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Combined effects of dissolved oxygen concentration and water temperature on embryonic development and larval shell secretion in the marine snail *Chorus giganteus* (Gastropoda: Muricidae)

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Abstract The present study was undertaken to determine the effects of both extracapsular oxygen concentration and temperature on embryonic development in *Chorus giganteus*. In normoxia increasing water temperature from 12°C to 18°C reduced by 15 days the median time required for the capsules to hatch. Hypoxia (oxygen content at 50% of air saturation) generated a low development rate and totally prevented both shell secretion and larval hatching from the egg capsule. Experimental transfer at weekly intervals, from normoxia to hypoxia and vice versa, induced a decrease and increase in the embryonic ash content, respectively, but did not affect the number of hatched larvae. Such an effect was more pronounced at 12°C than at 15°C or 18°C. The embryonic inability to produce a shell under hypoxia is likely to be a result of the low intracapsular oxygen concentration (IPO_2) generated as the combined effect of a low extracapsular oxygen concentration (environmental) added to the intracapsular embryonic oxygen demands, which lowers the IPO_2 still further. Under such conditions, a decrease in intracapsular pH is likely to take place, and, if so, embryos might divert carbonates away from shell calcification to balance such changes in pH.

Introduction

Both dissolved oxygen concentration and water temperature are determining factors for larval development and survival in mollusks (Pechenik 1987; Morgan 1995). Encapsulated development is common in gastropods and, compared to free development in the seawater column, is advantageous, since the capsule restricts predation of pelagic phases to the post-hatching larval stages, prevents bacterial attacks and acts as a filter for ultraviolet radiation (Pechenik et al. 1984; Morgan 1995; Rawlings 1996). Encapsulated development, however, constrains both the shape of the capsules and the number of embryos inside them, mainly due to the problems of oxygen supply it generates. Dissolved oxygen concentration has been regarded as an important factor for both the evolution of gelatinous egg masses and egg capsules (i.e. opisthobranchs and prosobranchs, respectively) and for the evolution of the dispersal patterns of propagules (Strathmann and Strathmann 1989; Cohen and Strathmann 1996; Lee and Strathmann 1998). Accordingly, Strathmann and Strathmann (1995) have shown that the diffusive supply of oxygen is a limiting factor for embryos clustered in gelatinous egg masses. The development rate in three opisthobranch species was retarded when embryos were exposed for 10–24 h to oxygen contents below 10% of air saturation, while embryos exposed to a low oxygen concentration hatched with shorter shells (Strathmann and Strathmann 1995). The restrictions of oxygen supply could also explain why among species of *Conus* the number of embryos increased in proportion to surface area of the capsule rather than to its volume (Perron and Corpuz 1982). Similarly, the concentration of embryos in egg masses of three species of gastropod mollusks decreased as mass thickness increased (Lee and Strathmann 1998). In an artificial gel matrix, simulating egg masses, the development rate of central embryos was retarded in relation to peripheral embryos, with increasing embryonic concentration and with increasing thickness of the gelatinous egg masses (Strathmann and Strathmann 1989). Similarly, Maeda-Martínez (1985) has shown that larvae of *Crepidula fornicata* submitted to environmental hypoxia reduced shell secretion. Moreover, egg capsules and egg masses are frequently fouled by micro-organisms, which affect intracapsular oxygen concentration and might under some circumstances generate critically low

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oxygen tensions, impairing development (Cohen and Strathmann 1996; Cancino et al. 2000).

Temperature also affects the rate of development in poikilotherms and interacts with dissolved oxygen concentration (Green and Carritt 1967). Growth rate in invertebrates usually correlates positively with temperature (Bayne 1976), but frequently maximal survival and optimal growth occurs within a limited temperature range. Reduced growth rate may result from too high a metabolic expenditure at temperatures above the optimal range of the species (Tettlebach and Rhodes 1981) and from other stresses occurring near the limit of tolerance. In the muricid *Thais haemastoma canaliculata* (Gray) survival to hatching was greater and development was faster at 28°C than at 22°C (Roller and Stickle 1989). Similarly, larvae of *Crepidula fornicata* (L.) and *C. plana* Say increased the rate of shell growth and decreased the time required from larval release to spontaneous metamorphosis when temperature was increased from 15°C to 29°C (Pechenik and Lima 1984; Lima and Pechenik 1985).

