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Oxygen-dependent asynchrony of embryonic development in embryo masses of brachyuran crabs

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Abstract Among brooding species, passive and active means to provide oxygen to embryos can be observed. Among passive oxygen providers, lower oxygen availability in the center than at the periphery of embryo masses seems to delay development of inner embryos. We investigated the differences in patterns of oxygen supply to the periphery and the center of embryo masses in two active oxygen providers, the brachyuran crabs *Cancer setosus* and *Homalaspis plana*, and evaluated the consequences on: (1) the proportion of time that early- and late-stage embryos were exposed to low or high oxygen partial pressure (PO_2), (2) oxygen consumption of the embryos from the center (inner) and the periphery (outer) of the embryo mass at those PO_2 levels that the embryos experience throughout development, and (3) development of inner and outer embryos. We found that oxygen availability in the embryo masses of brachyuran crabs exhibited dramatic contrasts between the periphery and the center during early development and that these differences decreased throughout embryonic development. These dissimilar patterns of oxygen availability produced differences in the proportion of the time that the embryos were exposed to high and low PO_2 levels throughout development. PO_2 affected oxygen consumption of the inner and outer embryos in the same fashion, but the oxygen demand of inner embryos was lower. Furthermore, development of inner embryos was delayed, in comparison to outer embryos of the same female. We suggest that the asynchrony in the development of inner embryos, in comparison to outer embryos, is due to oxygen limitation, since oxygen availability affects embryonic oxygen consumption. The differences between development of inner and outer embryos is relatively small, when compared to other marine invertebrates, probably because female crabs are able to adjust oxygen supply to the embryos according their needs, while passive oxygen providers are not. However, active oxygen provision may affect investment in reproduction. Our results could have important implications both on studies of larval development and survival and in understanding the life-history tradeoffs of aquatic invertebrates.

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Introduction

Different strategies can be observed among marine invertebrates to solve the oxygen-limitation problem of the embryo during development. On one extreme, broadcasting appears to be the simplest and cheapest way to ventilate a large number of embryos (Strathmann and Strathmann 1982). Among brooding species, passive and active means of oxygen provision to embryos can be observed. Embedding eggs in gel is a passive way to provide oxygen to inner embryos, since gel helps in oxygen diffusion (Strathmann and Strathmann 1995; Lee and Strathmann 1998). In spite of the addition of gel, some gelatinous embryo aggregations exhibit lower oxygen availability in the center than at the periphery (Chaffee and Strathmann 1984; Booth 1995; Cohen and Strathmann 1996). The number of embryos in the embryo aggregation and the spacing among them seem to play critical roles in determining differences in oxygen availability between the periphery and the center of gelatinous embryo masses (Strathmann and Strathmann 1995). Brachyuran crabs exhibit a high level of embryo packing and no gel, which is an adverse scenario for oxygenating inner embryos. However, brachyuran crabs

show active brooding behavior, mainly directed to supply oxygen to the embryos (Baeza and Fernández 2002). Oxygen supply to the center of the embryo mass of brachyuran crabs is low during early development (Naylor et al. 1999; Fernández et al. 2000) and increases as embryos develop and oxygen consumption by the embryos increases (Fernández et al., unpublished data). The main difference between active and passive oxygen providers is the pattern of change in oxygen gradients between the periphery and the center of the embryo mass, from early to late development (Cohen and Strathmann 1996; Fernández et al. 2000). The oxygen gradient between the periphery and the center becomes stronger as embryos develop among passive oxygen providers (passive brooders), while the opposite occurs among active oxygen providers (active brooders).

The differences in oxygen availability between the center and the periphery of embryo masses of passive oxygen providers seem to affect embryo development, since a delay in inner embryo development has been found in gelatinous embryo masses (Chaffee and Strathmann 1984; Strathmann and Strathmann 1995). Moreover, the delay becomes greater as embryos develop. This delay in development can be explained by the decrease in oxygen availability (Chaffee and Strathmann 1984; Strathmann and Strathmann 1995), which could lower oxygen consumption rates of embryos at low oxygen partial pressures (Patel and Crisp 1960; Wheatly 1981; Naylor et al. 1997; Booth 1995; Fernández et al. 2000). Embryos of brachyuran crabs do not seem to suffer low oxygen availability during late development (Naylor et al. 1999; Fernández et al. 2000), probably because the change in female patterns of oxygen supply throughout development compensates for the higher oxygen demand of late embryos (Fernández et al., unpublished data). However, during early development dramatic differences in oxygen availability between the center and the periphery of the embryo masses have been found in several crab species (Naylor et al. 1999; Fernández et al. 2000). As of yet, asynchronic development within the embryo mass has not been reported for brachyuran crabs. However, hatching over several days can be an indicator of asynchrony in development (Zeng and Naylor 1997; Pardo 1998).

