Vertical distribution, specific gravity, and free amino acids in anchoveta *Engraulis ringens* eggs under contrasting spawning habitat conditions

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ABSTRACT: We determined whether maternally influenced early life history traits varied for anchoveta eggs spawned in 2 contrasting environments: off northern (Iquique 23° S) and central (Talcahuano 36° S) Chile. Using eggs collected in the field, we showed that free amino acid contents, specific gravity, and vertical distributions of eggs in the water column changed as eggs developed. Specific gravity correlated negatively with free amino acid content. At the inter-population level, differences were determined in free amino acid egg contents (higher in Iquique), specific egg gravity (lower in Iquique), and vertical egg distributions (shallower in Iquique). Specific egg density along with the seawater density distribution and vertical mixing must be taken into account in both areas to explain the observed vertical distributions, as depicted by different egg buoyancy models. Overall, the results of this study suggest that free amino acids might play a role in egg development, potentially modulating specific gravity and egg position in the water column. The observed vertical distribution of eggs at each location and their changes throughout the season seemed to benefit young offspring, allowing them to avoid layers of stressful conditions especially in northern Chile, where a layer of minimum oxygen concentrations occurs near the surface.

KEY WORDS: Anchovies · Humboldt Current · Fish eggs · Vertical distribution · Amino acids · Egg buoyancy

1. INTRODUCTION

Changes in the extent of spawning habitats have been reported for several small pelagic fishes in recent decades in large marine ecosystems. In the Humboldt Current System, changes in main fishing and spawning areas of anchoveta *Engraulis ringens* were observed in central Chile during the 1990s from the documented spawning area, located between Valparaíso and Talcahuano (32–37° S) in

the 1970s and 1980s (Bernal et al. 1983), shifting southward to 38–39° S (Castro et al. 1997). More recent analyses of ichthyoplankton data from the fjord region (40–44° S) suggest that the spawning area has continued to expand southward (Bustos et al. 2008). Early life history traits of the offspring of this small pelagic fish, which has its main spawning zone in northern Perú (7–10° S; Ayon 2000), must be flexible enough to allow survival and growth under contrasting environmental conditions for it

to reside in such a wide latitudinal range and diverse habitats.

A number of recent studies have been dedicated to determining how anchoveta *E. ringens* copes with variations in environmental conditions along its latitudinal range (4–44°S). Along the Chilean coast, the peak spawning season extends from late austral winter to mid-spring, with a longer total spawning period in northern areas where the duration has lengthened during the last decades (Claramunt et al. 2014). Results from female gonad histology and plankton sampling have showed that oocytes and egg volumes increase with latitude in the Humboldt Current System (Castro et al. 2001, 2009, Llanos-Rivera & Castro 2004, Leal et al. 2009), along with a reduction in female fecundity (Castro et al. 2009, Leal et al. 2009). Egg incubations and larval rearing experiments showed greater larval length at hatch, greater yolk sac volumes of recently hatched larvae, and greater larval lengths at the end of the yolk period in individuals from the spawning area off central (36°S) versus northern (21°S) Chile (Llanos-Rivera & Castro 2006). At the same time, larvae from higher latitudes reared at the normal warmer temperatures of the north as well as at the normal cooler temperatures of the south grew faster than their northern counterparts, suggesting a wide scope of temperature responses in the southern larvae (range: 10–20°C) (Llanos-Rivera & Castro 2006). More recent studies have demonstrated that the concentration of biochemical components in anchoveta eggs differed between the northern and southern populations and that the biochemical composition of the eggs changed during the spawning season (Castro et al. 2009). How these changes in the biochemical composition of anchoveta eggs affect ecological traits of young offspring, such as their vertical distribution in the water column, have not yet been assessed.

Specific gravity and resulting buoyancy of fish eggs are life history traits that vary among populations spawning under different environmental conditions (Stenevik et al. 2008, Jung et al. 2012, Sundby & Kristiansen 2015). Specific gravity is determined initially during oocyte maturation in the gonads and in last stages when water enters the egg during the hydration phase as a result of the formation of an osmotic gradient due to the breakdown of vitellogenin, a large lipo-glyco-phosphoprotein, into free amino acids (Thorsen et al. 1996, Govoni & Forward 2008). During this stage, the overall volume of egg increases because the more permeable egg membrane allows water to enter. Once spawned, egg buoyancy in the water column results from the bal-

ance of compound classes with higher specific gravities (e.g. proteins, large structural carbohydrates) with seawater, which induces sinking, and lighter elements with lower specific gravities (e.g. water, lipids present in the eggs; Govoni & Forward 2008). During this stage, when the egg volume remains constant, aspects such as embryo volume, perivitelline space, and chorion thickness become important factors in egg density along with free amino acid dynamics between the remaining yolk and developing embryo (Jung et al. 2014).

In anchoveta eggs, a trend of increasing egg volume and lipid contents with increasing latitude has been reported. If buoyancy is determined by lipid content or egg volume (Kjesbu et al. 1992), anchoveta eggs at higher latitudes should show increased buoyancy compared to their northern counterparts. Accordingly, higher latitude eggs should show shallower vertical distributions than those located at lower latitudes (northern Chile). However, buoyancy and vertical positioning of eggs in the water column are also modulated by seawater density, an environmental factor known to decrease with latitude as a result of lower evaporation due to reduced solar radiation, increased rainfall, and runoff from the coast. If egg density exceeds that of seawater at higher latitudes, then eggs should show a deeper distribution at higher latitudes than at lower latitudes, a prediction that contrasts with that based on egg lipid contents. Information on the vertical distribution of young offspring is crucial for understanding the mechanisms that enable species to reside under different environmental conditions. The distribution of eggs and larvae may determine, for instance, whether young offspring will be transported to different locations at different depths, if they will be exposed to unfavorable environmental conditions (predators, oxygen minimum zones) at certain depths, whether hatched larvae will find adequate food at different depths, or whether their growth rates might be modified because of changes in temperature along the water column. In the Humboldt Current System, estimates of anchoveta egg vertical distributions and specific gravity have not yet been reported despite the key role of this species in the upwelling ecosystem (Cury et al. 2000, Krautz et al. 2007) and regional economy (worldwide, the largest small pelagic fishery).

The present study aimed to determine whether differences occur in the specific gravity of anchoveta eggs spawned in 2 areas at different latitudes and, if so, whether these differences modulated the vertical distribution of eggs at both locations and throughout the reproductive season. Contents of free amino

acids, as potential osmoeffectors, were also determined in eggs from both areas. The areas studied were located off northern (Iquique, 21°S) and central (Talcahuano, 36°S) Chile (see Fig. 1). Variations in egg specific gravity (ESG) during embryonic development were determined through egg incubation experiments at both locations. In parallel, environmental conditions were monitored during the spawning season via monthly cruises from mid-winter to late spring. Information on ESG during embryonic development and *in situ* seawater density profiles along with wind speed and egg size data were used to estimate mean vertical distributions of eggs at each location utilizing 2 buoyancy-dependent models (pelagic and bathypelagic models; Sundby 1991). These distributions were compared with egg profiles obtained from stratified zooplankton samplings. Given the extent of this species' latitudinal distribution, we analyzed our results in the context of potential implications (benefits and consequences) for anchoveta early life histories given potential changes in environmental conditions, either as a result of southward expansions of spawning habitat (as apparently occurred during the last decade) or predicted climate change scenarios.

2. MATERIALS AND METHODS

2.1. Ichthyoplankton collections, vertical distribution, and specific gravity of eggs off northern and central Chile

To assess the water column structure and vertical distribution of eggs and to collect eggs for incubations under controlled conditions, a series of monthly cruises were conducted in the coastal zone off Iquique (21°S, northern Chile) and Talcahuano (36°30′S, central Chile) from mid-winter (July) to late spring (December) 2011 (see Table 1). During each cruise, at least 4 stations were visited regularly (see Fig. 1). Water column temperature, salinity, and dissolved oxygen concentrations were obtained from CTD casts (Seabird 19+) down to 100 m depth. Monthly sea surface temperature (SST) was also obtained from satellite images (NOAA MODIS Aqua Globales) for each region during the sampling period.

Stratified zooplankton samples were collected with a Tucker trawl net (0.25 m^2 mouth opening, 300 µm mesh, equipped with a flowmeter to quantify the volume of water filtered), from the surface down to 100 m. The sampled depth strata were 0–5, 5–10, 10–15,

15–20, 20–30, 30–40, 40–50, 50–75, and 75–100 m. Stratified zooplankton samples were preserved in 10% buffered formalin. Eggs were identified, sorted, and weighed in the laboratory.

2.2. Specific gravity estimates of eggs from northern and central Chile

Additional zooplankton samples were collected at all stations in both spawning zones in 2011, using oblique tows, for ESG estimates in rearing experiments. These live zooplankton samples were rapidly transported to the laboratory (within 1 h after collection) in thermo-regulated coolers. There, the anchoveta eggs were separated and staged following Moser & Ahlstrom (1985).

Specific gravity estimates and egg development experiments were done using subsamples of eggs of the youngest possible stages (usually stages I–III, following Moser & Ahlstrom 1985). The ESG was estimated from measurements in a density gradient column (Coombs 1981) containing calibrated density floats (Martin Instruments). Once the selected eggs were placed in the density gradient column (5 eggs column^{-1}), their initial positions in the column were measured after 1 h, and their positions were re-measured every 3–4 h until hatching under constant temperature conditions in an incubation chamber. Incubating temperatures were within the range of those usually observed at the mean depth of egg collection for the spawning sites and were kept the same throughout the season at each location (Iquique: 16°C; Talcahuano: 12°C). Out of the 5 eggs initially placed in each column at both locations every month, all survived up to the last stages (except 1 in Iquique and 1 in Talcahuano, both in October). A second column was set in Talcahuano in October, obtaining a similar result (1 dead egg).

