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## Effects of temperature on development and survival of embryos and on larval production of *Chorus giganteus* (Lesson, 1829) (Gastropoda: Muricidae)

Efectos de la temperatura en el desarrollo y la supervivencia de embriones y en la producción larval de *Chorus giganteus* (Lesson, 1829) (Gastropoda: Muricidae)

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**Resumen.** - *Chorus giganteus* muestra un rápido desarrollo intracapsular en alta temperatura, pero esto tiene como consecuencia una alta mortalidad embrionaria y una baja producción larval. El presente estudio fue realizado para determinar la condición de cultivo óptima para asegurar un rápido desarrollo intracapsular y una alta producción larval. Embriones en tres estados de desarrollo intracapsular (segmentación [ES], segmentación avanzada [LS] y trocófora post ingestión de huevos nutricios [TPI]) fueron aclimatados a 12°C y luego incubados a 12, 15 y 18°C para evaluar la producción larval. El tiempo medio de eclosión incrementó con el aumento de la temperatura, pero el éxito en la eclosión de embriones disminuyó desde  $51 \pm 2\%$  en 12°C a  $8 \pm 1\%$  en 18°C en embriones ES y desde  $53 \pm 2\%$  en 12°C a  $20 \pm 0,2\%$  en 18°C en embriones LS. Esta declinación estuvo asociada a un incremento de embriones anormales, los cuales aumentaron desde  $14 \pm 2\%$  en 12°C a  $46 \pm 3\%$  en 18°C en embriones ES y desde  $8 \pm 1\%$  en 12°C a  $28 \pm 1\%$  en 18°C en embriones LS. En cambio, la eclosión de embriones TPI varió entre 65-70% y los embriones anormales solo representaron entre 13 y 20%. Por lo tanto, la probabilidad de producir y liberar larvas normales fue significativamente afectada por el efecto combinado de la temperatura y el estado embrionario inicial de cultivo. Una reducida habilidad para ingerir huevos nutricios quizás da cuenta de la variación en la producción larval.

Palabras clave: Desarrollo intracapsular, eclosión, trocófora, velígera, cultivo de moluscos

**Abstract.** - *Chorus giganteus* shows faster intracapsular embryonic development at high temperature but this is generally associated with a high embryonic mortality and low larval production. The present study was undertaken to determine the optimal combination of temperature and developmental stage for a successful (faster) larval production at high temperature. Embryos at three stages of intracapsular development (early stage [ES-embryos], late stage [LS-embryos] and trochophore post nurse-egg ingestion stage [TPI embryos]) were acclimated at 12°C then incubated at 12, 15 or 18°C to evaluate larval production. The median hatch time was accelerated by increasing water temperature. However, the hatching success of ES- and LS-embryos was compromised by higher temperatures, decreasing from a  $51 \pm 2\%$  at 12°C to  $8 \pm 1\%$  at 18°C in ES-embryos and from  $53 \pm 2\%$  at 12°C to  $20 \pm 0.2\%$  at 18°C in LS-embryos. The incidence of developmental abnormality increased with temperature from  $14 \pm 2\%$  at 12°C to  $46 \pm 3\%$  at 18°C in ES-embryos and from  $8 \pm 1\%$  at 12°C to  $28 \pm 1\%$  at 18°C in LS-embryos. By contrast, hatching success of TPI embryos ranged from 65-70% and the incidence of abnormality was only between 13 and 20%, with both factors being independent of temperature. Thus the probability of producing and releasing normal larvae was significantly affected by the combined effect of temperature and embryonic stage at the initiation of the culture. A reduced ability to ingest nurse eggs may account for the variation in larval production between the trials.

Key words: Intracapsular development, hatching, trochophore, veliger, molluscan aquaculture

## Introduction

Muricid snails represent an important resource of Chilean fisheries, with some species approaching over-harvesting due to high demands for these products in international markets. As a response to the limitations imposed on fishery of natural stocks, mass culture in hatcheries is

being considered as an alternative source for such products (DiSalvo 1988, DiSalvo & Carriker 1994, Leiva *et al.* 1998, Carrasco *et al.* 2006). However, efforts to culture the most valuable and most intensively researched muricid, *Concholepas concholepas*, produced discouraging results due to its extended planktotrophic larval phase and inadequacy to available culture methods