We investigated the differences in patterns of oxygen supply to the periphery and the center of the embryo masses in females carrying embryos in early and late stages of development, using two species of brachyuran crabs (*Cancer setosus* and *Homalaspis plana*), and evaluated the consequences on: (1) the proportion of time that early- and late-stage embryos were exposed to low or high oxygen partial pressure (PO_2), (2) oxygen consumption by embryos from the center (inner embryos) and the periphery (outer embryos) of the embryo mass at those PO_2 levels that the embryos experience throughout development, and (3) development of inner and outer embryos. Two indicators of developmental stage were used: (1) volume of the embryos and (2) proportion of the embryo occupied by yolk. In order to evaluate the effects of female size and embryo mass volume on the development of inner and outer embryos, each of these variables was correlated with the two indicators of development.

Materials and methods

Background information

Two species with large body size were selected: the Xanthidae *Homalaspis plana* (Milne-Edwards, 1834) and the Cancridae *Cancer setosus* (Molina, 1782). Both species exhibit similar ranges of body size and a peak in brooding during winter and early spring. *H. plana* is distributed between 0 and 53°S (Retamal 1981) and inhabits rocky substrata. *C. setosus* is found on soft bottom, between 0 and 46°S (Retamal 1981). Both species are subtidal and are exploited (Fernández and Castilla 1998). Experimental animals were collected by diving in Las Cruces (33°29′S; 71°38′W) and were maintained in 500-l tanks with circulating seawater (14°C) and constant aeration. The calibration for oxygen measurements and experiments were conducted at the constant temperature of 14°C. Crabs were feed ad libitum on fresh mussels (*Choromytilus chorus*). Size of brooding females ranged between 89 and 132 mm for *H. plana* ($\bar{x} = 104.7$, SD = 14.4) and between 98 and 138.5 mm for *C. setosus* ($\bar{x} = 118.5$ mm, SD = 12.6). Mean volume of the embryo mass of *H. plana* was 46.5 cm^3 (SD = 19.1) and of *C. setosus* was 72.3 cm^3 (SD = 31.4). Mean embryo volume of early-stage embryos was 0.06 mm^3 (SD = 0.014) for *H. plana* and 0.023 mm^3 (SD = 0.007) for *C. setosus*.

The developmental stage of a subsample of embryos was determined for all females used in this study: (1) immediately after collection, in females used to assess asynchrony in development (see subsection "Stage development and assessment of asynchrony") and (2) before experiments were conducted (see "Oxygen consumption by crab embryos" and "Oxygen availability at the periphery and in the center of the embryo mass"). The following stages were determined (Vargas 1995): stage I (embryos with uniformly distributed yolk, absence of cleavage and eyes, ca. 10 days of development); stage II (embryos with cleavage and yolk reduced to not less than 75% of embryo volume, ca. 25 days of development); stage III (embryo with visible but undeveloped eyes, and presence of pigments, ca. 45 days of development); and stage IV (embryo with well-developed eyes, pumping heart, and appendages, ca. 60 days of development, just before hatching). In some of the experiments these four stages were utilized, in others only two categories of embryo stage were used: early (stages I and II) and late (stages III and IV). Throughout this manuscript, we refer to inner embryos as those from the center of the embryo mass and to outer embryos as those from the periphery. We define periphery as the first 5 mm of the embryo mass from the border to the center and center as the 10 mm diameter sphere located exactly in the center of the embryo mass.

Oxygen consumption by crab embryos

In order to evaluate whether inner and outer embryos consume oxygen at the same rate and are affected similarly by PO_2, laboratory experiments were conducted. Treatments were: (1) position in the embryo mass (inner and outer), (2) stage of development (four stages), and (3) PO_2 (low, < 39.7 mmHg; high, > 119.1 mmHg). In this case, the stage of development of the embryo mass of each female was determined using outer embryos. The two PO_2 levels utilized were selected, because previous work on other crab species has shown that: (1) oxygen consumption is always affected at $PO_2 < 39.7$ mmHg and (2) PO_2 of > 119.1 mmHg oxygen consumption is not affected, regardless of the embryo stage (Naylor et al. 1999; Fernández et al. 2000; Baeza and Fernández 2002; Fernández and Pörtner, unpublished data).