The age (in hours) of the eggs at the start of each experiment was calculated from their observed stage of development and from a model (Lo 1985) that estimated age-at-stage of anchoveta eggs from north (Iquique; Eq. 1) (Soto 2001) and central Chile (Talcahuano; Eq. 2) (Claramunt et al. 2007) at different temperatures:

$$E_{i,T} = 9.8206 \, e^{(0.099T + 0.102i)} i^{1.815} \tag{1}$$

$$E_{i,T} = 9.214 \, e^{(0.117T + 0.170i)} i^{2.331} \tag{2}$$

The models included the 11 developmental stages (following Moser & Ahlstrom 1985), where $E_{i,T}$ is the age of stage i at temperature T (°C).

ESG data obtained at different ages (h) in the rearing experiments were utilized to fit a polynomial model that estimated specific gravity as a function of the egg age (Ospina-Álvarez et al. 2012):

$$D_E = a_0 + a_1 E + a_2 E^2 + ... + a_n E^n \qquad (3)$$

where D_E is the density of the egg at a given age (E), and a_0, a_1, a_2, and a_n are parameters from the model. Thus, with these models, specific gravity-at-age values may be estimated for staged eggs in the field if the seawater temperature at the depth from where the eggs were collected is known.

Knowing the original vertical distribution of different egg stages by sampled depth interval, and assuming that the estimated densities of the eggs are in equilibrium with the density of seawater (in eggs located below the mixing layer, in particular), we can estimate a mean weight depth of all staged eggs within each sampled strata utilizing the seawater density profile and finally estimate a profile of the distribution of the eggs along the water column. The estimated profile only takes into account the temperature and density of the seawater but does not include other forcing factors such as wind or bottom-generated turbulence at different depths, which need to be considered when turbulence induces vertical mixing (e.g. near surface).

2.3. Composition of free amino acids of eggs from northern and central Chile

During the 2012 spawning season, zooplankton samples were collected from both spawning zones with oblique tows in order to obtain anchoveta eggs for free amino acid (FAA) determinations. Samples were rapidly transported to the laboratory in thermo-regulated coolers, and anchoveta eggs were separated and staged following Moser & Ahlstrom (1985), who described 11 developmental stages. In the laboratory, groups of at least 25 eggs in developmental stages I–III (early stages, no embryo), usually the most abundant stages in the samples, were separated and stored in liquid nitrogen for later analyses. Most samples were collected in 4 mo throughout the first half of the spawning season (from July through October off Iquique and from August through November off Talcahuano). Off Iquique, a total of eight 25-egg stage I–III groups were obtained, whereas off Talcahuano, ten 25-egg stage I–III groups were obtained. In August 2012 off Talcahuano, along with egg stages I–III separated for ESG incubations in the density gradient columns and for the early egg FAA con-

tents, egg stages IV–VII (mid stages, early embryo), and VIII–XI (late stages, late embryo) were also abundant and hence egg samples of these stages were also obtained for amino acid determinations. These developmental stage groups had been utilized in other studies of this species (Krautz et al. 2010).

Amino acids were identified and quantified using RP-HPLC after precolumn derivatization with *o*-phthaldialdehyde (OPA) and 2-mercaptoethanol (Lindroth & Mopper 1979, according to Pantoja & Lee 1999). Amino acids were quantified and classified as essential FAAs (EFAAs) to fish (i.e. leucine, valine, isoleucine, lysine, phenylalanine, arginine, histidine, methionine, tyrosine, threonine) and as non-essential FAAs (NEFAAs) to fish (i.e. aspartic acid, glutamic acid, serine, alanine, glycine) (Wilson 1985).

2.4. Modeled vertical distribution of anchoveta eggs in Iquique and Talcahuano

Vertical distributions of eggs in 2011 were modeled for Iquique and Talcahuano. For eggs from Iquique, a pelagic egg model (Sundby 1991, Stenevik et al. 2001) was utilized given that maximum distributions were located at the shallower depths. In Talcahuano, we utilized a bathypelagic egg model (Sundby 1991) in months when the maximum abundances occurred at intermediate depths (August, October), and the pelagic egg model in November, the month in which the maximum abundance occurred at the surface layer (Sundby 1991, Stenevik et al. 2001). Both models include egg size estimations and specific density at age, and accordingly, we were able to model distributions only in those months for which we had data (Iquique: August, September, and October; Talcahuano: August, October, and November).

Estimating the vertical distribution of anchoveta eggs with the pelagic egg model (Stenevik et al. 2001) included, after standardizing egg abundances per seawater volume (e.g. eggs m^{-3}), estimating the fraction of eggs in each depth stratum over the depth range sampled. Then, the mean weight depth of all eggs was calculated as:

$$Z = \sum_{i=1}^{n} P_i Z_i \qquad (4)$$

where Z_i is the depth of the ith simple and P_i is the proportion of eggs at depth Z_i.

The spawning depth at each date and location was estimated considering the first 2 developmental stages (stages I–II; Moser & Ahlstrom 1985), to which the vertical distance ascended by the eggs was added, this based on the buoyancy measurements. The time

from spawning was estimated utilizing the 11-stage temperature development model (Lo 1985) previously described for anchoveta eggs from Iquique (Eq. 1; Soto 2001) and Talcahuano (Eq. 2; Claramunt et al. 2007).

Based on the buoyancy measurements, the ascent velocity of the eggs (w) (from the depth of spawning until they reach the equilibrium depth of neutral buoyancy) was estimated based on Sundby (1991) and Sundby & Kristiansen (2015), who applied Stoke's equation:

$$w = \tfrac{1}{18}\, g\, d^2 \Delta\rho\, v^{-1} \quad (5)$$

where g is the gravity acceleration, $\Delta\rho = \rho_w - \rho_e$ (ρ_w is the specific gravity of the mixed layer and ρ_e is the ESG), v is the molecular viscosity (Sverdrup et al. 1942, Petitgas et al. 2006) and d is egg diameter. The egg specific gravity values (ρ_e) were expressed as a function of the *in situ* temperatures at the depth of the eggs' locations, utilizing the table of gravity values at different temperatures provided by the density gradient column manufacturer (Martin Instruments). Because anchoveta eggs are ellipsoid, an estimated d value ranging between 0.08–0.09 cm (equivalent to the diameter of a sphere with the same volume of the anchoveta egg ellipsoids; Tanaka 1992) was utilized in Eq. (5) (see Table 1). Eq. (5) was utilized in all months because Reynolds numbers (Re), Re = wd/v, obtained were < 0.5.

The equilibrium vertical distribution of pelagic eggs (C_z) (with a specific density lower than that observed in the mixed layer) was calculated based on the equation proposed by Sundby (1983) and Sundby & Kristiansen (2015):

$$C_z = C_a e^{-(w/K)(z-a)} \quad (6)$$

where C_a is the concentration of the eggs at depth $z = a$ and K the mean eddy diffusivity coefficient of the upper mixed layer. K was estimated using the empirical relation given by Sundby (1983):

$$K = 76.1 \times 10^{-4} + 2.26 \times 10^{-4} \times W^2 \quad (7)$$

where W is the wind speed in m s^{-1} and K has the units m^2 s^{-1}. Wind data (m s^{-1}) utilized corresponded to those measured 5 h before the eggs were sampled in the field (Stenevik et al. 2001) and were obtained from meteorological stations at Diego Aracena Airport in Iquique (20.53° S) and at Carriel Sur Airport in Talcahuano (36.77° S).

The vertical distribution of anchoveta eggs was estimated with the bathypelagic egg model (Sundby 1991, 1997) for those dates when the depth of maximum abundance occurred at mid-depth (August and October 2011, Talcahuano), according to:

$$C_z = C_A e^{\left[-(m/2K)(z-z_A)^2\right]} \quad (8)$$

where C_A is the concentration of eggs at the depth of neutral buoyancy of the eggs, z_A. It seems from Eq. (8) that the bathypelagic eggs are vertically distributed as a normal distribution with a standard deviation:

$$\sigma = \left(K/m\right)^{1/2} \quad (9)$$

around the mean depth level z_A. When the velocity of the eggs is confined within the Stokes regime (Eq. 5), the expression for m in Eqs. (8 & 9) becomes:

$$m = \left(\tfrac{1}{18}\right) d^2 v^{-1} \rho_w N^2 \quad (10)$$

where N is the Brunt-Väisälä frequency (with the unit s^{-1}), v is the molecular viscosity (Sverdrup et al. 1942, Petitgas et al. 2006), d is egg estimated spherical diameter, and ρ_w is the water density. Brunt-Väisälä frequency values were 2.84×10^{-2} and 5.29×10^{-3} s^{-2} in August and October.

2.5. Modeled vertical distribution of eggs from specific density-at-age relationships off central Chile

We estimated the vertical distribution of eggs obtained at 2 additional oceanographic stations off Talcahuano (Dichato, Arauco Gulf) sampled in September 2015. Stratified zooplankton samples were collected utilizing a Tucker trawl net (0.25 m^2 mouth opening, 300 µm mesh, equipped with a flowmeter to quantify the volume of water filtered). Depth strata were sampled every 10 m from the surface down to 70 m (0–10, 10–20, 20–30, 30–40, 40–50, 50–60, 60–70 m). The samples were preserved on board in 4 % formalin for later egg identification at the laboratory, where anchoveta eggs were staged utilizing the Moser & Ahlstrom (1985) scale of 11 stages of development for anchovy eggs. The hydrographic characteristics of the water column were obtained from CTD profiles (Seabird 19+).

