Egg trait variation in anchoveta Engraulis ringens: a maternal response to changing environmental conditions in contrasting spawning habitats

L. R. Castro¹,²,*, G. Claramunt³, M. C. Krautz¹,⁴, A. Llanos-Rivera¹,⁵, P. Moreno³

¹Laboratorio de Oceanografía Pesquera y Ecología Larval, Departamento de Oceanografía, ²Centro FONDAP-COPAS, and ³Programa de Doctorado en Oceanografía, Universidad de Concepción, PO Box 160-C, Concepción, Chile
⁴Departamento de Ciencias del Mar, Universidad Arturo Prat, Casilla 121, Iquique, Chile
⁵Present address: Unidad de Biotecnología Marina, Facultad de Ciencias Naturales e Oceanográficas, Universidad de Concepción, PO Box 160-C, Concepcion, Chile

ABSTRACT: The Engraulis ringens distribution (4 to 42° S) covers a wide variety of environmental conditions. We assessed the coping mechanisms used by this anchoveta in different spawning habitats, reporting differences in egg traits between populations off northern (Iquique, 20° S) and southern Chile (Talcahuano, 36° S) and throughout the spawning season. Eggs were smaller off Iquique, declined in size during the reproductive season (both populations), and inter-population differences persisted throughout the spawning season. Batch fecundity (eggs per batch per female) and relative fecundity (eggs per female weight) were lower off Talcahuano during the peak spawning months. Thus, larger eggs spawned in the southern population seem to be produced at the cost of a reduction in fecundity. The eggs also differed biochemically between the 2 populations; total lipids and triacylglycerides were much higher off Talcahuano. The biochemical composition (both populations) also changed throughout the spawning season, with higher lipids early in the spawning season (July to August) off Talcahuano. Hatching success decreased during the spawning season, and correlated positively with egg size and lipid contents. Environmental conditions differed markedly between spawning areas and from mid-winter to late spring. Off Talcahuano, the temperature and winter-time water column productivity are lower and turbulence is higher, while eggs are larger, lipid contents higher, and batch fecundity lower. This mechanism seems to facilitate survival of young offspring in the more adverse winter conditions off Talcahuano. These egg characteristics are determined by the adult female reproductive system, representing a maternal influence on the early life-history traits of anchoveta that might enable rapid changes in population densities in some years of improved habitat conditions.

KEY WORDS: Anchoveta · Engraulis ringens · Upwelling · Reproductive strategy · Egg quality · Lipids · Humboldt Current · Small pelagic fishes

INTRODUCTION

Variations in egg size among populations located along a latitudinal gradient or during the spawning season have been reported for a number of demersal and pelagic fishes (Blaxter & Hempel 1963, Bagenal 1971, Blaxter & Hunter 1982, Thresher 1984, Chambers 1997). In general, larger eggs are spawned at higher latitudes, egg size tends to decrease as the spawning season progresses, and eggs produced in winter are larger than those spawned in summer (Ciechomski 1973, Clarke 1989, Claramunt et al. 1994, Rijnsdorp & Vingerhoed 1994, Chambers & Waiwood 1996, Plaza et al. 2002, Rideout et al. 2005). Whereas most authors have proposed egg size variability to be an adaptive trait to enhance offspring survival under different environmental conditions, others have suggested that egg size variation may be the result of the changing environmental temperature on the reproductive system of the adult females and, hence, not neces-
sarily an adaptive genetic response to facilitate off-
spring survival in different environments (Daoulas &
Economou 1986). These hypotheses, however, are not
usually tested. It has long been documented that larger
eggs usually produce larger and more robust larvae,
capable of searching larger volumes of water for
food, evading predators more successfully, or growing
faster. All these characteristics should reduce mortality
under adverse environmental conditions (Marteins-
dottir & Steinarsson 1998).

Compared with egg size variation studies, less attention
has been paid to assessing the quality of eggs in the
field that originate larvae of different sizes. Variations
in energetic content, the oil vesicle, or relative
concentrations of different biochemical components in
the eggs might play an important role in modulating
the offspring’s chances for survival (Chambers et al.
steiner & Patarnello 2005). The amount of lipid or pro-
tein reserves provided for the eggs or recently hatched
yolk sac larvae, for instance, may modify the hatching
success or the amount of energetic reserves available
in different periods during the spawning season.
Accordingly, the amount of reserves available to the
eggs is expected to be correlated with the environmen-
tal conditions the larvae will face during the spawning
season. These studies, although initiated some de-
decades ago, have only recently been applied to wild
small pelagic fish populations (Riveiro et al. 2000,
2004), probably due to advances in rearing methods
and the simplification of biochemical techniques for
aquaculture purposes.