The muricid *Chorus giganteus* (Lesson, 1829) is an endemic species from the Chilean coast, occurring subtidally on soft sediments and in areas with small rocks. During the breeding season *C. giganteus* produces dense clusters of egg capsules, which are attached to rocks (Gallardo 1981). In subtidal environments with fine sediments water movement is low, which is likely to generate hypoxic conditions, especially near the water–substratum boundary layer and in association with dense clusters of egg capsules such as those of *C. giganteus*. Furthermore, this species has been reported from Antofagasta (23°48′S; 70°32′W) to Valdivia (39°25′S; 73°10′W) (Osorio et al. 1979). In such a wide dispersion range it might encounter water masses with a large variety of temperatures and dissolved oxygen contents. In central Chile, near Concepción, for example, *C. giganteus* occurs mainly in bays such as the Golfo de Arauco, Concepción and Coliumo, all of which are characterized by a low temperature and a variable dissolved oxygen concentration. In the Golfo de Arauco oxygen reaches minimum values in the summer time, with values between 1 and 3 ml O_2 l^{-1} in seawater at a temperature between 10°C and 11°C (Ahumada 1976, 1994; Llancamil 1982). Under such conditions capsules located at the center of groups are likely to be subjected to hypoxic or even anoxic conditions for many weeks. In *C. giganteus* the entire embryonic development occurs inside the egg capsule, and, at completion, 150–300 lecithotrophic veliconch larvae of 900–1000 μm shell length emerge from each egg capsule (Gallardo 1981; Leiva et al. 1998; González and Gallardo 1999). These biological characteristics make this species ideal for experimental study of the effects of temperature and oxygen on embryonic development. The results of such a study are not only of theoretical importance, but could also be useful for the possible rearing of the species in hatcheries. *C. giganteus* is economically important in Chile and, due to overfishing of natural populations, the landings of this species have decreased by >95% in the decade 1987–1997 (SERNAP 1997; Gajardo et al. 2002). Therefore, studies of the biological requirements for intracapsular development, like the present one, are urgently needed in order to provide a basis for culturing of the species.

Materials and methods

Effects on intracapsular embryonic development

In October 1997, 321 capsules were collected at Metri, near Puerto Montt, southern Chile. The capsules were transported overnight in insulated boxes at 12°C to the Laboratorio Costero Lenga, in Talcahuano (36°45′S; 73°10′W), central Chile. They were randomly assigned to 12 groups, to be reared in 1.5-l glass aquaria in filtered seawater (5 μm) and subjected for 60 days to six treatments: three temperatures (12°C, 15°C or 18°C) at two oxygen concentrations [normoxia (100%) and hypoxia (~50%)], with two replicates each. Seawater during experiments was changed every 24 h, preventing the possible accumulation of excretory products. The normoxic condition was generated with constant air bubbling. The hypoxic condition was generated by bubbling nitrogen until 50% air oxygen saturation was obtained, the aquarium being sealed thereafter to prevent further gas exchange with air. Extremely low dissolved oxygen concentrations, resulting from embryonic metabolic oxygen consumption in a closed environment, was prevented by air bubbling until 50% oxygen saturation was reestablished 12 h after the water was changed. The initial dissolved oxygen concentration value used in hypoxia were on average: $49.96 \pm 0.15\%$ air saturation (equivalent to 3.09, 2.90 and 2.73 μl O_2 ml^{-1} at 12°C, 15°C and 18°C, respectively). The minimum oxygen saturation values achieved after 12 h were: $44.64 \pm 0.50\%$, $42.74 \pm 0.75\%$ and $37.94 \pm 3.14\%$ at 12°C, 15°C and 18°C, respectively (equivalent to 2.76, 2.48 and 2.07 μl O_2 ml^{-1} at 12°C, 15°C and 18°C, respectively). Every 2 weeks, three egg capsules were removed from each treatment, quantifying length, total dry weight, organic and inorganic matter content of embryos. For these, embryos were collected on pre-ashed fiberglass filters, washed in an ammonium formate solution isotonic with seawater and dried at 80°C to constant mass. The organic content was determined as the differences in dry mass before and after ashing the sample for 4 h at 500°C. Inorganic matter content (ash weight) was regarded as indicative of shell secretion.