Profiles of egg abundance per stage were obtained to determine potential changes in vertical distribution during development. Then, all egg ages were estimated using the relationship described in Eqs. (1) & (2) and their specific density was obtained with Eq. (3). Specific density-at-age was re-estimated using the temperature corrections provided by the density gradient column manufacturer (Martin Instruments) and for this, temperature values corresponded to those observed in the field. By knowing the original vertical distribution of the different aged eggs by

sampled depth interval, and assuming that the estimated density of the eggs was in equilibrium with the density of the seawater, we estimated a mean weight depth of all eggs within each sampled strata utilizing the seawater density profile in the field. Based on these estimations, new profiles describing the distribution of eggs along the water column were obtained at each location. The estimated profiles only considered the temperature and density of seawater and did not include other forcing factors such as wind or bottom-generated turbulence at different depths. Resulting profiles described well the observed peak abundance at mid-depth and the bottom secondary peak of egg abundance at the maximum depth sampled, suggesting that turbulence at the bottom might also be important, especially at the greatest depth sampled. Unfortunately, because we lacked egg size measurements from the 2 sampling locations in 2015, we could not apply Sundby's bathypelagic egg model to fit egg vertical distributions data in order to assess the potential importance of bottom turbulence.

3. RESULTS

3.1. Average seasonal vertical distribution of hydrographic characteristics, egg vertical distribution, ESG, and FAA content

Hydrographic characteristics of the water column differed markedly between locations. SST at the onset of the spawning season (August) was 15–16°C off the northern coast (Iquique) and 10–11.5°C off the central coast (Talcahuano) (Fig. 1). SST increased steadily through spring in both areas, but a difference of ~2°C persisted between areas. In the water column, while the highest mean seasonal temperature, salinity, and density (sigma-t) were highest in the surface layer off Iquique, the lowest average oxygen concentrations were in this area as well (Fig. 2). The vertical structure of the water column also differed between locations. The thermocline detected in both areas in the top 70 m depth was stronger in the shallower 20–30 m of the central zone. Similarly, while a clear halocline (and pycnocline) was observed in the central zone from the surface to deeper than 20 m depth, variations in the water column were minimal in the northern area. Although strong oxyclines were observed at both locations, the vertical extension was much shallower in the northern area, where concentrations lower than 1 ml O_2 l^{-1} were observed closer to surface in Iquique (20–30 m deep) than in the central zone (50–60 m deep).

Differences in the mean vertical distribution of eggs during the spawning season were observed in both areas. Off Iquique, eggs were located between the sea surface and 50 m deep, although abundances were higher mainly in the shallowest strata (surface to 15 m), where seawater density was slightly lower and the water was more oxygenated (>2 ml O_2 l^{-1}) (Fig. 3). Off Talcahuano, this vertical distribution went deeper (down to 75 m), with higher abundances
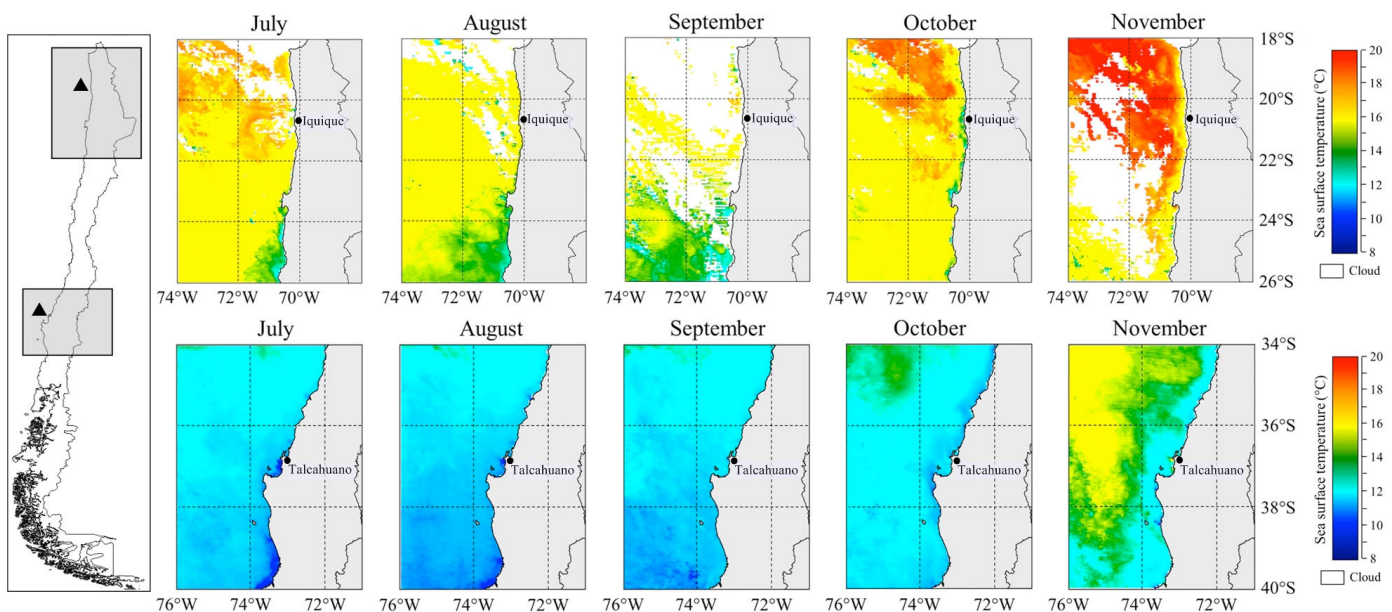

Fig. 1. Sampling areas for anchoveta *Engraulis ringens* eggs off northern (Iquique, 21° S) and central (Talcahuano, 36° S) Chile showing mean monthly sea surface temperature (°C) during the 2011 spawning season. (From NOAA MODIS Aqua Globales). Triangles in position map represent the actual location of the sampling zones
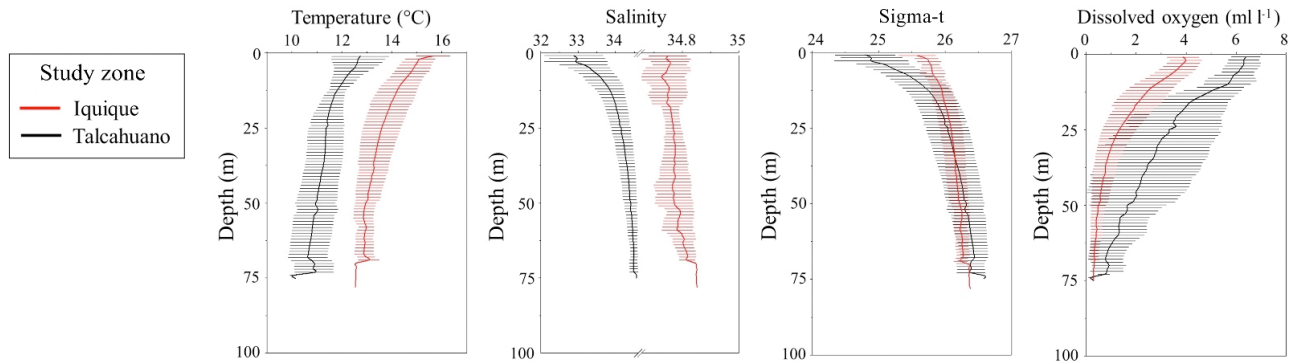
Fig. 2. Mean oceanographic characteristics (temperature, salinity, sigma-t, dissolved oxygen) along the water column during the 2011 anchoveta sampling season off Iquique and Talcahuano. Horizontal error lines: SD. Hydrographic data come from 4 stations at each location

from 10 to 75 m. This depth range was greater than that observed off Iquique and was located below the lowest salinity surface layer, below the pycnocline, and at oxygen concentrations above 2 ml O_2 l^{-1}.

3.2. Mean ESG determinations

ESG determinations were carried out twice a month at both localities from August through November, except in September in Talcahuano when only eggs older than stage III were collected. ESG changed as embryos developed (Fig. 4). In eggs from Talcahuano, where egg development is slower and egg duration takes longer (even at the same temperature; Castro et al. 2010) than at Iquique, ESG showed a small initial increase during the first day, a slight decrease in the following days, and finally a noteworthy increase during the last day before hatching. Eggs from Iquique showed an initial increase in specific gravity during the first day followed by a slight decrease in subsequent days. However, specific gravity increased before hatching in only some

months. Thus, the mean pattern of change in specific gravity during egg development in Iquique showed a last day period of increased variability instead of the increase in egg specific gravity observed in Talcahuano (Fig. 4).

When grouping ESG measurements into 3 embryonic developmental phases (early development, mid stages, late stages), we observed that mean ESG in different phases differed between Iquique and Talcahuano, with those from Iquique always being less dense, regardless of the developmental phase or month (Kruskal-Wallis, p < 0.05) (Table 1). When grouping the specific gravity data for all months and developmental stages during the sampling seasons, differences in mean specific gravity were apparent between anchoveta eggs from Iquique and Talcahuano (Kruskal-Wallis, p < 0.05) (Table 2).