Oxygen consumption rates of inner and outer embryos of each female were measured simultaneously using a double-wall, closed microchamber filled with 2 ml of filtered (filter size: 0.2 μm) seawater (with added antibiotic). A solution saturated with Na_2SO_3 was used for the calibration at 0% air saturation, and aerated water, for the calibration at 100% air saturation. A small number of embryos was removed from the periphery (or the center) of the embryo mass of brooding females and placed on a fine grid in the microchamber. Water was stirred inside the chamber (Naylor et al. 1997). PO_2 was monitored continuously with oxygen electrodes (Eschweiler M200; measuring range: 0–760 mmHg; resolution: 0.1 mmHg) until oxygen was depleted to 30 mmHg. At the end of the experimental period, the embryos were weighed (wet weight). Numbers of replicates for each combination of stage, position in the embryo mass, and PO_2 in each species are indicated in Fig. 1. For each species, oxygen consumption per unit of time and weight was estimated and compared between stages, positions in the embryo mass, and PO_2 using a nested analysis of variance (ANOVA). Data were transformed in order to meet the assumptions of the model. The Tukey test was used for a posteriori comparisons. Based on the homogeneous groups determined from these comparisons, the levels within the factor stage of development were determined for the other experiments.

Oxygen availability at the periphery and in the center of the embryo mass

In order to determine oxygen availability at the periphery and in the center of the embryo mass of females carrying early- and late-stage embryos, oxygen was monitored continuously under laboratory conditions in *H. plana* and *C. setosus*. Optic fibers with a tip diameter between 20 and 50 μm were used to continuously monitor oxygen availability; optic fibers do not affect female brooding behavior (Fernández et al. 2000; Baeza and Fernández 2002). In order to measure oxygen availability in the center of the embryo mass, a small hole (<1 mm) was drilled into the abdomen (V segment) of brooding females, and a plastic tube (of variable length depending on embryo mass diameter) was inserted and fixed to the abdomen. To measure oxygen at the periphery of the embryo mass a small hole was drilled on the border of the abdomen, and then the plastic tube was glued in place. The microoptode was inserted into the tube after calibration (0% air saturation: solution saturated with Na_2SO_3; 100% air saturation: aerated water from the tank where the experiment was conducted). The tip of the optic fiber reached 2–3 mm outside the catheter and was in contact with the embryos. Constant aeration was maintained during the whole experimental period. Females were placed in the experimental tank filled with a layer of shell hash, rocks, and unfiltered seawater. After 1 h of acclimation, PO_2 in the center of the embryo mass was monitored continuously for 2 h. The data were recorded once every 5 s during the experimental period. Four replicates for each treatment combination were conducted. The percentage of time that the embryos from the periphery and the center of the embryo mass (at each stage of development) spent at low PO_2 (39.7 mmHg) or high PO_2 (119.1 mmHg) was estimated. Two independent, nested ANOVAs were used to test for differences between species in the mean percentage of time that early- and late-stage embryos in the center and the periphery of the mass were exposed to low (or high) PO_2. Data were transformed to meet the assumptions of the ANOVA model.

Stage of development and assessment of asynchrony

In order to determine asynchrony in development between inner and outer embryos throughout development, the embryo masses of female crabs carrying embryos at different stages of development were sampled in both species. From each female, the following data were recorded: (1) stage of development of outer embryos, (2) largest and shortest diameter of a subsample of at least ten inner and ten outer embryos, (3) largest and shortest diameter of yolk in inner and outer embryos, (4) largest and shortest diameter of the embryo mass, and (5) size of the female. The developmental stage of outer embryos was used to identify the embryos of that female (regardless of the presence or degree of asynchrony). Embryos were staged into early or late development. These categories of embryo development were selected based on the results of oxygen consumption by crab embryos, and were also used in oxygen availability at the periphery and in the center of the embryo mass, so that the patterns of oxygen availability could be related with oxygen consumption and development. Within each category of stage of development, the volume of the embryos and the proportion of the embryos occupied by yolk were used as indicators of development. Using the longest and shortest diameter of each embryo, the mean radius of the embryos was calculated; the volume of the embryos was then estimated (using the volume of a sphere) for outer and inner embryos and for each female using the corresponding subsamples of embryos. The same approximation was used to estimate the volume of the yolk in inner and outer embryos. Then, the proportion of the egg volume occupied by yolk was calculated for each embryo. The mean percentage of difference between inner and outer embryos was calculated for each female and each response variable. Positive values indicate that volume of outer embryos is larger than volume of inner embryos. Since the volume of embryos increases with development (Wear 1974), larger embryos grown at the same temperature will correspond to more

