0.4 m^2 of the mouth area was submerged. The net was towed from astern for 5 min at speeds of 2-4 knots, and inside an arc to avoid interference from propeller wash. Hemiramphid larvae were sorted from plankton samples immediately after collection based on reference larval specimens from the South Australian Museum fish collection. The sorted larvae were either preserved in 80% ethanol for the examination of otoliths, or fixed in 10% formalin buffered with sodium β-glycerophosphate (1 g L^{-1}) and later preserved in 70% ethanol for the description of larval development (Chapter 4).

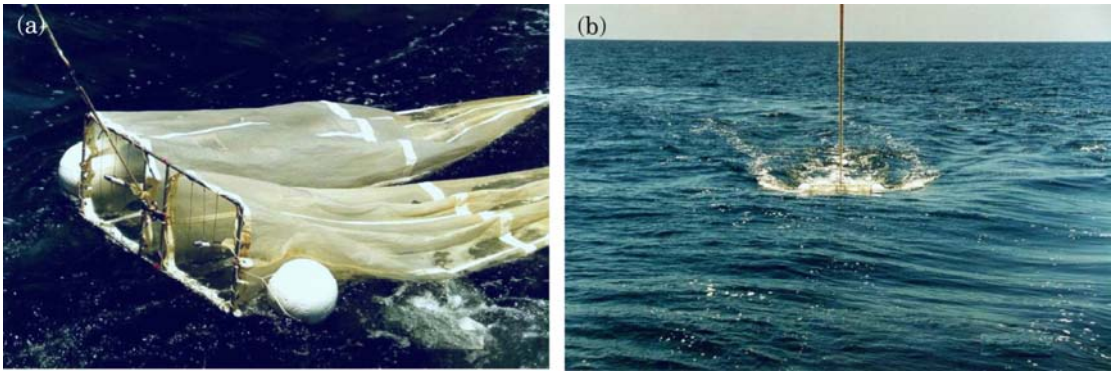

FIG. 5.3 (a) Hauling and (b) operation of the neuston net used to collect *Hyporhamphus melanochir* larvae.

5.2.3 DISTRIBUTION AND ABUNDANCE OF LARVAE

To determine the distribution and abundance of *H. melanochir* larvae, neuston tows were conducted at sites positioned throughout Gulf St Vincent (n = 57) aboard the 25 m RV *Ngerin* during daylight in December 1998 (cruise 1) and December 2000 (cruise 3) (cruise paths shown in APPENDIX D). Sites were positioned at every 7' latitude (*c*. 12.9 km) and 7' longitude (*c*. 10.7 km) within the gulf. Some sites in the northeast of the gulf could not be sampled as they were within a restricted military testing area. A calibrated flowmeter (General Oceanics, model 2030R) was mounted at the centre of each net mouth, and the average of the two readings used to determine the swept area according to the manufacturer's calculations. Since hemiramphid larvae are expected to be neustonic, abundances of *H. melanochir* larvae were standardised to area (1000 m^2) rather than volume of water filtered. In addition, opportunistic sampling of *H. melanochir* larvae was undertaken in Spencer Gulf and Gulf St Vincent in February 1999 (cruise 2) during a trawl survey for juvenile snapper (*Pagrus auratus*). Larvae collected on cruise 2 were collected using the sampling method described and were used to describe age and growth only (Section 5.2.6). The distribution and abundance of these larvae was not compared to larvae collected from cruises 1 and 3 since they were collected opportunistically and from different locations.

5.2.4 EFFECTIVENESS OF METHOD FOR COLLECTION OF LARVAE

To verify that *H. melanochir* larvae were concentrated in the neuston layer and that sampling should be done during daylight, a separate experiment was undertaken in the Bay of Shoals aboard the 6 m RV *Pagrus* in March 2000 (cruise path shown in APPENDIX D). The Bay of Shoals is a shallow (up to 8 m deep), sheltered bay located on the northern coast of Kangaroo Island, South Australia (FIG. 5.1). It has an area of *c*. 33 km^2 and is dominated by seagrass and algal beds interspersed with unvegetated sand patches (see Section 6.2.1 for a

detailed description). At the time of sampling, the bay was influenced by a tidal movement of 0.2-1.1 m.

A series of plankton tows were conducted (as described above) at 10 evenly distributed sites within the bay with and without the neuston arrangement (i.e. floats attached and detached), and during the day and night. When the floats were detached, the net sampled beneath the water surface at a depth of *c*. 1 m (subsurface samples). Sampling was carried out on two consecutive days. During each 24-h period, five sites were sampled during the day (day 1: from 1200-1600 hrs; day 2: from 1400-1630 hrs) and then again at night (night 1: from 2330-0330 hrs; night 2: from 0030-0430 hrs). At each site, both neuston and subsurface samples were collected, in alternating order between sites. The sampling method used in the Bay of Shoals was identical to that used in Gulf St Vincent, except for modifications of the net for subsurface sampling and that RV *Pagrus* could be operated in shallower water (i.e. <1-5 m) than RV *Ngerin*. Absolute abundances of larvae (1000 m^{-2}) were log(X+1)-transformed and subjected to a two-factor ANOVA with equal replication to test for significant factors (Zar, 1999).

5.2.5 SPATIAL ANALYSIS OF LARVAL ABUNDANCES

Moran's (1950) *I* statistic was used to estimate the spatial autocorrelation of larval abundances as a function of distance between pairs of sites within the irregular lattice described above for cruises 1 and 3 in Gulf St Vincent. Abundances were log(X+1)-transformed to standardise and normalise the distribution of abundances. Moran's *I* is defined by:

$$I = \frac{n\sum_i \sum_j W_{ij}(X_i - \overline{X})(X_j - \overline{X})}{S_0 \sum_i (X_i - \overline{X})^2} \qquad [5.1]$$

where n is the number of samples, X_i is the transformed abundance at site i, $\overline{X}$ is the mean of X over all sites, W_{ij} is the proximity of observations i and j, and $S_0 = \sum_i \sum_j W_{ij} (i \neq j)$. Values of I that exceed the expected value of -1/(n-1) indicate positive spatial autocorrelation, in which similar values (either high values or low values) are spatially clustered. The significance of I was tested by the standard Z-statistic at the $\alpha = 0.05$ level (Zar, 1999) under the assumption of randomisation. To detect spatial patterns and patch sizes, Moran's I was calculated separately for mutually exclusive distance classes (0-10.65 km, 10.65-21.3 km, etc.), with the minimum nearest neighbour (i.e. 10.65 km) specified as the lag distance. A spatial correlogram of I *v.* distance class was plotted to assist with the analysis of spatial

structure (Legendre & Fortin, 1989). All calculations for spatial analysis were carried out using an add-in program for Microsoft Excel, RookCase v 0.9.6. (Sawada, 1999).

5.2.6 AGE AND GROWTH OF LARVAE

Hyporhamphus melanochir larvae and early juveniles <36 mm L_B collected during the three cruises were used for the determination of age and growth. Body lengths of all collected larvae were measured to the nearest 0.1 mm by firstly capturing images of specimens at 6.5-40× magnifications with a video camera mounted on a Wild M3Z stereomicroscope, and then measuring cumulative distances with SigmaScan Pro® 5.0 automated image analysis software (SPSS Inc., 1999*a*).