A total of 15 amino acids were identified in anchoveta eggs from both locations, of which 10 were classified as EFAAs for fish (histidine, arginine, tyrosine, methionine, valine, phenylalanine, isoleucine, leucine, lysine, threonine) and 5 as NEFAAs (aspartic acid, glutamic acid, serine, alanine, glycine) (Wilson 1985) (Table 3). Eggs from Iquique (stages I–III) showed higher mean contents of amino acids than eggs from Talcahuano (Fig. 5). In eggs from Iquique, the highest mean contents were of histidine, lysine, and alanine; in eggs from Talcahuano, these were histidine, alanine, and leucine. The highest mean concentrations in eggs from Iquique were histidine, lysine, and alanine whereas histidine, alanine, and leucine were higher in eggs from Talcahuano (Table 3). The total concentrations of FAAs (pooled or
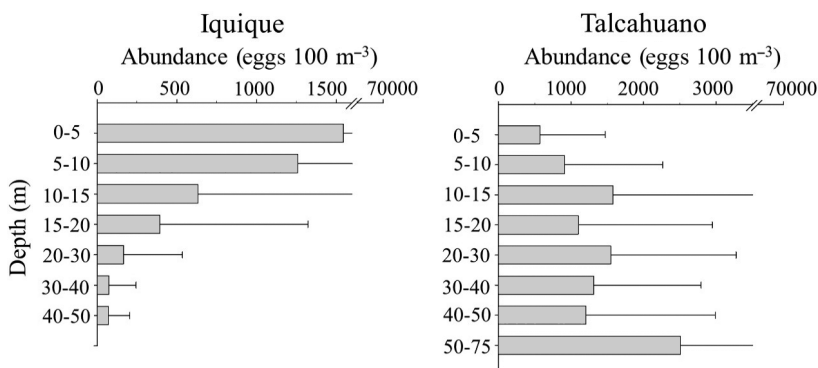

Fig. 3. Mean total anchoveta egg vertical distributions during the 2011 spawning season off Iquique and Talcahuano. Error bars: SD
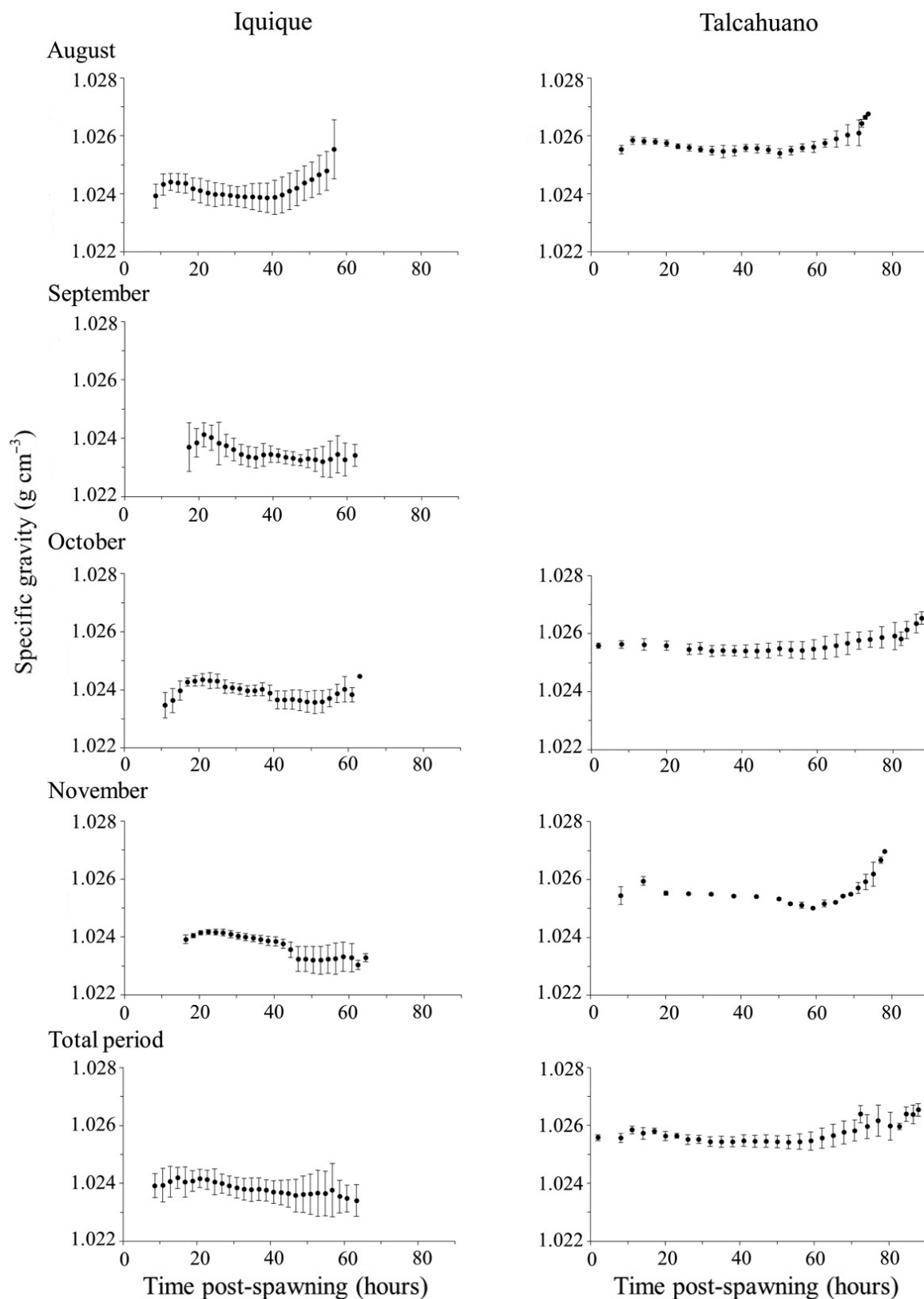
Fig. 4. Monthly and mean seasonal (bottom panels) specific gravity of anchoveta eggs determined from egg development experiments (5 eggs per density gradient column) off Iquique and Talcahuano during the 2011 spawning season (note that no data were collected in Talcahuano in September). Egg specific gravity estimates correspond to laboratory temperatures. Error bars: SD

Table 1. Monthly mean (SD) specific gravity of anchoveta eggs for developmental stages I–III, IV–VII, and VIII–XI, as determined from rearing experiments in density gradient columns off Iquique and Talcahuano, 2011. A total of 6 eggs per density gradient column were utilized. Egg specific gravity estimates correspond to laboratory temperatures

Month	Egg specific gravity (g cm^{-3})		
	Stage I–III	Stage IV–VII	Stage VIII–XI
Iquique			
August	1.02426 (0.0004)	1.02400 (0.0004)	1.02455 (0.0007)
September	–	1.02370 (0.0005)	1.02333 (0.0004)
October	1.02370 (0.0004)	1.02417 (0.0002)	1.02375 (0.0003)
November	–	1.02406 (0.0001)	1.02341 (0.0004)
Talcahuano			
August	1.02573 (0.0002)	1.02552 (0.0001)	1.02591 (0.0004)
September	–	–	–
October	1.02560 (0.0002)	1.02543 (0.0002)	1.02579 (0.0005)
November	1.02564 (0.0003)	1.02543 (0.0001)	1.02561 (0.0006)

Table 2. Seasonal mean (SD) anchoveta egg specific gravities estimated for all stages of development, obtained from specific density rearing experiments throughout the 2011 sampling season off Iquique and Talcahuano. Egg specific gravity estimates correspond to laboratory temperatures

Zone	Egg specific gravity (g cm^{-3})		
	Stage I–III	Stage IV–VII	Stage VIII–XI
Iquique	1.02404 (0.0005)	1.02398 (0.0004)	1.02362 (0.0006)
Talcahuano	1.02567 (0.0002)	1.02546 (0.0002)	1.02580 (0.0005)

averaged) expressed either per egg (ng FAA egg^{-1}) or standardized per weight of egg (µg FAA g^{-1} egg, to discard egg size effects) were always higher in eggs from Iquique than from Talcahuano.

During egg development experiments in the density gradient columns carried out in August 2012 in Talcahuano, a change in the specific gravity of eggs similar to the one described above was observed. Also, a decrease in FAA concentrations was observed in eggs of different developmental stages collected from the field on the same date (Fig. 6). Then, when the FAA concentrations for each developmental stage group (I–III, IV–VII, IX–XI) of eggs collected from the field were diagramed with the experimentally determined specific gravity measurements of eggs in the same developmental stages of the same month, a negative trend was observed between FAA concentrations and specific gravity (Fig. 6).