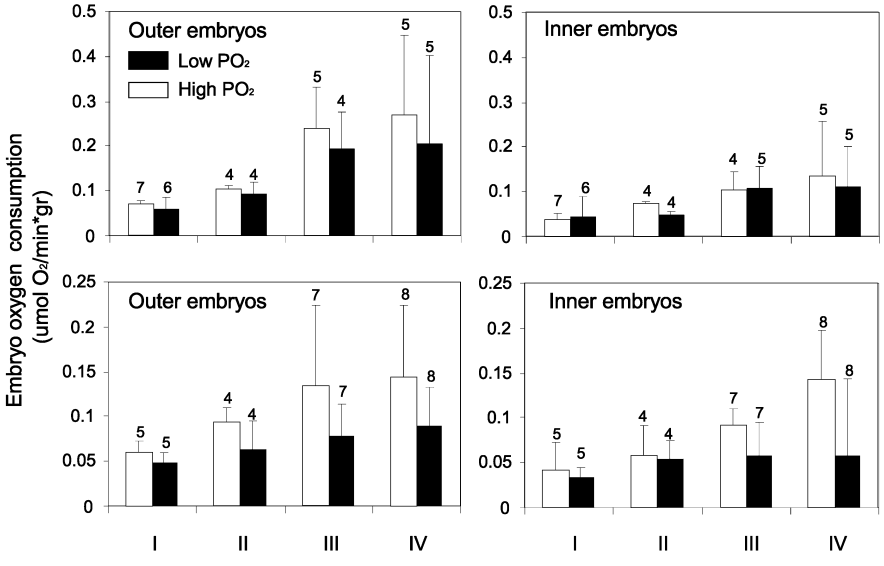

Fig. 1 *Cancer setosus*, *Homalaspis plana*. Mean oxygen consumption by crab embryos from the periphery (*outer embryos*) and from the center (*inner embryos*) of embryo masses of *C. setosus* (*upper panels*) and *H. plana* (*lower panels*) at low (<39.7 mmHg, *solid bars*) and high oxygen partial pressure (PO_2; >119.1 mmHg; *open bars*) for the different stages of development (four stages, *I–IV*). *Numbers* indicate replicates; *vertical lines* indicate one standard error

advanced stages. Negative values indicate that the proportion of yolk in inner embryos is larger. Since yolk is consumed as embryos develop, a lower proportion of yolk in the embryo corresponds to more advanced stages. Numbers of replicates for each combination of species and stage of development are shown in Fig. 4. Three different statistical analyses were performed. First, two-way ANOVAs were conducted to test for differences in the response variables between stages of development (early and late) and species. Data were not transformed since the assumptions of the ANOVA model were met. Second, to assess whether the mean difference in development between inner and outer embryos (using both indicators of development) was significantly different from zero (zero asynchrony), a one-sample *t*-test was used for each combination of species and stages. Significance levels were adjusted with a sequential Bonferroni correction (Péres-Neto 1999). Finally, both response variables were correlated against female size and embryo mass volume, since it is known that embryo mass size varies with female size (Hines 1982) and asynchrony increases with embryo mass sizes among marine invertebrates (Strathmann and Strathmann 1995).

Results

Oxygen consumption by crab embryos

The three factors analyzed had a significant effect on mean oxygen consumption by crab embryos in both species. Oxygen consumption by crab embryos of both species was lower at low PO_2 (Fig. 1, *Cancer setosus*: $F = 9.54$; $df = 1,38$; $P < 0.05$; *Homalaspis plana*: $F = 14.5$; $df = 1,29$; $P < 0.05$). Inner embryos exhibited significantly lower oxygen consumption than outer embryos, and this pattern was consistent in both species (Fig. 1, *C. setosus*: $F = 7.25$; $df = 1,38$; $P < 0.05$; *H. plana*: $F = 36.25$; $df = 1,29$; $P < 0.05$). Embryo oxygen consumption was different among embryo stages (*C. setosus*: $F = 3.63$; $df = 1,38$; $P < 0.05$; *H. plana*: $F = 3.67$; $df = 1,29$; $P < 0.05$), although slightly different patterns were found between species. In *C. setosus* oxygen consumption was lowest for stages I and II, intermediate for stage III and highest for stage IV (Fig. 1). Two homogeneous groups were found in *H. plana*; stages I and II did not exhibit differences among themselves, and no differences were found for the second homogeneous group composed by stages III and IV. Based on these results we categorized stages for the rest of the experiments into early (stages I and II) and late (stages III and IV). PO_2 did not have a differential effect on oxygen consumption by inner and outer embryos (interaction terms: $P > 0.05$) nor on oxygen consumption by early and late stages (interaction terms: $P > 0.05$).

Oxygen availability at the periphery and in the center of the embryo mass

Patterns of oxygen availability in embryo masses of females carrying early and late stages of embryo masses varied from the periphery to the center and throughout development in a similar fashion for the two studied species (Fig. 2). In both species oxygen availability at the periphery of the embryo masses exhibited levels close to normoxic conditions throughout development, the values recorded at the periphery were always higher than those seen in the center of the embryo masses even during late development (Fig. 2). Oxygen availability in the center of the embryo mass changed from regular, low-frequency PO_2 increments and an extremely hypoxic baseline at an early stage, to a nearly normoxic baseline for late stages, where regular PO_2 decrements occurred at different frequencies (Fig. 2). Although, in general, both species showed similar patterns of oxygen availability at the periphery and in the center, throughout development, small differences could be observed in the shape of the peaks between species (Fig. 2).