Of the ethanol-preserved specimens, a length-stratified subsample (n = 56) was used for ageing to ensure an unbiased description of growth of *H. melanochir* larvae, i.e. where possible three larvae were randomly selected from each 1-mm length class. Larvae were placed in a drop of water on a microscope slide and otoliths were teased out under cross-polarised light with electrolytically-sharpened tungsten needles. The otoliths were air-dried and mounted flat side up (i.e. convex side down) in Euparal™ mountant (Asco Laboratories, Manchester, U.K.) for microscopic examination. To prevent the positioned otoliths from being disturbed, the mounting procedure involved the marking of a circle of Euparal™ around the removed otoliths with a probe, leaving a dry well that gradually closed and thus encasing the otoliths. Prepared slides were left to dry horizontally.

Of the three pairs of otoliths, the lapilli were chosen for counting growth increments for the range of body lengths examined since the sagittae required significantly more preparation (by grinding) to reveal or improve clarity of increments, and the asterisci do not form until after hatching. Certain characteristics observed in the microstructure of the lapilli were assumed to represent specific events of the early life stages of *H. melanochir*, and the subsequent ageing of specimens in this study was reliant upon these assumptions. Specifically, these assumptions are: (i) the 3-5 faint increments encircling the core are formed during the embryonic stage; (ii) a prominent check *c.* 30 μm in diameter after the embryonic increments is the hatch mark, which appears to be in a slightly different focal plane; and (iii) regular unambiguous increments after the hatch mark are deposited at a rate of one per day (i.e. daily growth increments) (FIG. 5.4). The first two assumptions were verified by examining the characteristics of lapilli from three newly-hatched larvae with a known age of 0 d (donated by A. Jordan). Lapilli were viewed under transmitted light at 400× and 600× magnifications with an Olympus BX51 compound microscope. Counts of growth increments were done blind with respect to knowledge of L_B and replicated at least twice by the same reader.

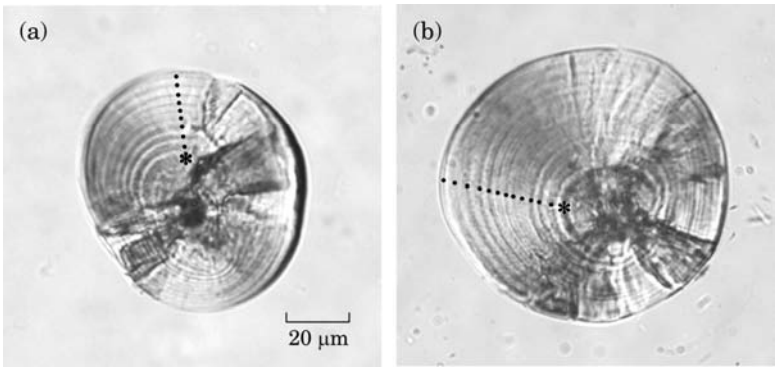

FIG. 5.4 Photomicrographs of lapilli from *Hyporhamphus melanochir* larvae, estimated to be (a) 9 and (b) 12 days of age after the presumed hatch mark (*). Each dot corresponds to the discontinuous zone of a daily growth increment (one daily growth increment = discontinuous zone + accretion zone). Both photomicrographs are at the same scale.

A Laird-Gompertz function was fitted to the age-at-length data to describe the growth of *H. melanochir* larvae, the equation for which is:

$$L_t = L_0 e^{k(1-e^{-Gt})} \qquad [5.2]$$

where L_t is the body length at age t; L_0 is the body length at $t = 0$; k is a dimensionless parameter, such that $kG = A_0$ is the specific growth rate at $t = 0$ ($A_t = A_0 e^{-Gt}$); G is the specific growth rate when $t = t_0$; and t_0 is the age at when the growth rate starts to decrease (i.e. the inflexion point of the curve; Ricker, 1979). The Laird-Gompertz growth function has been shown to provide an adequate fit for age-at-length data for larvae of many different species (Watanabe *et al.*, 1988; Dulčić, 1998; Ekau, 1998; Quiñonez-Velázquez, 1999; Gaughan *et al.*, 2001). Parameters were estimated by the Marquardt-Levenberg nonlinear least squares method in the Regression Wizard module in SigmaPlot® 8.0 (SPSS Inc., 2002). The S.E. of the fitted curve was calculated using the delta method (Seber, 1982).

5.2.7 BACK-CALCULATION OF HATCHING AND SPAWNING DATES

Back-calculated hatching dates were obtained by subtracting the predicted age calculated by the Laird-Gompertz growth function from the date of capture. Back-calculated spawning dates were obtained by further subtracting the egg incubation period from fertilization through to hatching. Crude estimates of the incubation period for *H. melanochir* eggs were obtained by modifying an egg development rate equation derived for another beloniform, *Belone belone* (Fonds *et al.*, 1974); *H. melanochir* eggs are similar to *B. belone* eggs in that they are large (*c.* 3 mm in diameter), demersal and covered with adhesive chorionic filaments (Collette *et al.*, 1984). Given that *H. melanochir* eggs take 29 d to hatch after fertilisation at

15.3°C (Jordan *et al.*, 1998), these values were used to recalculate the intercept of the rate equation for *B. belone* so that it can be specifically applied to *H. melanochir*, yielding:

$$D = e^{0.226(19.86-T)} + 7.57 \qquad [5.3]$$

where D is the incubation time (d) and T is water temperature (°C). The application of this equation to *H. melanochir* eggs relies upon the assumption that the linear regression coefficient of a temperature-dependent relationship that describes egg development for a particular species can be applied to closely related taxa without introducing an excessive error component (e.g. as for decapod species in Wear, 1974).

Throughout the duration of this study, juvenile *H. melanochir* >40 mm L_S were also collected by beach seining and dab netting to describe their growth and for the back-calculation of hatching dates. However, problems were encountered in obtaining estimates of age of juveniles. Despite the examination of different otoliths and section planes and attempts to validate growth increments, the vast majority of the sample was rejected as a result of otoliths being unreadable or the large variation in counts of growth increments. For these reasons, ageing data for juveniles are not presented here; instead, this study focuses on the growth of larvae to back-calculate hatching dates of *H. melanochir*.

5.2.8 WIND DATA

Real-time wind data for seven land-based measurement stations situated around Gulf St Vincent (FIG. 5.1) were obtained from the Bureau of Meteorology. Wind data consisted of three-hourly readings of surface wind direction and speed (m s^{-1}) during Oct 1-Dec 17, 1998 and Oct 1-Dec 7, 2000, which correspond to the presumed start of the spawning season through to the end of the respective sampling period. These data were compared with the distribution and abundances of larvae to predict the influence of prevailing winds and currents on larval transport.