3.3. Intra-seasonal variations in hydrographic characteristics and vertical egg distributions

Variations in the hydrographic characteristics of the depth range where eggs were found (Iquique

0–50 m; Talcahuano 0–75 m) differed among locations during the spawning season (Fig. 7). Whereas salinity did not change drastically off Iquique during the sampling season, temperature decreased early in spring due to upwelling intensification and then increased due to greater solar radiation at the end of spring. Density followed the changes in temperature, increasing early in spring and then decreasing late in the season. Although temperature varied little during the sampling season off Talcahuano, changes in salinity were more pronounced as a result of the combination of increased freshwater due to rain and runoff in winter and upwelling intensification in spring; both processes affected the changing pattern of density during the season (an increase from late winter to late spring). The evolution of dissolved oxygen followed a similar trend at both locations (decreased oxygen concentrations towards late spring), although the changes were more note-

Table 3. Mean (SD) concentrations of free amino acids (FAAs) in anchoveta eggs for developmental stages I–III, obtained for all sampling dates during the 2012 spawning season off Iquique and Talcahuano

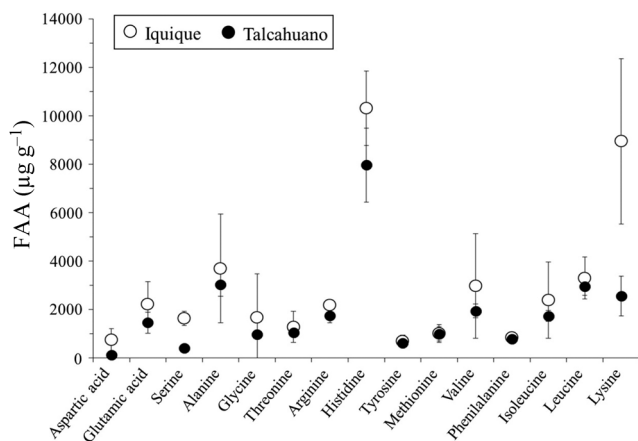| FAA	Mean concentration (µg FAA g^{-1} egg)	
	Iquique	Talcahuano
Non-essential FAAs		
Aspartic acid	740.2 (473.7)	123.6 (58.2)
Glutamic acid	2207.2 (944.8)	1451.3 (428.6)
Serine	1621.4 (292.8)	393.9 (128.8)
Alanine	3693.6 (2249.9)	3024.2 (483.5)
Glycine	1671.9 (1799.9)	972.5 (180.5)
Essential FAAs		
Arginine	2169.5 (178.1)	1733.5 (284.6)
Histidine	10302.3 (1543.0)	7957.6 (1521.6)
Tyrosine	694.7 (241.7)	602.3 (107.4)
Methionine	1010.0 (363.1)	991.6 (293.1)
Valine	2965.2 (2162.7)	1938.9 (276.7)
Phenylalanine	844.5 (80.5)	771.6 (93.4)
Isoleucine	2379.7 (1574.4)	1729.9 (223.2)
Leucine	3297.5 (867.9)	2947.3 (375.0)
Lysine	8941.5 (3411.6)	2557.9 (82.0)
Threonine	1277.6 (635.9)	1041.8 (165.1)
Sum	43816.9	28237.7
Average	3560.0 (498.0)	1882.5 (290.8)

Fig. 5. Free amino acid (FAA) mean concentrations in anchoveta eggs determined from field collected plankton samples during the 2012 spawning season off Iquique and Talcahuano. Error bars: SD

worthy off Talcahuano due to the resumption of upwelling early in spring. The absolute values of dissolved oxygen concentrations also differed strongly between locations: whereas the water column was well-oxygenated in winter off Talcahuano and low values occurred only late in spring, oxygen concentrations off Iquique remained steady with very low values ~2 ml l^{-1} or less, particularly from the start of spring onwards.

During the study season, changes in the vertical distribution of eggs also occurred at both sampled locations. Off Iquique, the depth range where maximum egg abundances occurred became shallower as the season progressed (from 5–20 m in August to 0–5 m in October); in November, when spawning was at its end and only a few eggs occurred, these were located at 5–10 m depth (Fig. 8).

Off Talcahuano, the change in depth followed the same pattern but, in this case, the depth range of maximum abundances started deeper (20–40 m) than off Iquique and remained deeper for almost the whole season until November, when maximum egg abundances reached the shallower layers (0–10 m). This common pattern of a shifting vertical distribution (from deeper waters to the surface layer) was more apparent when looking at the mean weighted depth (MWD) of the eggs in each area as the season progressed (Fig. 9).

3.4. Modeled egg vertical distributions off Iquique and Talcahuano in 2011

The vertical distributions of eggs at Iquique (August–October) and Talcahuano (November) were modeled using the pelagic egg model (Sundby 1991, Stenevik et al. 2001) considering that the maximum abundances occurred in the shallower layers. In August and October in Talcahuano, we used a bathypelagic egg model (Sundby 1991) instead because the maximum abundances occurred at intermediate
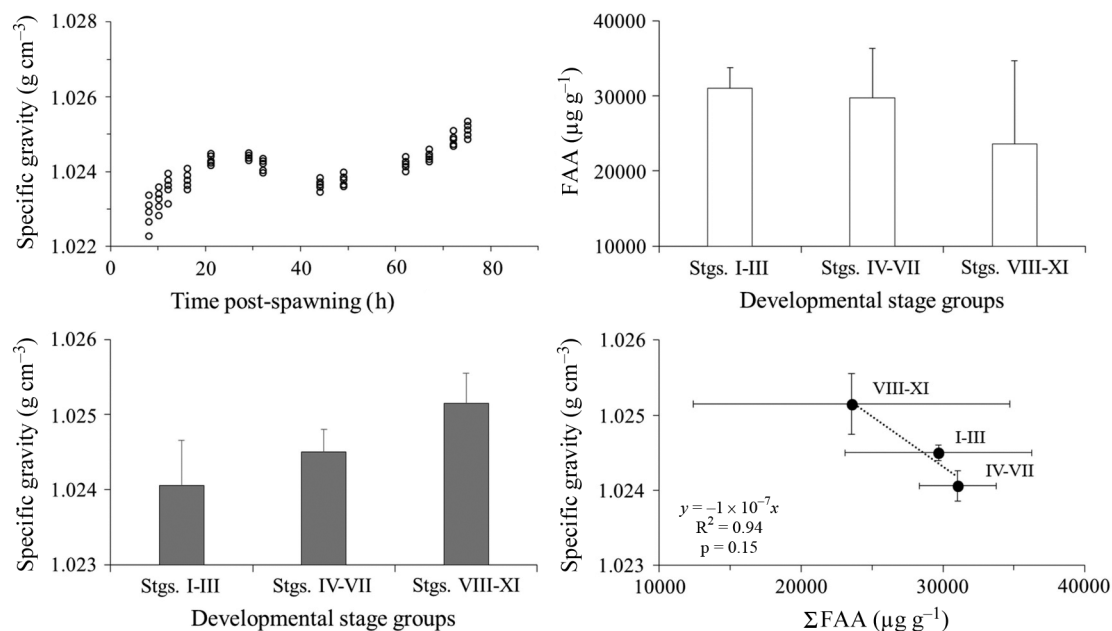

Fig. 6. Anchoveta egg specific gravity estimates from rearing experiments and free amino acid (FAA) concentrations obtained for the 3 groups of developmental stages (I–III, IV–VII, VIII–XI) collected from the field obtained during the August 2012 sampling season off Talcahuano. Egg specific gravity estimates correspond to laboratory temperatures. Error bars: SD
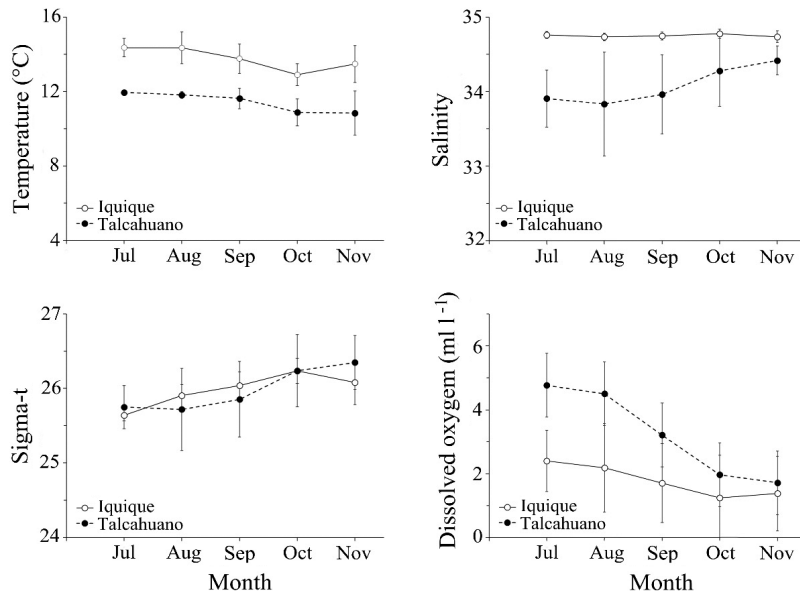
Fig. 7. Mean values of hydrographic characteristics within the strata where most anchoveta eggs were collected during the 2011 spawning season (0–50 m Iquique; 0–75 m Talcahuano). Error bars: SD

depths. The observed and some of the estimated parameter values for the pelagic egg model are shown in Table 4. The observed MWDs ranged between 9.1 and 11.3 m in Iquique and between 9 and 30 m in Talcahuano. The spawning depth ranges were narrower and shallower in Iquique (8.8–9.2 m) than in Talcahuano (22–48 m). The mean estimated spherical egg diameters were larger in Talcahuano than in Iquique. The difference between the egg and seawater density ($\Delta\rho$) ranged between 0.0016 and 0.0029 g cm^{-3} in Iquique and between −0.0012 and 0.0010 g cm^{-3} in Talcahuano (Table 4 shows only pos-