Lipid and protein contents are reported to be impor-
tant egg constituents and sources of stored energy in
fish eggs. For lipids the triacylglycerides (TAG) is
known to be a common form of energy, while, for pro-
tein, various free amino acids are important energy
sources during embryonic development (Fynh 1989).
Phospholipids are also a major lipid component in the
eggs of many marine fish species. Phospholipids are
important structural components of membranes and an
important source of energy during embryonic develop-
ment in species with lower TAG contents (Tocher et al.
1985, Fraser et al. 1988, Rainuzzo et al. 1992). Larvae of
many marine fish require highly unsaturated fatty
acids (HUFA) of the n-3 series such as eicosapentaenoic
acid [EPA: 20:5(n-3)] and docosahexaenoic
acid [DHA; 22:6(n-3)] (Rainuzzo et al. 1997, Sargent et
al. 1999). The fatty acid composition and hatching
success are related, for instance, in different cod popu-
lations (Pickova et al. 1997). Proteins, the largest com-
ponent of fish egg yolk, are transformed into embry-
sonic tissue and are consumed to provide energy
(Kamler 1992). Because of their apparent strong in-
verse relationship with embryonic development times,
protein contents have also been suggested to be a key
biochemical component in winter-spawned eggs of
Sardina pilchardus off the Iberian Peninsula (Riveiro et
al. 2000, 2004). For this small, winter-spawning pelagic
fish, the higher protein concentrations in the eggs at
the beginning of the cold months are thought to induce
higher embryonic developmental rates. Thus, proteins
could compensate for the lower temperatures that slow
development in early winter. All these studies point to
the existence of a relationship between the biochemi-
cal egg composition and the survival of young devel-
oping stages, thereby strongly suggesting a relation-
ship between the environment and egg quality via
maternal effects.

Along the Humboldt Current, the anchoveta En-
graulis ringens is distributed from 4 to 42° S; the envi-
ronmental conditions in this latitudinal range vary
dramatically. Three major stocks are recognized along
the Humboldt Current: off northern Peru (largest), off
southern Peru–northern Chile (mid-sized), and off
central Chile (smallest). Recent studies initiated to
determine how the early life stages of this species cope
with variations in environmental conditions (Castro et
al. 2000, Hernáñdez & Castro 2000, Llanos-Rivera &
Castro 2004, 2006) have identified early life-history
traits, such as egg size, larval size at hatching, yolk vol-
ume at hatching, and larval growth rates that vary
along its latitudinal range. In particular, the egg size,
larval length at hatching, and yolk sac volume of
recently hatched larvae increase with latitude, and
instantaneous yolk sac larval growth rates may differ
between latitudes (Llanos-Rivera & Castro 2006). Sea-
sonal variations in size have also been reported in eggs
of the southern (36°S) anchoveta population (Llanos-
Rivera & Castro 2004). At this latitude, larger eggs are
generally spawned in winter, and smaller eggs, in
summer. At lower latitudes, however, no information
is available on egg size variations throughout the spawn-
ing season of this species or on potential effects of egg
size differences on the survival of subsequent early life
stages.

In the present study, we report egg quality variations
throughout the spawning season for 2 anchoveta pop-
ulations located at different latitudes in the Humboldt
Current (21° S vs. 36° S). First, we assessed variations
in egg sizes of recently spawned eggs (<1 d) in the
plankton. Secondly, we determined whether variations
in the biochemical composition of these eggs occurred
during the season (total protein content, total lipid con-
tent, TAGs, cholesterol). Finally, we used rearing ex-
periments carried out under natural temperature con-
ditions to assess whether differences in hatching
success occurred in eggs produced at different times
during the spawning season that could suggest poten-
tial relationships among egg quality, offspring sur-
vival, and environmental conditions during spawning. This approach seeks to combine disciplines such as adult reproductive biology, early life histories of fish, biochemistry, and basic oceanography within a conceptual framework of reproductive strategies.

**MATERIALS AND METHODS**

**Field cruises, ichthyoplankton collections, and egg sorting.** During the 2003 spawning season of *Engraulis ringens*, short (4 to 6 h each) cruises were carried out to the Talcahuano (south, 36° S, n = 15), and Iquique (north, 20° S, n = 8) spawning areas (Fig. 1). In 2004, 7 and 8 cruises were carried out to the northern and southern spawning areas, respectively. During each cruise, the ichthyoplankton was gently collected in short (3 to 4 min) tows from the surface to 20–40 m depth, either with bongo or conical nets (300 µm, oblique tows, with flowmeters to quantify volume of water sampled). Sea surface temperature was recorded at each station. Ichthyoplankton samples were rapidly brought to the laboratory under temperature-controlled conditions and in the dark. Once in the laboratory, the anchoveta eggs were identified and quickly separated from the rest of the sample; subsamples were obtained for different analyses. Depending on their abundance, over 50 eggs per sampling date were preserved in 4% formaldehyde and then measured (widest and shortest axes) under a binocular microscope. The egg volume was estimated considering the egg to be ellipsoid in shape ($V = 4\pi \times a \times b \times c / 3$). Another subsample was utilized for biochemical analyses and egg wet weight determinations on a 0.0001 g analytical scale. The wet weight (WW) was later transformed into dry weight (DW) by utilizing a conversion factor obtained from dry and wet weight measures of egg subsamples collected from each area (13 subsamples of 50 eggs each from the northern area; 20 subsamples of 50 eggs each from the southern area) during the sampling season (mean ± SD DW/WW: north = 0.1273 ± 0.026; south = 0.1061 ± 0.0214). At least 50 eggs subsample$^{-1}$ were used for protein and lipid determinations. These subsamples were separated into single vials and deep frozen at −20°C for biochemical analyses. Finally, a fifth subsample of 100 one-day-old eggs was separated and incubated for hatching success determinations during 2004.

