J. Mar. Biol. Ass. U.K. (2006), **86**, 1253–1264
Printed in the United Kingdom

Reproductive tactics and larval development of bigeye flounder, *Hippoglossina macrops*, off central Chile

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Through a series of ichthyoplankton surveys carried out off central Chile, we characterize the offshore spawning and coastal nursery ground of the bigeye flounder *Hippoglossina macrops*. Eggs were collected in higher abundances over the continental shelf during late winter, over the shelf-break in mid spring, and at the continental slope in late summer, showing a seasonal depth gradient in spawning. No significant spawning was detected nearshore (less than 8 km offshore). Field data suggest a longitudinal progression from offshore to onshore of larval stages from preflexion to transformation stage. Finally, vertically stratified samples showed that eggs and larvae were present mainly in the upper 100 m. We discuss the potential onshore transport of early life stages of *H. macrops* through the compensatory flow of Equatorial Subsurface Waters that balances the surface offshore transport in the Ekman layer during upwelling events. Additionally, larval development from yolk-sac to transformation stage is briefly described.

INTRODUCTION

Transport and retention are physical processes responsible for moving early pelagic life stages from the spawning site toward an appropriate nursery ground and can play a role in the regulation of recruitment and year-class strength. In particular, flatfish show an array of strategies to arrive at, and/or maintain themselves in suitable nurseries (see Bailey et al., 2005, for a review). For example, winter flounder *Pseudopleuronectes americanus* enters shallow estuaries for spawning; furthermore, larvae are delivered to coves by tidal current (Chant et al., 2000). Conversely, flounder *Platichthys flesus* migrates from their estuarine habitat to spawning grounds over the continental shelf (Sims et al., 2004). Arrowtooth flounder *Atheresthes stomias* and Pacific halibut *Hippoglossus stenolepis* spawn along the continental slope and outer shelf in the Gulf of Alaska and the larvae are transported inshore over the continental shelf (Bailey & Picquelle, 2002). In areas where upwelling prevails seasonally or throughout the year, reproductive tactics of flatfish have been poorly investigated until the present study (Markle et al., 1992; Toole et al., 1997).

The bigeye flounder, *Hippoglossina macrops* Steindachner, 1876 inhabits muddy and soft bottoms from shallow waters to 600 m depth (Yáñez & Barbieri, 1974) in the eastern Pacific from Mazatlán (Mexico) to Punta Arenas (Chile) (Fischer et al., 1995). The species sustains an artisan fishery and is a common component of the by-catch of crustacean and hake fisheries of northern and central Chile (Arancibia, 1991; Villarroel & Acuña, 1999). Due to its economic importance, some aspects of the adult biology have been documented regarding feeding (Tomicic, 1973; Arancibia & Méndez, 1987) and parasitism (Riffo, 1991). Evidence from ontogenetic changes in distribution, feeding habits (Villarroel & Acuña, 1999, 2000) and changes in ectoparasitism incidence (González et al., 2001) suggest that this species lives in different habitats (depths) during its lifetime. Through gonad analysis spawning has been reported to occur between August and November in central Chile (Voigth & Balbontín, 1981). During this period, a high population density of mature specimens has been observed in deep waters (250–350 m, Villarroel et al., 2000). In spite of its commercial importance there is no information on development, distribution and abundance of eggs and other early life history stages in the literature.

The oceanography off central Chile (33°–40°S) where the bigeye flounder is abundant (Arancibia, 1992), is characterized by seasonal spring–summer coastal upwelling (September–March, with a peak in December–January) (Arcos & Navarro, 1986; Figueroa & Moffatt, 2000). During the upwelling season, strong south winds induce upwelling of deep waters (Equatorial Subsurface Water, ESSW) at the coast and offshore movement of the surface Ekman layer (Sub-Antarctic Surface Water, SASW). Conversely, during autumn–winter, SASW occurs at the coast and the ESSW deepens towards the continental slope (Bernal et al., 1983). It has been hypothesized that in coastal, wind-induced upwelling areas, bottom dwelling and mid-depth fish may adjust their reproduction to enhance transport of their early life stages to nearshore nursery zones (Vargas et al., 1997; Smith & Suthers, 1999; Landaeta & Castro, 2002).

Through a series of ichthyoplankton cruises in the central zone of Chile we evaluated patterns in horizontal

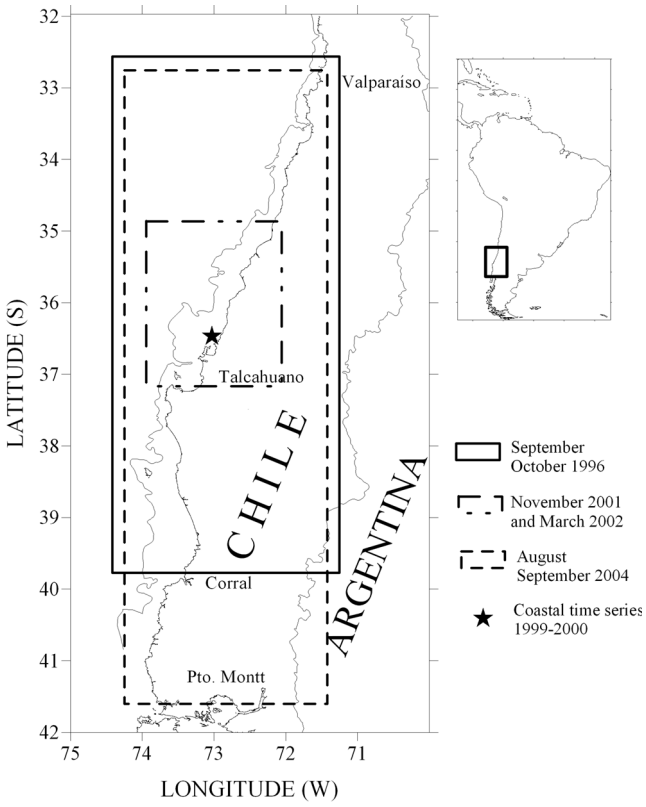

Figure 1. Survey area utilized during September–October 1996, November 2001, March 2002, August–September 2004, and the coastal time series (1999–2000). Bathymetric contour represents 200-m isobath.

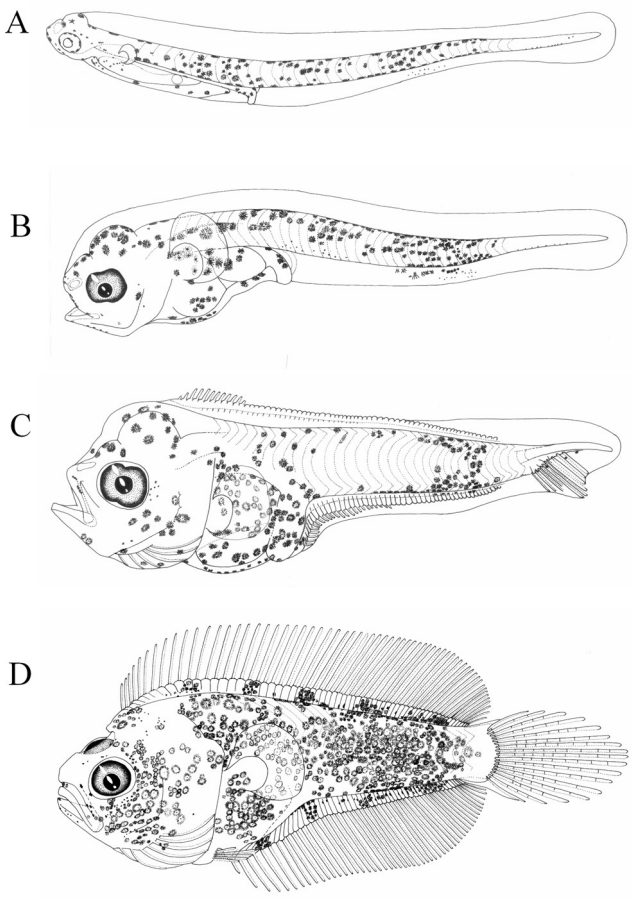

Figure 2. Larvae of bigeye flounder *Hippoglossina macrops* off central Chile: (A) yolk-sac larva (4.9 mm NL); (B) preflexion larva (5.8 mm NL); (C) flexion larva (6.5 mm NL); and (D) transformation stage (12.0 mm SL).

