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The effects of environmental factors on the embryonic survival of the Patagonian squid *Loligo gahi*

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Abstract

Seawater temperature and salinity are environmental variables that impose physiological limits for the embryonic development of marine invertebrates. For cephalopod species, these limits have rarely been established. This work presents experimental results on the embryonic survival of the Patagonian squid *Loligo gahi*, which is the last decades' most important loliginid species in terms of volume of commercial catches worldwide, as a function of seawater temperature and salinity. Reference magnitudes of surface seawater temperature and salinity within the area of distribution of the species were explored by analysis of satellite databases and published information. Embryos were incubated under eight constant regimes of temperature within 4–22 °C and four constant salinity regimes within 20–34.33‰ (12 °C). Also, to determine the effects of sudden temperature changes on embryonic survival, embryos were incubated at four variable regimes of temperature, with thermal shifts (6-day long 2-°C magnitude alterations of the incubation temperature) applied both at early and late stages of embryonic development. Embryonic survival was zero in incubations at constant temperature regimes ≤5° and at 22 °C, low at 6 °C, and high within 8–20 °C. A function was fitted by nonlinear regression to relate embryonic survival and mean incubation temperature. Thermal shifts applied in incubations at 20–22–20 °C variable regime of temperature provoked low embryonic survival compared to that observed in incubation at 20 °C constant regime. Embryonic survival was zero in incubations conducted at 20.0‰ and 34.3‰ salinity, and high at 26.4‰ and 32.8‰ salinities.

Keywords: Embryology; *Loligo gahi*; Patagonia; Salinity; Survival; Temperature; Thermal shift

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1. Introduction

The Patagonian squid, *Loligo gahi*, is a neritic species distributed in the South East Pacific Ocean from Perú (6°S) to Tierra del Fuego (55°S), and in the South West Atlantic Ocean from Tierra del Fuego to coastal (36°S) and slope (38°S) waters off Argentina (Castellanos and Cazzaniga, 1979; Roper et al., 1984; Vigliano, 1985; Cardoso et al., 1998) (Fig. 1). This species has an annual life cycle (Hatfield, 1991) throughout which individuals undertake off-shore and on-shore migrations (Castellanos and Cazzaniga, 1979; Chesheva, 1990; Hatfield et al., 1990; Rodhouse et al., 1992; Portela et al., 1994). Evidence obtained so far suggests that spawning occurs in coastal and inner-shelf waters off the Argentine Patagonia, Falkland (Malvinas) Islands and Chile (Hatfield and Rodhouse, 1994; Arkhipkin et al., 2000; Guerra et al., 2001; Barón, 2001). Females stick their egg masses to seaweeds, ropes and other hard structures resting on the sea bottom (Arkhipkin et al., 2000; Barón, 2001). These are formed by several cylindrical capsules joined to each other and to the substrate at their bases. Each one includes a spiral array of eggs covered by several gelatinous layers (Fig. 2). After hatching, paralarvae and juveniles move from near-shore spawning grounds to feeding grounds located on the continental shelf and slope, while mature individuals return to the coast to mate and spawn (Hatfield and Rodhouse, 1994; Arkhipkin and Middleton, 2002).

In the South West Atlantic, high commercial catches (~ 70.000 tn in 2000, FAO, 2002) make this species a valuable resource. Historically, the fishery has operated around the Falkland (Malvinas) Islands (Hatfield, 1992), and more sporadically on the Argentinean shelf south of 42°S (Portela, 1992). During the last two decades since the beginnings of its

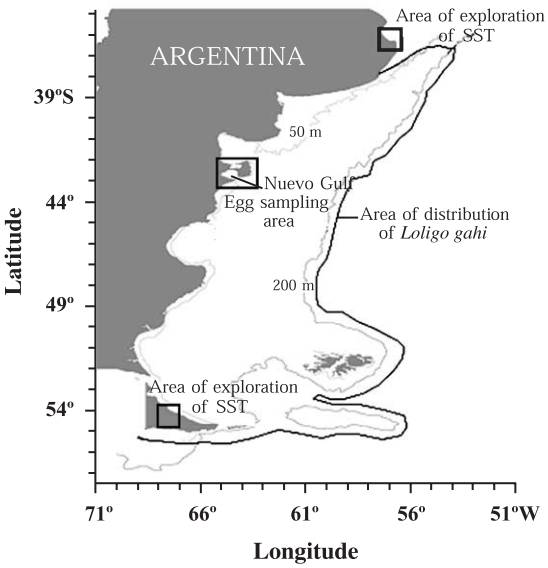

Fig. 1. Area of distribution of *Loligo gahi* in the South West Atlantic, areas of exploration of the surface seawater temperature and area of collection of egg masses.