**Biochemical egg composition.** The biochemical composition of field-collected anchoveta eggs was determined throughout the spawning season for the southern stock in 2003 and for both stocks in 2004. The biochemical analyses included the concentration of total proteins and lipids (total lipids, TAGs, cholesterol). The analyzed samples consisted of recently fertilized eggs (prior to embryonic development); these eggs were younger than 1 d old at the environmental temperatures of collection (Stages 1 to 4 after Fisher 1958). Because protein and lipid contents per egg should co-vary with egg volume (i.e. µg protein egg$^{-1}$; µg lipid egg$^{-1}$), to compare the biochemical content of the eggs between populations and among months avoiding the effect of egg volume variability, all the biochemical compounds were standardized to egg dry weight (i.e. µg protein mg$^{-1}$ egg DW; µg lipid mg$^{-1}$ egg DW).

**Protein content:** Anchoveta egg subsamples were macerated in PBS (phosphate-saline buffer; 10 mM, pH 7.4) using a glass tissue homogenizer. Each tube was centrifuged at 6600 × g for 5 min (4°C); the supernatant was removed, aliquoted, and frozen at −20°C. Total fish egg proteins were determined after Lowry et al. (1951). Briefly, a 100 µl aliquot of supernatant and NaOH 1 M was added to vials of 1.5 ml, and incubated for 30 min at room temperature. Then, we added 1 ml of Lowry reagent, incubating this for 10 min. Finally, we added 100 µl of Folin Ciocalteu’s Phenol Reagent
N2 through the sample. Vials were chilled on silica gel and the solvent proportions were 0.8:1:2 (water:methanol:chloroform). After adding the solvents, the tubes were centrifuged at 1400 × g for 5 min to facilitate phase identification. The chloroform phase was transferred to previously weighed glass vials. Samples were filtered through glass wool to remove any impurity. Glass vials were placed over a glass vials. Samples were filtered through glass wool to remove any impurity. Glass vials were placed over a thermoblock at 36°C to avoid humidity accumulation. Chloroform was completely evaporated by bubbling nitrogen through the sample. Vials were chilled on silica gel and the solvent proportions were 0.8:1:2 (water:methanol:chloroform). After adding the solvents, the tubes were centrifuged at 1400 × g for 5 min to facilitate phase identification. The chloroform phase was transferred to previously weighed glass vials. Samples were filtered through glass wool to remove any impurity. Glass vials were placed over a glass vials. Samples were filtered through glass wool to remove any impurity. Glass vials were placed over a thermoblock at 36°C to avoid humidity accumulation. Chloroform was completely evaporated by bubbling nitrogen through the sample. Vials were chilled on silica gel to avoid humidity and weighed on an analytic balance (sensitivity: 0.1 g). Vials were kept frozen at –20°C until further analysis.

**Triacylglycerides (TAG) and total cholesterol:** The dry lipid sample was reconstituted with 300 μl isopropanol p.a. (Merck). Aliquots of 10 μl were obtained to determine TAGs and total cholesterol through enzymatic and colorimetric reactions. TAG determinations were performed using an enzymatic kit TG PAP 150 (bioMérieux). Cholesterol was determined using an enzymatic colorimetric kit CHOD-PAP (Spinreact).

**Hatching success:** The egg subsamples collected in the field on each date were used for experimental setups (2 replicates, 1 l glass flasks with 100 eggs each, maintained in a thermo-regulated bath). The 1 d old eggs that were suspended in the incubation flasks were incubated in a 10:14 h light:dark photoperiod at the in situ seawater temperature. Every day one-third of the water content of the flasks was replaced. The number of eggs hatched was determined by checking sections (front, medium and posterior) of about 0.2 g each, from which at least 100 hydrated oocytes were observed. Batch fecundity was estimated as the average of these 3 subsamples. While in the northern region 99 and 96 females were analyzed in August 2003 and 2004, respectively, in the southern region, 100 and 96 females were analyzed during the same months and years.

**Environmental conditions.** Daily sea surface temperature records (January to December) were obtained for both areas from tidal gauge stations operated by the Servicio Hidrografico y Oceanografico de la Armada (SHOA) located off Talcahuano and Iquique. Daily mean wind data (direction, intensity) were obtained from stations operated by the Dirección Meteorologica de Chile (DMC) at the local airports Diego Aracena (Iquique) and Carriel Sur (Talcahuano). The wind data were utilized to estimate a turbulence index (m³ s⁻³) and the predominant south and north wind components, which induce upwelling and the seaward Ekman transport of eggs and larvae from the coast (south winds) and shoreward egg and larval transport and downwelling at the coast (north winds).

### RESULTS

#### Intra-seasonal variations

In September 2003, the eggs from the northern population were larger than in other months; otherwise, egg sizes in both areas tended to decrease from the beginning to the end, as revealed by the significant (p < 0.001) and negative slopes in the regressions between egg volume and month of the spawning season in both areas (Tables 1 & 2, Fig. 2).