and vertical distribution of *Hippoglossina macrops* eggs and larvae. Additionally, larval development from yolk- to transformation stage is briefly described.

MATERIALS AND METHODS

Field work

Plankton samples were obtained over the continental shelf off central Chile between 33° and 41°30′S (Figure 1) in four cruises: September–October 1996, November 2001, March 2002, and August–September 2004. All samples were preserved in 10% formalin buffered with sodium borate.

During the austral spring 1996, a grid of 74 stations that covered variable distances from nearshore to a maximum of 110 km (60 nautical miles, (n.m.)) offshore was used. Throughout the survey, conductivity–temperature–depth

Tabe 1. *Range of body length (notochord length in yolk-sac, preflexion and flexion larvae, and standard length in postflexion larvae) and range and mean ±SD of body proportions (as a percentage of body length) of* Hippoglossina macrops *larvae.*

	Yolk-sac (N=6)	Preflexion (N=17)	Flexion (N=18)	Postflexion (N=20)	Transformation (N=13)
Body length (BL) (mm)	4.4–5.7	4.5–7.8	7.7–9.4	8.2–11.6	11.1–16.6
Head length (HL) (% BL)	37.5–43	34–42.3	27.5–45.7	38.9–53.8	38–52.2
	39.6 ±1.9	38.4 ±2.2	38.4 ±3.8	44.8 ±3.8	42.9 ±3.7
Eye diameter (% HL)	33.3–50	29.6–50	27.9–37.5	25–35.8	23.6–30
	42.9 ±6.5	36.6 ±5.4	31.9 ±2.4	30.7 ±2.7	28 ±1.8
Snout length (% HL)	16.7–33.3	11.1–29.4	15.2–23.9	12.5–25	16.5–22.6
	25.8 ±6.5	20.7 ±5.1	18.4 ±2.3	17.8 ±3.2	18.6 ±1.6
Body depth (% BL)	7.7–14.1	7–26.4	25.9–32.4	34.8–47.5	38–43.4
	9.7 ±2.2	14.4 ±5.4	29.6 ±1.8	39.8 ±3.2	40.9 ±1.5
Preanal length (% BL)	37.5–43	34–42.3	27.5–45.7	38.8–53.8	38.4–52.2
	39.6 ±1.9	38.4 ±2.2	38.4 ±3.8	44.8 ±3.8	42.9 ±3.7

N, sample size; SD, standard deviation.

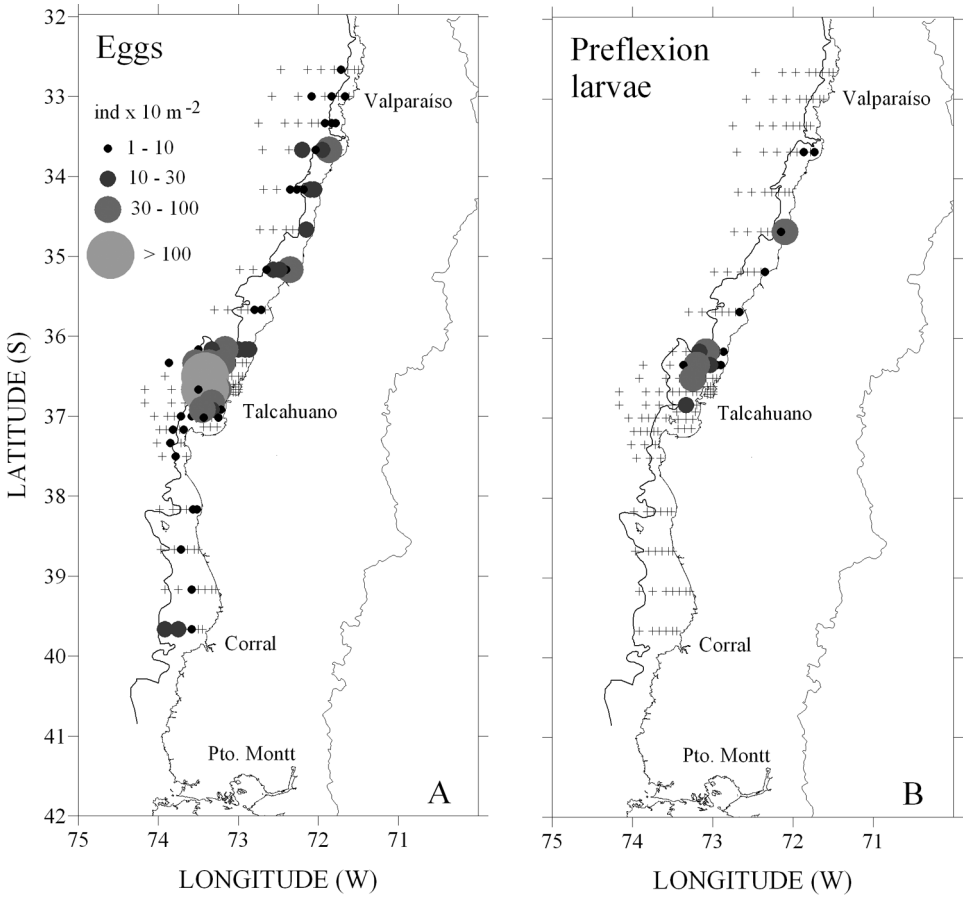

Figure 3. Horizontal distribution of eggs and preflexion larvae of *Hippoglossina macrops* (ind×10 m^{-2}) during October 1996 off central Chile.

(CTD) (Sensordata SD2003) casts to 100 m were conducted. Oblique tows were carried out at each station during day and night hours using a 1 m^2 Tucker trawl (250 μm mesh) with a General Oceanics flowmeter mounted in the frame of the net. Towing speed was ~2–3 knots. Volume filtered in each tow (range=69–706 m^3/tow; mean=348 m^3/tow) was estimated taking into account mouth area and towing duration. Two strata (0–40 and 40–100 m deep) were sampled. Onboard, the samples were divided using a Folsom splitter and one subsample (i.e. 50% of the whole) was preserved.