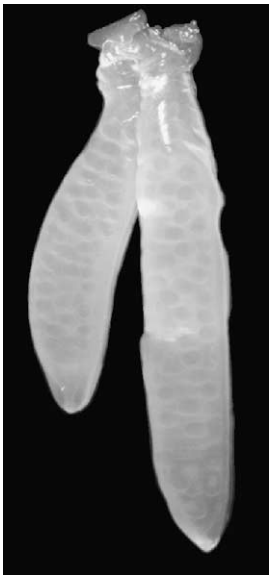

Fig. 2. Photograph of two egg capsules of *Loligo gahi*.

industrial exploitation (1983–2003), annual catches of *L. gahi* have shown wide fluctuations (Agnew et al., 2000; FAO, 2002), a feature that is common to other cephalopod fisheries and that has been related to environmental variability in a number of species (Roberts and Sauer, 1994; Waluda et al., 1999, 2001a,b; Sakurai et al., 2000; Sims et al., 2001). Seawater temperature and salinity have been shown to be among the most important environmental variables that may determine adult, paralarval and egg mass distribution, as well as embryonic and paralarval development and survival of several cephalopod species (Vecchione, 1981, 1991; D'Aniello et al., 1989; Fagundez and Robaina, 1992; Roberts and Sauer, 1994; Lima, 1999; Sakurai et al., 1996, 2000). Therefore, these variables may be particularly useful for the development of models to predict commercial catches (Agnew et al., 2000; Waluda et al., 2001a,b; Georgakarakos et al., 2002).

In marine waters, typical temperature-versus-depth profiles show a surface layer tens of meters thick, called the mixed layer, occurring because surface winds play an important role in keeping the water well mixed and maintaining a nearly isothermal condition (Knauss, 1978). If the water column is not too deep, tidal currents may produce turbulence in the bottom layers resulting in additional mixing of the water column. In this scenario, if strength of tidal current and wind stress are high, which is the case in most of the coastal Patagonian shelf (Glorioso and Flather, 1995), the mixed layer may extend to the bottom. Since current knowledge suggests that female *L. gahi* attach their egg masses to objects resting on the sea bottom in shallow waters (Hatfield and Rodhouse, 1994; Arkhipkin et al., 2000; Guerra et al., 2001; Barón, 2001), these might experience approximately the same temperature as that occurring at surface. In coastal waters off Falkland (Malvinas) Islands, current knowledge of *L. gahi* (Arkhipkin et al., 2000) suggests that highest concentrations of egg masses are associated to seawater temperatures of 7.5–7.8 °C and 33.8‰ of salinity.

Along the Patagonian coastline, Barón (2001) reported the finding of *L. gahi* egg masses at temperatures varying between 5 and 19.3 °C. Furthermore, Barón (2002) experimentally determined that embryos of this species develop normally between 5 and 20 °C. The aim of the present study was to establish the percent survival of the embryos of *L. gahi* as a function of different seawater temperature (constant and variable) and salinity (constant) regimes, based on controlled incubations in laboratory.

2. Materials and methods

2.1. Exploratory analysis of surface seawater temperature

To establish reference temperatures for experiments on the embryonic survival at different constant and variable temperature regimes, we explored the surface seawater temperature (SST) time series in two coastal areas (depth ≤50 m) of the Argentine Continental Shelf (ACS) using remotely sensed data. These areas were approximately 0.4° latitude by 0.4° longitude each, and were, respectively, located at the northern and southern extremes of the species distribution (35.8–36.2°S, 56.7–57.1°W and 53.8–54.2°S, 66.9–67.3°W, respectively, Fig. 1). For both areas, we obtained a 2-year period (1996–1997) daily average SST series, based on data downloaded from a 9 × 9 km spatial resolution satellite image database (NOAA/NASA, AVHRR Pathfinder). Then, we estimated SST for any given time of the year by least-square nonlinear regression of the function.

$$\mathrm{SST}(t) = T_0 + T_1 \cos[w(t - t_0)],$$

to the average SST series corresponding to each area, where T_0 is the annual mean temperature, T_1 is the amplitude of the seasonal cycle, $w = 2\pi/365.25\ \mathrm{day}^{-1}$, t is the time in days counted from 1 January and t_0 is the phase that coincides with the day of the year in which temperature is maximum (T_0, T_1 and t_0 are parameters to be estimated) (Rivas, 1994). Based on this function, we calculated SST anomalies as the difference between any observed SST and that estimated for a given day, and defined a "thermal deviation" as a continuous series of anomalies of the same sign (positive anomaly series: warming, negative anomaly series: cooling; Fig. 3). Therefore, every thermal deviation had assigned a magnitude, duration and sign. It should be noted that the actual duration and magnitude of a thermal deviation from the estimated SST(t) function may have been frequently underestimated because of the discontinuity in satellite SST data series due to cloudiness. Box plots were outlined with Statistica 5.0 to analyze the maximum magnitude of the thermal deviations in each given area as a function of their durations in days. Based on this analysis, we estimated the magnitude and duration of thermal deviations occurring in the field to be able to apply realistic thermal shifts in experiments with variable regimes of temperature. Due to physiological limitations imposed by temperature, we expected positive thermal deviations (warming) to affect embryonic survival at the northern (warmest) area of SST exploration, and negative thermal deviations (cooling) to affect embryonic survival at the southern (coldest) area. Therefore, for the northern area, we based our analysis on

