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Malformations in the embryonic development of *Engraulis ringens* (Clupeiformes) in a spawning area off central–southern Chile: description and rates

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Variations in the rate of embryonic malformations of the anchoveta Engraulis ringens *in a spawning area off central–southern Chile were assessed. Eggs in stages of development subsequent to blastodisc formation were collected and preserved. Several severe malformations were identified and grouped by: (1) proliferation of disorganized tissue in early and late developmental stages; (2) irregular blastopore closure; and (3) notochordal deformation, of which the second had the highest incidence among the embryos. The average rate of malformation was 5.64%, a value close to that reported for other Clupeiformes and below the rate of 10%, indicating the influence of purely endogenous factors. The expected relationship between temperature variations and malformation rates was not observed, probably because the natural temperature range experienced by the embryos was above that at which severe malformation had previously been observed in the laboratory. The relationship between egg size and malformation rates over the spawning season was not determined; however, a relationship between egg size and malformation rates was detected among the samples from each sampling date, which suggests that malformations may be attributable to endogenous factors rather than to environmental factors acting directly on the eggs in the plankton.*

Keywords: anchoveta, central–southern Chile, embryo, malformation rate

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INTRODUCTION

The early mortality of eggs and larvae among marine fish species is high. At the intraspecific level, mortality rates due to exogenous and endogenous factors are both temporally and geographically variable (Bunn *et al.*, 2000). Exogenous sources of mortality include environmental biotic factors, such as predation, and abiotic factors, such as temperature fluctuations, abrupt changes in salinity and transport to unsuitable areas for development, among others. Endogenous sources include inherited genetic abnormalities or a reduction in egg quality originating from the parental condition prior to spawning. However, there is usually no single source of natural mortality in these stages, but rather interactions among these sources. Independent of their origin, the presence of embryos with malformations is a natural characteristic of the majority of fish populations, including in the absence of the aforementioned exogenous factors (Makhotin *et al.*, 2001).

The incubation of eggs collected from the natural environment, which include both healthy embryos and those with alterations in their development, allows establishment of the relationship between the frequency of malformations, hatching rates (egg mortality) and variations in larval mortality (Von Westernhagen, 1988; Avery *et al.*, 2009). This relationship indicates that malformations can have direct repercussions on the viability of embryos and on their natural mortality. However since it is not possible to achieve this type of experiment in all scenarios, a description and quantification of abnormalities in the natural environment would be valuable as a first phase.

Most studies evaluating malformations in natural fish populations (Grauman, 1982; Cameron *et al.*, 1996; Dethlefsen *et al.*, 1996) are based on the four-stage scale proposed by Von Westernhagen (1970), who carried out the first estimations of malformations in species such as cod (*Gadus morhua*) and flatfish (*Pleuronectes platessa, Platichthys flesus*) in the Baltic Sea. This scale includes all the principal

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events occurring in early fish ontogeny and corresponds to: Ia. Early cleavages until the building of the blastodisc; Ib. Epiboly, the building of the embryonic shield and the axes; II. Differentiation of the embryo, formation of the head and growth around the yolk (up to 180°); III. Embryo between 180° and 270° around the yolk with further differentiation, heart beat, further expression of the sense organs, primordial fins present; and IV. Embryo between 270° and 360° around the yolk, tail free, eye pigmentation and body movements. For different species and habitats the general trend is a decrease in malformation rates as embryos progress in development, with higher values in the first stages of development (Cameron *et al.*, 1992).

The anchoveta *Engraulis ringens* Jenyns (Clupeiformes, Engraulidae) is a heavily exploited neritic coastal fish species with a short life cycle, rapid growth rate and high rate of natural mortality (Cubillos *et al.*, 1999, 2002). This species inhabits a wide latitudinal range from 4°S to 42°S (Serra *et al.*, 1979), where differentiated spawning regions have been identified. An important anchoveta spawning area is located off the coast of central Chile, with high concentrations of eggs in the plankton (11–100 eggs.0.05 m^{-2}, Cubillos *et al.*, 2007) during the winter and early spring. In this area the superficial temperatures range between 12 and 15°C, then the egg development may take two to three days (Tarifeño *et al.*, 2008). Information in relation to egg mortality occurring in this period is scarce. The only evaluation, for the 1995–1996 spawning season, found a mean daily egg mortality of 91.4%, which is much higher than the rate estimated for the larval stage (Castro & Hernández, 2000).

The anchoveta is characterized by planktonic eggs (0.31–0.36 mm^3) whose development concludes with the hatching of labile larvae less than 3 mm in length (Llanos-Rivera & Castro, 2006). The eggs have a transparent chorion, which facilitates observation and the identification of deviations from normal embryonic development. Another biological trait of the anchoveta is seasonal variation in size and quality of eggs, as measured by rates of successful hatching, with the largest and best quality eggs occurring at the beginning of the spawning season (Castro *et al.*, 2002, 2009).