The effects of oxygen and temperature on intracapsular development were tested by using a two-way analysis of variance (ANOVA) and an a posteriori Tukey test.

Embryonic shell secretion

With the aim of contributing to understanding the effects the former experimental treatments had on larval shell secretion, egg capsules were collected in October 1998, from snails that spawned in tanks with running seawater at the Laboratorio Costero Lenga of Universidad Católica de la Santísima Concepción, central Chile. A total of 273 capsules laid within a 2 week period were held for 30 days at 12°C. During this time the embryos developed from eggs to trochophore post-ingestion, and the embryonic shell began to calcify (Leiva et al. 1998). At this stage the egg capsules were randomly assigned to the following treatments: 12°C, 15°C or 18°C with two constant oxygen concentrations (normoxia and hypoxia, as above) per temperature and with three replicates per treatment. Three further groups of egg capsules in each temperature were subjected for 1 month to alternating normoxia and hypoxia, with transfer at weekly intervals. Afterwards the capsules were maintained in normoxia until larval hatching. Once a week three egg capsules, one per replicate, were removed from each treatment, quantifying only the inorganic matter content of embryos, as described above. The experimental oxygen conditions were generated as explained before. Similarly, dissolved oxygen concentration was reestablished in hypoxia by air bubbling 12 h after water change,

which, under all three experimental conditions, took place every 24 h. Dissolved oxygen concentration was measured with a Strathkelvin 781b oxygen meter. The effects of extracapsular oxygen concentration and temperature on shell secretion were tested by using a two-way ANOVA and *t*-test.

The time taken at each temperature for 50% of the capsules to hatch was regarded as median hatching time (MHT). Total number of larvae produced at each temperature condition was also determined.

Results

Embryonic development

The initial embryonic stage in the egg capsules used in the present experiments was a trochophore post-ingestion. Total length of the embryo (shell length when present) increased linearly with time in all treatments, but with a different rate in normoxia and hypoxia. In normoxia the growth rate was similar between treatments (6.2 μm day^{-1} at 12°C, 5.2 μm day^{-1} at 15°C, 5.7 μm day^{-1} at 18°C), with no significant difference in total length of the pre-hatching embryos under the three temperature conditions studied (Table 1). In normoxia a similar percentage of egg capsules hatched at the three temperatures studied (70%, 65% and 78% at 12°C, 15°C and 18°C, respectively). In hypoxia, however, the embryos remained in the pre-calcified stage, and not a single veliger hatched under any of the temperature conditions. The veliger larvae reached the tetralobulated-velum stage typical of veliconch larvae and showed a normal foot and operculum, but lacked a shell. Since the growth rate in hypoxia was lower than in normoxia (1.7 μm day^{-1} at 12°C, 2.8 μm day^{-1} at 15°C, 1.12 μm day^{-1} at 18°C), oxygen concentration generated significant differences in total length of pre-hatching embryos (Table 1).

Initial total dry mass of embryos varied on average between 66.9 and 58.8 μg at 12°C and 18°C, respectively (sum of organic and inorganic matter in Fig. 1A and C, respectively). In normoxia, at 12°C and 15°C, the mass of the embryo remained constant around 66.1 and 63.1 μg, respectively, but at 18°C increased to 84 μg (Fig. 1A–C). In hypoxic conditions the final average dry mass of the embryos was 57.4 and 45.1 μg at 12°C and 18°C, respectively (Fig. 1A, C). The statistical analysis detected significant differences in total dry mass between oxygen levels (normoxia vs. hypoxia), but not between temperatures (Table 1).