itive values to be utilized in the pelagic egg model). The registered $\Delta\rho$ estimated values fell within the ranges reported in other areas (Stenevik et al. 2001). The anchoveta egg ascent velocities (w) in Iquique ranged between 0.047 cm s^{-1} (1.7 m h^{-1}) and 0.68 cm s^{-1} (2.5 m h^{-1}), with the slowest speed estimated in August. The w estimated in November in Talcahuano was 0.0045 cm s^{-1} (1.6 m h^{-1}), and was very similar to that observed in Iquique in August. The calculated w values are comparable to others reported for pelagic eggs (Stenevik et al. 2001). While in Iquique (August–October) and Talcahuano (November) the wind speed values (W), measured 5 h previous to sampling on all sampling dates (not shown in the table) were low (Iquique [Aug–Oct], range: 1.7–5.8 m s^{-1}; Talcahuano [Nov] range: 0.0–5.1 m s^{-1}), in Talcahuano earlier in the season (August, October) these wind speeds were higher (range: 0.5–11.0 m s^{-1}). Finally, the estimated anchoveta egg vertical distributions (pelagic egg model; C_z, Eq. 6) in Iquique (all months) and Talcahuano (November) coincided with the observed surface maximum abundances (Fig. 10). In August and October, the estimated depths of maximum egg abundances in Talcahuano (bathypelagic egg model; C_z, Eq. 8) also coincided with the observed maximum abundances in the field but at mid-depth. However, in August 2011, the modeled distribution did not show the secondary increase in egg
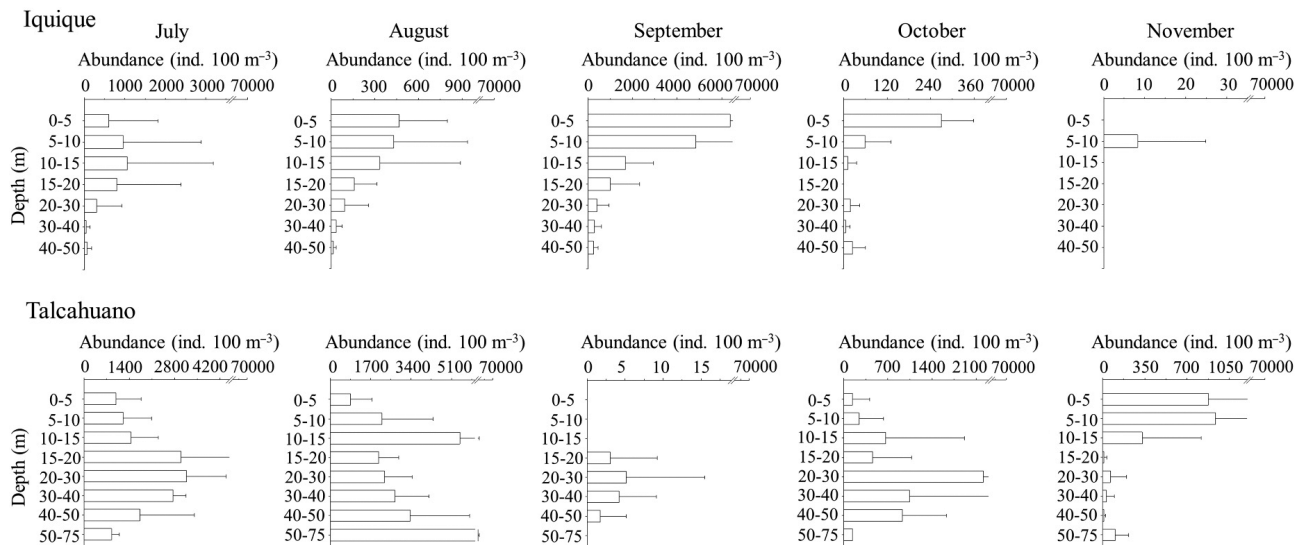

Fig. 8. Monthly mean anchoveta egg vertical distributions during the 2011 spawning season off Iquique and Talcahuano. Error bars: SD
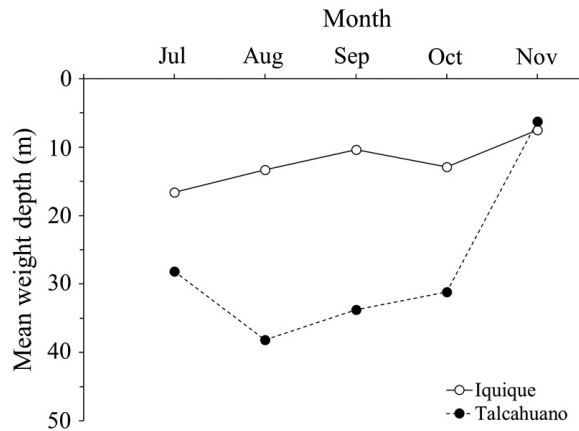
Fig. 9. Seasonal monthly mean weight depths (MWD) for anchoveta egg vertical distributions during the 2011 spawning season off Iquique and Talcahuano

abundance towards the bottom depicted by the field data (Fig. 8).

3.5. Observed egg vertical distributions at the Talcahuano zone in 2015

Vertical distributions of eggs *in situ* of all developmental stages (I–XI) were determined at 2 oceanographic stations (Dichato and Arauco Gulf) off Talcahuano in September 2015, early in the spawning season. For these 2015 eggs, we did not include egg size measurements to fit the models described in the previous section. The observed *in situ* egg vertical distributions were similar between the stations sampled in 2015 and showed that the initial stage I occurred near the surface, then most eggs descended to 10–20 m depth, where they resided until stage IX (some approached the surface again during stage

VIII off Dichato), and finally, from stage X onwards, the eggs descended to layers between 30–70 m (Fig. 11). Utilizing the age-at-stage relationship and seawater density *in situ* profiles for each station, the estimated MWD distribution per stage showed a steady increase in depth of residence for the eggs as they developed (Fig. 12). When the vertical distributions of all egg stages were pooled (either from the observed abundances or estimated using the age-at-stage relationship and *in situ* density profiles), the subsurface intermediate depth peak in egg abundance was observed at the base of the pycnocline in both sampled locations (Fig. 13), the same as in the first spawning months (August–October) off Talcahuano in 2011 (Fig. 8). This coincidence in distribution with the previously modeled distributions (with the bathypelagic egg model in 2011) suggests the main physical forcing factor determining the vertical egg distributions might be operating similarly during the first months of the spawning season among locations and years in the Talcahuano area. Interestingly, in both stations sampled in September 2015, a secondary peak occurred at the maximum depth sampled at both locations, the same as occurred in August 2011 off Talcahuano.

4. DISCUSSION

The present study aimed to determine whether maternally influenced early life history traits varied between anchoveta eggs spawned off northern and central Chile (Iquique and Talcahuano, respectively). We used eggs collected in the field in these zones to assess vertical distributions and characteristics such as specific gravity and FAA contents because of their

Table 4. Mean weight depth (MWD), mean estimated spawning depth and mean measured egg dimensions (length, height, width), estimated spherical diameter (*d*), the difference between the egg specific and seawater density (Δρ), mean terminal velocity (cm s^{-1}) (Eq. 5). Values in parentheses are SD The egg specific gravity values utilized in Δρ were estimated utilizing *in situ* temperatures at the depth of the egg locations

| Month	Observed MWD (m)	Mean estimated spawning depth (m)	Mean dimensions (cm)	*d* (cm)	Δρ (g cm^{-3})	Mean ascent velocity (cm s^{-1})
Iquique						
August	11.3	–	0.12 × 0.06 × 0.06	0.077 (0.002)	0.0016–0.0017	0.047 (0.0023)
September	9.1	8.8	0.13 × 0.06 × 0.06	0.078 (0.002)	0.0022–0.0028	0.068 (0.0075)
October	9.3	9.2	0.13 × 0.06 × 0.06	0.078 (0.003)	0.0021–0.0029	0.062 (0.0099)
Talcahuano						
August	30	22	0.15 × 0.07 × 0.07	0.087 (0.002)	–	–
October	27	35	0.15 × 0.06 × 0.06	0.086 (0.001)	–	–
November	9	48	0.15 × 0.06 × 0.06	0.086 (0.001)	0.0005–0.0015	0.045 (0.0063)
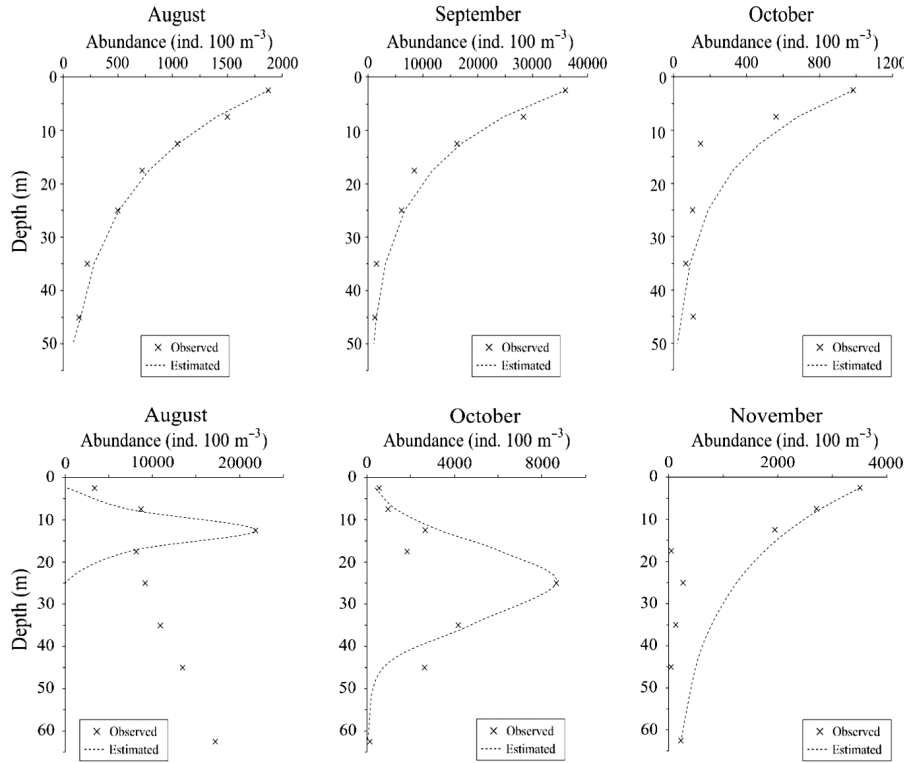
Fig. 10. Observed (2011) and estimated abundance of anchoveta eggs (ind. 100 m^{-3}) resulting from the pelagic egg model (Eq. 6) in Iquique (August–October) and in Talcahuano (November), and from the benthopelagic egg model (Eq. 8) in Talcahuano (August–September). The egg specific gravity values (ρ_e) utilized in the models were corrected as a function of the *in situ* temperatures at the depth of the egg locations