(DiSalvo 1988). The muricid *Chorus giganteus* (Lesson, 1829), seems more suitable to mass culture than *C. concholepas* since the whole embryonic development takes place in 60-100 days. It occurs within the egg capsule containing nurse eggs, which are consumed by the ingesting trochophore stage (González & Gallardo 1999). Further Martínez *et al.* (2008) conclude that these larvae have the ability to obtain exogenous dissolved food and to incorporate it into metabolizable compounds. At completion 150-300 lecithotrophic larvae of 900-1000 µm shell length, emerge from each egg capsule (Gallardo 1981, Leiva *et al.* 1998, Cancino *et al.* 2003). The larvae settle and metamorphose spontaneously in 2-5 days after hatching (Leiva *et al.* 1998). Moreover, metamorphosis can be successfully induced by KCl soon after hatching (Gallardo & Sánchez 2001), thus avoiding the complicated steps of hatchery feeding and larval management as in *C. concholepas*. Furthermore juveniles and adults are easily grown in laboratory conditions (Gutiérrez & Gallardo 1999, Navarro *et al.* 2002, Gallardo *et al.* 2004, Carrasco *et al.* 2006). In relation to larval production, *C. giganteus* shows faster intracapsular embryonic development at high temperature (18°C > 9°C) but this is generally associated with a high embryonic mortality and low larval production (Cancino *et al.* 2003). Cancino *et al.* (2003) suggest that the high mortality and low hatching constrain of high temperature can be solved if egg capsule rearing at high temperature begins when embryos have reached advanced developmental stages. The present study was undertaken to determine the optimal combination of temperature and developmental stage for a successful (faster) larval production.

## Material and methods

Whelks (*Chorus giganteus*) were collected from the Gulf of Arauco, Chile (37°10'S; 74°25'W) and maintained with *ad libitum* food (*i.e.* Mytilidae) in flow-through seawater at the Laboratorio Costero Lengua of the Universidad Católica de la Santísima Concepción. Three experiments, each one using egg capsules containing embryos at different stages, were conducted between March and December 1998. One or two weeks after spawning, capsules were acclimated for 2, 3 or 4 weeks at 12 ± 1°C in experiments I, II and III, respectively and then incubated at temperatures of 12, 15 or 18°C until hatching. In each experiment, embryonic stages were classified as early cleavage stage (ES-embryos; Exp I), late stage (LS-embryos, Exp II) and trochophore post nurse-egg ingestion stage (TPI-embryos; Exp III), characterised by total ingestion of nurse-egg and by initiation of protoconch calcification according to González & Gallardo (1999). One hundred and seventy three, 585 and 273 capsules were used in experiments I,

II, and III, respectively. Capsules were distributed almost equally in two replicate treatments in Exp I and in three replicate treatments in Exp II and III.

When the hatching process began in each experiment, both the daily number of capsules hatched and the number of larvae released were determined. The median hatching time (MHT; time taken for release 50% of larvae or 50% of capsules hatched) was registered at each temperature. In all experiments, the numbers of normal and abnormal hatching larvae were also determined. A larva was considered to be 'normal' if it showed four fully developed velar lobes and a calcified protoconch characterized by a dark brown colour. Finally, the probability of releasing a normal veliconch larva ( $P_{RL}$ ) was calculated for the three experiments from the following equations:

$$P_{RL} = P_H \times P_N$$

where  $P_H$  is the egg capsule hatching probability and  $P_N$  is the probability of generating a normal larva per capsule.  $P_H$  was determined as the ratio of spontaneously hatching capsules over the initial number of capsules in the experiment;  $P_N$  was determined as the ratio of normal veliconch larvae hatched over the total number of larvae in each egg capsule.

The estimates of different parameters for each temperature were compared using a one way analysis of variance (ANOVA) and *a posteriori* Tukey test, when appropriate. The combined effect of temperature and initial embryonic stage on the probability of a normal veliconch larva being released ( $P_3$ ) was tested using a two-way analysis of variance (ANOVA).

## Results

### Time and success in hatching

Increased temperature accelerated the rate of embryonic development, and lowered the hatching time in all experiments (Table 1). For example, time to hatching 50% of larvae was delayed by nearly 30 days in 12°C compared with 18°C in experiment II (Fig. 1).