During the November 2001 and March 2002 surveys, a grid composed of 11 transect lines with six or seven stations per line was sampled. A CTD cast (Seabird SB-19) was conducted at each station to 250 m or near the bottom. Oblique tows at speeds of 2–3 knots were carried out using a 1 m^2 Tucker trawl (300 μm mesh) with a General Oceanics flowmeter mounted in the frame of the net to estimate filtered volume. Volume ranged between 17 and 589 m^3/tow (mean=142 m^3/tow) during November 2001, and between 12 and 217 m^3/tow (mean=96 m^3/tow) during March 2002. Multiple trawls were carried out at each station for more intensive depth sampling. Depth

Table 2. *Mean, standard deviation (SD) and maximum values (ind×10 m^{-2}) of early stages of bigeye flounder* Hippoglossina macrops *off central Chile during all cruises studied.*

	September–October 1996			November 2001			March 2002			August–September 2004		
	Mean	SD	Maximum	Mean	SD	Maximum	Mean	SD	Maximum	Mean	SD	Maximum
Eggs	26.72	30.27	114.99	34.77	34.79	139.4	23.11	19.41	82.12	30.11	16.91	54.35
Preflexion larvae	13.62	15.36	74.38	5.17	4.13	14.69	4.81	3.44	14.94	11.31	3.35	18.46
Postflexion larvae	–	–	–	4.57	2.49	9.48	6.44	5.07	15.51	–	–	–
Transformation	–	–	–	4.19	2.52	7.99	3.82	0.77	5.33	–	–	–

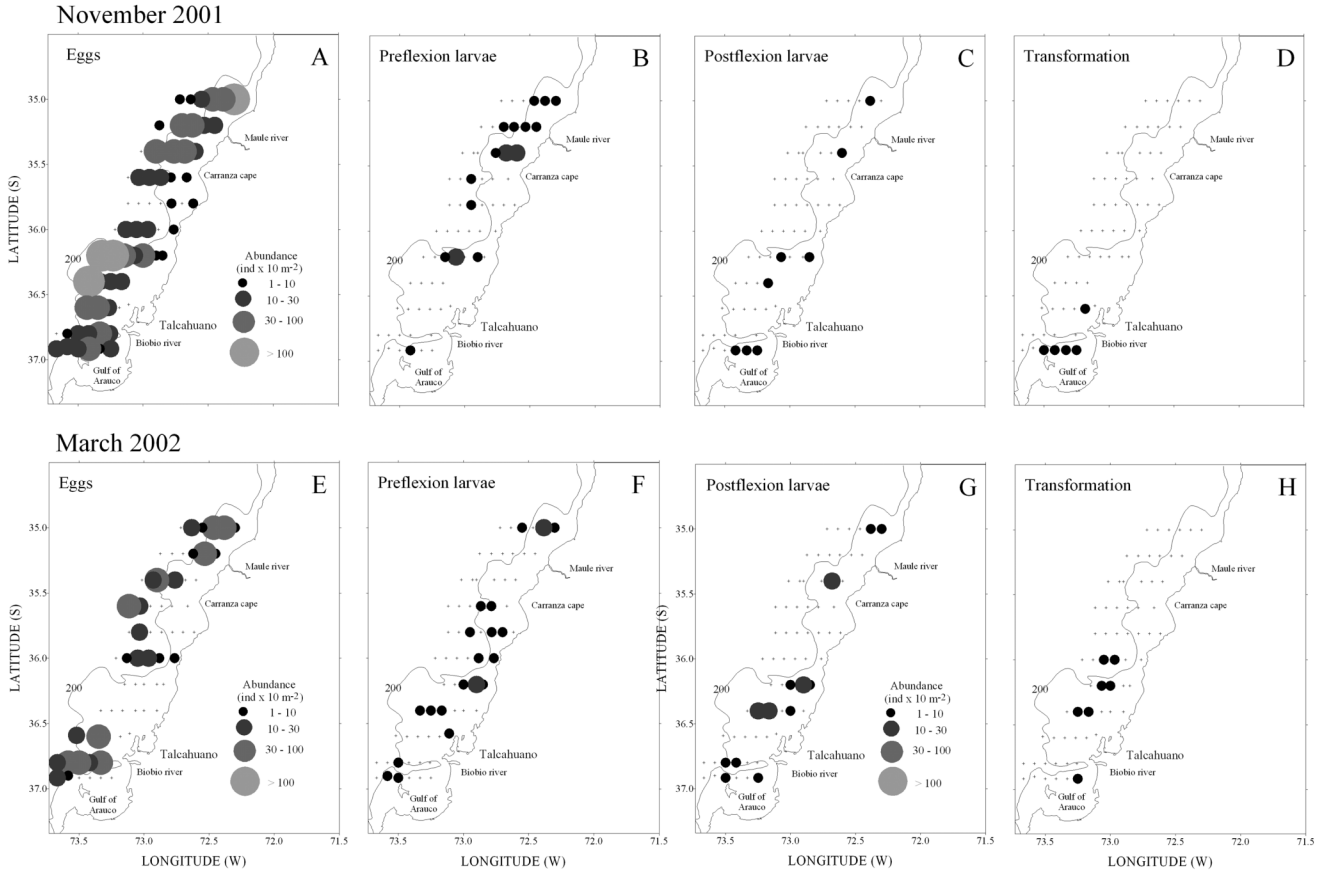

Figure 4. Horizontal distribution of (A) eggs; (B) preflexion larvae; (C) postflexion larvae; (D) transformation larvae of *Hippoglossina macrops* (ind×10 m^{-2}) during November 2001; and (E) eggs; (F) preflexion larvae; (G) postflexion larvae; (H) transformation larvae of *Hippoglossina macrops* (ind×10 m^{-2}) during March 2002, off Talcahuano, central Chile.

strata were variable, ranging from 0–25, 25–50, 50–75 and 75 to 100 m deep over the shelf, and 0–50, 50–100, 100–150 and 150–250 m deep at the shelf break area. Total samples were preserved.

Finally, during late August–September 2004, 78 stations were surveyed between Valparaíso (33°S) and Puerto Montt (41°30′S) over the continental shelf during day and night hours. At each station a CTD cast (Seabird SB-19) and an oblique Bongo (60 cm diameter, 300 μm mesh, with General Oceanics flowmeter) tow to 80 m depth or near the bottom, were conducted during day and night hours. Towing speed was 2 knots. Filtered volume ranged from 7 to 200 m^3/tow (mean=48 m^3/tow). One sample was preserved in 10% formalin buffered with sodium borate and the other one was preserved in 90% ethanol.

To analyse the temporal spawning pattern of bigeye flounder in the coastal area, a nearshore station (less than 8 km offshore from Talcahuano, ~90 m depth) was visited monthly between July 1999 and September 2000 following the same sampling method used in the September 2004 survey (i.e. zooplankton sampling with Bongo nets).