There are few studies in Chile that evaluate the frequency of embryonic malformations of native fish in the natural environment and even fewer that assess the incidence of malformations on the subsequent embryonic viability. Based on samples of ichthyoplankton collected in the area between the mouth of the Itata River and Coliumo Bay in the spawning zone of the anchoveta off central Chile, the present study seeks: (i) to characterize embryonic malformations in *Engraulis ringens*; (ii) to determine the frequency of malformations during the different stages of embryonic development; and (iii) to evaluate variations throughout the reproductive season of this species.

MATERIALS AND METHODS

Study area

Sampling of ichthyoplankton was carried out from May through to December 2007 on-board the research vessel 'Kay Kay II' of the Universidad de Concepción, at three oceanographic stations located between the mouth of the Itata River and Coliumo Bay (36°36′–36°49′S, 72°90′–72°96′W, Figure 1), an area characterized by a high abundance of anchoveta eggs and larvae (Cubillos *et al.*, 2007). In each station the zooplankton sampling were realized with a Bongo net (300 μm mesh size, 60 cm mouth diameter, equipped with a flowmeter), by gentle oblique towing from 30 m deep to the surface. Given the high sensitivity of early developmental stages of fish to mechanical stress, the velocity of the sampling was kept below 1.5 knots (Cameron *et al.*, 1992). Once on-board, the complete sample obtained from one cod-end of the net was diluted in black plastic buckets (30 l) with seawater from the same sampling area (live sample) and transferred rapidly (less than two hours) to the Marine Biology Laboratory of the Universidad de Concepción. The sample from another cod-end of the net was preserved on-board for later analyses (preserved samples). Information on oceanographic conditions was obtained from each sampling station through CTD casts (SBE 19 plus).

Description of malformations in embryonic development

Detailed observations and descriptions of normal embryonic development and malformations at different stages of development were made from the live eggs collected in the field (live samples). Development was considered defective if stages deviated from normal development and morphological differentiation. The description of normal embryonic development of *Engraulis mordax* by Moser & Ahlstrom (1985) was used as a reference, as it is a more detailed description than that of Fischer (1958) for *Engraulis ringens*.

In addition to the descriptions of malformations present in the samples, normal eggs and eggs with malformed embryos were separated and incubated under controlled conditions. To do this, each egg was individually placed in a 10 ml culture chamber with 8 ml of seawater (filtered 0.5 μm, UV), kept in an environmentally controlled incubator room at a constant temperature of 12°C, with a photoperiod of 12 h light and 12 h darkness. To verify embryonic viability, observations were made every 6 h under a stereomicroscope (40×) equipped with a Canon camera.

Effect of preservation on embryonic development and estimating the malformations rate

Malformation rates of ichthyoplankton samples were estimated by observing samples preserved in formaldehyde. Klinger & Van den Avyle (1993) indicated that this fixative at 5% does not cause deformation of fish embryos. Nevertheless, it was necessary to assess the effect of 5% formaldehyde on the embryonic morphology of the anchoveta. To do this, the following experimental protocols were implemented in order to discount the possible sources of bias in the estimation:

1. Live eggs classified as either normal or abnormal were ordered by stage, photographed and then preserved individually in 5% formaldehyde. The samples were reviewed after one month for possible morphological alterations and photographed again. This procedure determined whether the fixative itself induced abnormalities in

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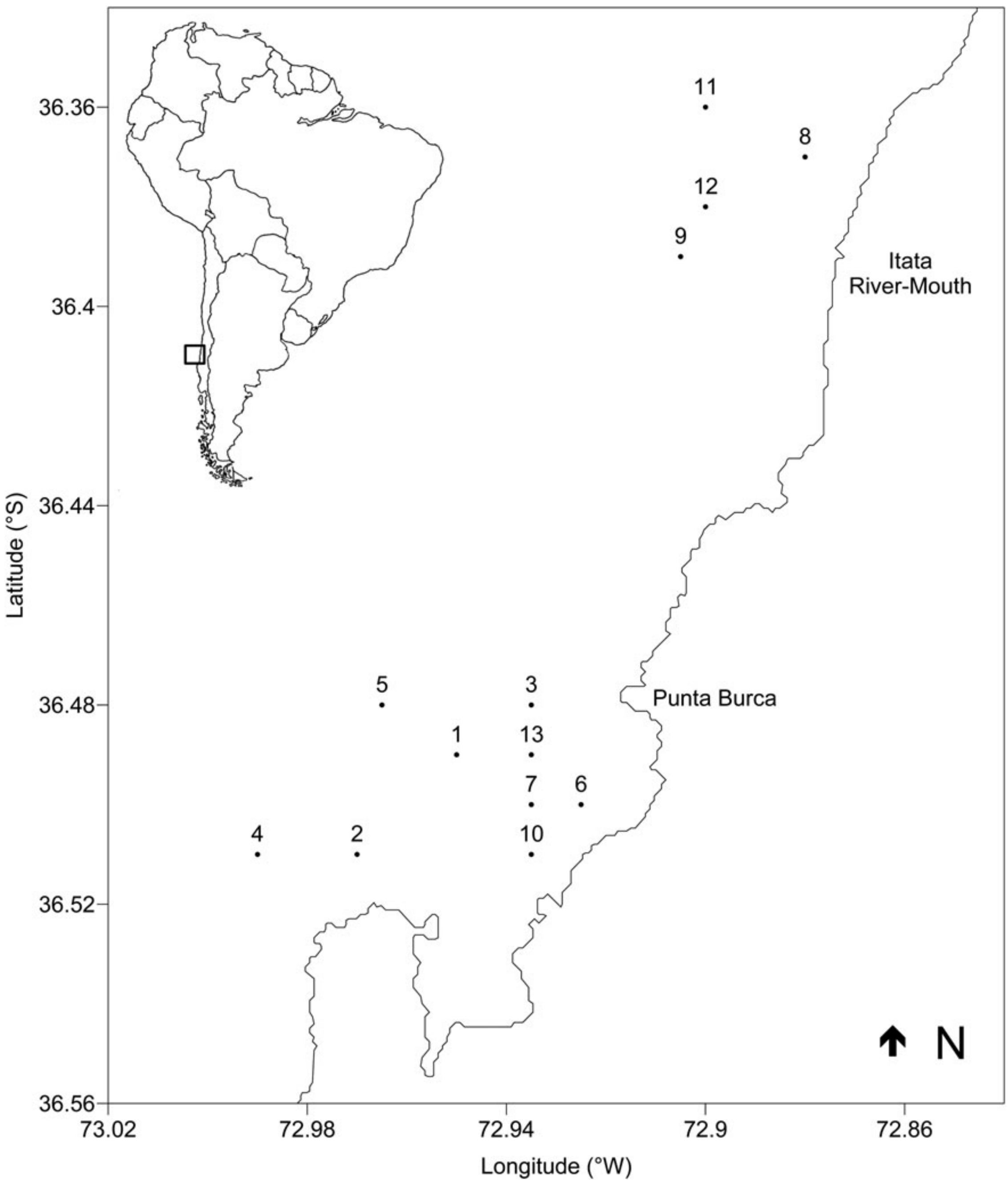