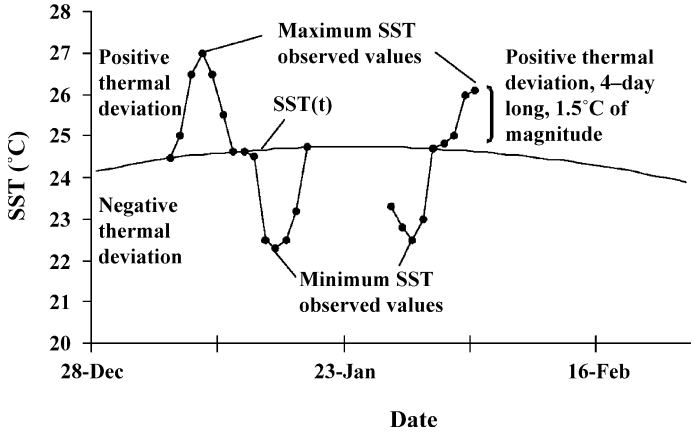

Fig. 3. Schematic representation of the sign, duration and magnitude of a thermal deviation of the observed SST with respect to that predicted by the SST(t) function.

maximum magnitudes of positive thermal deviations, and for the southern one, we examined negative thermal deviations.

2.2. Incubation experiments

Egg masses of *L. gahi* were obtained in March, June, September and October 2002 from artificial collectors (Fig. 4) deployed in Nuevo Gulf (42°50′S, 65°00′W; Fig. 1) at depths between 15 and 25 m. Immediately after collection, these were kept in incubators under controlled temperature, salinity and oxygen levels, resembling the in situ environmental conditions. Embryos were incubated using a computerized temperature control system

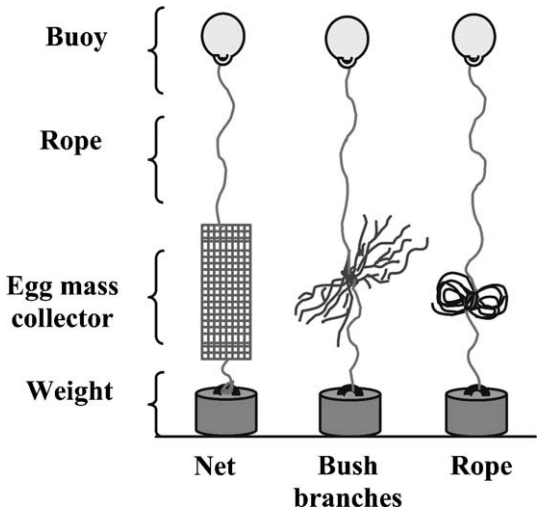

Fig. 4. Schematic representation of the artificial collectors used to obtain *Loligo gahi* egg masses.

capable to maintain and record three distinct thermal regimes simultaneously with 0.1 °C accuracy. Incubators were equipped with aeration, commercial heaters and temperature sensors. A specially designed software program allowed the regulation of the frequency and duration of the heating pulse as a function of the difference between the temperature recorded by the sensor and that previously set in the program. Every 10 min, the program recorded the temperature of each incubator. The natural photoperiod was simulated with a photocell exposed to the natural day–night cycle and connected to a low-power lamp placed just above the incubators. Incubations of *L. gahi* embryos were conducted at eight constant and four variable temperature regimes, and at four constant salinity regimes (Fig. 5). In all cases, the experimental unit was an egg capsule containing a variable number of live embryos (40–120). Three replicates (capsules) per treatment were used for each experiment. At the beginning of the experiments, the number of embryos, and their stages of development (according to the scale established by Barón (2002) for the species), were registered for each capsule (replicate). Experiments ended when no live embryo remained in all of the three replicates (all had either hatched or died). Percent survival was calculated for each capsule as the ratio between the number of embryos that hatched and the number of embryos at the beginning of the incubation, multiplied by 100.