potential osmoregulatory role during egg development. Our results showed that the egg amino acid content, specific gravity, and vertical distribution in the water column changed as eggs developed; that these characteristics differed for populations located in contrasting environmental conditions; and that they varied throughout the spawning season. The results of this study, thus, suggest that FAAs might play a role during egg development, modulating the ESG and therein affecting the depth of the eggs in the water column. However, to better determine the vertical egg distribution, besides the ESG, other physical characteristics of the water column, such as seawater density, vertical distribution, and wind-
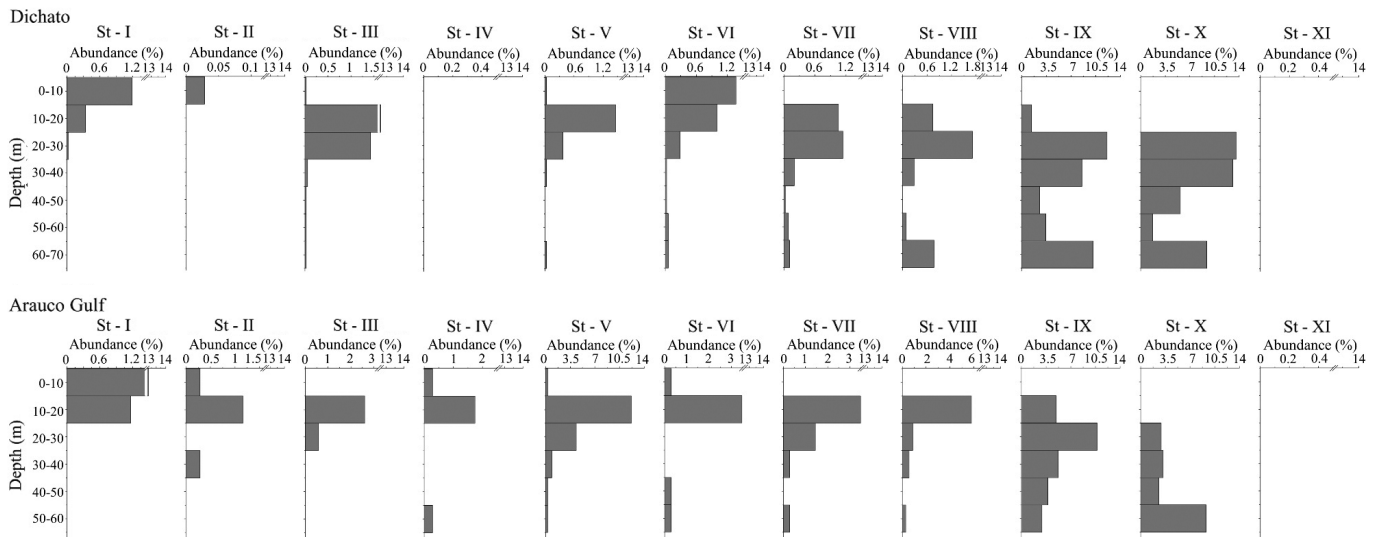

Fig. 11. Abundances of anchoveta eggs at each developmental stage (I–XI) at the different sampled depths off Dichato and in the Arauco Gulf, at the Talcahuano zone, 2015. Values per depth stratum are expressed as percentages of all stages pooled along the sampled water column
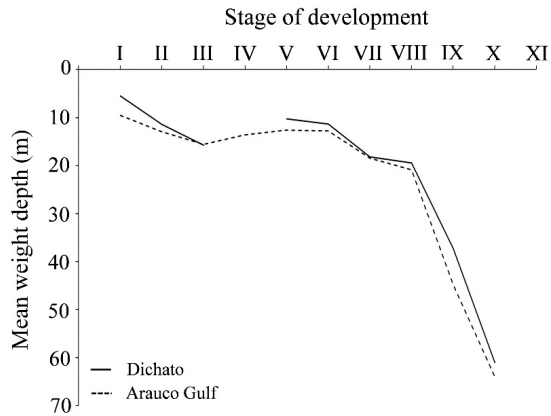
Fig. 12. Mean weight depths for each developmental stage of anchoveta eggs estimated from the age-at-stage relationship, density-at-age relationship, and seawater density profiles off Dichato and in Arauco Gulf, at the Talcahuano zone, 2015

induced turbulence at sea surface, had to be included as drivers in egg buoyancy models (Sundby 1983, 1991). The resulting vertical distributions, different between locations, seemed beneficial to the young offspring because it allows these early life stages to avoid strata with adverse environmental conditions (e.g. oxygen minimum zones off Iquique). In Talcahuano, instead, where winds are stronger and an oxygen minimum zone is not present in winter, eggs and larvae are not subjected to potentially adverse environmental conditions due to oxygen limitation and they may reside in deeper waters of lower turbulence. Observed differences in egg vertical distribution have been reported previously among developmental stages and also among different dates during the spawning season for other anchovies (Tanaka

1992, Coombs et al. 2004, Sabatés et al. 2008, Ospina-Álvarez et al. 2012).

Changes in specific gravity during egg development is a common characteristic in the pelagic eggs of many teleost fishes. In our results, eggs from northern and central Chile followed a similar pattern: the increased specific gravity occurring during the first stages of development was followed by a weak but steady decrease and then a new, greater increase in specific gravity that occurred as the hatching stage approached. This pattern of variation has been observed repeatedly in species such as cod in the Baltic or in different fjords along Norway (Nissling & Westin 1991, Nissling & Vallin 1996, Jung et al. 2012), anchovies in the Gulf of Lyon (Ospina-Álvarez et al. 2012), and sardines and anchovies in the Bay of Biscay (Coombs et al. 2004), among others. However, this pattern of specific gravity variation during egg development may not be the rule in all fish populations. For instance, in some fishes, increased egg density at the beginning or end of the embryonic period has sometimes not been observed (e.g. mackerel *Scombrus scombrus*, Coombs 1981; cod *Gadus morhua*, Nissling & Vallin 1996). In our study, there were 2 occasions off Iquique in which the pattern of specific gravity variation through egg development did not show the increase at the end of development before hatching. Thus, our results confirmed that slight variations in the general patterns of specific gravity change during development occur not only among species but also sometimes within a single population at different times during the spawning season.

Changes in amino acid contents occurred while the eggs developed, resulting in a decrease particularly at older stages close to hatching. Previous studies of
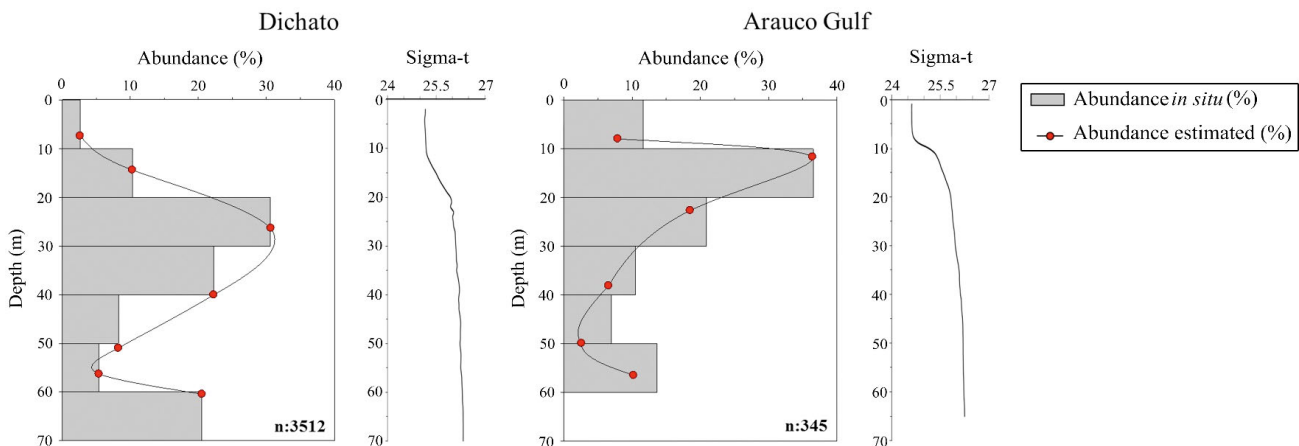

Fig. 13. Observed abundances (%; horizontal bars) of all observed developmental anchoveta egg stages pooled per depth interval (expressed as percentages along the water column) and seawater density profiles, off Dichato and in Arauco Gulf, at the Talcahuano zone, 2015

anchoveta eggs off Talcahuano have already shown that FAA contents decline with egg development (Krautz et al. 2010). These results corresponded to our measurements of changes in specific egg density and with the marked density increase in older-stage eggs measured experimentally in density gradient columns. Other studies have demonstrated that FAAs play a role in osmoregulation, modulating the amount of water in the eggs and affecting their specific gravity (Govoni & Forward 2008, Jung et al. 2012). During oocyte development, FAA contents initially increase as a result of the hydrolysis of large yolk proteins (e.g. vitellogenin); this precedes the hydration phase in the ovary and is a prerequisite for the subsequent increase in egg buoyancy in seawater (Seoka et al. 2003). Once spawned, the osmoregulatory capacity of the eggs changes during development, occurring initially as an outflow of water from the eggs and later, once the osmoregulatory capacity increases, as an ingress of water back to the eggs with a consequent reduction in density (Jung et al. 2014). At later stages of development, the yolk volume decreases along with the FAA concentrations in the yolk because of their catabolism to fulfill embryo energetic demands and their structural use in new large proteins necessary for embryonic growth (Finn et al. 1995). Thus, the relative reduction in FAAs in the yolk and increase in large proteins in the embryo, both particularly fast at the end of egg development, seem to play a key role in increased pelagic ESG close to hatching (Jung et al. 2014). Interestingly, our observations of the vertical distribution of eggs during different stages showed that older eggs were located deeper in the water column at the Dichato and Arauco Gulf stations in 2015, agreeing with our predictions based on increased specific gravity with advanced egg development.