In both normoxia and hypoxia the average organic matter content (ash-free mass) per embryo decreased slightly with time at almost every temperature and oxygen level studied, but at 18°C and normoxia it slightly increased from 53.1 to 66.4 μg $embryo^{-1}$ (Fig. 1A–C). However, the differences among treatments were not statistically significant (Table 1).

Inorganic matter content of embryos (ash mass) increased in normoxia from initial average values between 6.9 and 5.7 μg $embryo^{-1}$ to 21.3 and 18.3 μg $embryo^{-1}$ at 12°C and 18°C, respectively. In hypoxia, however, the average inorganic matter content per embryo decreased in all conditions studied (Fig. 1A–C). The statistical analysis detected significant effects of oxygen concentration, but none due to water temperature (Table 1).

Shell secretion

The initial embryonic stage in the egg capsules used in this experiment was pre-veliger (Leiva et al. 1998), the stage at which shell calcification begins. As before, the inorganic matter content of embryos (ash content) significantly increased in normoxia at all water temperatures studied, but embryos in hypoxia failed to do so (Fig. 2A–C). The statistical analysis showed significant effects of oxygen concentration on inorganic matter content throughout the experiment, while significant effects of temperature were detected only on day 7 (Table 2).

The experimental transfer from normoxia to hypoxia on day 7 was followed by a significant decrease in ash content at 12°C and 15°C ($t = 6.08$, $P < 0.05$ and $t = 3.64$, $P < 0.05$, respectively), while a transfer from hypoxia to normoxia on day 14 was followed by an increase in ash content at 12°C and 15°C ($t = -3.08$, $P < 0.05$ and $t = -3.42$, $P < 0.05$, respectively) (Fig. 2D, E). At 18°C significant changes in ash content were detected only after

Table 1 *Chorus giganteus*. Two-way ANOVA on the effects of temperature and environmental oxygen concentration on total length, dry weight, organic and inorganic matter content of the pre-hatching embryos (on day 45). Temperatures: 12°C, 15°C and 18°C; oxygen concentrations: normoxia and hypoxia (100% and 50% of air saturation, respectively)

Source	*df*	MS	*F*	*P*	*df*	MS	*F*	*P*
	Embryonic length				Organic matter			
Temperature (T)	2	0.00074	0.870	0.434	2	0.01266	3.148	0.069
Oxygen (O)	1	0.07646	88.857	<0.001***	1	0.00485	1.207	0.287
T×O	2	0.00169	1.971	0.165	2	0.00125	0.313	0.735
Error	20	0.00086			17	0.00402		
	Total dry weight				Inorganic matter			
Temperature	2	0.00243	0.57225	0.57475	2	0.05491	2.729	0.094
Oxygen	1	0.02525	5.9336	0.026*	1	1.41618	70.373	<0.001***
T×O	2	0.00044	0.10549	0.90046	2	0.00895	0.449	0.648
Error	17	0.00425			17	0.02012		