In 2003, the number of biochemical determinations throughout the spawning season allowed statistical tests comparing monthly mean values of protein, lipid, TAG, and cholesterol in the eggs at the beginning (July/August) versus the end of the spawning season (October/November) for the southern population. For

<table>
<thead>
<tr>
<th>Month</th>
<th>North 2003</th>
<th>South 2003</th>
<th>North 2004</th>
<th>South 2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jul</td>
<td>0.29 ± 0.02 (40)</td>
<td>–</td>
<td>0.29 ± 0.03 (162)</td>
<td>0.33 ± 0.03 (407)</td>
</tr>
<tr>
<td>Aug</td>
<td>0.27 ± 0.02 (70)</td>
<td>0.38 ± 0.03 (100)</td>
<td>0.29 ± 0.03 (57)</td>
<td>0.33 ± 0.03 (410)</td>
</tr>
<tr>
<td>Sep</td>
<td>0.31 ± 0.03 (43)</td>
<td>0.36 ± 0.03 (200)</td>
<td>0.28 ± 0.02 (108)</td>
<td>0.32 ± 0.03 (408)</td>
</tr>
<tr>
<td>Oct</td>
<td>0.25 ± 0.02 (152)</td>
<td>0.36 ± 0.03 (100)</td>
<td>0.27 ± 0.05 (50)</td>
<td>0.31 ± 0.03 (292)</td>
</tr>
<tr>
<td>Nov</td>
<td>–</td>
<td>0.35 ± 0.02 (200)</td>
<td>0.26 ± 0.02 (49)</td>
<td>0.31 ± 0.03 (296)</td>
</tr>
</tbody>
</table>

Table 1. *Engraulis ringens*. Mean volume (mm³), standard deviations, and number of anchoyeta eggs measured (in parentheses) off northern (Iquique) and southern (Talcahuano) Chile during the winter spawning seasons of 2003 and 2004. –: no data.
2004, such analyses were only possible for proteins in eggs of this same population (Table 3). Protein content in eggs from the southern population differed slightly from the beginning (July/August) to the end (October/November) of the spawning season in 2003 (t-test, \( p = 0.023 \)); however, this decrease in protein concentrations was not significant in 2004 (t-test, \( p = 0.452 \)) (Fig. 2). Protein concentrations from the northern population in 2004, although variable at the beginning of the season, showed a clear decrease trend throughout the 3 mo of data available.

The highest concentrations of total lipids in eggs from the northern area occurred at the beginning (July) of the spawning season in 2004. In the southern area, higher lipid concentrations also occurred early in the season, but their highest values occurred in August of both years (Fig. 2), decreasing later towards the end of the season. TAG and cholesterol concentrations per milligram of egg dry weight from the southern populations showed fairly similar trends of a decrease in concentrations throughout the spawning season in both years. In the northern population this trend was not observed.

In 2003, eggs from the northern area had successful hatchings from about 62 to 89%, and the maximum success coincided with the dates of maximum egg size (September; Table 4). During 2004, in turn, hatching success for eggs of both populations tended

---

### Table 2. *Engraulis ringens*. Regression parameters between egg volume and month (beginning in July, \( t = 0, 1, 2, 3, \) and 4) off northern (Iquique) and southern (Talcahuano) Chile during the winter spawning seasons of 2003 and 2004. All p-values are highly significant (\( p < 0.001 \))

<table>
<thead>
<tr>
<th>Location</th>
<th>Intercept</th>
<th>Slope</th>
<th>N</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>North</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>0.380</td>
<td>−0.012</td>
<td>305</td>
<td>2.46 × 10^{−16}</td>
</tr>
<tr>
<td>2004</td>
<td>0.367</td>
<td>−0.010</td>
<td>426</td>
<td>5.219 × 10^{−20}</td>
</tr>
<tr>
<td><strong>South</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>0.414</td>
<td>−0.006</td>
<td>600</td>
<td>1.50 × 10^{−6}</td>
</tr>
<tr>
<td>2004</td>
<td>0.377</td>
<td>−0.006</td>
<td>1813</td>
<td>2.025 × 10^{−34}</td>
</tr>
</tbody>
</table>

---

### Table 3. *Engraulis ringens*. Mean concentration, standard deviation, and number of replicates utilized (in parentheses) for total protein, total lipid, triacylglycerides (TAG), and cholesterol in anchoveta eggs collected off northern (Iquique) and southern (Talcahuano) Chile during the winter spawning seasons of 2003 and 2004. –: no data