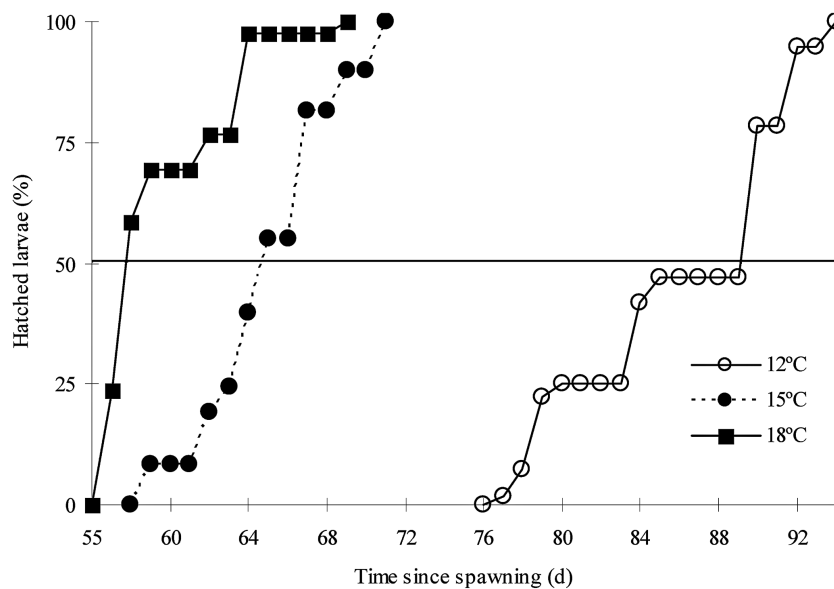
The percentage of hatched capsules significantly decreased as temperature increased, in experiments I and II (exp I,  $F(2.3) = 108$ ;  $P < 0.01$ ; exp II,  $F(2.6) = 5.14$ ;  $P < 0.05$ ; Fig. 2). This suggested that successful embryonic development in *C. giganteus* was restricted in 18°C. However, deleterious effects of the high temperature were absent when this was applied to capsules containing embryos in the ingesting trochophore stage (exp III), where a similar percentage of hatched capsules were obtained at all temperatures ( $F(2.6) = 0.73$ ;  $P = 0.51$ , Fig. 2).

**Table 1**

**Hatching time of *C. giganteus* larvae in each experiment as function of temperature. IHT= Initial hatching time; MHT = median hatching time (time taken for release 50% of larvae or capsules hatched); LHT = Last hatching time**

Tiempo de eclosión de larvas de *C. giganteus* en cada experimento en función de la temperatura. IHT = Tiempo inicial de eclosión; MHT = Tiempo medio de eclosión (tiempo requerido para que el 50% de las larvas o cápsulas eclosionen); LHT = Tiempo de la última eclosión

Culture temperature	Experiment 1 (ES-embryos)			Experiment 2 (LS-embryos)			Experiment 3 (TPI-embryos)		
	IHT	MHT	LHT	IHT	MHT	LHT	IHT	MHT	LHT
12°C	68	78	85	77	87	94	62	68	70
15°C	64	68	86	59	64	71	53	56	68
18°C	60	63	86	57	58	69	53	54	62

**Figure 1**

**Effect of temperature on the hatching time of *C. giganteus* larvae in experiment II. Time since spawning refers to days after the egg capsules were laid. MHT = median hatching time (time taken for release 50% of larvae or capsules hatched)**

Efecto de la temperatura sobre el tiempo de eclosión larval de *C. giganteus* en el experimento II.

Tiempo se refiere a los días después de la postura de las cápsulas. MHT = Tiempo medio de eclosión (tiempo requerido para que el 50% de las larvas o cápsulas eclosionen)