NS not significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

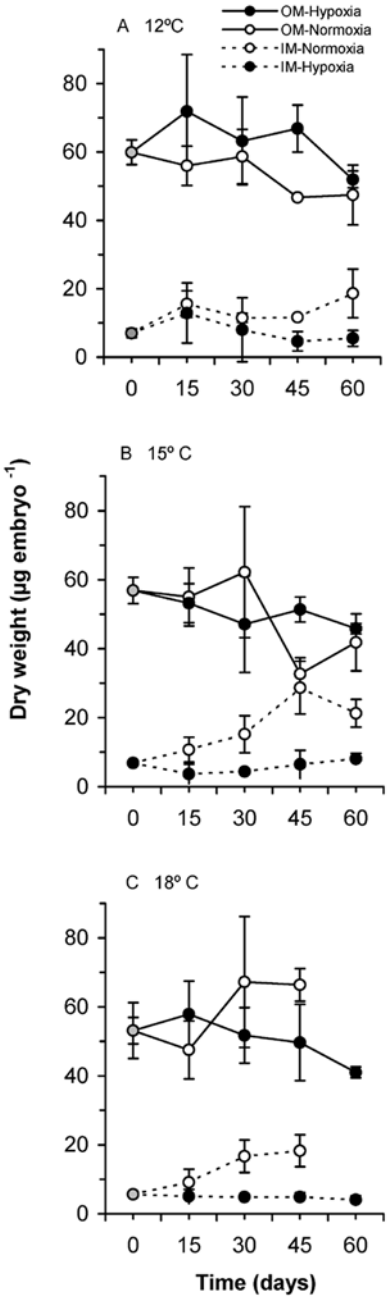

Fig. 1A–C *Chorus giganteus*. Organic and inorganic matter content per embryo (*OM* and *IM*, respectively) under two dissolved oxygen concentrations as a function of time at 12°C (**A**), 15°C (**B**) and 18°C (**C**). Values given are averages (±1 SD, $n=3$)

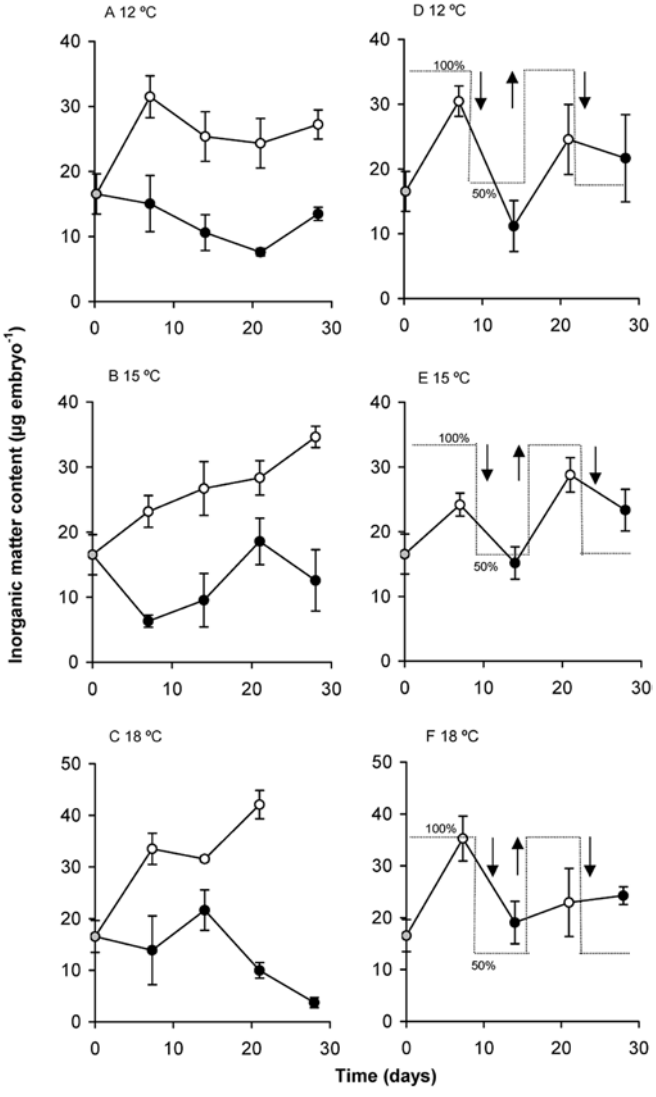

Fig. 2A–F *Chorus giganteus*. Inorganic matter content per embryo under three dissolved oxygen concentrations as a function of time and temperature (12°C, 15°C and 18°C). The left panels (**A–C**) represent ovicapsules that were kept in constant normoxia (100% air saturation, *open circles*) or in constant hypoxia (50% air saturation, *filled circles*). The right panels (**D–F**) represent ovicapsules that were transferred from normoxia to hypoxia (*downward pointing arrows*) and vice versa (*upward pointing arrows*) at weekly intervals. Values given are averages (±1 SD, $n=3$)

the first transfer ($t=-3.25$, $P<0.05$), but not after later ones. Note that the first transfer to hypoxia (day 7) was followed by a larger decrease in ash weight than the second one (day 21), and such a decrease was more pronounced at 12°C than at the higher temperatures. Similarly, the increase in ash content after transfer to normoxia on day 14 was more pronounced at 12°C and 15°C than at 18°C. The above suggests that embryos became less responsive to the changes in oxygen concentration as development proceeded. After the final transfer to normoxia, on day 28, the embryos continued developing until hatching.