5.3 RESULTS

5.3.1 COLLECTION OF *H. MELANOCHIR* EGGS

In this study, plant material was collected by divers and by using a beam trawl for the search of *H. melanochir* eggs. Although a much greater area could be sampled using a beam trawl with equal effort, the amount of material collected by both sampling methods was restricted by dive limits and the time and labour required sorting for eggs. The total volume of plant material collected by divers in a benthic area of 9-15 m^2 was 30-50 L. For beam trawl samples, a maximum volume of *c.* 70 L of plant material could be thoroughly sorted per

person in a day. The mean towing duration required to collect this volume at a speed of 0.83 m s^{-1} was 45 s, which is equivalent to a swept area of only 45 m^2. The plant material collected by both methods invariably consisted of combinations of drifting and attached macroalgae, and the seagrasses *Z. capricorni* or *Z. tasmanica* ('garweed' or 'eelgrass'), *Posidonia* spp. ('tapeweed'), *Halophila* spp. ('paddleweed') and *Amphibolis* sp. ('wireweed'). After thorough searching of a total amount of 960 L of plant material on 12 occasions (120 L by one person from six diving samples and 840 L by two people from six beam trawl samples), no *H. melanochir* eggs were found. The only evidence of *H. melanochir* spawning over seagrass in S.A. was the discovery of *H. melanochir* eggs attached to seagrass and algae (FIG. 5.5) in a market fish bin, which were collected by a commercial fisher from the Bay of Shoals whilst dab netting the parent fish.

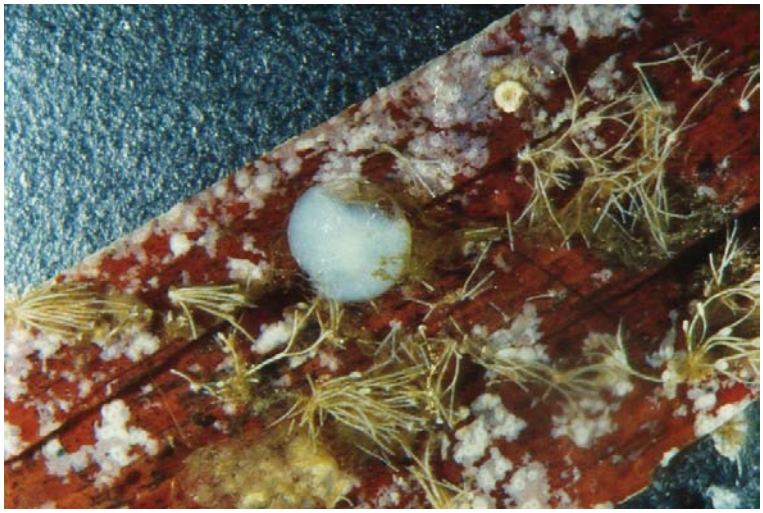

FIG. 5.5 A *Hyporhamphus melanochir* egg attached to the algal epiphyte *Jania* on seagrass *Posidonia*.

5.3.2 DISTRIBUTION AND ABUNDANCE OF LARVAE

In the Bay of Shoals, the abundance of *H. melanochir* larvae was significantly greater in the neuston than in subsurface samples (mean abundance of 5.1 larvae 1000 m^{-2} *v.* 0.7 larvae 1000 m^{-2}; $n = 40$, i.e. 10 sites × 2 sampling methods × 2 times of day; ANOVA, $F_{1,36} = 10.7$, $P<0.01$) (FIG. 5.6, TABLE 5.2). Although more larvae were collected during the day than at night (mean: 3.7 larvae 1000 m^{-2} *v.* 2.1 larvae 1000 m^{-2}), no significant differences were found in abundance with the time of day or with the interaction of time of day × sampling gear type. These results indicate that *H. melanochir* larvae are concentrated in the neuston during both day and night.

Totals of 108 and 317 *H. melanochir* larvae were collected during cruises 1 and 3 in Gulf St Vincent, respectively, with frequencies of occurrence of 49 and 79%. The corresponding mean abundances were 4.8 and 12.2 larvae 1000 m^{-2} of surface water, with maxima of 40 and

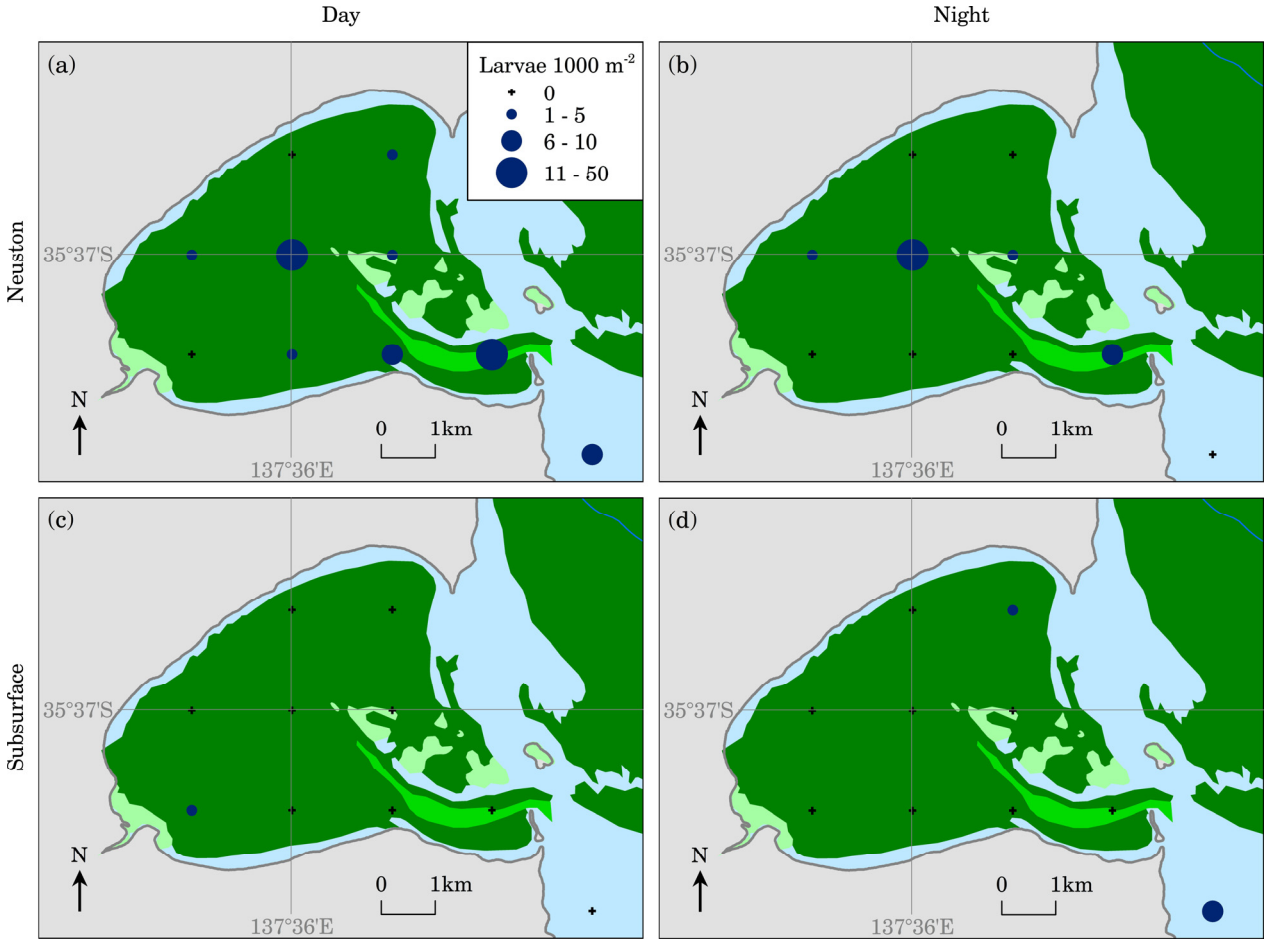

FIG. 5.6 Abundance of *Hyporhamphus melanochir* larvae in the Bay of Shoals during Mar 3-5, 2000, collected with a neuston net and subsurface net during day and night. (a) neuston at day; (b) neuston at night; (c) subsurface at day; (d) subsurface at night. Densities of larvae are the same as for the legend in (a). Also shown are the densities of seagrass habitat: sparse; medium; dense; patchy (from Edyvane, 1999).