At the laboratory, all samples were analysed, and all eggs and larvae of bigeye flounder were identified and counted. Egg and larval densities were expressed as ind×10 m^{-2} for examination of the horizontal pattern and as ind×1000 m^{-3} for vertical distribution. The standard length (SL) of undamaged larvae (N=229) was

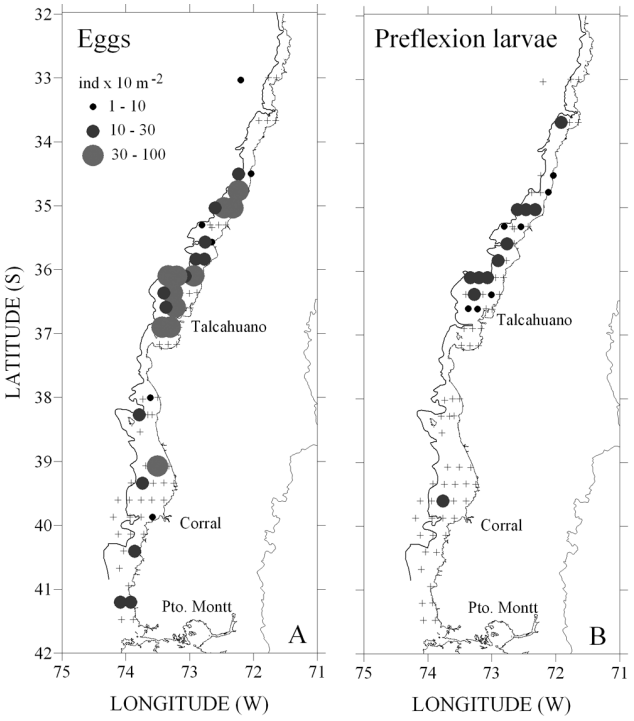

Figure 5. Horizontal distribution of eggs and preflexion larvae of *Hippoglossina macrops* (ind×10 m^{-2}) during September 2004 off central Chile.

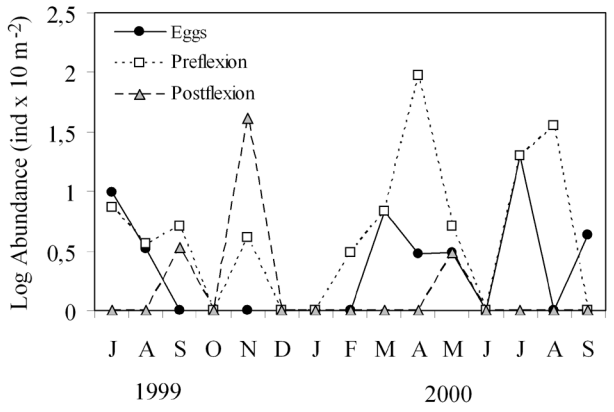

Figure 6. Log-transformed abundance (ind×10 m^{-2}) of eggs, preflexion and postflexion larvae, collected in a coastal station (see Figure 1 for location) between July 1999 and September 2000.

measured to the nearest 0.1 mm, and the developmental stage was classified as preflexion, postflexion (flexion and postflexion larvae pooled) and metamorphosing or transformed individuals. Vertical distribution analysis was limited to total larvae due to low number of individuals per ontogenetic stage. This also precluded an analysis of the vertical migration for each larval stage. Parametric tests were used on log-transformed abundance when assumption of homogeneity of variance was not rejected (Levene's test). Otherwise, non-parametric tests were run using STATISTICA software.

Eggs and larval identification

Eggs and larvae of bigeye flounder, *Hippoglossina macrops*, were identified using the serial method (Neira et al., 1998), by positively identifying the largest available larva, or the smallest juvenile, using known adult characters such as fin meristics, and subsequently linking this specimen with progressively smaller specimens by using general morphological and pigment characters until a developmental series is assembled. The body length (BL) corresponds to notochord length (NL) in preflexion larvae and to SL in flexion, postflexion and transformation specimens. Morphometric measurements of body depth (BD), head length (HL), and preanal length (PAL) were converted to percentage of BL (Table 1). Egg and yolk-sac larval identifications were confirmed through laboratory reared eggs collected with oblique Bongo net tows at 18.5 km (10 n.m.) offshore Dichato, Chile (36.5°S) during October 2004. Eggs and yolk-sac larvae were maintained in 1 l glass jars with ultraviolet-irradiated and filtered seawater (0.5 μm) at 12°C and a photoperiod of 12:12. One-third of the seawater was replaced daily. Larvae were maintained without food until death.

RESULTS

Description of early life stages

Among species of *Hippoglossina*, *H. macrops* has comparatively large eggs, measuring 1.54–1.59 mm in diameter, vs 1.22–1.38 mm in *H. stomata* (Moser & Sumida, 1996) and 0.86–1.12 mm in *H. oblonga* (Miller & Marak, 1962; Fahay, 1983). They have homogeneous yolk, narrow perivitelline space and a single oil globule (0.23–0.26 mm diameter). Larval *H. macrops* at yolk-sac, preflexion, flexion and postflexion stages are briefly described below, focusing on changes in body shape, pigmentation and fin development. Yolk-sac larvae (Figure 2A) are elongate with a gut with a pronounced bending at its end (preanal distance 40% of notochord length, Table 1) and measure 4.4–4.8 mm NL at hatch (N=6). They show heavy pigmentation composed mainly of dendritic melanophores scattered around the trunk and tail, except for the posteriormost part of the tail, in the last 2–3 myomeres. Melanophores are more concentrated near mid-tail, forming a bar. Dendritic melanophores are scattered on the brain, under the snout, in the nuccal region, ventrally on the yolk-sac and under the intestine. Small punctate melanophores are observed anteriorly under the pectoral fin area, in a patch in the anal finfold near the midpoint between the anus and the tip of the tail, and in a series at the ventral margin of the notochord tip; in a few specimens, one or two small melanophores are observed laterally at the tip the notochord. Larvae with yolk-sac ranged between 4.4 and 5.7 mm NL (Table 1).

In preflexion larvae (Figure 2B) the body becomes deeper anteriorly, at the cephalic and gut regions (BD=14.4% BL, range 7–26.4%, N=17). Preanal length changes little. Three small spines are observed on the upper portion of the preopercular bone (Figure 2C). The pigmentation becomes more marked as new melanophores appear and increase in size. Dendritic melanophores develop on top of the brain, on the sides of the trunk at pectoral fin level, on the sides of the gut, dorsally to the intestine and ventrally under the mid- and hindgut. Small melanophores develop ventrally on the lower jaw and on the tail behind the intestine. Some of the melanophores in the patch at the anal finfold become dendritic. Melanophores form also on the inner and outer surface of the pectoral-fin base.

Flexion occurs at ~7.7–9.4 mm (Figure 2C). The body continues to grow deeper in the head and anterior trunk region (BD=29.6% BL, range 27.5–45.7%, N=18). Preanal distance remains at ~38% BL as flexion and gut coiling (which began during preflexion stage) take place. The three preopercular spines remain visible, hypural plates develop, and the dorsal and anal fins begin to form during preflexion stage. The dorsal and anal anlagen appear in the finfold, separated from the body, as elongate thickenings that extend posteriorly and divide into segments corresponding to the individual pterygiophores. Fine filaments extend from the dorsal and ventral anlagen toward the margins of the body. The dorsal and anal rays begin to form from the anterior to the posterior end on both fins, even before the anlagen have fully developed posteriorly. Dorsal- and anal-fin pterygiophores, which began to form within the finfold separate from the body, complete their development in the postflexion stage by growing toward the margins of body on both fins. The pigmentation is distributed as in previous stages. The melanophores in the blotch on the anal finfold decrease in number; they become embedded in the pterygiophores of the anal fin. The melanophores on the ventral margin of the notochord tip also begin to disappear, with those remaining larger than in previous stages. Except for a few