<table>
<thead>
<tr>
<th>Location</th>
<th>Total protein (µg protein mg−1 egg DW)</th>
<th>Total lipid (µg lipid mg−1 egg DW)</th>
<th>TAG (µg TAG mg−1 egg DW)</th>
<th>Cholesterol (µg cholesterol mg−1 egg DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>South, 2003</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jul</td>
<td>215.48 ± 41.76 (4)</td>
<td>391.32 ± 175.50 (3)</td>
<td>18.89 ± 7.36 (2)</td>
<td>8.32 ± 3.27 (3)</td>
</tr>
<tr>
<td>Aug</td>
<td>249.26 ± 81.00 (9)</td>
<td>894.29 (1)</td>
<td>15.18 ± 9.05 (7)</td>
<td>8.62 ± 5.25 (7)</td>
</tr>
<tr>
<td>Sep</td>
<td>203.14 ± 49.66 (3)</td>
<td>404.31 ± 253.46 (2)</td>
<td>9.09 ± 1.46 (2)</td>
<td>3.76 ± 3.72 (2)</td>
</tr>
<tr>
<td>Oct</td>
<td>222.81 ± 23.29 (2)</td>
<td>373.80 (1)</td>
<td>1.98 (1)</td>
<td>3.74 (1)</td>
</tr>
<tr>
<td>Nov</td>
<td>231.50 ± 11.66 (3)</td>
<td>217.39 ± 121.89 (3)</td>
<td>2.82 ± 0.41 (2)</td>
<td>8.79 ± 3.73 (3)</td>
</tr>
<tr>
<td><strong>North, 2004</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jul</td>
<td>202.65 ± 95.45 (4)</td>
<td>147.32 ± 87.23 (7)</td>
<td>10.86 ± 4.12 (4)</td>
<td>2.95 ± 0.99 (3)</td>
</tr>
<tr>
<td>Aug</td>
<td>154.20 ± 56.07 (2)</td>
<td>110.98 ± 56.32 (2)</td>
<td>13.25 ± 3.14 (7)</td>
<td>4.67 ± 2.11 (6)</td>
</tr>
<tr>
<td>Sep</td>
<td>122.82 ± 45.81 (3)</td>
<td>118.96 ± 95.38 (1)</td>
<td>12.64 ± 4.16 (2)</td>
<td>5.34 ± 4.49 (2)</td>
</tr>
<tr>
<td>Oct</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>South, 2004</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jul</td>
<td>220.05 (1)</td>
<td>117 (1)</td>
<td>23.81 (1)</td>
<td>9.71 (1)</td>
</tr>
<tr>
<td>Aug</td>
<td>200.91 ± 34.85 (4)</td>
<td>341.34 ± 193.60 (2)</td>
<td>34.75 ± 2.37 (2)</td>
<td>5.77 ± 0.54 (2)</td>
</tr>
<tr>
<td>Sep</td>
<td>–</td>
<td>196.77 ± 12.60 (2)</td>
<td>17.36 ± 0.99 (2)</td>
<td>4.80 ± 1.19 (2)</td>
</tr>
<tr>
<td>Oct</td>
<td>232.72 ± 9.47 (2)</td>
<td>190.33 ± 22.12 (2)</td>
<td>23.47 ± 6.24 (2)</td>
<td>4.96 ± 2.07 (2)</td>
</tr>
<tr>
<td>Nov</td>
<td>198.57 (1)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

---

Fig. 2. *Engraulis ringens*. Intra-seasonal variation in anchoveta egg volume (mm³) during the 2003 and 2004 spawning seasons off northern and southern Chile
to decrease as the spawning season progressed, with the values of hatching success registered for the southern populations being higher than those for the northern eggs (Fig. 3).

Inter-population variations

Results from \( t \)-tests for comparisons of slopes and intercepts (given in Table 2) showed significant (\( p < 0.05 \)) differences in egg volume between populations as the spawning season progressed in both years (\( t_{\text{calc}} \) slopes = 3.67 and 3.12; \( t_{\text{calc}} \) intercepts = 11.97 and 7.99 for 2003 and 2004, respectively). Within each zone, intercepts and slopes did not vary between years in the northern population (\( t_{\text{calc}} = 1.42 \) and 1.02 for slopes and intercepts, respectively); however, a significant difference in the intercepts (\( t_{\text{calc}} = 0.65 \)), but not in the slopes (\( t_{\text{calc}} = 9.87 \)), was detected in the southern population.

When mean values are compared, eggs spawned by the southern population were larger than those from the northern area in both years (Kruskal-Wallis, \( H_{2003} (1, N = 905) = 558; H_{2004} (1, N = 1823) = 439 \)). Differences in egg size also occurred inter-annually in each population (Kruskal-Wallis \( H_{\text{North}} (1, N = 732) = 36.24, p < 0.001; H_{\text{South}} (1, N = 2413) = 546.98, p < 0.001 \)) (Fig. 4).

Comparisons of biochemical characteristics of the eggs between populations were carried out only for 2004 because these characteristics were not determined for the northern population in 2003. All egg characteristics showed the same trend of higher values in the southern populations (Fig. 4). This trend was statistically significant for egg volume (\( t \)-test, \( p < 0.001 \)), total lipids (\( t \)-test, \( p = 0.02 \)), and TAG (\( t \)-test, \( p < 0.001 \)), but not for mean egg dry weight (\( t \)-test, \( p = 0.136 \)), total protein (\( t \)-test, \( p = 0.81 \)), or cholesterol (\( t \)-test, \( p = 0.18 \)).

Hatching success, egg size, and biochemical composition relationships

In order to evaluate the potential relationship between hatching success, egg size, and the different biochemical characteristics of the eggs over their entire range of variability, data obtained in 2004 from the northern and southern populations were pooled (Fig. 5). Positive relationships were observed between hatching success (HS, %) and egg size (\( V \)) (\( HS = -212.91 + 921.04 V; R^2 = 0.86; p < 0.001, N = 9 \)). Lipid (\( L \)) (\( HS = 26.51 + 0.22 L; R^2 = 0.84; p < 0.05, N = 6 \)) and TAG concentrations (\( HS = 32.87 + 1.86 \text{TAG}; R^2 = 0.67; p < 0.05, N = 6 \)) were also positive and significantly correlated. Although the relationship between total proteins (Pr) and hatching success followed the same trend (positive relationship), it was not significant (\( HS = 0.34 + 1.81 \text{Pr}; R^2 = 0.46; p < 0.14, N = 6 \)).