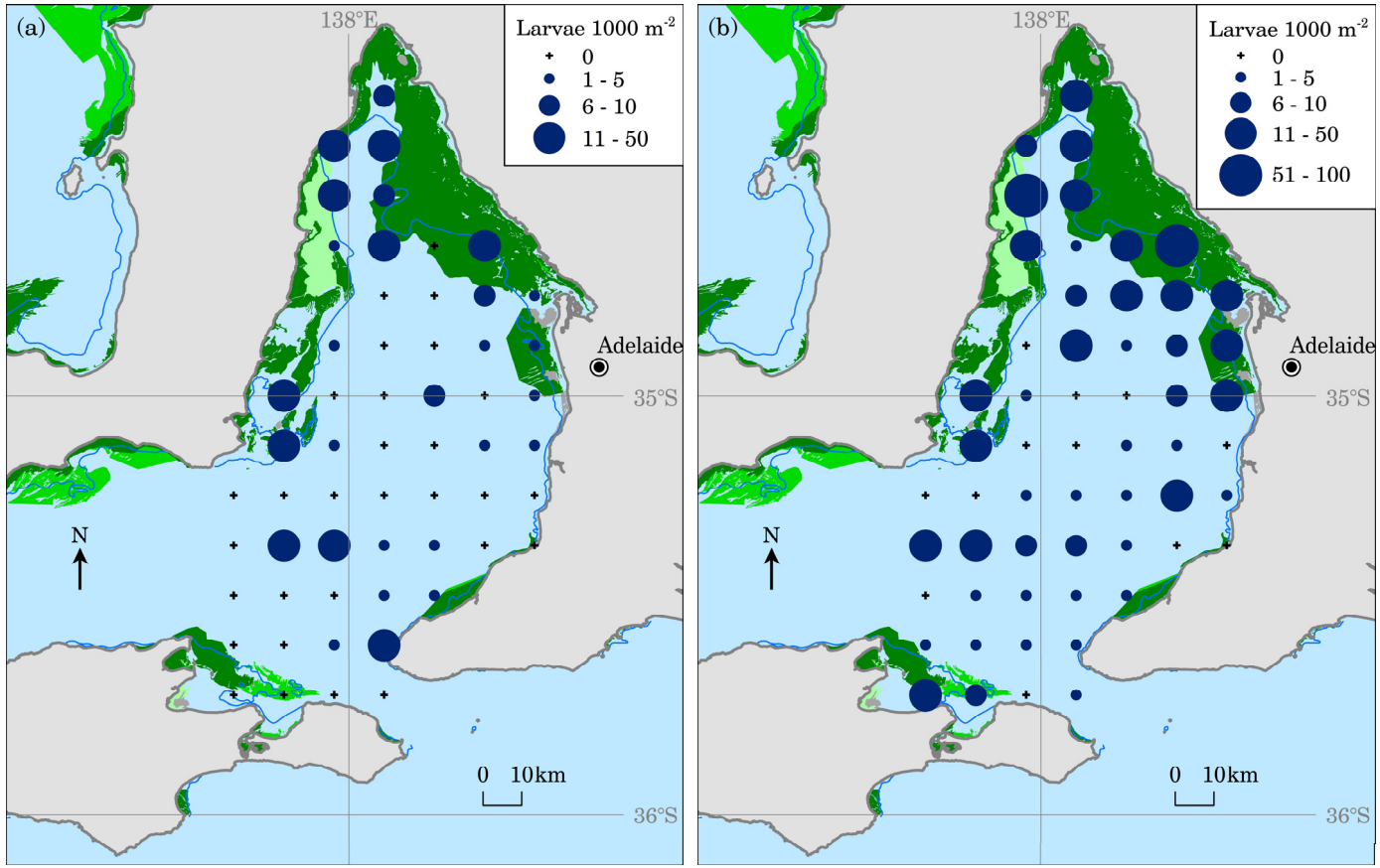

FIG. 5.7 Distribution and abundance of *Hyporhamphus melanochir* larvae in Gulf St Vincent during (a) Dec 14-17, 1998 (cruise 1) and (b) Dec 4-7, 2000 (cruise 3) aboard RV *Ngerin*. Also shown are the densities of seagrass habitat: sparse; medium; dense; patchy (from Edyvane, 1999).

TABLE 5.2 Summary of two-factor ANOVA with replication to test for significance of type of sampling gear and time of day on abundance of *Hyporhamphus melanochir* larvae.

Source of variation	SS	d.f.	MS	*F*
Time of day	0.066	1	0.066	1.258
Sampling gear	0.561	1	0.561	10.709**
Interaction	0.166	1	0.166	3.172
Within (error)	1.885	36	0.052	
Total	2.678	39		

**P<0.01.

84 larvae 1000 m^{-2} [FIG. 5.7(a), (b)]. In general, the greatest abundances of larvae were concentrated in the upper region of the gulf where extensive dense seagrass beds also occur [FIG. 5.7(a), (b)]. The other region where larvae were abundant was in the middle of the entrance to the gulf. The size compositions of the samples from cruises 1 and 3 combined were clearly dominated by larvae <12 mm L_B (80% of samples) (FIG. 5.8); juveniles >22 mm L_B were infrequently caught in the plankton net due to their greater avoidance capacity. Consequently, the size distributions of larvae are skewed to the left. Only a few larvae were