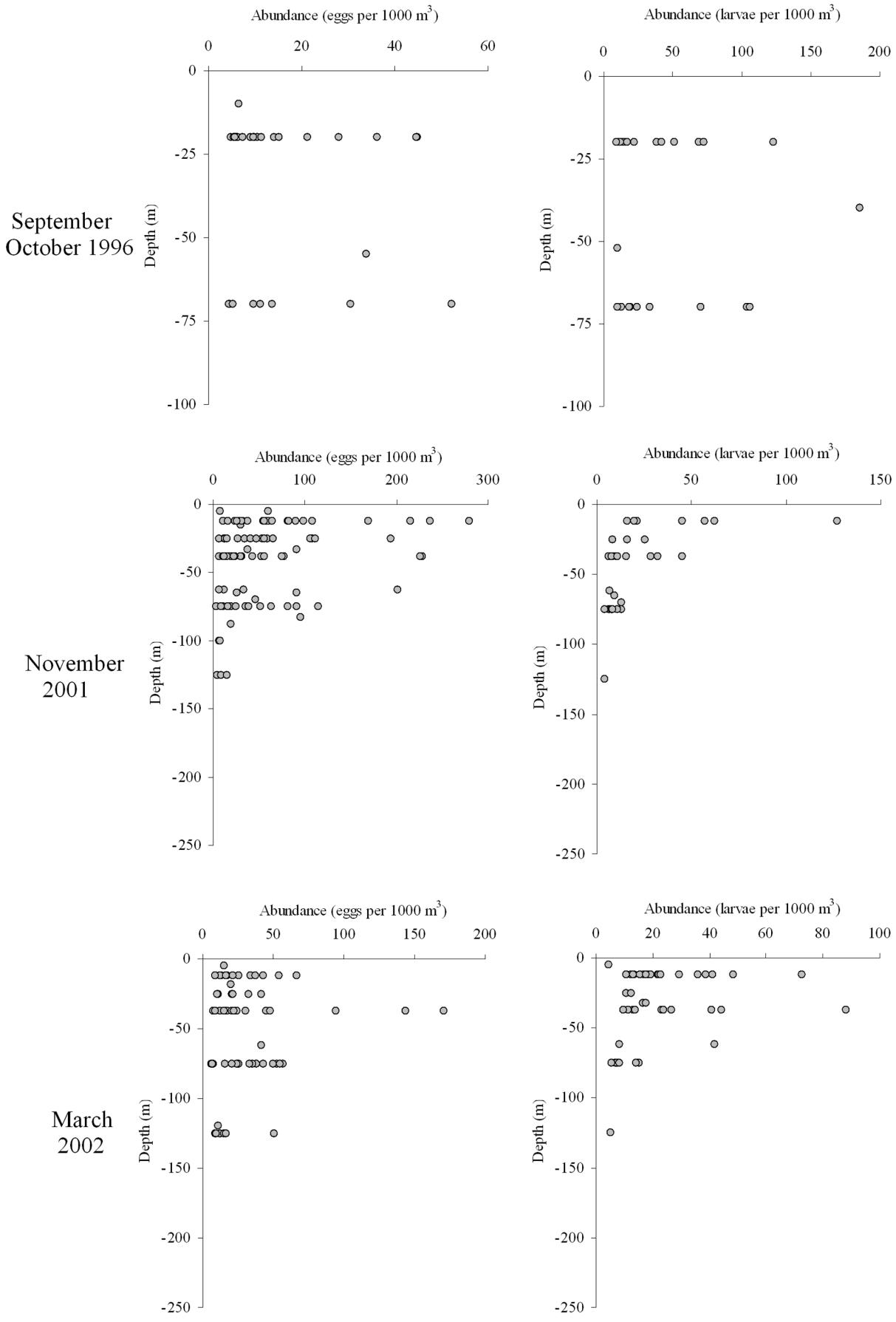

Figure 7. Mean depth (m) of eggs (left) and larval *Hippoglossina macrops* (right) during October 1996, November 2001 and March 2002 off Talcahuano, central Chile. Abundance expressed as individuals per 1000 m^3.

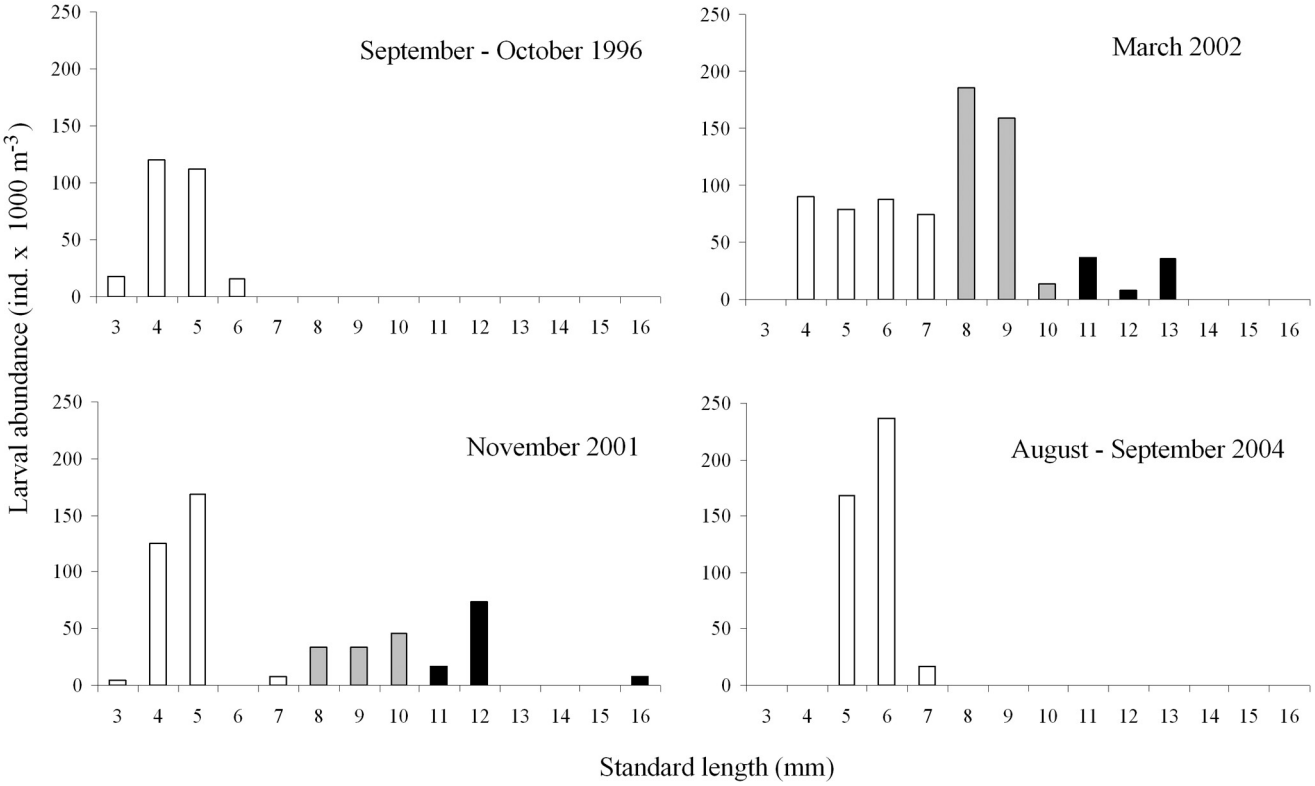

Figure 8. Size–length frequency distribution of preflexion (white bar), postflexion (grey bar) and transformation (black bar) larvae of *Hippoglossina macrops* off central Chile.

scattered small melanophores located behind the eye, almost all melanophores on the body are dendritic.