Batch and relative fecundity

Mean batch fecundity estimated in females from the northern population was higher (13,425 and 10,087 eggs in 2003 and 2004, respectively) than in southern females (6,491 and 8,325 eggs in 2003 and 2004, respectively) during both years. The same trend was observed in relative fecundity with 562 and 412 eggs g\(^{-1}\) in northern females and 398 and 390 eggs g\(^{-1}\) in southern females, in 2003 and 2004, respectively (Fig. 6). The regressions between female weight and batch fecundity were all significant (\( p < 0.001 \)) for all years and areas, and no differences were detected between years in each zone (Table 5). Accordingly, we pooled the data for both years and compared the regressions between zones. Significant differences were observed between the slopes of the pooled re-

### Table 4. *Engraulis ringens*. Mean percentage of hatching success for anchoveta eggs collected off northern (Iquique) and southern (Talcahuano) Chile during the winter spawning seasons of 2003 and 2004. –: no data

<table>
<thead>
<tr>
<th>Month</th>
<th>2003 North</th>
<th>2004 North</th>
<th>2004 South</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jul</td>
<td>85.0 ± 12.1</td>
<td>65.6 ± 26.6</td>
<td>–</td>
</tr>
<tr>
<td>Aug</td>
<td>61.8 ± 17.8</td>
<td>55.3 ± 26.5</td>
<td>99.5 ± 0.7</td>
</tr>
<tr>
<td>Sep</td>
<td>88.9 ± 14.3</td>
<td>38.6 ± 15.0</td>
<td>78.5 ± 16.7</td>
</tr>
<tr>
<td>Oct</td>
<td>71.5 ± 36.3</td>
<td>31.2 ± 33.2</td>
<td>69.3 ± 20.0</td>
</tr>
<tr>
<td>Nov</td>
<td>–</td>
<td>–</td>
<td>63.2</td>
</tr>
</tbody>
</table>

Fig. 3. *Engraulis ringens*. Intra-seasonal variation in hatching success (%) during the 2003 and 2004 spawning seasons off northern and southern Chile
gressions ($t$-slope test: $t = 2.76; t_{0.05(2),359} = 1.97$), the slope values for the northern females being higher than those for their southern counterparts.

**Environmental conditions**

A clear seasonal pattern in environmental characteristics was observed for both years and at both locations. The highest sea surface temperatures and most frequent southern, upwelling-favorable winds occurred in summer, and the lowest temperatures and least frequent upwelling winds occurred in winter, coinciding with the anchoveta spawning season (Fig. 7). The seasonal changes in environmental conditions were more marked at the southern latitudes, especially in wind-related characteristics; from summer to winter the wind directions changed from south to north and the wind intensity increased from about 3 m s$^{-1}$ daily to >5 m s$^{-1}$.

Besides the range in seasonal fluctuations, the magnitude of the environmental variables between sites was also different. Sea surface temperature was always about 3 to 4°C higher in the more northern area (Iquique). For most of the year, winds off Iquique were from the southern quadrants, inducing year-round coastal upwelling; in the southern area, southern winds only predominated in spring and summer. Finally, wind-induced turbulence due to strong wind events that might have mixed the water column did not occur off Iquique, but they did occur off the southern location. During the main winter anchoveta spawning season, therefore, the environmental conditions for the early life stages were less harsh in the northern than in the southern zone.
DISCUSSION

The reproductive strategies proposed for anchovies living under different environmental conditions have traditionally included rapid growth rates, young age of maturity, and high fecundity associated with abundant but small and energetically cheap eggs. In our study, we show that anchoveta *Engraulis ringens* egg size is smaller off northern than off southern Chile, that the egg size declines during the reproductive season in both populations, and that the inter-population differences remain almost unaltered throughout the spawning season. Seasonal variations in egg size have also been reported for several small pelagic species with protracted or repeated spawning periods such as *Engraulis anchoita* (Ciechomski 1973), *Scombrus scombrus* (Ware 1977), *Engraulis mordax* (Hunter & Leong 1981), *Encrasicholina purpurea* (Clarke 1989), and *Sardinops sagax* (Claramunt et al. 1994). Changes in...
egg size during the spawning season have also been reported for the anchoveta *E. ringens*, but only in the higher latitude population (Castro et al. 2001, Llanos-Rivera & Castro 2004). For this species, recent reports also show that the initial differences in egg size between the northern and southern populations off Chile propagate to the following exogenous-feeding larval stage (post yolk sac larvae) (Llanos-Rivera & Castro 2006). In both populations, initial winter egg sizes were highly correlated with larval length at hatching, yolk sac volume at hatching, and larval length at yolk absorption; all these traits may positively affect the survival chances of the young larvae. Since larger egg sizes occurred in those individuals spawned in the middle of winter, when the environmental conditions were harshest, especially in the southern populations, we propose that variations in the egg volume may represent an indirect maternal effect to facilitate subsequent larval survival during the winter season.