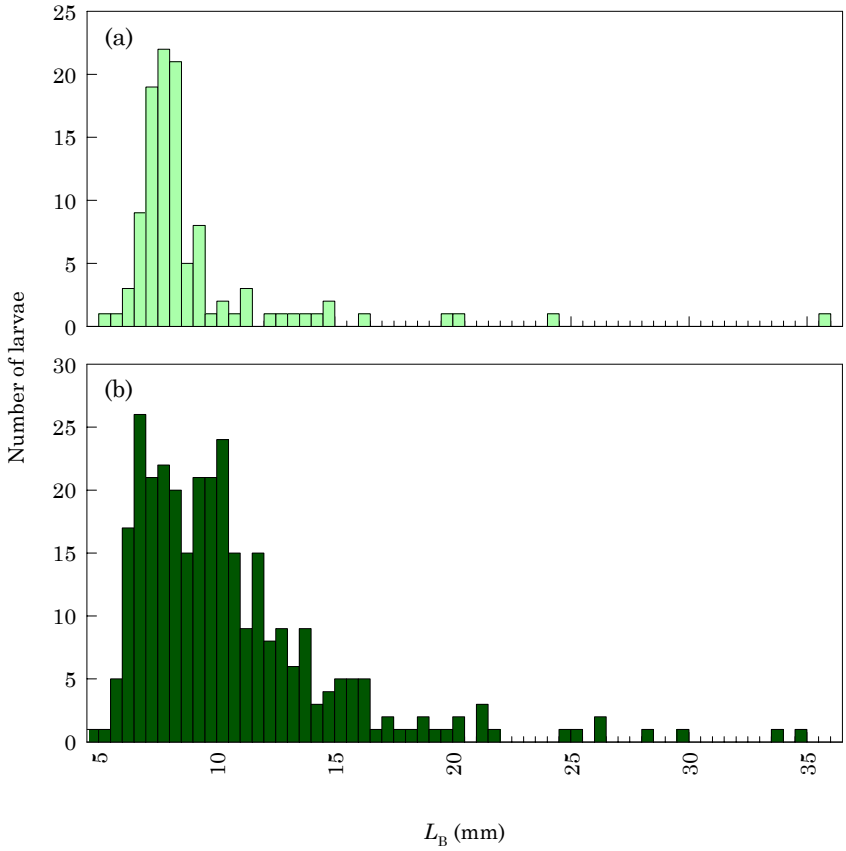

FIG. 5.8 Length-frequency distribution of *Hyporhamphus melanochir* larvae from Gulf St Vincent collected on (a) Dec 14-17, 1998 (cruise 1, n = 108) and (b) Dec 4-7, 2000 (cruise 3, n = 310). Note: some larvae from cruise 3 could not be measured due to damage or poor preservation.

measured to be less than the expected minimum size of *c.* 6.4 mm L_B (Noell *et al.*, 2003), possibly as a result of excessive shrinkage.

5.3.3 SPATIAL ANALYSIS OF LARVAL ABUNDANCES

Moran's *I* statistic for log(*X*+1)-transformed larval abundances for cruise 1 (*I* = 0.347, *Z* = 2.210, *P*<0.05) and cruise 3 (*I* = 0.423, *Z* = 2.670, *P*<0.05) indicate positive spatial autocorrelations within Gulf St Vincent that were significantly different from a random spatial distribution of larvae. The correlogram of Moran's *I v.* distance classes demonstrates a similar spatial structure of larval abundances for both cruises, with significant positive spatial autocorrelation for distance classes 1-2 for cruise 1 and distance classes 1-3 for cruise 3, which correspond to patch sizes of 0-21.3 km and 0-31.95 km, respectively (FIG. 5.9).

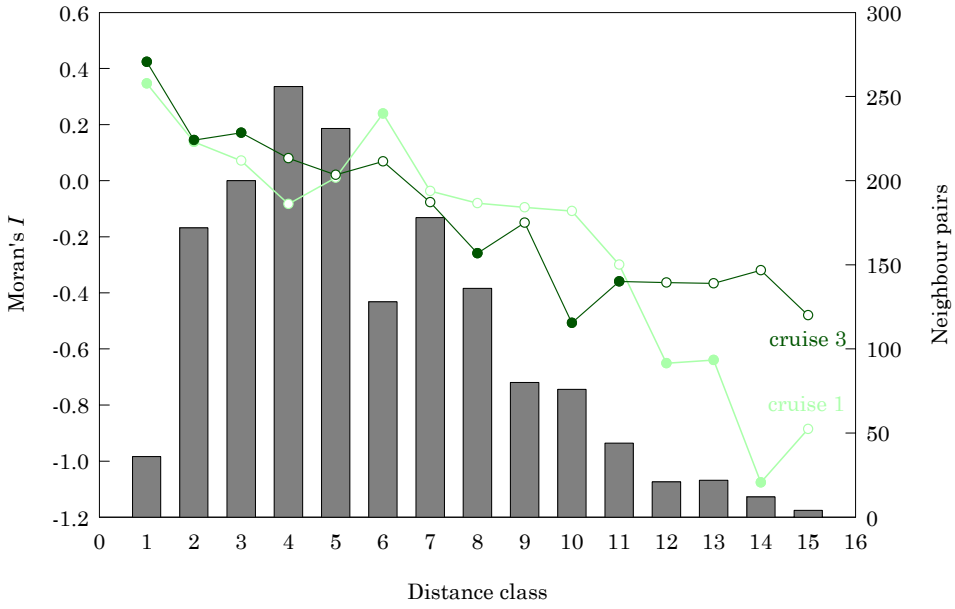

FIG. 5.9 Correlogram of Moran's *I* statistic *v.* distance class for abundances of *Hyporhamphus melanochir* larvae in Gulf St Vincent on Dec 14-17, 1998 (cruise 1) and Dec 4-7, 2000 (cruise 3). The width of each distance class is 10.65 km. Autocorrelation values significant at α = 0.05 are indicated with solid circles. Histogram shows the number of neighbour pairs at each distance class (2nd *Y*-axis).

5.3.4 AGE AND GROWTH OF LARVAE AND BACK-CALCULATED SPAWNING DATES

Estimates of the parameters of the Laird-Gompertz growth function and the fitted growth curve demonstrate that this function adequately describes the growth of *H. melanochir* larvae, accounting for 97% of the variance in length up to 36 mm L_B (TABLE 5.3, FIG. 5.10), although the predicted length at hatching (5.1 mm L_B) was slightly less than that observed (mean = 5.8 mm L_B). The specific growth rate of *H. melanochir* larvae for the size range examined increased from 0.50 mm d^{-1} at age 0 d to the inflection point of 1.09 mm d^{-1} at age 30 d (31.6 mm L_B) (TABLE 5.4).

TABLE 5.3 Estimates of Laird-Gompertz growth function parameters for *Hyporhamphus melanochir* larvae collected from gulf waters of South Australia.

Parameter	Value	S.E.	*n*	r^2
L_0	5.078	0.344	56	0.971
k	2.819	0.155		
G	0.035	0.005		

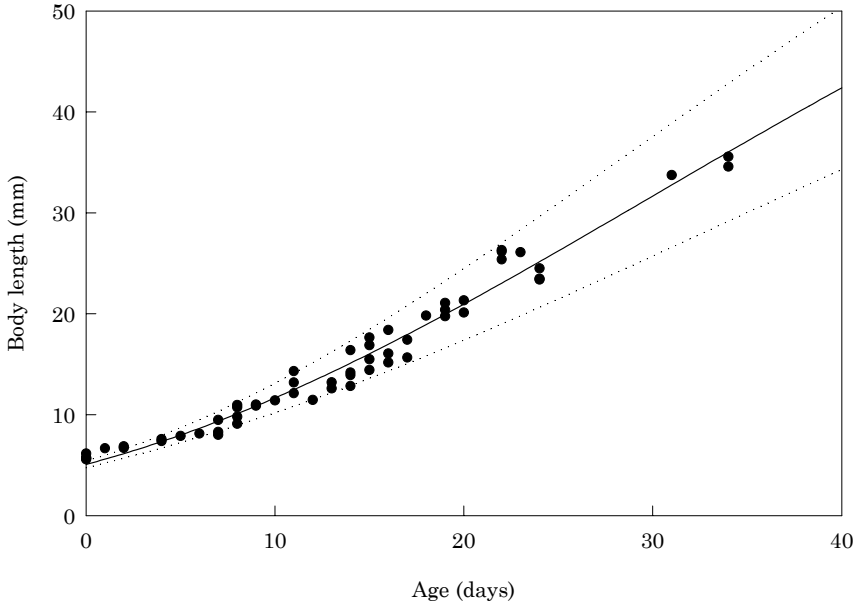

FIG. 5.10 Estimated age (± 1 S.E.) v. body length for *Hyporhamphus melanochir* larvae from Gulf St Vincent and Spencer Gulf, South Australia. The curve was fitted using the Laird-Gompertz growth function. Parameters of the model are shown in TABLE 5.3.