2.2.1. Embryonic survival at different constant temperature regimes

In all of the experiments conducted at constant temperature regimes, salinity was maintained at 33.6–33.8‰ by periodically adding distilled water to the incubators up to a marked level to compensate for evaporation. Basing on the incubation results, a composed

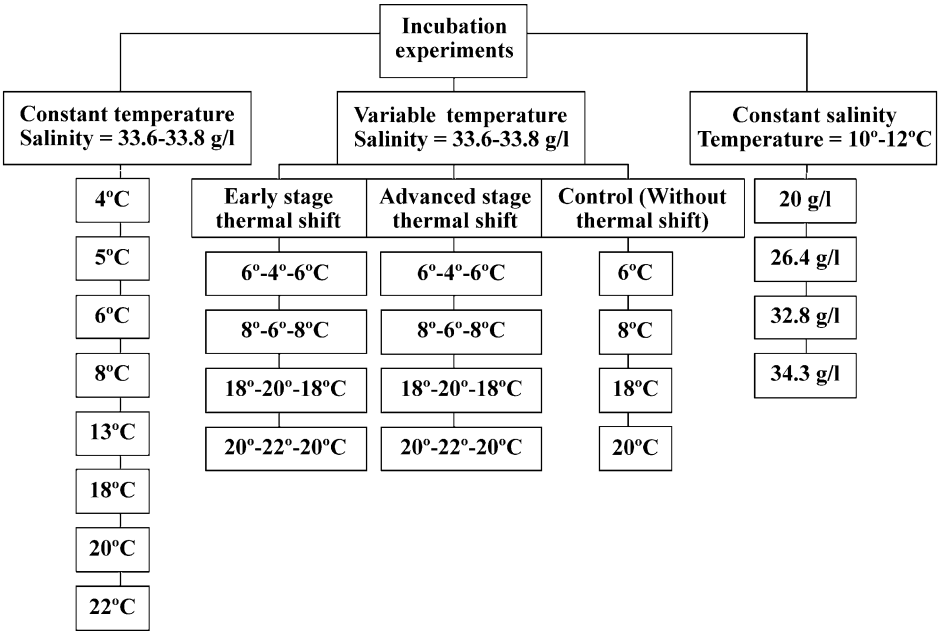

Fig. 5. Schematic representation of the experimental design of incubations at different thermal and salinity regimes conducted in this study.

function of survival on incubation temperature was obtained by nonlinear regression of two logistic curves, one for the first half of the temperature interval analyzed (4°–13 °C) and the other for the second half (13°–22 °C), to the survival values observed at constant temperature regimes. Using two logistic models resulted in a better fitting to data than utilizing a single model (e.g. quadratic), allowing a more realistic description of the embryonic survival as a function of incubation temperature. It should be noted that mean incubation temperatures hereafter reported in the text (4, 5, 6, 8, 13, 18, 20 and 22 °C) are nominal representations of the actual incubation temperatures used to establish the function (4.07 ± 0.44 °C, 5.02 ± 0.08 °C, 5.9 ± 0.1 °C, 8.47 ± 1.1 °C, 13.01 ± 0.08 °C, 17.87 ± 0.05 °C, 19.98 ± 0.05 °C, 21.77 ± 0.08 °C, Mean ± S.D.).

2.2.2. Embryonic survival at different variable temperature regimes

In experiments conducted at variable temperature regimes, embryos were kept in an incubator set at a given constant temperature during 5 days or more, depending on their developmental stage at the beginning of the incubation, and on the developmental stage at which the thermal shift was applied (early or late embryogenesis), then were moved into another incubator with a distinct constant temperature over a 6-day period, and then were moved back into the original incubator until they hatched or died. Control experiments were conducted by maintaining egg capsules within the first incubator during the whole incubation. In all cases, experiments were replicated with embryos at stage 21 or earlier (early embryogenesis) and at stages 26–27 (late embryogenesis) from the scale of Barón (2002) (Fig. 5). Survival of embryos incubated under a given variable regime of temperature at early and late developmental stages and those incubated at control conditions (without thermal shift) were contrasted with Kruskal–Wallis tests. When significant differences were found, a posteriori multiple comparison of the average ranges were conducted to contrast particular pairs of treatments (Zar, 1984).

2.2.3. Embryonic survival at different constant salinity regimes

For incubations conducted to test the effect of salinity on survival, salinity regimes of 20.0‰, 26.4‰, 32.8‰ and 34.3‰ were chosen based on published information on the characterization of seawater salinity at the shelf and river mouths off the Patagonian region (Guerrero and Piola, 1997; Esteves et al., 2000). Relatively high-salinity seawater (34.33‰) was obtained from Las Grutas Beach (Río Negro Province, 40°48′S, 65°05′W). Salinity was determined using an inductometric salinometer (Plessey, Model: 6230 N), and stock solutions were prepared by dilution using distilled water. During all incubations, temperature was maintained at nearly constant conditions (10–12 °C).