Table 5. *Engraulis ringens*. Regression parameters between weight and batch fecundity in northern (Iquique) and southern (Talcahuano) female anchoveta during August 2003 and 2004. All p-values highly significant (p < 0.001)

<table>
<thead>
<tr>
<th></th>
<th>Intercept</th>
<th>Slope</th>
<th>N</th>
<th>$R^2$</th>
<th>$F$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>North</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>–1237.7</td>
<td>615.8</td>
<td>99</td>
<td>0.54</td>
<td>114.6</td>
<td>$4.02 \times 10^{-18}$</td>
</tr>
<tr>
<td>2004</td>
<td>–4164.1</td>
<td>584.2</td>
<td>96</td>
<td>0.20</td>
<td>23.6</td>
<td>$4.74 \times 10^{-6}$</td>
</tr>
<tr>
<td><strong>South</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>122.0</td>
<td>395.1</td>
<td>100</td>
<td>0.58</td>
<td>134.5</td>
<td>$4.34 \times 10^{-20}$</td>
</tr>
<tr>
<td>2004</td>
<td>600.2</td>
<td>360.9</td>
<td>68</td>
<td>0.56</td>
<td>85.3</td>
<td>$1.68 \times 10^{-13}$</td>
</tr>
</tbody>
</table>

![Fig. 7. *Engraulis ringens*. Sea surface temperature (SST, °C), wind speed (N–S component, m s$^{-1}$), and turbulence index (m$^3$ s$^{-3}$) in the northern and southern spawning areas in 2003 and 2004. Negative wind speeds (from the south) induce upwelling (see 'Results: Environmental conditions'). Vertical lines are standard deviations](image-url)
Given the usual positive relationship between fecundity and female size, females have to be larger and possess larger ovaries to contain more and larger oocytes. Batch fecundity (number of eggs spawned per batch per female) and relative fecundity (number of eggs spawned per female weight) during the peak spawning season were lower in the southern than in the northern population. In other words, the cost for spawning larger eggs in the southern population seems to be a reduction in the female’s batch fecundity. Batch fecundity and egg size, therefore, represent traits in reproductive strategy that clearly differ between anchoveta populations along the Humboldt Current and that probably vary according to the environmental characteristics of the different reproductive habitats.

Analyses of the biochemical characteristics of eggs during the spawning season also showed differences between populations. Total lipid contents and TAGs occurred in much higher concentrations in the southern eggs that in those from the northern stock. Lipids are some of the most important nutritional components of broodstock that affect egg quality (Rainuzzo et al. 1997). Lipids are used as an energetic source for metabolism during embryonic development, and TAGs have been cited as the most common form of energy stored in eggs, as well as in later life stages, of most marine fish (Cowey et al. 1985). During starvation, TAGs are used before phospholipids (Sargent et al. 1999), which are important structural membrane components, although the latter may also be important sources of energy during embryonic development in species with less TAG (Tocher et al. 1985, Fraser et al. 1988, Falk-Petersen et al. 1989, Rainuzzo et al. 1992). Thus, our results showing higher lipid and TAG contents in eggs produced during winter in the southern area, where temperatures are lower and, thus, embryonic developmental times are longer (Tariñeno et al. 2007), and feeding conditions are poorer (i.e. less plankton production, strong turbulence, lower temperature), seem to support the idea that increased lipid and TAG concentrations in eggs may be beneficial for embryos and for later larval survival.

Seasonal changes in the biochemical composition of fish eggs have also been reported for other clupeiforms in upwelling areas. In Sardinia pilchardus eggs, for instance, Riveiro et al. (2000, 2004) reported that the organic content (lipids, carbohydrates, proteins) decreased as the spawning season progressed from winter to mid-spring off the Iberian Peninsula. This decrease was accompanied by an increase in the environmental temperature and seston in the water column (a proxy for larval food) and was interpreted as part of a reproductive strategy in which better quality eggs were spawned under winter conditions (low temperature, less abundant larval food). In S. pilchardus, proteins correlated negatively with embryonic developmental times and, hence, were proposed to be potentially more important than lipids and carbohydrates, because eggs with higher protein concentrations could compensate for the lower temperature at the beginning of the cold months that retarded development. Our results coincided almost entirely with those reported for S. pilchardus. In Engraulis ringens, estimated developmental times and the tendency for protein concentrations to decrease also changed during the spawning season, although the latter was not significant.

High intra-seasonal variability was observed in our hatching success estimates for both areas. Despite this variability, a clearly decreasing trend in hatching success occurred as the spawning season progressed. Lipids and TAGs also showed the same trend over the season and were highly correlated with hatching success when the entire dataset from both areas was pooled. The biochemical content of the eggs, which is determined while energy is accumulated and allocated to oogenesis (Chambers et al. 1989), therefore, seems to play an important role in determining the survival chances of the different cohorts spawned during the season. In periods of the reproductive season in which egg quality is high (i.e. high lipid contents), hatching success is also high, independent of whether this occurs in the northern or southern population. These results are particularly interesting, especially when considering inter-annual variations, and they might explain the abrupt changes in recruitment observed from one year to another despite large reductions in biomass. This explanation, therefore, is an alternative to the traditional hypotheses dealing with improved habitat conditions for larval growth and survival proposed to explain population booms in clupeiforms.