During postflexion, the body continues to grow deeper anteriorly, and also on the anterior portion of the tail (BD=39.8% BL, range 34.8–47.5%, N=20). Dorsal and anal rays extend considerably increasing the total depth of the body. The most anterior dorsal rays do not elongate as happens in the larvae of other species of *Hippoglossina*. Pelvic fin buds develop at ~8 mm standard length. Dendritic melanophores on the body increase in size and number and myomeres become barely visible because they are obscured by the increasing pigmentation. Preopercular spines are not visible during this stage.

Transformation begins at ~11 mm SL. During this process (Figure 2D) body depth continues to increase, as do the lengths of the dorsal- and anal-fin rays (BD=40.9% BL, range 38.4–52.2%, N=13). The largest transformation specimen examined (16.6 mm SL) had not developed rays on the pectoral fins yet. Toward transformation the right side becomes paler as melanophores on the body and pterygiophores begin to decrease in number and size. On the left side, the melanophores continue to increase in number and size to cover almost the entire body, except for the tip of the tail and caudal fin; a few small, elongate and isolated melanophores develop between pterygiophores of the dorsal and anal fins. As eye migration takes place (11 mm SL), series of five dorsal- and four anal-fin blotches of small dendritic melanophores develop on the left (eyed) side of the pterygiophores. This pattern is the same as observed in transformation specimens of *H. stomata* (Moser & Sumida, 1996). Meristic attributes of adult *H. macrops* are: dorsal 63–78, anal 49–52, vertebrae 37 (9–10+27–28), pectoral 11, pelvic 6, caudal 17, (Chirichigno, 1974; Pequeño & D'Ottone, 1987).

The larvae of *Hippoglossina macrops* show few differences from *H. stomata* (Sumida et al., 1979) and *H. oblonga* (Leonard, 1971; Fahay, 1983); *H. macrops* do not develop a pigment patch dorsally on the median finfold, have fewer spines on the preoperculum and lack noticeably elongate rays anteriorly on the dorsal fin. *Hippoglossina macrops* hatches at a larger size than the other two: ~4 mm vs 3 mm; also, it may transform at a slightly larger size: in *H. stomata* eye migration begins at ~9 mm and transformation is complete at ~12 mm, while in *H. oblonga* eye migration begins at ~10 mm and transformation is complete at >12 mm. Larval *H. macrops* are quite different from other flounder larvae present in the same area, i.e. *Paralichthys microps* and *P. adspersus*. Both *Paralichthys* larvae have smaller size at hatching (1.5 and 1.7 mm NL, respectively), have sphenotic spines, and show elongated anterior dorsal-fin rays (Zuñiga & Acuña, 1992) that are absent in bigeye flounder larvae.

Egg and larval horizontal distribution

During the early austral spring 1996 cruise, eggs of *Hippoglossina macrops* were found over the continental shelf and near the shelf break of central Chile. A spawning area was detected (up to 115 eggs×10 m^{-2}) over the continental shelf out to the shelf break off the Talcahuano area and inside the Gulf of Arauco (Figure 3A). Egg abundance between 36° and 37°S was significantly higher than observed in the northern (32°–36°S, *t*-test, $P=0.004$) and

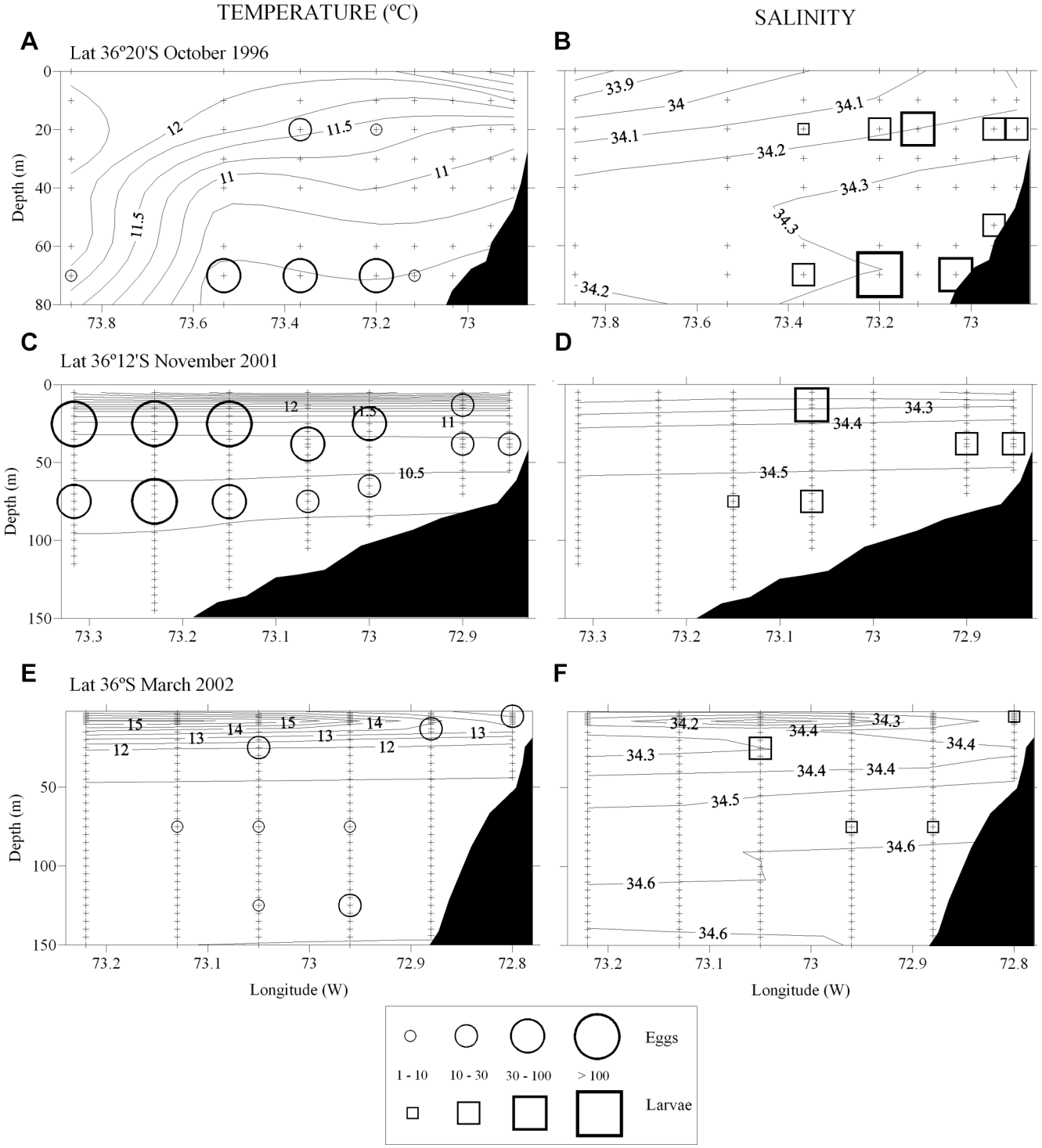

Figure 9. Vertical sections of temperature (°C) and salinity showing hydrographic conditions during October 1996 (active upwelling), November 2001 (relaxation) and March 2002 (upwelling) off central Chile. Overimposed are vertical distribution of eggs (dots) and larvae (squares). Abundance in ind×1000 m^{-3}. The cross indicates the position of conductivity–temperature–depth casts.

southern areas (37°–40°S, *t*-test, $P=0.039$). Bigeye flounder preflexion larvae were collected only over the continental shelf along the northern and central areas (up to 75 larvae×10 m^{-2}; Figure 3B, Table 2). Larvae occurred closer to the coast compared with the eggs off the Talcahuano area, and no differences in total larval abundance were detected between northern and central areas (Mann–Whitney test, $U=20$, $P=0.051$).