At inter-population levels, egg differences in FAAs, specific gravity, and vertical distributions have also been reported. Differences in FAAs between buoyant and non-buoyant eggs of *Anguilla japonica* were observed by Seoka et al. (2003), whereby pelagic eggs were observed to contain much higher FAAs and water content than demersal eggs. Differences in FAA contents have also been determined for fish eggs spawned in brackish versus marine waters (Thorsen et al. 1996, Goarant et al. 2007). Other studies have reported combined data on specific gravity and vertical distributions for populations in different habitats (Coombs et al. 2004, Jung et al. 2012) or for initial and late-spawned batches of eggs within a reproductive season (Kjesbu et al. 1992, Nissling et al. 1994). However, studies that complement FAA data with ESG and the observed vertical distribution in the water column are scarce. In our study, differences were observed between eggs from both populations in terms of FAA content, mean specific gravity, and vertical distributions. Interestingly, the 3 characteristics assessed seemed to match: higher FAA contents occurred in eggs from Iquique, where lower specific gravity eggs were found and where eggs showed a shallower distribution in the water column. However, the sole potential relationship between FAA contents and specific density in eggs between populations may not necessarily explain the vertical distributions of the eggs in the field at each location. In fact, the observed differences in egg vertical distribution between populations seem to result from physical processes operating with different intensities at each location, as revealed by the models fitted to the vertical distributions at each zone. At Iquique, where the density of eggs was lower than the density of water at the mixed layer, eggs occurred in higher abundance closer to the surface and variations in the spread of eggs from the surface downwards result mainly from differences in wind speed, as predicted by the Sundby (1991) pelagic egg vertical distribution model. In Talcahuano (August, October), instead, where density of the eggs was higher than the density of the water at the mixed layer but lower than the density of the seawater at the bottom layer, the eggs remained at mid-depth closer to the base of the pycnocline, also as the bathypelagic egg vertical distribution models predicted (Sundby 1991).

Changes in the vertical distribution of eggs occurred as the spawning season progressed in the north (Iquique) and central (Talcahuano) Chilean localities (Fig. 8). To understand these changes, at least 2 aspects should be taken into consideration: changes in the egg density and changes in the physical characteristics of the water column such as the vertical distribution of seawater density and wind-induced turbulence. In both locations, there was a slight decrease in egg specific density as the season progressed (Table 1) and also a marked increase in seawater density (Fig. 7)—both of which should increase egg buoyancy ($\Delta\rho$, see Table 4) and facilitate the rise of the eggs towards shallower depths, as observed in our study (Fig. 8). In both areas, the upwelling process (almost continuous during the year in Iquique but strongly seasonal in Talcahuano) plays a role in determining the water column characteristics. In Talcahuano, the freshwater inputs due to rainfall and riverine input probably have a larger effect in winter, lowering the seawater density at the

surface and hence decreasing egg buoyancy, thereafter inducing egg deepening to intermediate waters as described in the bathypelagic egg vertical distribution model results.

Several biological characteristics of the eggs, some maternally inherited, have been reported to potentially affect egg buoyancy — among others, lipid, protein and FAA contents, egg size, and chorion thickness (Govoni & Forward 2008) — and interestingly, some of the different biochemical components of the eggs might counterbalance each other in the determination of their specific gravity. In this study, we determined that FAA content differed between eggs collected in northern and central spawning zones. Protein and lipid contents have also been previously reported to differ between Iquique (less abundant) and Talcahuano (Castro et al. 2009). While some of the differences between populations seem to match our observations regarding differences in egg specific density (e.g. lower FAA and higher protein contents in Talcahuano should increase egg density), others do not agree with the predicted effects in egg density (e.g. higher lipid content and larger egg size in Talcahuano should decrease egg specific density). We do not know the exact relative importance of each component in terms of buoyancy, but from the results of this study, FAAs, through their effect on the water balance during egg development and their influence in larger protein formation, seem to have an important effect (see linear relationship in Fig. 6). Other factors that have been reported to have an effect on egg buoyancy but were not considered in this study are chorion thickness (Nissling et al. 1994) and the presence of transmembrane water channels that facilitate water transport (aquaporins) (Cerdà et al. 2017). To date, no information exists on either of these factors for anchoveta eggs, and consequently, we cannot predict their potential effects on eggs from different habitats. There are other aspects observed in the present study that we initially did not resolve with the models or the information so far obtained. For instance, the presence of secondary peaks in egg abundance deep in the water column during the winter months in Talcahuano. The buoyancy theory indicated that high egg abundances close to the bottom might occur if the density of the eggs at those depths was higher than that observed in the water column at that same depth (Sundby 1983, 1991). In our study, the seawater density did not change much towards higher depths below the pycnocline. However, the specific density of old-age eggs did, as revealed in the density gradient column experiments, reach density values higher than younger stages. Unfortu-

nately, we lacked specific density estimates for the oldest egg stages in August 2011 to prove the bathypelagic egg model (Sundby 1991) but from the other months' estimates of old eggs, it may well be that these specific densities could have been larger than those occurring in bottom seawater. If we were to prove this, the deep secondary egg abundance peak might have been old eggs (close to hatching) as was observed off Dichato and the Arauco Gulf in September 2015. Under this scenario, the results would suggest that, for some single species such as anchoveta in central Chile, different models should be applied to the different egg stages if their specific density changes markedly among dates or seasons.

The observed differences in early life history traits such as egg FAA content and specific gravity may have major implications for the ecology of species under different environments, particularly because of their effect on the vertical distributions of the eggs. Eggs located at different layers may be susceptible to advection to different zones if current direction varies with depth, or to retention in specific areas where benign habitat conditions exist (Stenevik et al. 2001, 2008, Parada et al. 2003, 2012, Soto-Mendoza et al. 2012). Similarly, since the biotic and abiotic characteristics of the water column change with depth, the residence of eggs in certain layers may expose them to either favorable or unfavorable conditions for development and survival. Residing in shallow waters may provide eggs with a warmer environment that might increase development rates and provide a food-rich habitat for young larvae that need to find food quickly during the first days after hatch. However, the presence of eggs in this shallow water may expose the young offspring to higher solar UV radiation that may have negative effects on the embryo or may transport eggs away from the coast in the Ekman layer (Stenevik et al. 2001, Vásquez et al. 2015). A deeper residence, in turn, might bring eggs and larvae back to the coast in coastal upwelling areas, but increase their chances of contact with deep-dwelling predators (e.g. euphausiids) or expose eggs and larvae to very low oxygen concentration layers where conditions are less favorable for development (Nissling & Vallin 1996, Stenevik et al. 2001, Krautz et al. 2007). In our study, the vertical distribution of the anchoveta eggs in each location and their changes throughout the season seem beneficial to the young offspring because they facilitate the avoidance of oxygen minimum zones during the upwelling season (particularly important in summer off Iquique) or the low-salinity surface layer found off Talcahuano in winter. However, locating the eggs close to the

surface in the northern zone may be costly because of potential offshore transport away from the coast in the Ekman layer. Offshore transport in this surface layer is comparatively much slower than in the southern latitudes of the Humboldt Current (Bakun 1996). Moreover, given the higher biological production and warmer temperatures in this surface layer, egg development times are reduced and larval growth rates increased, maximizing chances for offspring survival. Because eggs transported offshore increase in specific gravity as they develop, their potential deepening during the later stages and the initiation of diel vertical migration of larvae might retard their offshore transport or even bring them back to the coastal zone, as proposed for the Benguela upwelling zone for other small pelagic fishes (Stenevik et al. 2001).

In summary, the observed variability in biochemical egg composition and other maternally inherited egg traits that influence the ESG, and interaction among specific egg density, seawater density, and vertical mixing during egg development suggest that early life stages of anchoveta *Engraulis ringens* may survive in habitats of contrasting environmental characteristics. The flexibility expressed in these traits may also be particularly useful in maintaining populations in habitats affected by strong variations in environmental characteristics such as in years of anomalous environmental conditions (e.g. El Niño). They may facilitate colonization of new spawning habitats that might become available under longer-term climate change periods, and also help to explain the observed latitudinal changes in spawning habitats of the anchoveta during the last decades.

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