The anchoveta life cycle in the southern distribution area along the Humboldt Current includes a series of sequential events (winter reproduction, spring–early summer juvenile growth, late summer–early fall lipid accumulation) and a reproductive strategy based on winter spawning. Three non-independent hypotheses may explain the timing of winter reproduction: (1) winter spawning facilitates egg and larval retention at the coast, as a result of northern winds inducing shoreward egg and larval transport (Castro et al. 2000, Castro 2001); (2) winter spawning may be beneficial for larvae and juveniles, allowing them to take advantage of the high plankton production in spring and early summer when juvenile anchoveta reach high growth rates (Cubillos et al. 2001); and (3) winter spawning may be favored as result of reserves accumulated in late summer for winter reproduction (Hunter & Leong 1981). All 3 of these hypotheses involve different pro-
cesses in the life history of the fish, but they all finally rely on the premise that acceptable offspring survival can be achieved during the winter spawning months. Since the feeding environment seems not to be the best for young larval success (less food available than in spring and summer upwelling months, higher water column turbulence due to winter storms), the parental population has to provide mechanisms to facilitate egg and young larval survival under these environmental conditions. Accordingly, maternal influences such as those reported in the present study should not be surprising, especially in areas close to the species’ southern distribution limit.

In the present study, we were interested in assessing early life-history characteristics that vary along the species distributional range in response to latitudinal changes in environmental conditions. Also, we were interested in determining whether differences exist in the early life-history traits of individuals spawned in different months of the spawning season. Our overall results from this and previous studies identified several egg and larval characteristics that vary between populations located at different latitudes. In some cases, these characteristics were not independent of each other, but were derived from an initial difference in egg volume. Additionally, our results showed that differences also existed in the biochemical characteristics present in the egg stage, such as lipids. When considered within the context of the normal environmental conditions faced by the population at different latitudes, the importance of these early life-history traits becomes more apparent. High turbulence, low temperature, and lower food availability are typical environmental conditions of early winter in the southern population. In other words, larger egg sizes and increased lipid contents, with the consequent reduction in batch fecundity, seem to be the mechanisms by which the survival of young offspring is facilitated in the more adverse southern, winter environmental conditions. These egg characteristics are tailored to the reproductive system of the adult female, and, as such, these characteristics represent one clear type of maternal influence on the early life-history traits of the anchovies that might enable rapid changes in population densities in years of improved habitat conditions, either for females or young stages.

Acknowledgements. Funding for the present study was provided by FONDECYT (Grants 1030819 and 1070502 to L.R.C. and G.C.). We acknowledge M. Braun (IFOP) and L. Cubillos (U. de Concepción) for providing the samples of hydrated females for partial fecundity estimations. A.L.-R. and M.C.K. were supported by the Graduate School of the Universidad de Concepción, and M.C.K. is currently the beneficiary of a CONICYT Doctoral Fellowship. We acknowledge collaboration in different phases of this research from the following: M. Gonzalez (MS) of the Department of Human Immunology, U. de Concepción, for laboratory facilities; Drs R. Quinones and R. Gonzalez for sharing equipment at the Coastal Marine Station in Dichato, U. de Concepción; Dr. E. Tarifeño for valuable comments made during the study; S. Soto and students from LOPEL and U. Arturo Part for help in the field work; Servicio Hidrografico y Oceanografico de la Armada (SHOA) for SST data; Dirección Meteorológica de Chile for wind data; the crew of the RV ‘Kay Kay’, and J. Marileo for maintaining the facilities at the Coastal Marine Station in Dichato, U. de Concepción.

LITERATURE CITED

- Bagenal TB (1971) The interrelation of the size of fish eggs, the date of spawning and the production cycle. J Fish Biol 3:207–219
- Castro LR (2001) Studies on environmental conditions and larval survival of the southernmost anchoveta stock of the Humboldt Current during the winter spawning season. GLOBEC Int News 7:15–17
- Ciechomska J (1973) The size of the egg of the Argentine anchovy Engraulis anchoita in relation to the season of the year of spawning. J Fish Biol 5:393–398
- Clarke TA (1989) Seasonal differences in spawning, egg size, and early development time of the Hawaiian anchovy of nebu, Engraulis chinensis purpurea. Fish Bull (Wash DC) 87:593–600
- Cubillos LA, Arcos DF, Bucary DA, Canales MT (2001) Seasonal growth of small pelagic fish off Talcahuano, Chile (37°S, 73°W): A consequence of their reproductive
strategy to seasonal upwelling? Aquat Living Resour 14: 115–124

Daoulas C, Economou AN (1986) Seasonal variation of egg size in the sardine, Sardina pilchardus Walb., of the Saro

hyppoglossus) eggs from planktonic sampes in northern Norway. Mar Biol 101:553–556

Fraser AJ, Gamble JC, Sargent JR (1988) Changes in lipid content, lipid class composition of developing eggs of cod


Hernández E, Castro L (2000) Larval growth of the anchoveta Engraulis ringens during the winter spawning season off central Chile. Fish Bull (Wash DC) 98:704–710

Fish Bull (Wash DC) 77:641–652

(Wash DC) 79:215–230


Ware DM (1977) Spawning time and egg size of Atlantic mackerel, Scomber scombrus, in relation to the plankton. J Fish Res Board Can 34:2308–2315

Editorial responsibility: Otto Kinne, Oldendorf/Luhe, Germany

Submitted: September 22, 2007; Accepted: January 8, 2009

Proofs received from author(s): March 31, 2009