During the mid-spring (November) 2001 cruise, eggs were detected at 75% of the sampled stations, with high abundance (>30 eggs×10 m^{-2}) along the shelf break and peak abundances of ~130 eggs×10 m^{-2} over the mid-shelf at 35°S and over the outer shelf further south (Figure 4A). Larvae were collected in low abundances (<30 ind×10 m^{-2}). Preflexion larvae were found mostly north of 36.5°S (Figure 4B) and postflexion larvae (>10 mm

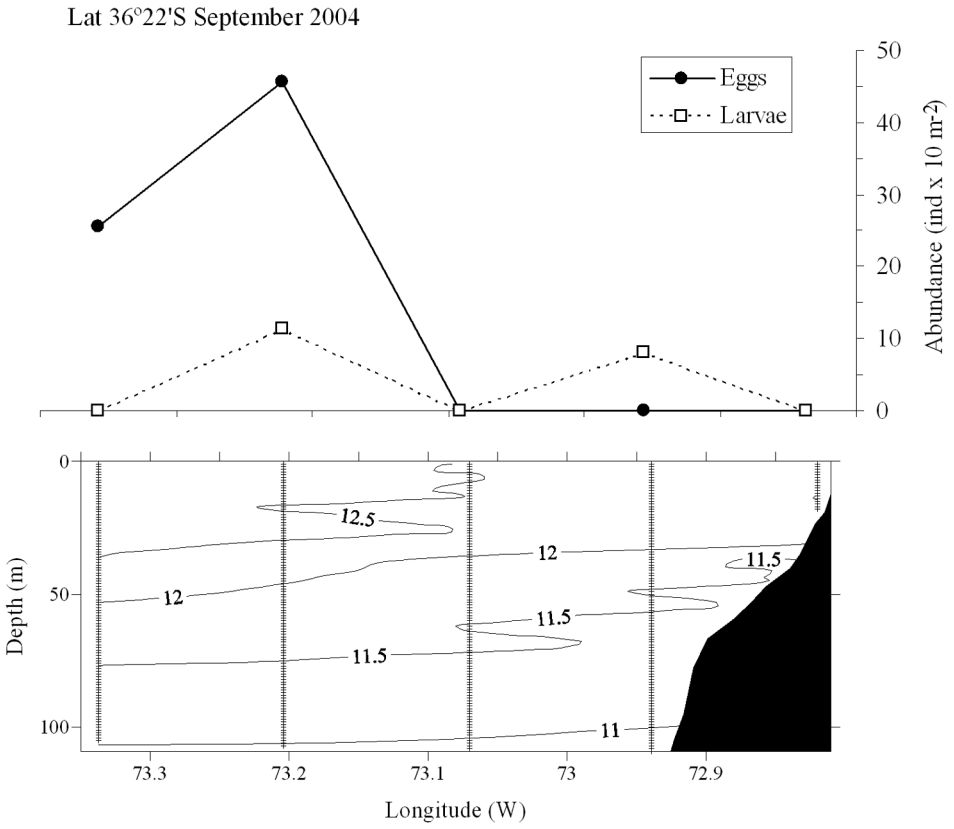

Figure 10. Vertical section of early stages abundance (top) and temperature (°C) (down) showing non-upwelling conditions during September 2004 off central Chile.

SL) were collected somewhat more shoreward along the entire sampling area and the transformed stage mostly inside the Gulf of Arauco (Figure 4C,D).

A different spatial pattern was noticed during the late summer (March) 2002 survey (Figure 4E). The eggs tended to be more offshore compared to previous cruises (over the slope and shelf break), and over the Biobio canyon (off the River Biobio). Although the peak egg density seemed lower than during the 1996 and 2001 surveys (<83 eggs$\times 10\,m^{-2}$), no significant differences were detected between years (one-way analysis of variance (ANOVA), $F=2.11$, $P=0.125$). All larval stages occurred in low abundance (<16 ind$\times 10\,m^{-2}$) over the shelf break and continental shelf, and nearer to the coast than eggs (Figure 4F–H). Also, transforming larvae appear to have been more widely distributed and farther from shore compared with 2001.

During late winter 2004, eggs and early larval stages were detected over the continental shelf (Figure 5A,B). Eggs were collected most frequently between 34 and 37°S, with peak abundance of ~40–50 eggs$\times 10\,m^{-2}$. No significant differences were detected north to 37°S (north-central area) and south to 37°S (south area) in 2004 (Mann–Whitney test, $U=86$, $P=0.663$), nor between years in egg abundance over the entire study area (1996–2004, one-way ANOVA, $F=1.776$, $P=0.155$). Early larval stages were almost absent in the southern area, and sparsely represented over the continental shelf between about 34° and 37°S (mean ±SD, preflexion larvae: 11.31 ±3.35 ind$\times 10\,m^{-2}$, Table 2).

Coastal time series

At the nearshore station off Talcahuano eggs were collected in low abundance (mean ±SD, 3.08 ±5.26 eggs$\times 10\,m^{-2}$) during austral winter (July–September) and early autumn (April–May) (Figure 6). Preflexion larvae, on the other hand, were present almost throughout the year, with maximum abundance in April and August 2000 (91.84 and 34.56 larvae$\times 10\,m^{-2}$, respectively). Post-flexion larvae were captured only in September, November and May (2.41, 40.23 and 2.05 ind$\times 10\,m^{-2}$, respectively).

Vertical distribution of eggs and larvae

The vertical distribution patterns of eggs and larval *H. macrops* were analysed from vertical series of Tucker tows integrated over all geographical areas separately for cruises carried out during September–October 1996, November 2001 and March 2002. Two vertical distribution patterns of eggs and larvae were noticed during the surveys. During September–October 1996, eggs and larvae were evenly distributed throughout the water column and no differences in abundance were detected between shallow (0–40 m) and deep (40–100 m) strata (for eggs, *t*-test, $P=0.627$; for larvae, Mann–Whitney test, $U=88$, $P=0.923$; Figure 7). During November 2001, eggs were more abundant in the upper 50 m of the water column (four strata: one-way ANOVA, $F=16.044$, $P<0.001$; Tukey honestly significant difference (HSD)

test), as well as total larvae (Mann–Whitney test, $U=22$, $P=0.001$). Egg abundance during late austral summer (March 2002) was significantly different among strata (Figure 7; one-way ANOVA, $F=3.346$, $P=0.043$) with higher abundance in the upper 100 m (Tukey HSD test). During this cruise larvae were most abundant in the upper 50 m (Mann–Whitney test, $U=40$, $P=0.001$).

Larval size distribution

Larvae were predominantly preflexion stage, with modes between 4 and 6 mm SL, except in March 2002 when postflexion larvae were more abundant (Figure 8, Table 2). During late winter and early spring (September–October 1996, August–September 2004) all larvae were preflexion stage and hence smaller (<8 mm, NL) than during late spring and late summer (November 2001, March 2002) when pre- and postflexion larvae occurred (one-way ANOVA, $F=49.05$, $P<0.001$; Tukey HSD test). During September–October 1996, larval size ranged between 3.4 and 5.8 mm NL; similarly, during August–September 2004 larvae 4.5 to 6.8 mm NL were collected. The largest individuals were collected in November 2001 and March 2002 (16.6 and 13.8 mm SL, respectively) (Figure 8).