Fig. 1. Area off central Chile sampled during 2007. The numbers correspond to each sampling; further details are presented in Table 1.

healthy embryos or imposed difficulties in sorting abnormal eggs.

2. To complement this, and to verify the general level of possible differences in estimated frequencies between live and preserved samples, the percentages of abnormal eggs in the samples from May and June 2007 were compared. For both dates, a sub-sample was examined from both the live and fixed replicates.

Temporal and spatial variation in the malformations rate

Each of the monthly samples of ichthyoplankton preserved on-board was analysed in the laboratory with a stereomicroscope (40×) equipped with a Canon camera. The anchoveta eggs were identified separately and a sub-sample was extracted, with varying numbers of eggs depending on their total abundance. Counts were made for both healthy and anomalous eggs at each stage of development. In addition, a sub-sample of eggs with normal and abnormal embryos was photographed and then, using the software Image J, the longest (a) and shortest (b) axes of each egg were measured and the egg volume calculated using the following equation for ellipsoids: $(4/3)\pi ab^2$. Egg size was recorded for all sampling dates except in June, when only normal eggs were measured.

To compare the percentage of estimated malformations in live versus preserved samples among sampling months, stage of development and egg size, the contingency test was applied using χ^2 statistics with PAST software (Hammer *et al.*, 2001). The other statistical analyses (*t*-test, ANOVA, regression) were carried out with Statistica 6.0 (StatSoft, 2004).

RESULTS

Malformations in embryonic development

The embryos obtained from plankton just a few hours after fertilization allowed for the observation of stages after the formation of the blastodisc (Stage III). Starting from this stage, several developmental abnormalities were identified, the most severe being in the early stages of development and occurring mainly in processes of cellular proliferation and organization that give rise to the embryo. These malformations were described, with emphasis on deviations from the normal pattern of embryonic development reported by Moser & Ahlstrom (1985) for *Engraulis mordax*. The same embryos, live and formaldehyde preserved (Table 1), were used to illustrate the abnormalities.

Embryos that develop abnormalities in phases III to VII exhibit retarded growth leading to the subsequent death of the egg. Embryos with abnormalities arising in Stages VIII to XI can continue their development, but the resulting anomalous larvae die during hatching.

Effect of preservation on embryonic development and estimation of malformations rate

No alterations were observed for any developmental stage, for normal or abnormal embryos, as a result of preservation in 5% formaldehyde (Table 1). The only perceptible effect was an increase in the perivitelline space and the loss of transparency in the embryonic tissue, making the detailed description of the abnormality more time-consuming.

The comparison of the number of abnormal embryos in live and preserved samples in May and June 2007 (Figure 2) indicated that there were significant differences in the estimations in only one of the two sampled stations in June ($\chi^2_{25.05.07} = 1.68$, $P = 0.13$; $\chi^2_{25.06.07(E1)} = 23.52$, $P < 0.001$; $\chi^2_{25.06.07(E2)} = 2.70$ $P = 0.10$). At that single station, the samples preserved in 5% formaldehyde had a significantly lower percentage of malformations (4.9%) than those identified in the live sample (malformation rate of 13.7%, Figure 2).