Based on measurements taken onboard RV *Ngerin* during each research cruise, mean sea surface temperatures (± 1 S.D.) during December and February were 19.4°C (± 1.1°C, $n = 30$) and 22.7°C (± 1.7°C, $n = 30$), respectively. For the development of *H. melanochir* eggs, these water temperatures equate to incubation periods of 16 and 12 d. The subtraction of estimated age and incubation period from the date of capture provides back-calculated spawning dates. The spawning date distributions of the collected larvae suggest that spawning occurred from mid October to early December and during all of January (FIG. 5.11). Both of these periods fall within the assumed spawning season from October-March, inferred from the reproductive biology of female *H. melanochir* (Chapter 2).

5.3.5 WIND DATA

Based on wind data from land stations situated around Gulf St Vincent, the prevailing winds between October and the end of each cruise in December appear to be predominantly from a SE to SW direction (FIG. 5.12 and FIG. 5.13). During November-December of 1998

and 2000, in particular, these directions accounted for 67-69% of all wind readings (unpubl. data).

TABLE 5.4 Growth rate (mm d^{-1} and % d^{-1}) of *Hyporhamphus melanochir* larvae from gulf waters of South Australia estimated from the Laird-Gompertz growth function.

Body length (mm)	Estimated age (d)	Growth rate (mm d^{-1})	Growth rate (% d^{-1})
5.1	0	0.50	9.84
8.0	5	0.66	8.26
11.7	10	0.81	6.94
16.0	15	0.93	5.83
20.9	20	1.02	4.89
26.2	25	1.08	4.11
31.6	30	1.09	3.45
37.1	35	1.07	2.90
42.3	40	1.03	2.44

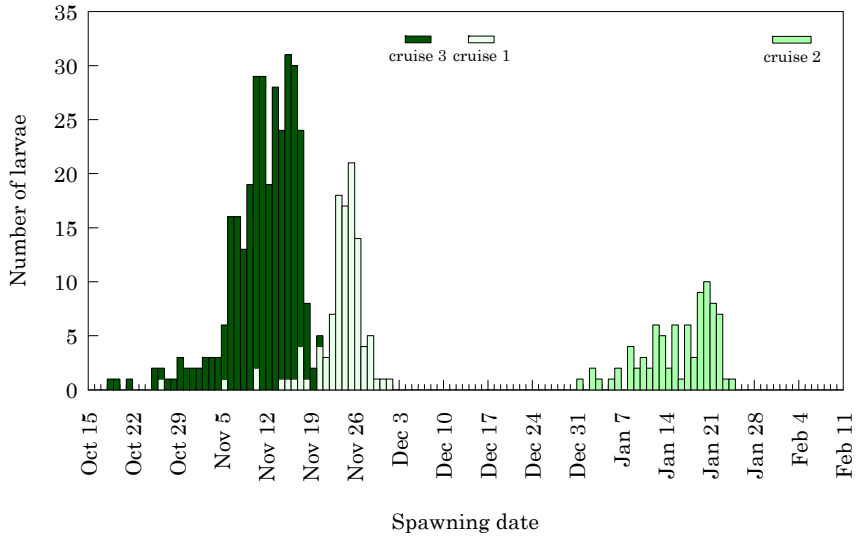

FIG. 5.11 Spawning date distributions for *H. melanochir* larvae collected from Gulf St Vincent during Dec 14-17, 1998 (cruise 1) and Dec 4-7, 2000 (cruise 3), and from Spencer Gulf and Gulf St Vincent during Jan 31-Feb 5, 1999 (cruise 2). Spawning dates were back-calculated by subtracting the estimated age and the predicted egg incubation period from the date of capture. Data are presented in the same calendar year. Horizontal bars indicate the duration of cruises during which samples were collected.

5.4 DISCUSSION

Surveys were conducted to sample eggs and larvae of *H. melanochir* in Gulf St Vincent of S.A. to predict spawning dates and to assess the importance of seagrass for spawning. No eggs of *H. melanochir* were found among plant material collected either by SCUBA divers or with a beam trawl. This was surprising but on review the amount of plant material sorted is a trivial fraction of the seagrass beds in Gulf St Vincent. The maximum volume of plant