3. Results

3.1. Exploratory analysis of surface seawater temperature

A summary of the results of the analysis of SST satellite data during the period 1996–1997 in the two areas explored is shown in Fig. 6. In the northern area, temperature ranged between a maximum of 26.7 °C and a minimum of 8.2 °C. Box plots show that the

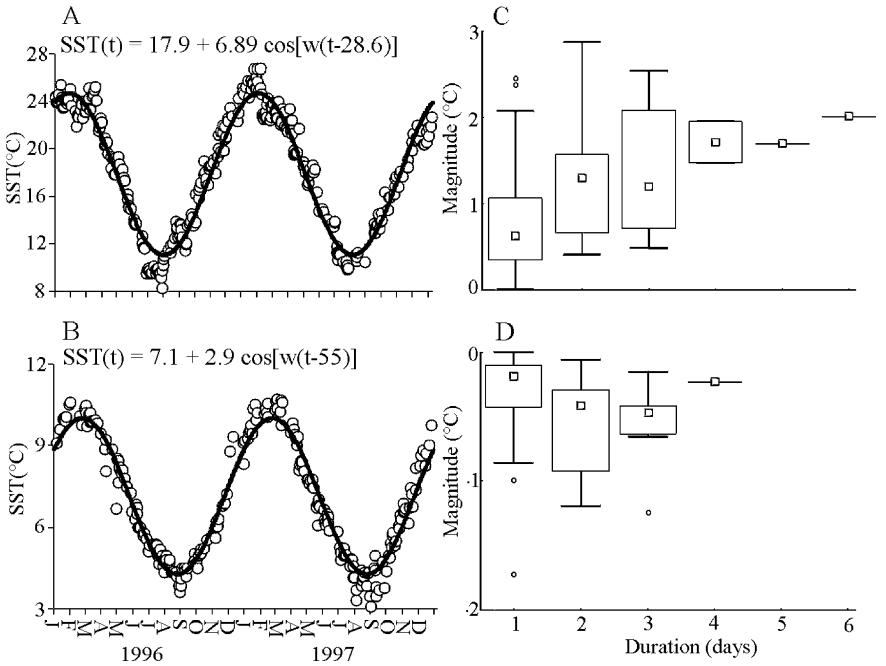

Fig. 6. Biannual series of surface seawater temperature (SST) and SST(t) functions fitted by regression to data at the Northern (A) and Southern (B) areas of SST exploration, and box-plots showing the maximum magnitude and duration of the thermal deviations at the Northern (C) and Southern (D) areas of SST exploration. Mark: median, box: inter-quartile range (IQR), whiskers: non-outlier range (range of values between 2nd quartile-1.5 × IQR and 3rd quartile + 1.5 × IQR), circles: outliers (observed values beyond non-outlier range).

maximum magnitude of a positive thermal deviation (warming) relative to the SST(t) function fitted to data was about 2–3 °C and its maximum duration was 6 days. In the southern area, SST ranged between a maximum of 10.7 °C and a minimum of 3.1 °C. Box plots show that short cooling events were frequent in that area, and that the maximum observed magnitude of a SST thermal deviation was approximately of 1.9 °C, and the maximum observed duration was 4 days. In the southern area, SST series were more discontinuous (SST data available only for 32% of days during the period analyzed) than in the northern area (41%). As mentioned earlier, since SST data is rather scattered, it is likely that the magnitude and duration of the thermal deviations were underestimated. Based on these results and considerations, it was considered that thermal deviations 2 °C in magnitude and 6 days in duration were representative of maximum SST deviations occurring in both areas. Therefore, these were the magnitude and duration of the thermal shifts applied to the embryos of *L. gahi* during the incubations at variable temperature regimes.

3.2. Incubation experiments

3.2.1. Embryonic survival at different constant temperature regimes

The experimental conditions applied during incubations at constant temperature regimes and the survival rates associated to them are shown in Table 1 and Fig. 7. Between 8 and

Table 1
Percent survival of *Loligo gahi* embryos incubated at eight constant temperature regimes, and time elapsed since the beginning of incubation until all embryos hatched or die

Treatment (°C)	Embryonic stage at incubation beginning	Mean percent survival (all replicates)	Incubation duration (days)
4	< 17	0	84
5	18	0	121
6	18	36.5	99
8	15	98.8	60
13	15	94.3	48
18	15	99.1	26
20	15	92.3	26
22	13	0	23

20 °C, mean embryonic survival was high (90–100%), hatching paralarvae showed a normal morphological aspect and swam actively. Below 8 °C and above 20 °C, survival decreased abruptly, being zero at 4, 5 and 22 °C. At 4 °C, all embryos remained in early developmental stages since the beginning of incubation until they died, the only observable evidence of organ formation being the rudiments of the pigmented eyes. At 5 °C, all of the embryos reached late stages of development (26–28, see Barón, 2002), but showed malformations (e.g., eyes further apart from each other and the internal yolk sacs broader than in morphologically normal embryos) and no one succeeded to hatch. Also, they were poorly active when stimulated by light during the incubation. At 6 °C, survival was low (30–40%), but normally developed embryos became active swimming paralarvae. At 22 °C, all embryos developed to stage 26 (Barón, 2002) showing reduced mantle and an unusually long external yolk sack, but no one hatched.