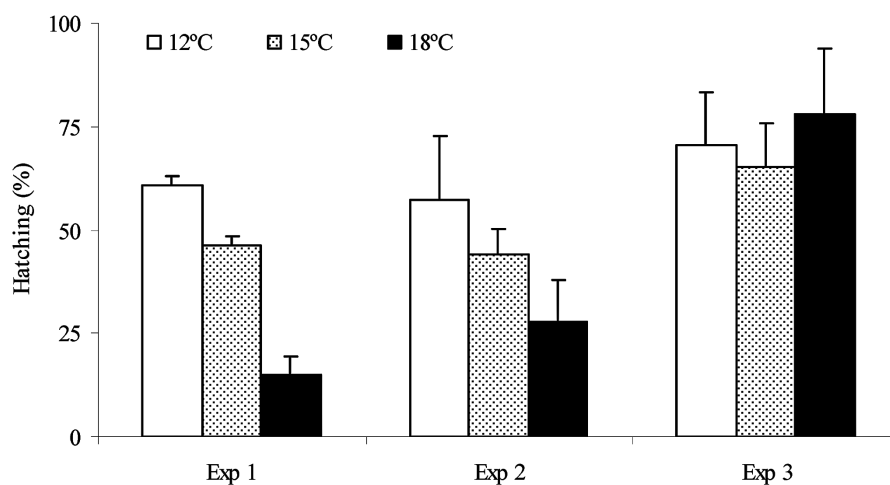


Figure 2

Effect of temperature on the percentage of hatched *C. giganteus* capsules in each experiment.

Exp. I started with ovicapsules containing embryos in early cleavage stage, Exp. II started with embryos in late cleavage stage, and Exp. III started with trochophore post nurse-egg ingestion stage. Values are shown as mean  $\pm$  1 SD (n = 3)

Efecto de la temperatura sobre el porcentaje de cápsulas eclosionadas de *C. giganteus* en cada experimento.

Exp. I comenzó con cápsulas que contenían embriones en segmentación temprana, Exp. II comenzó con embriones en segmentación avanzada, y Exp. III comenzó con embriones en estado de trocófora post ingestión de huevos nutricios. Los valores son mostrados como el promedio  $\pm$  1 DE (n = 3)

Table 2

Total number of cultured *C. giganteus* capsules, percentage of hatchling, number of hatched larvae and percentage of abnormal embryos in each experiment as a function of temperature

Número total de cápsulas cultivadas de *C. giganteus*, porcentaje de eclosión, número de larvas eclosionadas y porcentaje de embriones anormales en cada experimento como función de la temperatura

Stage	Temp. (°C)	Total N° of capsules	% hatchling	N° of hatched larvae per replicate	% of abnormal embryos
ES-embryos	12	28	61 $\pm$ 2	120 $\pm$ 56	14 $\pm$ 3
	15	20	46 $\pm$ 2	81 $\pm$ 42	26 $\pm$ 1
	18	6	15 $\pm$ 5	26 $\pm$ 17	46 $\pm$ 3
LS-embryos	12	93	57 $\pm$ 16	3,302 $\pm$ 296	8 $\pm$ 1
	15	76	44 $\pm$ 6	2,857 $\pm$ 359	12 $\pm$ 3
	18	48	28 $\pm$ 10	1,196 $\pm$ 358	27 $\pm$ 1
TPI-embryos	12	14	70 $\pm$ 13	564 $\pm$ 95	13 $\pm$ 11
	15	17	65 $\pm$ 11	631 $\pm$ 259	20 $\pm$ 1
	18	12	78 $\pm$ 16	466 $\pm$ 139	17 $\pm$ 4

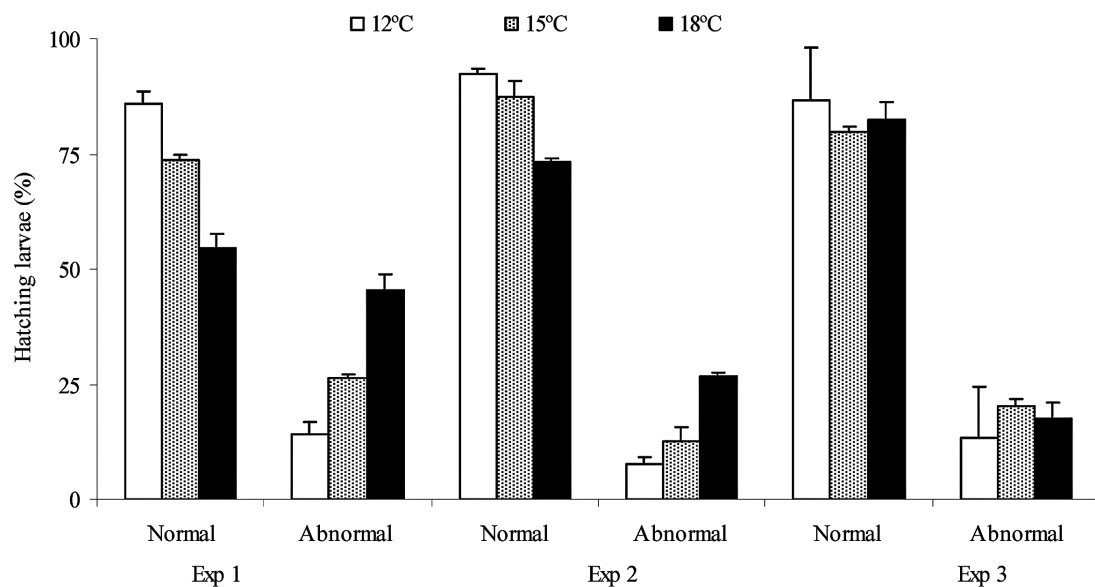
### Embryonic abnormalities and larval production