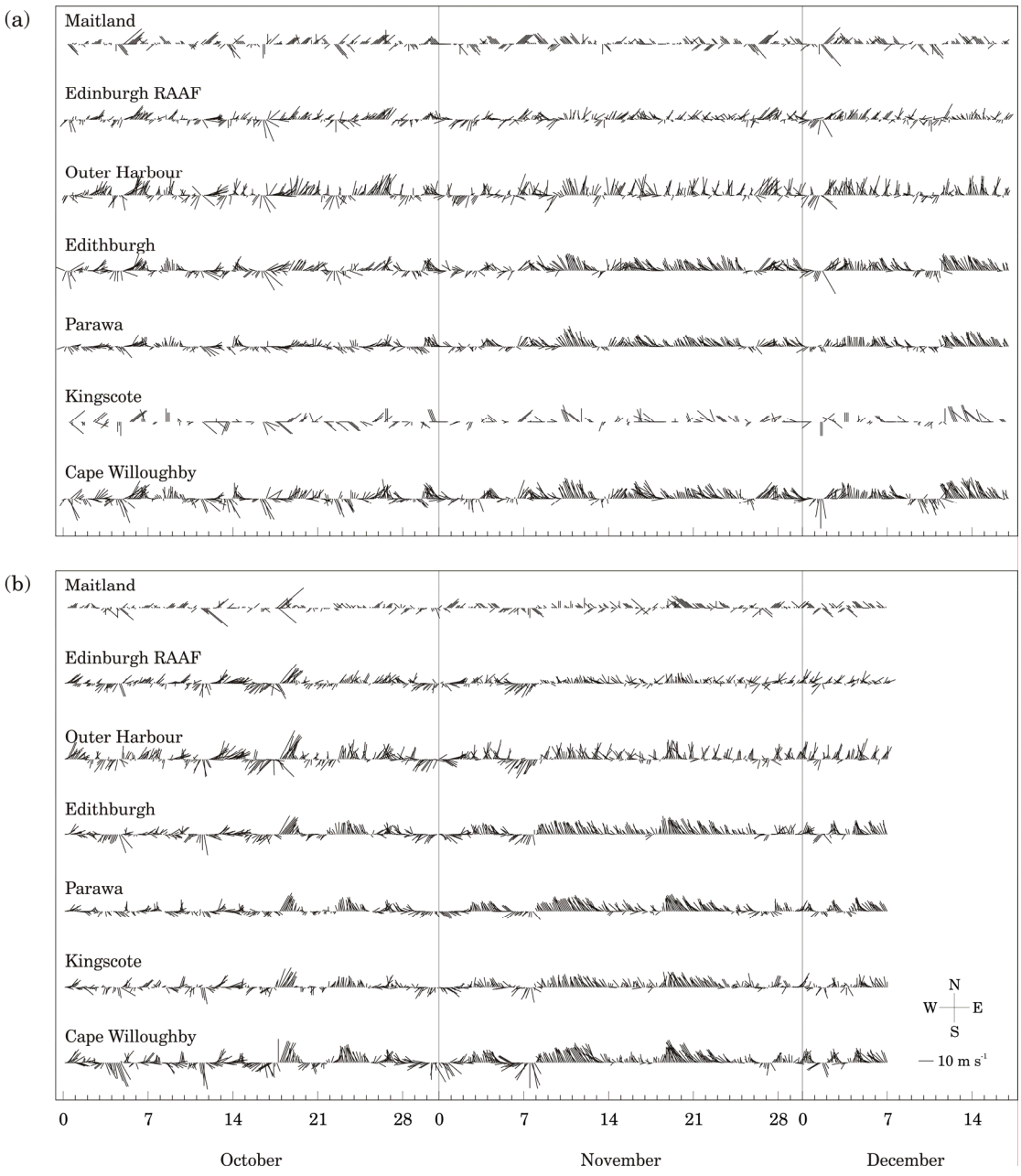

FIG. 5.12 Incident wind vectors (m s^{-1}, degrees True) recorded at three-hourly intervals from seven land-based wind measurement stations situated around Gulf St Vincent during (a) Oct 1-Dec 17, 1998 and (b) Oct 1-Dec 7, 2000.

material that can be sorted by one person in a day is 70 L, which on average is equivalent to 45 m^2 of vegetated habitat, compared to the estimated 2436 km^2 (= 2.4 x 10^9 m^2) that seagrass occupies in the Gulf St Vincent (Edyvane, 1999). Floating plant material is not considered as important as the extensive seagrass beds and drifting algae below the surface for the attachment of *H. melanochir* eggs since the amount of floating material that would be required to support *H. melanochir* eggs at the surface appeared to be insufficient to explain the observed abundance of larvae (the same reasoning was given by Tanaka & Oozeki, 1996, for the Pacific saury). One could suggest that, from the lack of *H. melanochir* eggs found

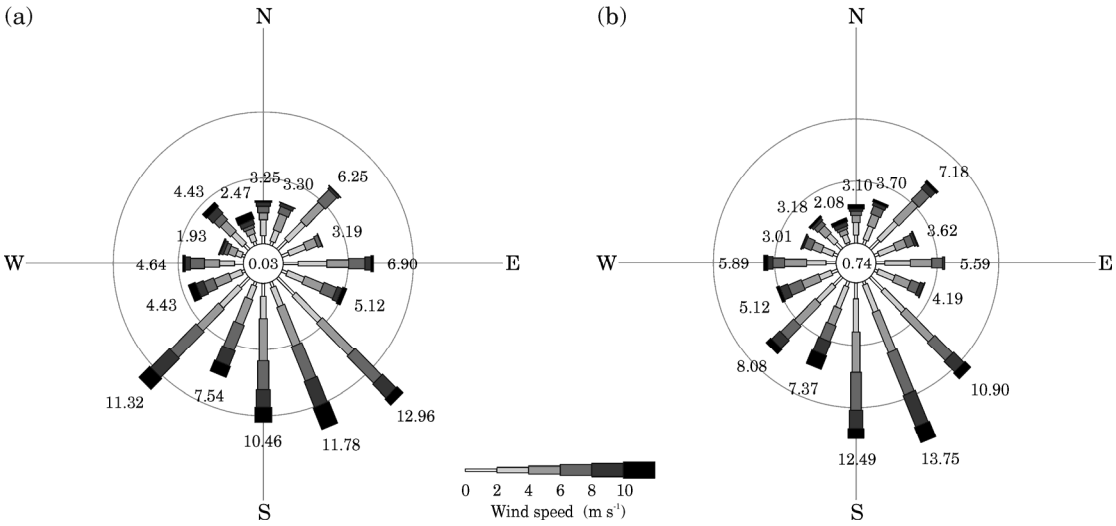

FIG. 5.13 Percentages of winds (directions and speeds) recorded at three-hourly intervals from seven land-based wind measurement stations situated around Gulf St Vincent for (a) Oct 1-Dec 17, 1998 (n = 3723 readings) and (b) Oct 1-Dec 7, 2000 (n = 3650 readings). Calms included at centre of wind rose. Rings are at 5% intervals. Wind flow is *from* the directions shown.

among seagrass and algal samples, spawning may occur at greater depths than those sampled (<10 m) in this study. However, this would appear unlikely for the following reasons: in a depth-stratified survey (2-5, 5-8 and 8-11 m) conducted in Great Oyster Bay, eastern Tas., the abundance of *H. melanochir* eggs increased at shallower depths (Jordan et al., 1998); female garfish (including running ripe fish) tend to concentrate at depths of 1-5 m in inshore waters of S.A. during the spawning season (Ye *et al.*, 2002*a*); and *H. melanochir* eggs were discovered by a fish processor among a commercial catch of garfish taken from the Bay of Shoals, where depths do not reach 10 m.

The size and number of eggs produced, the duration of their incubation period, and their habitat vary greatly among species and all of these traits have important consequences for survival (Fuiman, 2002). For *H. melanochir*, batch fecundity is constrained by the small body cavity for the production of large eggs. Whilst there may be a relationship between low abundance of *H. melanochir* eggs in vegetation and the species' low fecundity due to large egg size, the observation that *H. melanochir* eggs are demersal may be more significant. For species that produce demersal eggs, it is rarely possible to sample this stage of the life cycle because the habitats over which spawning occurs are poorly known or cannot be sampled easily or effectively (Pepin, 2002).

Although *H. melanochir* eggs could not be found among diving or beam trawl samples, the incidental finding of eggs attached to seagrass and algae among market fish from the Bay of Shoals provided direct evidence of *H. melanochir* spawning over this type of habitat in South Australia. *Hyporhamphus melanochir* eggs have also been found along the east coast of Tas., attached to filamentous drift algae (Jordan *et al.*, 1998). The observation that eggs of

both samples were heavily entangled and attached to filamentous algae or epiphytic algae on the leaves of *Posidonia* suggests that spawning is not as dependent on seagrass *per se* as the relatively large surface area that seagrass and algae collectively provide for attachment of *H. melanochir* eggs. The suggestion that some structure is required for successful egg development is supported by Jordan *et al.* (1998), where it was noted that eggs that were attached to artificial substrate in rearing experiments fully developed through to the larval stage while unattached eggs did not survive. Also, Jones (1990) found *H. melanochir* eggs adhering to set gill nets in Baird Bay, South Australia.

In any region and moment of time, there is a complex array of predator species and sizes that can consume eggs and larvae. Consequently, there is a general lack of information on the community of predators that consumes fish eggs and larvae, especially the quantified effects of predators on a particular prey species (Houde, 2002). Nevertheless, it is generally accepted that predators inflict a heavy toll on the early stages of fishes and are likely to be the most significant cause of mortality (Bailey & Houde, 1989). Whilst predation on most fish eggs tends to be size-specific, the susceptibility of the relatively large *H. melanochir* eggs to predation is expected to be greatly reduced, owing to a number of ecologically favourable characteristics. The demersal eggs become attached to vegetation before they reach the bottom, thus becoming somewhat hidden or protected from predators, especially where parental care is reduced or absent (Potts, 1984). Furthermore, the observation that *H. melanochir* eggs have the tendency to become relatively isolated from one another when attached to vegetation (Jordan *et al.*, 1998), rather than being clumped together, suggests an adaptation that minimises the risk of mass predation.