The functions fitted to experimental data on the embryonic survival at different incubation temperatures were $S = 100/(1 + \exp^{(-(-62.96 + 10.58 \times -T))})$ ($r^2 = 0.995$) for incubation temperatures within 4–13 °C, and $S = 100/(1 + \exp^{(-(132.27 + 6.50 \times -T))})$

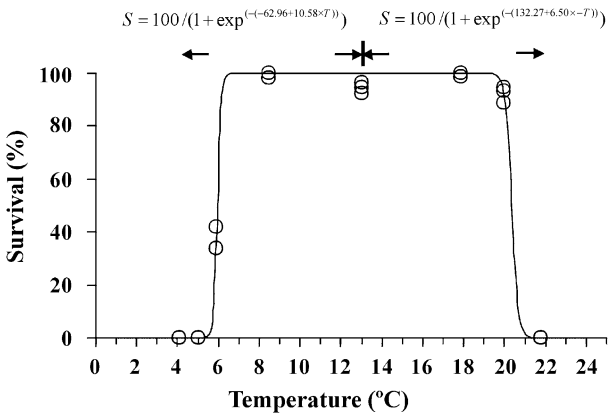

Fig. 7. Embryonic survival of the embryos of *Loligo gahi* incubated at different constant temperature regimes and functions of thermo-dependent survival fitted to data.

Table 2
Percent survival of *Loligo gahi* embryos incubated at four variable temperature regimes and their respective control (constant) temperature regimes, and time elapsed since the beginning of incubation until all embryos hatched or die

Treatment (°C)	Embryonic stage at		Mean percent survival (all replicates)	Incubation duration (days)
	Incubation beginning	Thermal shift		
6–4–6	18	21 (early)	26.4	99
6–4–6		26 (late)	27.6	99
Control: 6			36.5	99
8–6–8	17	18 (early)	93.3	63
8–6–8		27 (late)	99.1	61
Control: 8			97.1	46
18–20–18	15	18 (early)	96.4	28
18–20–18		26 (late)	97.4	26
Control: 18			99.1	26
20–22–20	15	19 (early)	0	23
20–22–20		26 (late)	79.2	23
Control: 20			92.3	26

($r^2 = 0.994$) for incubation temperatures within 13–22 °C, where S is the percent of embryonic survival and T is the mean incubation temperature (Fig. 7).

3.2.2. Embryonic survival at different variable temperature regimes

Survival of embryos of *L. gahi* incubated at different variable temperature regimes is shown in Table 2. Statistical comparisons of survival in incubations under three different treatments: (1) with no thermal shift (control incubations), (2) thermal shift applied at early developmental stages (< stage 21) and (3) thermal shift applied at late developmental stages (≥ stage 26) are presented in Table 3. No statistical differences were found between treatments in incubations at 6–4–6 °C (6 °C control) regime. Also, no significant differences in embryonic survival were found between treatments of the 8–6–8 °C (8 °C control) experiment. Paralarvae from the control treatment started to hatch first, those subjected to thermal shift at a late developmental stage started to hatch 10 days later, and those subjected to thermal shift at an early developmental stage started to hatch 20 days later. In all cases, paralarvae were morphologically normal and swam actively. In 18–20–18 °C (18 °C control) experiment, paralarvae started to hatch first in the treatment with

Table 3
Results of Kruskal–Wallis tests for percent survival of *Loligo gahi* embryos between incubations with no thermal shift (control incubations), thermal shift applied at early developmental stages and thermal shift applied at late developmental stages

Temperature regime (°C)	*H*	*P*
6–4–6 (control: 6 °C)	4.728	0.094
8–6–8 (control: 8°C)	5.422	0.067
18–20–18 (control: 18 °C)	5.241	0.073
20–22–20 (control: 20 °C)	7.448	0.024

H: Kruskall–Wallis statistic, *P*: probability ($\alpha = 0.05$) (Sokal and Rohlf, 1979).

Table 4
Percent survival of *Loligo gahi* embryos incubated at four constant salinity regimes, and time elapsed since the beginning of incubation until all embryos hatched or die

Treatment (‰)	Embryonic stage at incubation beginning	Mean percent survival (all replicates)	Incubation duration (days)
20.0	15	0.0	38
26.4	16	98.0	56
32.8	15	98.8	67
34.3	15	0.0	90

thermal shift at a late developmental stage, while those of the other two treatments (thermal shift at an early developmental stage and control) started to hatch 3 days later. Survival differences were also not significant between treatments, and paralarvae were normal. In 20–22–20 °C (20 °C control) incubations, survival was zero when thermal shift was applied to embryos at early developmental stages, with all the embryos being malformed by the end of the experiment. When thermal shift (20–22–20 °C) was applied to embryos in late developmental stages, premature hatching was observed. Therefore, hatching paralarvae showed large amounts of vitelline reserves in their external yolk sac. Nevertheless, mean survival was relatively high, no malformations were observed and paralarvae were capable to swim actively. Statistical analyses suggests that survival was significantly different between treatments. When comparing particular pairs of treatments by a posteriori test, significant differences were found between control and early embryonic stage treatments. However, no significant differences were found when comparing control versus late embryonic stage treatments, and late versus early embryonic stage treatments.