As temperature was increased the total larval production decreased in experiments I and II (Table 2). The total number of veliconch larvae was reduced from 3,373 at 12°C to only 156 in 18°C and from 9,156 at 12°C to 3,505 at 18°C in experiment I and II, respectively. The negative effect of high temperature on larval production decreased when the embryos were subjected to higher temperature after the ingesting trochophore stage had been reached. Accordingly, in experiment III a similar number of veliconch larvae were produced at the three temperatures (Table 2). Both, temperature and the initial embryonic stage affected the proportion of normal to abnormal veliconch larvae generated (Fig 3). The percentage of abnormal larvae increased with increase in temperature; significant differences were found in experiment I ( $F(2.6) = 5.14$ ;  $P < 0.05$ ), and II ( $F(2.6) = 65.9$ ;  $P < 0.001$ ). However, in experiment III there were no significant differences among the numbers of normal or abnormal larvae generated at the three temperatures ( $F(2.6) = 0.79$ ;  $P = 0.49$ ) (Fig 3). Thus the probability of producing and releasing normal veliconch larvae ( $P_{RL}$ ) was significantly affected by the combined effect of temperature and embryonic stage at the initiation of the culture (Table 3).

A similar larval production could be obtained at 12, 15 or 18°C when the embryos are reared in high temperature from trochophore post ingestion stage (Fig. 4).

### Discussion

The present study confirms that an accelerated embryonic development in *Chorus giganteus* at a high temperature constrains other physiological processes resulting in a reduced probability for reaching the veliconch larval stage. In other marine snails, increasing temperature generally accelerated the rate of shell growth and decreased the number of days required from larval release to spontaneous metamorphosis (Pechenik & Lima 1984, Lima & Pechenik 1985). This could be a desired effect for rearing in hatchery conditions, since shortening the time of intracapsular development may reduce culture costs. However, in *C. giganteus* the physiological cost of faster intracapsular embryonic development at 18°C led to the production of veliconch larvae of smaller size and fewer in number, as well as to the production of a high percentage of abnormal larvae and a lower spontaneous capsule hatching.



**Figure 3**

Effect of temperature on the proportion of normal and abnormal hatched larvae of *C. giganteus* in experiments I, II, and III. Values are shown as mean  $\pm$  1 SD ( $n = 3$ )

Efecto de la temperatura sobre la proporción de larvas eclosionadas normales y anormales de *C. giganteus* en los experimentos I, II y III. Los valores son mostrados como el promedio  $\pm$  1 DE ( $n = 3$ )