The effects of oxygen concentration were clearly detected in the number of normal and abnormal embryos generated per capsule (Fig. 3; Table 3). The number of abnormal embryos (those lacking a shell after 42 days in culture, plus embryos that did not consume or consumed only a few nurse-eggs) hatching per capsule was low in normoxia and similarly low in the capsules subjected to experimental transfer from normoxia to hypoxia and vice versa (Fig. 3). In both treatments, normal embryos were dominant (Fig. 3), with no significant differences in the number hatching per egg capsule (Table 4). All embryos were abnormal in continuous hypoxia (Fig. 3; Table 4).

Table 2 *Chorus giganteus*. Two-way ANOVA on the effects of temperature and environmental oxygen concentration on inorganic matter content, as a function of time. Temperatures: 12°C, 15°C and 18°C; oxygen concentrations: constant normoxia, transfer normoxia–hypoxia and vice versa, and constant hypoxia

Source	*df*	MS	*F*	*P*	*df*	MS	*F*	*P*
	7 days				14 days			
Temperature (T)	2	0.094	4.246	0.038*	2	0.226	2.527	0.111NS
Oxygen (O)	2	0.471	21.357	< 0.001***	2	0.362	4.047	0.037*
T×O	4	0.011	0.479	0.7507NS	4	0.084	0.939	0.466NS
Error	13	0.022			16	0.089		
	21 days				28 days			
Temperature	2	0.061	3.275	0.064NS	1	0.001	0.035	0.8556NS
Oxygen	2	0.445	24.065	< 0.001***	1	0.141	8.014	0.022*
T×O	4	0.037	2.024	0.139NS	1	0.007	0.379	0.554NS
Error	16	0.019			8	0.141		

NS not significant, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$

Temperature had a significant effect on the MHT determined under normoxic conditions (Fig. 4). As expected, development was faster; therefore, MHTs were smaller at higher temperatures, e.g. 54 days were required at 18°C for 50% of the capsules with successful development to hatch, while 69 days were required at 12°C.

Discussion

Dissolved oxygen concentration plays an important role in controlling the intracapsular development of *Chorus giganteus*. Temperature is also important, but only seems to control the speed at which development takes place, explaining why hatching occurs earlier at a higher than at a lower temperature. Although temperature affects the amount of oxygen that dissolves in seawater at air saturation (Green and Carritt 1967), no significant temperature–oxygen interactions were detected in any of the experiments carried out in the present study, leaving oxygen as the key-factor controlling intracapsular development.

Prolonged hypoxia produced two effects on the embryonic development of *C. giganteus*.

1. Shell secretion was inhibited, which could result from a limiting intracapsular PO_2. Cancino et al. (2000) have reported that ovicapsules of *C. giganteus* placed in hypoxic water (oxygen at 50% of air saturation) reached a stable intracapsular oxygen concentration value of only 1.5 ml O_2 l^{-1} (around 25% of air saturation at 12°C). Such a low intracapsular oxygen concentration could be limiting for the embryos. If, under such conditions, anaerobic metabolic pathways are used by the embryos, the acidic end-products generated could induce a lowering in pH. This could be partially compensated for by mobilization of $CaCO_3$ away from calcification (De Zwaan 1983). Secondly, if the intracapsular fluid becomes acidic, it might prevent the utilization of carbonates for shell formation, since in a low pH condition the balance of the carbonate equation will be displaced towards carbon dioxide and carbonic acid rather than