Temporal and spatial variations in the malformations rate

The abundance of anchoveta eggs was variable among the sampled months and stations, and there were occasions when no eggs were found in the plankton. Mean incidence of embryonic abnormalities in the natural environment was 5.6% ± 3.7 (SD) and was not constant in the different stages of embryonic development (Figure 3). The highest malformation rate was concentrated in the early Stages III and IV (20–30%), followed by the Stages VII and VIII (20 and 15%, respectively). The mean value per stage was 10% (±11.6 SD), with a variation range between total absence of malformations in Stage XI and 31.1% in Stage IV (Figure 3).

Malformation rates were variable throughout the reproductive season of the anchoveta. Contrary to expectations, no trend was observed over time in terms of significant increases or decreases in the percentage of malformations (Figure 4), although there were significant differences in the presence of abnormal embryos among sampling dates ($P < 0.001$). Correlating these values with surface temperature at sampling time, an inverse linear trend was observed but it was not significant ($F_{(1,11)} = 0.57$, $P = 0.46$). In effect, environmental temperature only explained 5% of the temporal variability in the percentage of malformations. Similarly, no relationship was detected between surface salinity and malformation rates ($F_{(1,2)} = 2.72$, $P = 0.241$). With respect to the spatial scale, the analysis indicated that there was no spatial trend in the malformations rates (Table 2). Stations with over >5% of the eggs showing malformations were found both near the mouth of the Itata River and off Coliumo Bay, an area of reduced influence of riverine freshwater discharges, particularly in 2007, a La Niña year with very low rainfall.

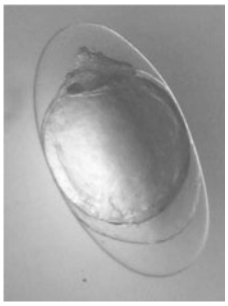
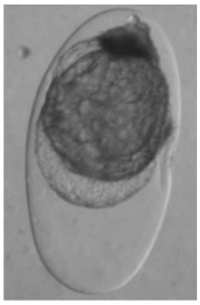
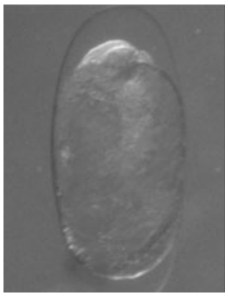
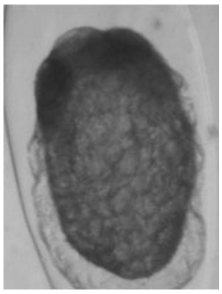
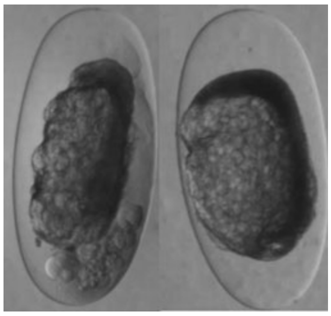
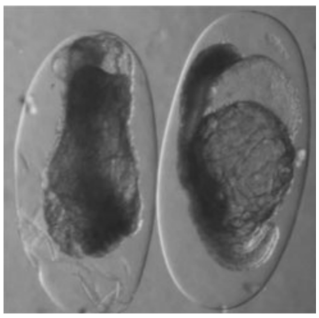
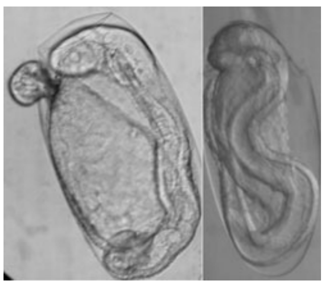
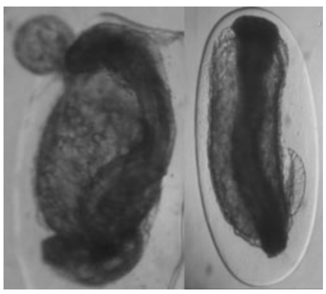
The anchoveta egg size during the sampling period varied significantly among months ($F_{(4,912)} = 14.47$, $P < 0.001$, Figure 5), with the highest values (May) prior to the main spawning period (July–September). Pooled data indicated statistical differences in volume between normal (0.349 mm^3) and abnormal (0.339 mm^3) eggs (*t* value = 3.80, $P < 0.001$), with abnormal eggs being smaller than the normal ones, except in December (Figure 5).

DISCUSSION

This study evaluated the incidence and type of natural malformations in anchoveta (*Engraulis ringens*) embryos during the spawning season in the area off central Chile. The malformations observed were similar to those described in other species, and the frequencies were in the range reported for other coastal areas. This is the first report about malformations in this spawning area, and it could be particularly useful for future monitoring of the study area to determine, for instance, whether malformation rates are related to changes in environmental quality, such as changes in hydrographic conditions or the presence of xenobiotics that could alter embryonic development and increase the percentage of malformations in samples from natural environments (Bodammer, 1993; Von Westernhagen & Dethlefsen, 1997; Von Westernhagen *et al.*, 2001).