Hyporhamphus melanochir larvae were most effectively sampled using a neuston net and its effectiveness presents a standard methodology suitable for the annual monitoring of year classes. The distribution and abundances of larvae throughout Gulf St Vincent indicated a non-random spatial structure, where similar abundances are spatially clustered. Furthermore, despite the greater number of larvae collected in cruise 3 than cruise 1, a similar spatial pattern and patch size were apparent. Most larvae were collected in the upper region of the gulf, which is almost entirely occupied by seagrass habitat. The neuston net collected *H. melanochir* from newly-hatched larvae through to transforming larva and early juvenile stages (see description of larval development in Noell, 2002 and Chapter 4). Transforming (or metamorphosing) larvae and juveniles were less frequently encountered, probably as a result of cumulative mortality and increased avoidance capacity (Sandknop *et al.*, 1984). It is important to note that the abundances of larvae for the entire size range should be treated as relative rather than absolute since even the smallest larvae are competent swimmers and thus could have some avoidance capacity (Jordan *et al.*, 1998). No apparent association was found between the size of larvae and where they were collected from.

In the newly-hatched larvae of *H. melanochir*, the free-swimming yolk-sac, pre-flexion and flexion stages are avoided, as the developing embryo undergoes these during the prolonged egg incubation period. Despite the trade-off in having relatively few eggs available for the production of offspring, a long incubation period and well formed larvae are typical early life traits of Beloniformes that present a number of ecological advantages to the larvae in terms of mortality from starvation, predation and advection (Collette *et al.*, 1984). As demonstrated in *Hemiramphus sajori* larvae (Kawamura et al., 1990), it is assumed that the similarly well-developed *H. melanochir* larvae have fully functioning sensory organs and advanced swimming ability (routine and burst swimming speed) soon after hatching. Therefore, *H. melanochir* larvae are better equipped than planktonic larvae at locating and obtaining food over larger areas, detecting predators (as well as plankton nets) and initiating an appropriate evasive response, and locating and remaining in a suitable habitat (Fuiman, 2002).

The use of otoliths to age larval and early juvenile *H. melanochir* relied upon the assumption that the increments of the lapilli were deposited at a rate of one per day. Unfortunately, it was not possible to validate this assumption in this study since, at these early life stages, *H. melanochir* were too fragile to be marked with oxytetracycline or similar dye and to be subsequently held for a specified number of days after being marked (personal observation). However, the use of lapilli to determine the age (in days) of other species found in temperate Australian waters and the similarity in the otolith increment structure among these species support the assumption that daily growth increments are also deposited in the lapilli of *H. melanochir* (greenback flounder *Rhombosolea tapirina* and longsnout flounder *Ammotretis rostratus*, Jenkins, 1987; blue grenadier *Macruronus novaezelandiae*, Gunn *et al.*, 1989; jack mackerel *Trachurus declivis*, Jordan, 1994; Spanish mackerel *Scomberomorus maculatus*, Peters & Schmidt, 1997; ling *Genypterus blacodes*, Morioka & Machinandiarena, 2001).

Hyporhamphus melanochir larvae exhibit increasingly fast growth from 0.5 mm d^{-1} immediately after hatching to greater than 1.0 mm d^{-1} at 20 d old. The vast majority of *H. melanochir* larvae collected during cruises 1 and 3 measured <12 mm in body length. The lapillar otoliths from these larvae typically have up to 10 daily increments, which presumably corresponds to the maximum number of days spent adrift since hatch. In addition to the age of larvae, a 12-16-d incubation period for eggs was added for the back-calculation of spawning dates of *H. melanochir* in the gulf waters of South Australia. Using this method, it is estimated that spawning occurred from mid October to early December and during all of January for the larvae collected. Both of these periods fall within the assumed spawning season from October to March, inferred from the reproductive biology of female *H. melanochir* (Chapter 2). It is important to note that the ages/spawning date distributions

reflect the fast growth of larvae and the size/age of maximum catchability, not the true pattern of spawning or the actual duration of the spawning season. The estimation of spawning dates should be interpreted cautiously since it is based upon the assumption that the incubation time for egg development of *H. melanochir* undergoes the same rate of exponential decay with increasing temperature as for the related needlefish, *Belone belone* (Fonds *et al.*, 1974). Nevertheless, it is expected that the significantly higher water temperatures in the South Australian gulfs (up to *c.* 23°C in this study) relative to Tasmanian waters (*c.* 15°C), where it took 29 days for larvae to hatch (Jordan *et al.*, 1998), would greatly accelerate *H. melanochir* egg development (see review by Pauly & Pullin, 1988). Although incubation periods for eggs were calculated separately for different water temperatures in December and February, larvae collected at these times were assumed to exhibit similar growth; subsamples of age-at-length data from all cruises provided a good overall fit of the Laird-Gompertz growth curve.

Until eggs can be sampled effectively, considerable extrapolation is required to predict spawning location. It remains to be established whether eggs become attached to seagrass and algae on the fixed substratum or to floating or drifting vegetation. If the *H. melanochir* eggs are in a fixed position until hatch, then it is predicted that most of the larvae collected will be collected near the spawning site. However, if eggs are transported on drifting objects, the distance that eggs/larvae move from spawning to the time that they are sampled would be greater. In addition to the age of the larvae and possibly the incubation period of the egg stage, local environmental processes (e.g. wind) and swimming behaviour are considered to be influential in the transportation of larvae. For example, larvae of a related species, *Belone belone*, can easily maintain a swimming speed of 1 body length s^{-1} (Rosenthal & Fonds, 1973). Whether swimming behaviour of *H. melanochir* larvae is random or can be predicted is unknown. A better understanding of these factors would enable better prediction of spawning locations within Gulf St Vincent.

During November and December, prevailing winds for the whole gulf were generally from a southerly direction. Local wind direction and speed are probably the most important factors that influence the general circulation of water in Gulf St Vincent (Bye, 1976). Therefore, it is likely that these southerlies, combined with the Coriolis effect and land boundaries, influence the clockwise gyre in the upper gulf, which in turn may explain the retention of larvae following spawning over the extensive seagrass beds in this region. A notable exception to the concentration of larvae in the upper region was small numbers found at the entrance to the gulf between Yorke Peninsula, Kangaroo Island, and Fleurieu Peninsula. With southerly and south-easterly winds predominantly registered specifically for the lower region of the gulf (Parawa, Kingscote, and Cape Willoughby wind stations), it is likely that this patch of larvae originated from the northeast coast of Kangaroo Island, where dense seagrass beds also occur (FIG. 5.7).

The broad-scale distribution and abundance of larvae suggest that spawning of *H. melanochir* does take place over or adjacent to extensive seagrass areas. However, this study has not been able to confirm that. The proposed remains supported by anecdotal evidence and the literature on the eggs of most beloniform species, which are demersal, covered with filaments, and are reliant upon seagrass beds and/or macroalgae for their attachment. The notable absence of eggs of *H. melanochir* in archival plankton collections held at SARDI Aquatic Sciences (taken by horizontal subsurface and vertical tows) further suggests that these eggs are not collected in pelagic samples but rather like other beloniforms the eggs are demersal.