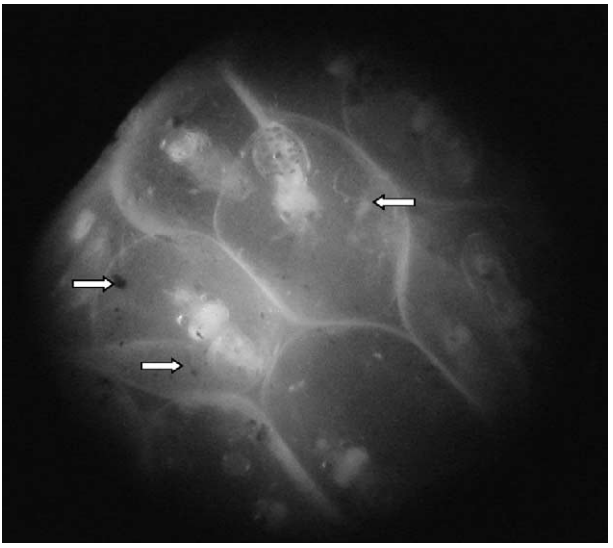

Fig. 8. Aspect of the embryos incubated at 34.3‰ salinity showing generalized ink liberation within the chorionic capsules (indicated by arrows).

3.2.3. Embryonic survival at different constant salinity regimes

Survival of *L. gahi* embryos in incubations at distinct constant salinity regimes is presented in Table 4. In the experiment conducted at 20‰ salinity regime, survival was zero in all replicates. All of the eggs showed a large intra-chorionic space since the first week of incubation. By the end of the experiment, chromatophore formation and heart beating was evident in most of the embryos, but mantle and arms were not yet developed. At 26.4‰ salinity, regime survival was high (90–100%). Hatchlings were morphologically normal and swam actively. At 32.8‰, salinity regime survival was high (90–100%) and hatching paralarvae were capable to actively swim. Finally, in the incubation conducted at 34.3‰ salinity regime, embryonic development was apparently normal until the last developmental stage before hatching. However, hatching did not occur. Embryos were unable to break their chorionic membranes, remaining at this stage for approximately 50 days, until they completely consumed their vitelline reserves and died. Generalized ink liberation was observed by the end of the experiment (Fig. 8).

4. Discussion

Correlation between the catch level and the seawater temperature on the spawning and feeding grounds (generally coincident with the fishing grounds) has been demonstrated for several squid species (*L. opalescens*, Mc Innis and Broenkow, 1978; *Loligo vulgaris reynaudii*, Roberts and Sauer, 1994; *Loligo bleekeri*, Lima, 1999; *Todarodes pacificus*, Sakurai et al., 2000; *Illex argentinus*, Waluda et al., 2001a,b). Particularly for waters of the South West Atlantic, Waluda et al. (2001a) found a positive correlation between the proportion of the "hatching grounds" with optimal temperatures for embryonic development (16–18 °C) in a given year and catch level during the next year. These authors suggested that for *I. argentinus* SST influence on recruitment is greater when it affects the earliest life-cycle stages. *L. gahi* is a squid adapted to spawn at low temperatures (Arkhipkin et al., 2000). For this species, there is growing evidence that spawning occurs in coastal waters (Hatfield et al., 1990; Hatfield and Rodhouse, 1994; Arkhipkin et al., 2000; Barón, 2001). In the sampling area of this study (Nuevo Gulf), SST reaches its minimum (~ 10 °C) and maximum (~ 18 °C), respectively, in August and February. The results of incubations at constant temperature regimes show that *L. gahi* successfully completes its embryonic development within average temperatures of 6–20 °C, with optimal survival within 8–18 °C. Taking into account these results and field observations reported by Barón (2001), it is possible to state that seawater temperature does not limit the embryonic survival in the study area. The curve of embryonic survival as a function of average temperature during incubation exhibits a central range of optimum, nearly constant survival. Above or below this range, survival rapidly decreases with increasing or decreasing temperatures. Even though the causes of embryonic death outside the range of normal embryonic development are not yet known, increased membrane permeability and blocking of ion pumps, reduction of energy liberation below basal needs, depolymerization or melting of nuclei acids, disequilibria of coupled enzyme reactions, limits imposed by kinetics and inactivation of enzyme proteins could be some of the responsible mechanisms, while hypoxia may be an important secondary effect (Prosser and Heath, 1994).