In this study, embryos from plankton allowed observation of stages subsequent to the formation of the blastodisc. Several malformations were identified and described that were consistently present throughout the sampling period. In general, these were more severe in the early phases of development; a trend observed in other species of clupeiforms (*Sprattus* and *Sardina*) and considered a generalized condition in fish embryos (Cameron & Berg, 1992; Cameron *et al.*, 1992). The malformations observed in anchoveta were similar to those of other fish species, which can be grouped by: (1) the proliferation of disorganized tissue (bubbles) in early and late stages of development; (2) the irregular closure of the blastopore; and (3) the deformation of the notochord (Cameron *et al.*, 1992). The latter two are produced during key periods in embryonic development, such as epiboly, which involves the advance of the germinal disc and tail elongation (Kunz, 2004).

Table 1. Description of normal and abnormal embryonic development of *Engraulis ringens*. Photographs correspond to same egg after and before formalin preservation.

Stage	Description	Malformation	Live	Preserved
III	The segmentation cavity arises in this phase (a space formed between the blastodisc and the yolk mass); the blastoderm is observed as tissue rather than as a group of individual cells. The edge of the blastodisc swells and forms the germinal disc that, upon extending inwardly, forms the embryonic shield that defines the future embryo axis	In abnormal development, cellular proliferation is observed without the organized formation of the blastodisc, giving rise to an undifferentiated apical cell cluster. The cellular divisions are incomplete and irregular, leading to deformations of the blastomere and abnormal shapes of the blastodisc		
IV	At the beginning of this stage, the germinal disc covers approximately a third of the yolk and the embryo is starting to form. At the end of this stage, the germinal disc covers two-thirds of the yolk and the head region is already visible	The anomaly observed corresponds to retardation in the development of the embryo, as a result of which the lengthening of the germinal disc is not observed. This anomaly can also be accompanied by atypical apical cellular proliferation		
V	This stage begins with the germinal disc covering over two-thirds of the yolk and ends with closure of the blastopore	Disorganization of the caudal region is observed in anomalous embryos. In contrast to the previous stage, there is advancement of the germinal disc, but formation of the caudal region is not complete and the subsequent closure of the blastopore does not occur. In some cases, the embryonic axis is unstructured and incomplete		
VII	At the beginning of this stage, the free tip of the embryo's tail begins to separate from the yolk. During this stage the tail initially appears rounded and then thin and elongated. At the end of this stage the free end of the tail is half the length of the head of the embryo (distance from the tip of the mouth to behind the cerebellum)	These characteristics are observed in anomalous embryos, but notochord development is abnormal, with the embryonic axis twisted, unformed or without formation of the tail and scarce cephalic differentiation		
VIII–XI	These stages are characterized by the degree of advancement in the length of the embryo tail, from the upper half of the head to three-quarters of the length of the yolk sac. The organogenesis of the embryo is gradually completed (sense organs, the outlines of the pectoral fins, etc.)	In anomalous embryos, the tail does not elongate parallel to the embryonic axis, but is instead twisted in the caudal region and different degrees of notochord twisting can also be observed. In some cases this can be accompanied by the rupture of the chorion and evagination of the yolk		

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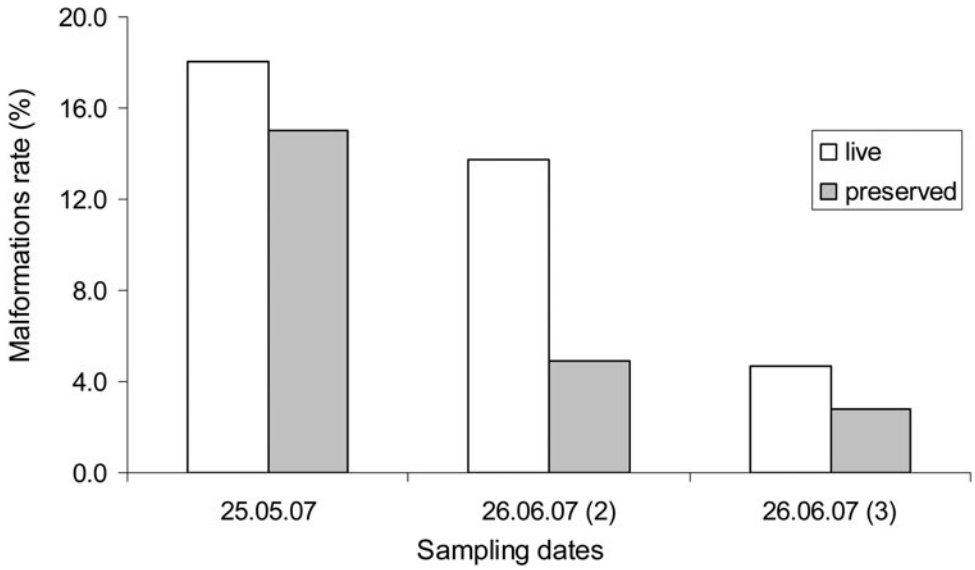

Fig. 2. *Engraulis ringens* malformation rates estimated from live and formaldehyde preserved ichthyoplankton samples. Data correspond to the two stations sampled in May and June of 2007.