Early stages and physical variables

The hydrographic characteristics of the water column off the Talcahuano area were consistent with the two main oceanographic seasons previously reported for the area. During the austral spring 1996 and late summer 2002, waters with temperatures <12°C and salinities >34.3, typical of ESSW, ascended from below 100 m to shallower depths and approached the shore as a result of wind-induced coastal upwelling (Figure 9A,B,E&F). In both periods ESSW of low temperature, salinity >34.3 and oxygen concentrations around 1 ml O_2 l^{-1} were recorded especially over the inner shelf (Vargas & Castro, 2001; Landaeta & Castro, 2002). During March 2002, warm surface waters (>15°C) present over the slope (Figure 9E) were also detected. Particularly in summer 2002, eggs of the bigeye flounder were located throughout the water column and continental shelf compared to larval stages, suggesting that the pre- and postflexion larvae collected over the shelf may have been transported in association with the ESSW. However, the mechanisms by which transforming larvae are transported/retained in the mid-shelf are still unknown (Figure 4H).

During November 2001, a strong vertical stratification was recorded throughout the sampling area (Figure 9C,D), typical of a relaxation of the upwelling process. A surface layer of ~30 m with higher temperature (between 12 and 15°C) and lower salinities (<34.3) indicated the presence of SASW over the shelf and shelf-break. Below this layer, cooler and saltier waters were detected. Higher abundances of eggs (>100 eggs×1000 m^{-3}) were recorded at the surface and mid-depth (0–50, 50–100 m) offshore waters, whereas the larvae were found near the coast in low densities (<10 larvae×1000 m^{-3}) (Figure 9B,C, Table 2).

A well-mixed water column was evident during the August–September 2004 cruise (Figure 10). The SASW (>11°C) was observed above 100 m depth, a usual hydrographic condition during winter (Castro et al., 2000). Eggs were more abundant (>40 eggs×10 m^{-2}) over the mid-continental shelf, with a similar trend in the larval distribution but with lower abundance (<10 larvae×10 m^{-2}) (Figure 10, Table 2).

DISCUSSION

This is the first study on the early life history (development, distribution and abundance) of the bigeye flounder off central Chile. The results presented here show several important features. First, there seems to be a seasonal depth gradient in the spawning: over the continental shelf (Figure 5) during late winter, over the shelf-break during mid spring (Figures 3 & 4A–D, Table 2), and at the continental slope during late summer (Figure 4E–H). Second, the data suggest the possibility of a shoreward progression of larval stages. Third, no important spawning events were detected nearshore (less than 8 km offshore) off Talcahuano during 1999–2000 (Figure 6). Fourth, the eggs and larvae were found to occur in the top 100 m (Figure 7).

The depth-related spawning pattern may be the result of several mechanisms. A seasonal deepening of the adult distribution throughout the spawning months may have occurred (i.e. between August and March, Voight & Balbontín, 1981). In an analysis of the demersal fish assemblage off the Talcahuano area, Arancibia (1992) described a change in the depth of adult *Hippoglossina macrops* from 100–200 m in November 1984 to 150–350 m depth during March 1984. Also, a progressive deepening in the distribution with increasing age has been inferred through changes in ectoparasites (González et al., 2001) and feeding (Villarroel & Acuña, 1999; Villarroel et al., 2000) of female bigeye flounders. Reproductive events may have been occurring at different depth-ranges as the spawning season advances, and are evidenced by an offshore egg distribution during late summer. Alternatively, the eggs may have been transported offshore through an Ekman layer during upwelling events; however, this latter mechanism does not explain the coastward movement of larval stages.

The offshore spawning suggested by our results is different than that observed for *Hippoglossina oblonga*, *H. stomata* and other flatfish, which tend to move to shallow waters to spawn (Leonard, 1971; Sumida et al., 1979; Minami & Tanaka, 1992; Porter, 2005), but is similar to that described by Bailey & Picquelle (2002) for the arrowtooth flounder *Atheresthes stomias* and Pacific halibut *Hippoglossus stenolepis* in the Gulf of Alaska. Studies off the Talcahuano area on the early life history of hake *Merluccius gayi* (Vargas et al., 1997; Vargas & Castro, 2001; Landaeta & Castro, 2006), squat lobster *Pleuroncodes monodon* (Gallardo et al., 1994; Roa et al., 1995; Yannicelli et al. 2006) and lightfish *Maurolicus parvipinnis* (Landaeta & Castro, 2002) show reproductive tactics similar to those observed in our results for *Hippoglossina macrops*. In these species, spawning occurs offshore and at depths above 100 m during the wind-induced upwelling season (September to March).

Transport of larval stages from the shelf break to nearshore probably occurs through the compensatory onshore

flow (~50 m thickness) that balances the surface offshore transport in the Ekman layer. In the study area the offshore Ekman layer is 20 m thick and Ekman transport is directed northward (Sobarzo & Djurfeldt, 2004). Additionally, there is a nearshore longitudinal pressure gradient directed southward that supplies water to the coast (Sobarzo & Djurfeldt, 2004). This latter physical mechanism may explain the southward progression of larval stages of *H. macrops* detected during November 2001 and March 2002, and their maintenance over the continental shelf and the shallow Gulf of Arauco (Figures 4 & 5) used as a nursery area for other demersal fish and crustaceans (Castillo et al., 1991; Landaeta & Castro 2006; Yannicelli et al., 2006). Upwelling fronts during active upwelling events may also prevent advective losses of larvae located onshore (Vargas & Castro, 2001; Sobarzo & Djurfeldt, 2004).

The distributional features are consistent with the transport of flounder larvae to a food-rich environment over the continental shelf with high primary (Daneri et al., 2000) and secondary production (Grunewald et al., 2002) in the area off Talcahuano. Interestingly, this area, where the transformation stages were collected, is a nursery ground of juvenile squat lobster *Pleuroncodes monodon*, the most important food item for juvenile bigeye flounders (<11 cm size, Villarroel & Acuña, 1999).

Finally, *Merluccius gayi*, *P. monodon* and *H. macrops* are important components of the bentho-demersal assemblage of the southern Humboldt Current (Arancibia, 1992) and it seems plausible that they share similar reproductive tactics to ensure successful transport to an area where enrichment processes, concentration of food organisms and larval retention occur to enhance larval and juvenile survival (Bakun, 1996).

The authors thank Wolfgang Schneider (Universidad de Concepción, Chile), who provided physical information from surveys made during November 2001 and March 2002 (project FONDECYT 1010900), and Luis Cubillos (Universidad de Concepción, Chile) for data collected during August–September 2004 (project FIP 2004-03). Physical and biological data from the September–October 1996 survey was obtained through the project FIP 96-11 awarded to Leonardo Castro. Rodrigo Veas (Universidad de Concepción, Chile) analysed temperature profiles from August–September 2004. Three anonymous referees made important contributions to an early version of this manuscript. During the writing of this work the first author was supported by a CONICYT doctoral scholarship (AT-4040126).

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Submitted 1 December 2005. Accepted 9 August 2006.