Based on incubations without quantitative control of embryonic survival, Barón (2002) estimated that the embryonic development of this species completes normally within the range of 5–20 °C. In contrast, in the present study, embryonic survival was zero at 5 °C. However, Barón (2002) used embryos obtained from waters of the Beagle Channel (55°S) for incubations at 5 °C, which may be genetically adapted to develop at the markedly lower mean water temperatures typical from that latitude. The latitudinal variations in the proportions of two alleles of an isozyme, each of which, respectively, confers a better adaptation to warm and cold environments, has been cited as one of the possible causes of adaptation of a species to different temperatures within its range of latitudinal distribution (Powers and Place, 1978). Arkhipkin et al. (2000) found live embryos developing at temperatures within 6.5–8 °C in shallow waters around Falkland (Malvinas) Islands, and reported that their occurrence was more frequent above 7.5 °C. Also, these authors found that natural embryonic mortality in winter was fourfold that of spring and summer, which reveals that temperature has a marked effect on embryonic survival in Falkland (Malvinas) waters. Accordingly, the function of embryonic survival obtained in our study shows that embryonic survival is nearly 100% within 6.5–8 °C, and much lower at slightly lower temperatures (e.g. 63% at 10 °C). For other squid species studied so far, minimum temperatures required for normal embryonic development are higher. For example, the embryos of *L. bleekeri* do not develop at temperatures lower than 7 °C, and its optimum thermal range for development is 11–18 °C (Lima, 1999). For *L. vulgaris reynaudii*, no embryos develop normally at 7 °C, and only 50% succeed to hatch at 9 °C (Oostuizien et al., 2002). For *I. argentinus*, the temperature range for normal development is 10–25 °C, but optimum temperatures for development are within 15–23 °C (Sakai et al., 1999). Finally, in *T. pacificus* the range for normal embryonic development is 14–26 °C, but highest survival occurs at 14.7–22.2 °C (Sakurai et al., 1996).

The length and magnitude of the thermal shifts applied to the embryos of *L. gahi* in incubations at variable temperature regimes were established based on an exploratory analysis of the SST oscillations in the latitudinal extremes of the species geographic distribution. Since this analysis was based on satellite SST data, quite discontinuous at the southern area of the study due to cloudiness, the length and magnitude of the thermal shifts may have been underestimated. Thermal shifts 6-day long and 2 °C of magnitude had negative effects on survival only when they were applied to embryos in early development close to the upper temperature limit for normal development (20–22–20 °C thermal shift). In this experiment, statistical differences were found only when comparing the control treatment (>90% survival) with the early developmental stage treatment (0% survival). Although the a posteriori comparison of average ranges failed to detect a significant difference when comparing embryonic survivals after thermal shifts applied at early and late developmental stages, this difference was substantial (Table 2). This suggests that the embryos at early stages of development are more vulnerable to the stress imposed by a thermal shift than those at late stages. Since duration of embryogenesis in cephalopod species is shorter at high than at low incubation temperatures (Boletzky, 1987; Barón, 2002, 2003), the length of a thermal shift (6 days in these incubations) represents a longer fraction of embryogenesis when incubation temperatures are high, and consequently, it can be more critical for survival than a shift of the same length and magnitude applied at low temperature.

Our results show that in incubations at 10–12 °C embryonic survival is high within 26.4–32.8‰, and zero at 20‰ and 34.3‰ salinities. At 20‰ salinity, the large intra-chorionic space observed since the first week of incubation could have been caused by osmosis through the chorionic membrane. On the other hand, at 34.3‰ salinity, the generalized ink liberation could have been the result of the stress suffered by the embryos due to starvation and the inability to break the chorionic membrane. Salinity ranges for embryonic development are widely variable between cephalopod species, even for those of the same genus and family. For example, the ranges reported for *Loligo vulgaris* (34–42‰) (D'Aniello et al., 1989) and *Sepioteuthis sepioidea* (27–38‰) (Fagundez and Robaina, 1992) markedly differ from that found in this study. The embryos of *Sepia officinalis* do not hatch at salinities lower than 23.9‰, only a few hatch at 26.5‰ and most do it at salinities above 29.8‰ (Paulij et al., 1990). In *Lolliguncula brevis*, a loliginid of estuarine habits, adults stand salinities as low as 10‰, while paralarvae and egg masses can only be found in environments with salinities above 21‰ (Hixon, 1980; Vecchione, 1991). The latter suggests that early life stages of this and many other cephalopods are probably confined to narrower salinity ranges than adults. This gives particular relevance to experimental results like those presented here. Further studies should be conducted to determine embryonic survival at salinities within the ranges not covered in the present study (20–26‰ and 33–34‰). Furthermore, since the energy budget of a given species for osmoregulation and thermoregulation determines a limited number of combinations of temperature and salinity that allow survival (Wheaton, 1978), it will also be important to determine the embryonic survival under different combinations of both of these two environmental variables.

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