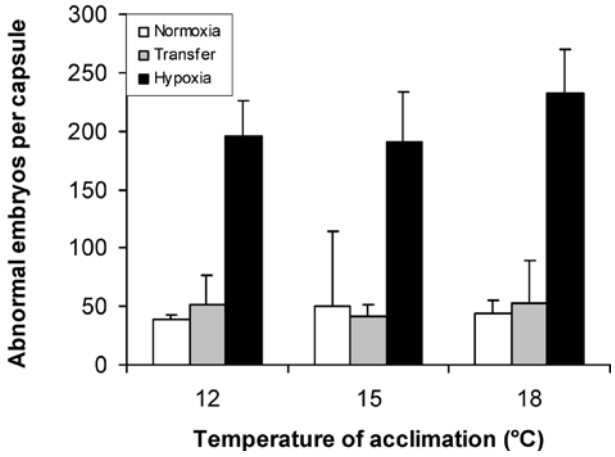

Fig. 3 *Chorus giganteus*. Number of abnormal pre-hatching embryos per capsule, under three dissolved oxygen concentrations as a function of temperature. Values given are averages (±1 SD, $n = 3$). Dissolved oxygen conditions as in Fig. 2

Table 3 *Chorus giganteus*. Two-way ANOVA on the effects of temperature and environmental oxygen concentration on the number of abnormal embryos (without a calcified protoconch). Water temperatures: 12°C, 15°C and 18°C; oxygen concentrations: constant normoxia, transfer normoxia–hypoxia and vice versa, and constant hypoxia

Source	*df*	MS	*F*	*P*
Temperature (T)	2	0.015	0.172	0.843 NS
Oxygen (O)	2	1.775	20.114	< 0.001***
T×O	4	0.062	0.702	0.600 NS
Error	18	0.088		

NS not significant, $*P < 0.05$; $**P < 0.01$; $***P < 0.001$

Table 4 *Chorus giganteus*. Average number of hatching larvae (±1 SD), as a function of temperature and oxygen concentration. *Numbers within parentheses* are total number of larvae and total number of capsules hatched, respectively

Temperature	Normoxia	Transfer	Hypoxia
12°C	564 ± 95 (1485, 14)	590 ± 209 (1771, 20)	0
15°C	631 ± 259 (2100, 17)	559 ± 377 (1667, 20)	0
18°C	466 ± 139 (1399, 12)	401 ± 373 (1203, 15)	0

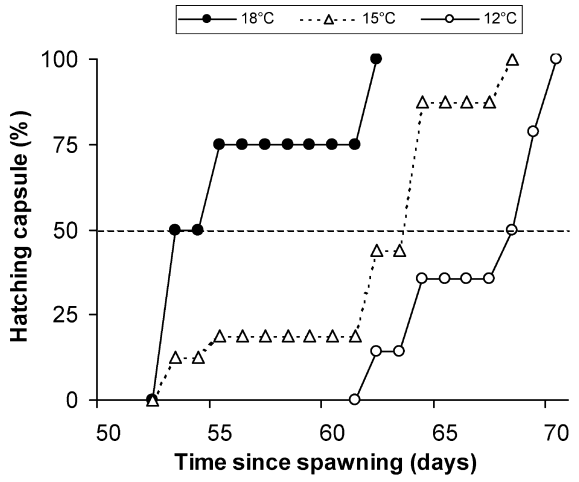

Fig. 4 *Chorus giganteus*. Cumulative percentage of hatched capsules in normoxia, as a function of time, under three temperature conditions: 12°C ($n = 12$ capsules), 15°C ($n = 25$ capsules) and 18°C ($n = 12$ capsules). Time refers to days after the egg capsules were laid. The time required for 50% of the capsules to hatch is regarded here as median hatching time (*dashed line*)

towards carbonates. Similar effects on shell deposition have been reported in the intertidal bivalve *Cerastoderma edule* when it was exposed to air during low tide (Richardson et al. 1981). Similarly, Maeda-Martínez (1985) demonstrated that larvae of *Crepidula fornicata* exposed to low environmental PO_2 reduce their rate of calcium shell deposition, while calcium concentration increases in the larval tissue, suggesting that calcium carbonate is being used to balance the drop in pH generated by the acidic end-products of anaerobic metabolism.