Most studies evaluating malformations in fish are based on the four-stage scale proposed by Von Westernhagen (1970), who carried out the first estimations of malformations in species such as cod (*Gadus morhua*) and flatfish (*Pleuronectes platessa, Platichthys flesus*) in the Baltic Sea. Within this stage-scale, the stage with the highest frequency of malformations is the second, which includes processes such as the differentiation of the embryo before closure of the blastopore until it encircles the yolk sac. This embryological process is included in Stages IV to VII considered in this study; which coincidentally exhibit the highest malformation rates in the anchoveta. The success of early embryonic development stages (prior to the closure of the blastopore) is believed to be associated with the parental condition, considering that all the physiological and reproductive traits of the parents affect the quality of the progeny (Cameron *et al.*, 1992; Schreck *et al.*, 2001). Moreover, alterations in later stages, especially notochordal torsion, have been

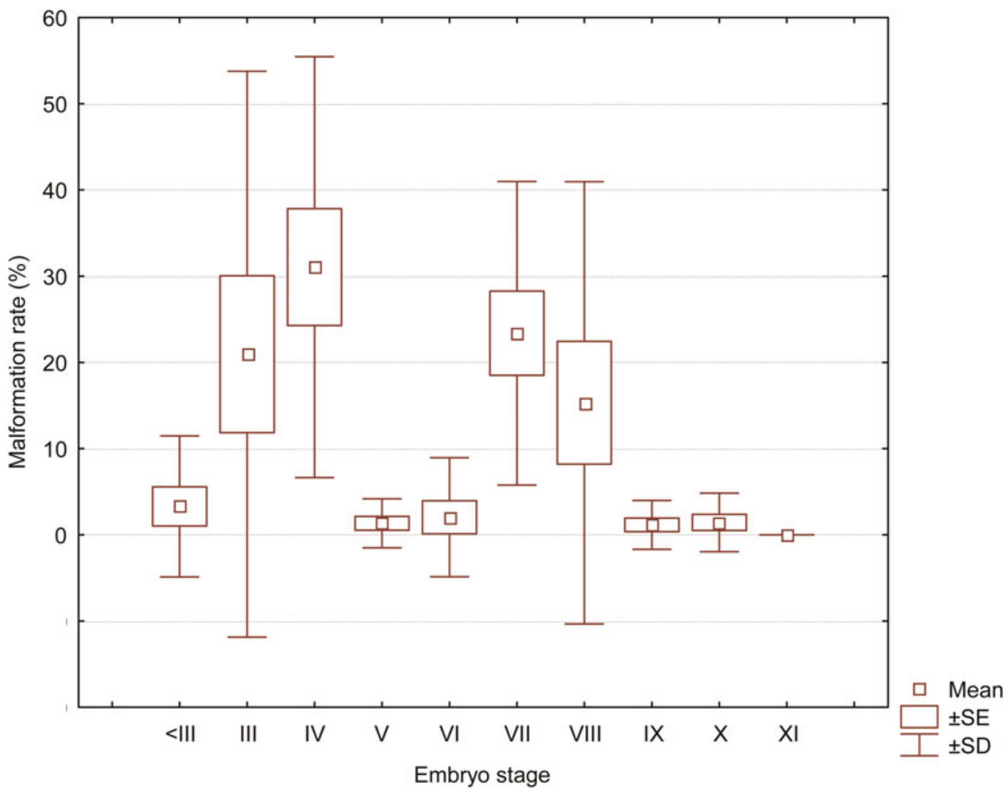

Fig. 3. Malformation rates (%) at different stages of the embryonic development of *Engraulis ringens:* values are calculated means considering all samples.

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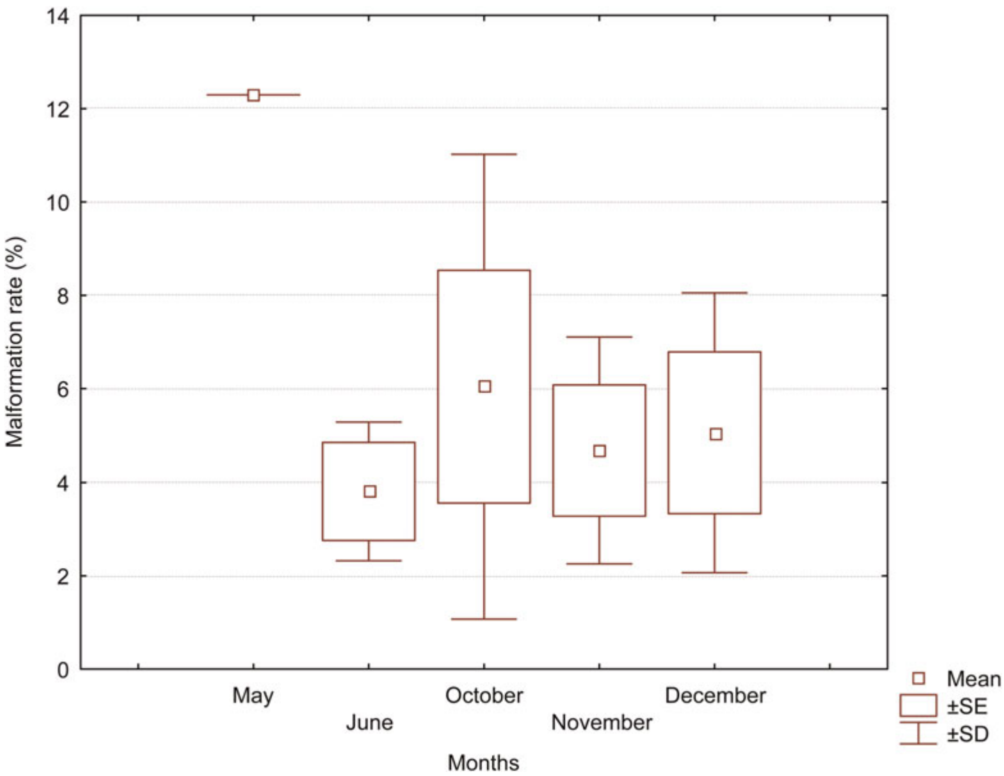