The differences in the patterns of oxygen availability over time between the periphery and the center of the embryo mass and throughout development strongly influenced the mean proportion of time that embryos were exposed to different levels of PO_2 in different parts of the embryo mass. Oxygen availability for central embryos shifted from predominantly hypoxic exposure during early development to predominantly normoxic exposure during late development (Fig. 3). Significant interactions in the ANOVA between developmental stage and posi-

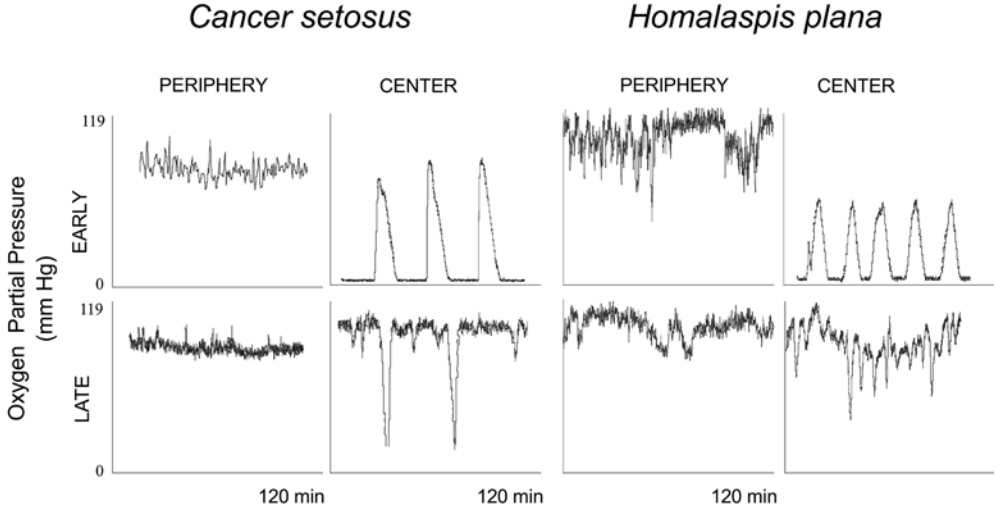

Fig. 2 *Cancer setosus*, *Homalaspis plana*. Patterns of oxygen partial pressure for early (*upper panels*) and late (*lower panels*) stages of embryo development at the periphery and in the center of the embryo masses of *C. setosus* and *H. plana*. The patterns shown here are for one female of each species and embryo developmental stage, but these results were consistent across replicates, and a summary of the information for all females analyzed is shown in Fig. 3

tion in the embryo mass precluded us from testing for the effect of these factors on the mean proportion of time that the embryos were exposed to high or low oxygen partial pressures (high PO_2: $F = 108.12$; $df = 1,12$; $P < 0.05$; low PO_2: $F = 165$; $df = 1,12$; $P < 0.05$). The interaction between these two factors was due to the lack of differences in the mean proportion of time that early and late embryos at the periphery of the embryo mass were exposed to high oxygen partial pressures, while in the center strong differences were found (Fig. 3). The general patterns found in the mean proportion of time that the embryos of both species spent at low PO_2 for the two developmental stages analyzed and the two positions in the embryo mass did not differ between species (Fig. 3; $P > 0.05$). However, embryos of *C. setosus* were exposed to high oxygen partial pressures for a significantly higher proportion of time than embryos of *H. plana* (Fig. 3; $P < 0.05$).

Assessment of asynchrony

The percent difference of the volume between outer and inner embryos was not significantly different between stages or between species (Fig. 4; Table 1). However, it is important to emphasize that the mean percent differences between the volume of outer and inner embryos were always positive. In spite of the lack of difference between stages and species, mean percent difference of the volume between outer and inner embryos was significantly different from zero for all combinations of species and stages (*t*-tests always < 0.05 after Bonferroni correction). The mean percent differences in the