2. Under hypoxia hatching was prevented. Plug degradation in ovicapsules seems to be a sudden event, rather than a gradual process (Pechenik 1975). The mechanisms by which gastropod larvae are released from the capsules are mainly: (a) osmotically induced uptake of water (Hawkins and Hutchinson 1988; Kennedy and Keegan 1992), (b) direct mechanical action of the enclosed embryos on the opercule (Vaughn 1953) and (c) chemical dissolution of the opercule (Hancock 1956; Pechenik 1975). Mechanism b has been reported as one of the hatching mechanisms used by *C. giganteus* (Leiva et al. 1998), but also the effect of hypoxia on development could have impaired the production of a hatching enzyme, as in c. Therefore, either or both b and c could be true in this case.

Transfer of egg capsules from normoxia to hypoxia and vice versa at different times during development induced changes in the ash content of embryos, demonstrating that the effects of hypoxia on shell secretion are both induced in a short time and reversible. This is consistent with the idea that the lack of calcification is a result of low intracapsular PO_2 levels affecting embryonic metabolism. Further studies are required to understand the actual mechanisms and processes involved in shell calcification. Interestingly, our results suggest that the response of embryos to a changing oxygen environment depends on temperature and that such a response is greater at earlier development stages. This effect can be explained through the effects of temperature on development, as follows. At a lower temperature, development takes place at a lower rate (present study; Cancino, unpublished data). Embryos subjected to a change in oxygen concentration might respond more readily to the changing environment because they are at an earlier developmental stage than embryos kept at a higher temperature.

Similar effects of hypoxia in development have been reported for other mollusks. Larvae of *Crassostrea virginica* fail to develop a shell if reared in seawater with oxygen content below 1% of air saturation (Baker and Mann 1994), while Strathmann and Strathmann (1995) have shown that opisthobranch embryos briefly exposed to a low oxygen concentration hatched with shorter shells. They also showed that increasing the external oxygen concentration was sufficient to rescue embryos within gelatinous masses in which rates of development were limited by the rate of diffusive oxygen exchange. Thus, the rescue showed that oxygen supply was the limiting factor rather than waste removal. Several studies have reported retarded development of embryos located in the center of egg masses and have suggested that oxygen supply could be the limiting factor for embryonic development in egg masses of different animals (Chaffee and Strathmann 1984; Lucas and Crisp 1987; Seymour and Roberts 1991; Strathmann and Strathmann 1995; Cohen and Strathmann 1996). Recently, Cohen and Strathmann (1996) have shown that the film of micro-organisms that usually cover the egg masses also affects the supply of oxygen to embryos of marine invertebrates. Cancino et al. (2000) showed that sessile Protozoa attached to the outer wall of ovicapsules of *C. giganteus* significantly reduced intracapsular oxygen concentration, preventing shell secretion, as in the present study under hypoxia.

The high sensitivity of intracapsular development to low oxygen supply in *C. giganteus*, reported in the present study, is rather surprising taking into account that members of this species attach their egg capsules in clusters to small subtidal rocks (Gallardo 1981). In such areas oxygen supply may be low due to proximity to the water–substratum boundary layer. Furthermore, within the distribution range known for this snail (Osorio et al. 1979), the species is likely to encounter seawater with very low values of oxygen content. In the area near Concepción in central Chile, for example, where natural populations of the snails occur at present, oxygen contents as low as 1.2 ml O_2 l^{-1} (5.357E-05 mol) have been reported to last for several months in spring time (Ahumada 1994). In a recent study Cancino et al. (2000) reported that egg capsules of *C. giganteus* in seawater with an oxygen concentration of 3.12 ml O_2 l^{-1} (1.429E-04 mol) have an internal oxygen tension of 1.56 ml O_2 l^{-1} (6.964E-05 mol), which still is above the minimum oxygen level registered during some weeks in

the natural environment during the spring and summer (Ahumada 1976, 1994; Llancamil 1982).

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