Fig. 4. Variations in malformation rates during the spawning season of *Engraulis ringens* off central–southern Chile.

described in embryos incubated under extreme environmental conditions, such as low salinity levels (De Ciechomski, 1966).

Analysis of live samples is generally recommended for detecting malformations over the course of embryonic development given that the translucence of embryonic tissue allows for a better appreciation of the changes that occur (Cameron *et al.*, 1992). However, the recommended approach to analysis, which includes identifying, sorting and observing numerous samples, requires long periods of observation by specially trained personnel. Consequently, an alternative is the examination of preserved samples that were collected by means of low velocity trawls to avoid injury by mechanical stress (De Ciechomski, 1966). Our results concur with those of Fischer (1958) in that the only perceptible effect of 5% formaldehyde was an increase in the periviteline space and some tissue opacity. Comparison of the estimated number of embryonic malformations in live samples and samples preserved in buffered 5% formalin indicated that preserved material could result in underestimates of the incidence of malformations. However, in only one of the sampled stations (June) was there a statistically significant difference in these estimations. This suggests that while observation of live samples is ideal, preserved samples can provide a good estimation of the incidences of malformations present in the natural environment, especially when the research focus is evaluating variations over time or spatial scales.

Overall analysis of the results indicates that the average malformation rate for the anchoveta in central Chile is 5.6%, which is close to the rates reported for other clupeiform species. In uncontaminated water, malformations reached 5%

Table 2. Sampling dates, surface temperature, number of eggs analysed and malformation rates in *Engraulis ringens* during 2007.

Sampling number*	Date	Surface temperature (°C)	Number analysed	Normal (%)	Abnormal (%)
1	25 May 2007	10.0	122	87.7	12.3
2	25 June 2007	9.0	576	95.1	4.9
3		10.0	580	97.2	2.8
4	10 October 2007	11.5	1279	99.2	0.8
5		11.8	6780	96.9	3.1
6	19 October 2007	11.0	332	88.6	11.4
7		11.0	112	91.1	8.9
8	26 November 2007	13.5	184	94.0	6.0
9		14.0	1859	98.1	1.9
10		14.0	178	93.8	6.2
11	11 December 2007	11.0	188	97.9	2.1
12		11.5	74	91.9	8.1
13		12.0	3378	95.1	4.9

*, the numbers correspond to sampling locations in Figure 1.

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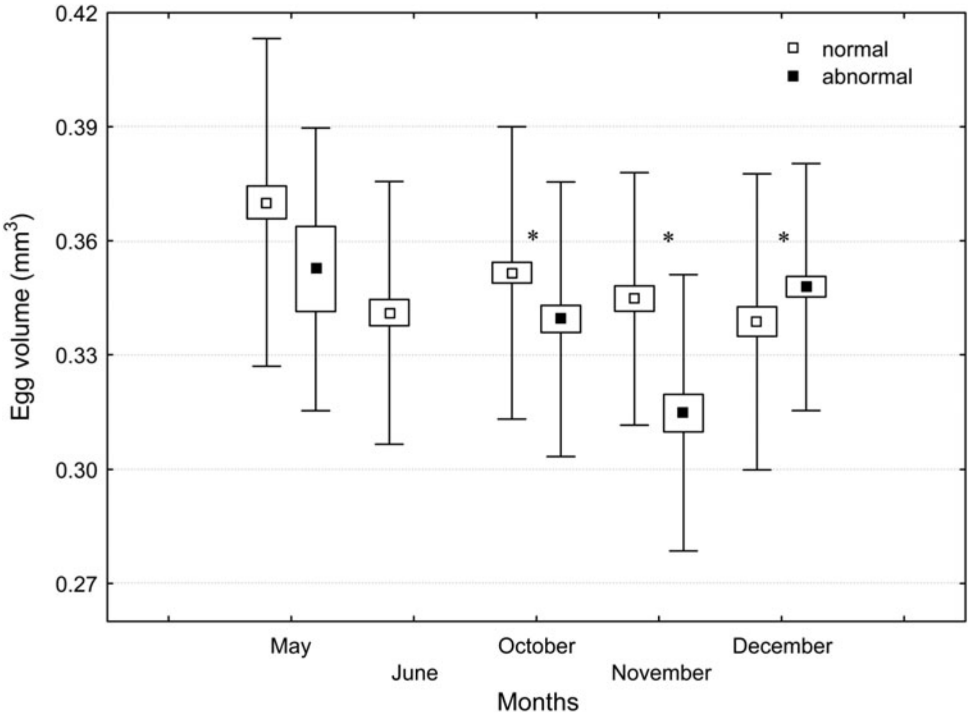