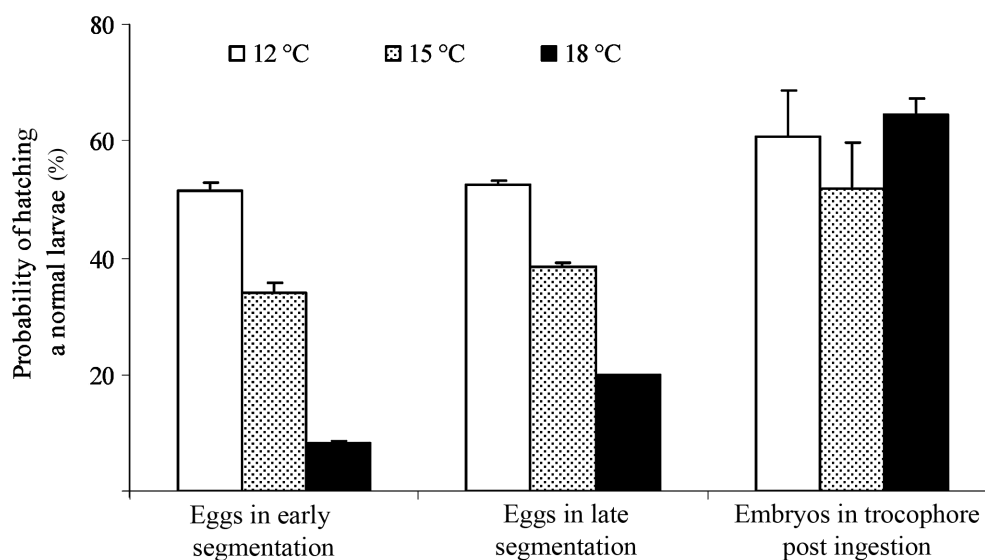
**Table 3**

**Two-way ANOVA on the effects of temperature and initial embryonic stage on the probability of producing and releasing normal larvae in *C. giganteus***

Análisis de varianza (ANDEVA) de dos vías para los efectos de la temperatura y del estado de desarrollo sobre la probabilidad de producir y liberar larvas normales de *C. giganteus*

Source of Variation	DF	MS	<i>F</i>	<i>P</i>
Initial embryonic stage	2	0.13	154	< 0.001
Temperature	2	0.19	228	< 0.001
T x E	4	0.05	61	< 0.001
Error	18			

DF: Degree of freedom; MS: Mean square; *F*: F statistic; *P*: Probability

**Figure 4**

**Probability of producing and releasing normal larvae (P3) of *C. giganteus* as a function of the combined effects of temperature and initial embryonic stage at which culture started. Values are shown as mean  $\pm$  1 SD (n = 3)**

Probabilidad de producir y liberar larvas normales (P3) de *C. giganteus* en función del efecto combinado de la temperatura y del estado de desarrollo embrionario inicial de cultivo. Los valores son mostrados como el promedio  $\pm$  1 DE (n = 3)

The constraints imposed on *C. giganteus* by temperature depend on the developmental stage at which the higher temperature was applied. We have shown that increasing temperature from 12 to 18°C did not affect the embryonic development when high temperature was applied after the post ingestion trochophore stage. This suggests that 18°C was not a limiting temperature for *C. giganteus* developing after nurse egg ingestion. A great number of nurse eggs were not consumed by other embryos when high temperature was applied early after spawning. These nurse eggs were agglutinating within capsule till hatching. This phenomenon was infrequent at 15°C and never happened when development occurred at 12°C. Thus, the negative effects of higher temperature observed in the present study are likely to be due to constraints in the intake of nurse eggs. The abnormal larvae probably consume only a few eggs compared to normal larvae. Gonzalez & Gallardo (1999) described a high variability in the nurse egg ingestion by embryo of *Chorus giganteus* at 15°C (mean:  $13.7 \pm 6.5$  nurse egg, minimum: 1, maximum 36). Further studies are required to determine how increased temperature interferes with nurse egg consumption.

The present results also contribute to understand the factors controlling spontaneous capsule hatching. In molluscs ovicapsule plug degradation seems to be a gradual process. There are three main mechanisms by which gastropod larvae are released from the capsules: (a) osmotically induced uptake of water (Hawkins & Hutchinson 1988, Kennedy & Keegan 1992), (b) direct mechanical action of the enclosed embryos on the opercle (Vaughn 1953), and (c) chemical dissolution of the opercle (Pechenik 1975). Mechanisms (b and c) have been reported as one of the hatching mechanisms used by *Chorus giganteus* (Leiva *et al.* 1998, González & Gallardo 1999). We show that high number of abnormal embryos was associated with a reduced larval hatching. It was frequently observed at 18°C, so spontaneous larval hatching from ovicapsules was prevented.

The present results must be, however, evaluated in relation to the origin of the parental broodstock. Eggs produced by *C. giganteus* in this study may have been adapted to lower temperature ranges given their origin in the Gulf of Arauco which is subject to low temperatures produced by the Humboldt Current. Populations from areas with different thermal conditions may respond differently to temperature variation due to local genetic differences produced by the low larval dispersal typical of the species (Gajardo *et al.* 2002).

We conclude that the conditions for rearing ovicapsules of *C. giganteus*, with the aim of maximising the production of veliconch larvae in the shortest possible time should include rearing at low temperature (12°C) by 21 days until the post ingestion trochophore stage has been reached, followed by rearing at 15°C-18°C during 36 days until larval hatching takes place.

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