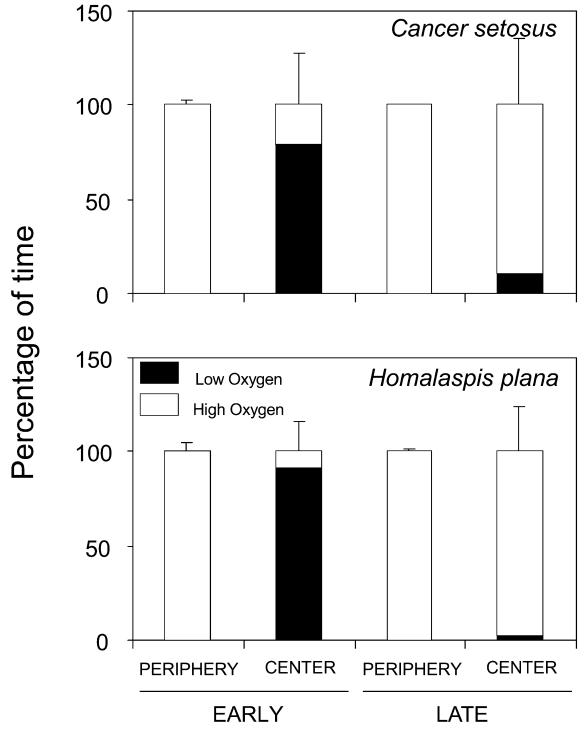

Fig. 3 *Cancer setosus*, *Homalaspis plana*. Mean percentage of time that early and late developmental stages of crab embryos located at the periphery and in the center of the embryo mass were exposed to low (< 39.7 mmHg; *solid bars*) and high oxygen partial pressure (PO_2; > 119.1 mmHg; *open bars*) in the two studied crab species, *C. setosus* (*upper panel*) and *H. plana* (*lower panel*). *Vertical lines* indicate one standard error

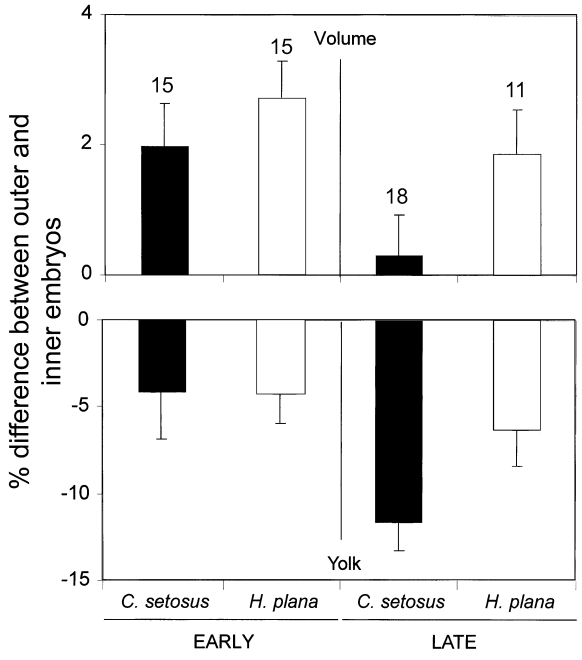

Fig. 4 *Cancer setosus*, *Homalaspis plana*. *Upper panel*: mean difference (%) between the volume of embryos from the periphery (*outer embryos*) and the center (*inner embryo*) of early (*left*) and late (*right*) embryos of *C. setosus* (*solid bars*) and *H. plana* (*open bars*). *Positive values* indicate that the volume of outer embryos is larger than the volume of inner embryos. *Lower panel*: mean difference (%) in the fraction of the embryo occupied by yolk between outer and inner embryos for the two species. *Negative values* indicate that the fraction of yolk in inner embryos is larger. *Numbers* indicate replicates; *vertical lines* indicate one standard error. Results of statistical analyses are reported in Table 1

Table 1 *Cancer setosus*, *Homalaspis plana*. Results of the two-way ANOVA conducted to test for differences in: (1) mean percentage differences in embryo volume between outer and inner embryos and (2) mean percentage differences in the proportion of the embryo occupied by yolk between outer and inner embryos. Both variables were compared between species (*C. setosus* and *H. plana*) and between stages of development (early and late)

Factor	*F*-ratio	*df*	*P*
Mean difference (%) in embryo volume between outer and inner embryos			
Species	0.97	1	0.33
Stage	0.68	1	0.41
Species×Stage	0.008	1	0.92
Error		50	
Mean difference (%) in the proportion of the embryo occupied by yolk between outer and inner embryos			
Species	1.19	1	0.28
Stage	7.41	1	< 0.008
Species×Stage	1.31	1	0.25
Error		53	

proportion of embryonic volume occupied by yolk varied between early and late stages in a consistent fashion in both species (Fig. 4; Table 1). Values were always negative, indicating that, in general, inner embryos had more yolk than outer embryos. The mean percent differences in the proportion of embryonic volume occupied by yolk were also significantly different from zero for all combinations of species and stages (*t*-tests always < 0.05 after Bonferroni correction). Neither, the volume of the embryo mass nor female size explained the percent differences in the volume between outer and inner embryos or the percent differences in the fractional embryo volume occupied by yolk (Table 2).