Fig. 5. Variation in the volumes (mm³) of normal and abnormal eggs of *Engraulis ringens* during different months of the spawning season off central–southern Chile. *, significant differences at 0.05 level between volumes of normal and abnormal eggs.

in *Anchoviella* (Engraulidae), 12% in *Sardina pilchardus* (Klumpp *et al*., 2002) and 9% in *Engraulis encrasicolus* (Yannoupulos & Yannoupolus, 1981). Studies in marine waters without contaminants, suggest that rates under 10% are attributable to purely endogenous factors (Klumpp & Von Westernhagen, 1995).

There are few reports on malformation rates and their relationship to the reproductive season. Our study was conducted in the months before (May, June) and after (October–December) the main anchoveta spawning season (July–September). Malformation rates were variable during the sampled period, with the highest value (12%) in May. There was no evident difference between these two periods. A seasonal variation in the size and quality of eggs has been described in anchoveta, with larger and better quality eggs at the beginning of the spawning season between July and August (Llanos-Rivera & Castro 2004; Castro *et al*., 2009). Accordingly, our expectations were that the incidence of malformations would increase towards the end of the spawning season, but this was not observed as the highest malformation rate was prior to the beginning of spawning period in this area (Cubillos *et al*., 1999) which might be explained as the product of the final portion of females spawning in the previous period (2006–2007). Nevertheless, an interesting result in relation to egg quality was that within each month the anomalous embryos were those from smaller sized eggs, indicating that the presence of abnormalities could be due to a parental effect in the sense that the egg size and quality is determined during oogenesis in the female gonad prior to the oocyte hydration and the spawning event (Leal *et al.,* 2009) and hence, they could be considered the product of an endogenous factor.

Another factor that has been recognized as inducing embryonic malformations is temperature (Koo & Johnston, 1978; Wiegand *et al.,* 1989), an environmental factor that can also enhance the effect of other deleterious effects such as the presence of contaminants. In *Limanda limanda,* for instance, a relationship was found between the presence of anomalies and the synergic effect of contaminants and temperature, which were explained as variations in environmental temperature making embryos more vulnerable to the impact of contaminants. In this species, a fraction of the inter-annual variations in malformations (between 6% and 21%) was explained by the combined action of contaminants and temperature (Von Westernhagen & Dethlefsen, 1997). Tarifeño *et al.* (2008) suggested that the optimum temperature along the Chilean coast for normal anchoveta embryo development was about 15°C. They also reported detrimental effects on embryos reared at temperatures lower than 10°C. In their study, embryo development was arrested at Stage III, larval hatching did not occur at 5°C, and at 8°C only some embryos started their development but only reached Stages VIII–X without successful hatching. Furthermore, the embryos exhibited severe malformations (e.g. twisted notochord, disaggregated yolk) indicating abnormal and unsuccessful development. Our results did not indicate a significant relationship between malformation rates and surface temperature, despite the fact that the surface temperatures registered in our study (ranging from 9°C to 14°C) were below the optimum temperature for embryo development. Comparison of malformation rates in embryos from the two sampling locations (near the mouth of the Itata River and the vicinity of Coliumo Bay) did not indicate that values of temperature and salinity we observed, constitute important environmental factors inducing deformations because both locations showed similar malformation values despite their contrasting freshwater influence. The potential negative effects of low temperature or low salinity values on malformations of anchoveta embryos cannot be identified either considering that temperature of the water column during the winter and spring 2007 were lower and salinities were higher than normal years due to the presence of La Niña event over the region (Castro *et al.*, 2010, Iriarte *et al.*, 2012). Experimental studies conducted independently during the same spawning season, 2007 reported malformation rates in winter lower (July 2007 = 2.6%, August 2007 = 1.4%, Krautz *et al.,* 2013) than those observed by us in this study in May when the temperature was higher, supporting the hypothesis that the low temperature values observed this year in this region did not increase malformation rates in the region.

In summary, the results of the present study indicate that the average malformation rate in the anchoveta in the sampled area was below 10%, within the range reported for other species. The expected relationship between temperature variations and malformation rates was not observed, probably because the natural temperature range experienced by the embryos in our study was above that at which severe malformations have been observed in the laboratory. We were unable to determine the relationship between egg size and malformation rates over the spawning season as our samples corresponded to the month prior to and after the main spawning season. However, a relationship between egg size and malformation rates was detected in each sampling period, which suggests that malformation could be attributable to endogenous factors (maternal effect) rather than to direct environmental factors acting on the eggs in the plankton.

In conclusion, the type of malformations observed in the anchoveta were consistent with those described for other fish species. The average anchoveta malformation rate in the sampled area was below 10%, within the range reported for other species in pristine areas. A relationship between egg size and malformation rates was found among the samples for each sampling date, which suggests that malformations could be attributable to endogenous factors rather than to environmental factors acting directly on the eggs in the plankton.

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