Discussion

Our results clearly show that oxygen availability in the embryo masses of brachyuran crabs exhibit dramatic contrasts between the periphery and the center during early development and that these differences decrease throughout embryonic development. These patterns of oxygen partial pressure may be produced by dissimilar in the patterns of oxygen supply by females carrying early- and late-stage embryos (Baeza and Fernández 2002), which, in turn, generates differences in the proportion of time that the embryos are exposed to low or high PO_2 levels throughout development. The differences in the fraction of embryonic volume occupied by yolk between inner and outer embryos might be due to oxygen limitation, since oxygen availability affects embryonic oxygen consumption. The differences between development of inner and outer embryos, as assessed by the fraction of embryonic volume occupied by yolk, are relatively small when compared to those of other marine invertebrates (Chafee and Strathmann 1984; Strathmann and Strathmann 1995; Cohen and Strathmann 1996). We think that the differences between inner and outer embryos are low, because female crabs are able to adjust oxygen supply to the embryos according to their needs (Baeza and Fernández 2002). The implications of our results are several. First, we provide new evidence of the constraints of large body size on brooding, this time for active brooders. Second, the necessity of providing oxygen to the brood seems to have an effect on development, which could have consequences on larval development and survival. Finally, this simple physiological constraint on brood care may have important life-history consequences as well as ecological and evolutionary implications.

The patterns of oxygen availability in early and late stages of embryo masses follow the same trends that have been described previously for other brachyuran crabs (Naylor et al. 1999; Fernández et al. 2000; Baeza and Fernández 2002). These patterns are linked to dramatic differences between the mean proportion of time that early-stage embryos from the periphery and from the center are exposed to low or high oxygen conditions in both species. It is interesting to point out that in spite of variation in the shape of the peaks of oxygen partial pressure during early development between species, no differences were found in the mean proportion of time that the embryos were exposed to low oxygen partial pressure between species. Thus, no difference in development between inner and outer embryos is expected between species, if only oxygen supply matters. In fact, development differences of inner and outer embryos were found between species. This can also be explained by the lack of interspecific variation in other factors, such as embryo oxygen demand, which would affect the gradient in PO_2 in the embryo mass, and embryo size, which would affect the spacing among embryos. Neither differences in embryo size nor in embryo oxygen consumption were found between these two species (Fernández and Pörtner, unpublished data).

At normoxia the transition from stages I and II to stages III and IV was associated with the largest increment in oxygen consumption (the increase in oxygen consumption between early- and late-stage embryos may be underestimated, since we used wet weight). The rise in oxygen consumption may be associated with the morphological and functional changes that take place (development of the circulatory system, neuromotor system, eyes, appendages, etc.). Hypoxia had a significant effect on embryo oxygen consumption. However, hypoxia did not have a differential effect on oxygen consumption between early- and late-stage embryos, in spite of the elevated rate of metabolism of the latter, nor on outer embryos compared to inner embryos for the two studied species. The lower oxygen consumption under hypoxia could lead to retarded development. Thus, the lower rate of oxygen consumption of inner embryos could be due to differences in development with respect to outer embryos. Retarded development is a plausible explanation for the lower oxygen consumption

Table 2 *Cancer setosus*, *Homalaspis plana*. Results of correlation analyses conducted to assess the relationship between embryo mass size and female size (carapace width) and the two indicators of asynchrony between outer and inner embryo development: (1) percent difference between inner and outer embryo volume (*embryo volume*) and (2) percent difference in the proportion of embryos occupied by yolk (*proportion of yolk*)

Variable 1	Variable 2	*Cancer setosus*	*Homalaspis plana*
Embryo mass volume	Embryo volume	$r = -0.07$; $P = 0.69$; $n = 33$	$r = 0.032$; $P = 0.88$; $n = 25$
	Proportion of yolk	$r = -0.04$; $P = 0.81$; $n = 33$	$r = 0.093$; $P = 0.66$; $n = 24$
Female size	Embryo volume	$r = -0.05$; $P = 0.79$; $n = 33$	$r = 0.04$; $P = 0.86$; $n = 24$
	Proportion of yolk	$r = 0.11$; $P = 0.56$; $n = 33$	$r = 0.27$; $P = 0.21$; $n = 23$

of inner embryos, given the systematic trend towards positive differences in embryo volume and negative differences in the proportion of the embryos occupied by yolk between inner and outer embryos. We think that the cumulative effect of exposure to hypoxia during early development results in a delay that is not compensated for late in development, when PO_2 in the center is high.

Different trends for each of the response variables analyzed to assess asynchrony were observed. On the one hand, no differences in volume between outer and inner embryos were found throughout development, although in both species and stages the difference in volume between inner and outer embryos was significantly different from zero (and positive), which is an indicator of asynchrony in development. On the other hand, the percent difference in the proportion of yolk increased with embryo development. These findings indicate that even when oxygen becomes available during late development, yolk is consumed at a lower rate by inner embryos, increasing the difference in development between inner and outer embryos. Differences in embryo size between inner and outer embryos have been reported for other brachyuran crabs (Wear 1974), and, although the differences were not significant for the two species studied here, the consistent positive difference suggests that a differential trend in development of inner and outer embryos may be a common pattern. Although the size at hatching of outer embryos may not be substantially larger than that of inner embryos, this difference may have an effect on survival of subsequent stages (e.g. zoeae or megalopae). For instance, Pardo (1998) suggested that survival of late hatchers (when hatching occurs within two to four consecutive days) is lower than that of early hatchers. The consequences of oxygen limitation during embryo development on larval development and survival need to be studied.

Female crabs seem to provide oxygen according to oxygen demand by the embryos, but this supply is not sufficient to make development perfectly synchronic. However, the delay in development of inner embryos observed among brachyuran is smaller than that reported for other marine invertebrates. This indicates that active mechanisms are more efficient in supplying oxygen to the embryos than passive methods facilitating oxygen diffusion to the center of the embryo mass. However, the costs associated with oxygen provision are substantial (Fernández et al. 2000; Baeza and Fernández 2002). Moreover, energy investment in oxygen provision could have important effects on egg production, female growth, and survival. Thus, it can be proposed that production of eggs may not only be limited by the space available for yolk accumulation (Hines 1982, 1986), but also by the capacity of females to supply oxygen according to embryo needs. Since oxygen supply must increase as embryo mass size (fecundity) increases, the average 10% investment in eggs (Hines 1982, 1986) may be limited by the capacity of females to supply oxygen to large embryo masses, as well as by the effect of hypoxia on the embryos and the consequences on later survival.

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References

Baeza A, Fernández M (2002) Active brood care in *Cancer setosus* (Crustacea: Decapoda): the relationship between female behaviour, embryo oxygen consumption, and the cost of brooding. Funct Ecol 16:241–251

Booth D (1995) Oxygen availability and embryonic development in sand snail egg masses. J Exp Biol 198:241–247

Chaffee C, Strathmann R (1984) Constraints on egg masses. I. Retarded development within thick egg masses. J Exp Mar Biol Ecol 84:73–83

Cohen C, Strathmann R (1996) Embryos at the edge of tolerance: effects of environment and structure of egg masses on supply of oxygen to embryos. Biol Bull (Woods Hole) 190:8–15

Fernández M, Castilla JC (1997) The Chilean artisanal stone crab (*Homalaspis plana*) fishery: catch trends in open access zones and the effect of management areas in Central Chile. J Shellfish Res 16:371–377

Fernández M, Bock C, Pörtner H (2000) The cost of being a caring mother: the ignored factor in the reproduction of marine invertebrates. Ecol Lett 3:487–494

Hines AH (1982) Allometric constraints and variables of reproductive effort in brachyuran crabs. Mar Biol 69:309–320

Hines AH (1986) Larval patterns in the life history of brachyuran crabs (Crustacea, Decapoda, Brachyura). Bull Mar Sci 39:444–466

Lee C, Strathmann R (1998) Scaling of gelatinous clutches: effects of sibling competition for oxygen on clutch size and parental investment per offspring. Am Nat 151:293–300

Naylor JK, Taylor E, Bennett D (1997) The oxygen uptake of ovigerous edible crabs (*Cancer pagurus*) (L) and their eggs. Mar Freshw Behav Physiol 30:29–40

Naylor JK, Taylor EW, Bennett DB (1999) Oxygen uptake of developing eggs of *Cancer pagurus* (Crustacea: Decapoda: Cancridae) and consequent behaviour of ovigerous females. J Mar Biol Assoc UK 79:305–315

Pardo LM (1998) Composición bioquímica y viabilidad larval de *Petrolisthes violaceus* (Anomura: Porcellanidae) eclosionadas en distintos tiempos. BSc thesis, Universidad Católica del Norte, Coquimbo, Chile

Patel B, Crisp D (1960) Rates of development of the embryos of several species of barnacles. Physiol Zool 33:667–680

Péres-Neto PR (1999) How many statistical tests are too many? The problem of conducting multiple ecological inferences revisited. Mar Ecol Prog Ser 176:303–306

Retamal M (1981) Catálogo ilustrado de los Crustáceos Decápodos de Chile. Gayana Zool 44:1–110

Strathmann R, Strathmann M (1982) The relation between adult size and brooding in marine invertebrates. Am Nat 119:91–101

Strathmann R, Strathmann M (1995) Oxygen supply and limits on aggregation of embryos. J Mar Biol Assoc UK 75:413–428

Vargas LM (1995) Descripción del desarrollo embrionario de *Cancer polyodon* Poepping, 1836 (Crustacea: Decapoda: Brachyura) y efectos de la temperatura, bajo condiciones de laboratorio. BSc thesis, Universidad Católica del Norte, Coquimbo, Chile

Wear R (1974) Incubation in British decapod Crustacea, and the effects of temperature on the rate and success of embryonic development. J Mar Biol Assoc UK 54:745–762

Wheatly MG (1981) The provision of oxygen to developing eggs by female shore crabs (*Carcinus maenas*). J Mar Biol Assoc UK 61:117–128

Zeng Ch, Naylor E (1997) Rhythms of larval release in the shore crab *Carcinus maenas*. J Mar Biol Assoc UK 77:451–461