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EFFECTS OF REPRODUCTIVE STAGE AND TEMPERATURE ON RATES OF OXYGEN CONSUMPTION IN *PARALITHODES PLATYPUS* (DECAPODA: ANOMURA)

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ABSTRACT

Paralithodes platypus is a large decapod that inhabits Alaskan and North Pacific waters. Females exhibit a biennial spawning cycle, requiring two years for production of fully mature oöcytes. We used respirometry and video recording to determine: 1) metabolic rates of brooding and post-brooding females, embryos, and larvae at different temperatures, 2) if females exhibit active brood care, 3) oxygen availability within the clutch, and 4) the timing of larval hatching. The rates of oxygen consumption (MO_2) of brooding females was significantly higher than that of post-brooding females at night, but was similar during the day and increased significantly with temperature. MO_2 of crab embryos did not differ with position in the clutch, whereas MO_2 of zoeae averaged 4-fold higher than that of embryos. Larvae from the periphery of the embryo mass, either top or bottom, hatched prior to larvae from the middle of the clutch. Oxygen availability in the embryo mass varied significantly with position in the clutch; saturation was highest at the top (~ 91%), and lowest at the middle (~ 66%). Flapping of the pleon in brooding females was coincident with sudden increases in oxygen availability at the bottom of the embryo mass. The percentage of time that brooding females were engaged in flapping of the pleon was 30% higher at night than during the day. Our results address the cost of brooding in a lithodid crab and the effects of temperature on this behavior. The importance of pleonal flapping to ventilation of the embryo mass and alternative hatching mechanisms is discussed.

KEY WORDS: blue king crab, brooding cost, oxygen availability, *Paralithodes platypus*, respirometry

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INTRODUCTION

The blue king crab (BKC), *Paralithodes platypus* Brandt, 1850, is a large anomuran decapod that inhabits the Bering Sea, Gulf of Alaska, Southeast Alaska, and western Pacific Ocean near Japan and Russia (Stevens, 2006a). This species of king crab has been commercially harvested since 1966, with peak annual landings of about 5000 tons (valued between US$10 and 25 million) during the 1980s (Stevens, 2006a). Since then, the population has decreased drastically, a decline that has been attributed to their commercial harvest. Thus, as with many of the other king crab fisheries (Lovrich, 1997), the BKC fishery collapsed during 1998 and has remained closed (NPFMC, 2002).

Adult female BKC exhibit a biennial spawning cycle, requiring two years for production of fully mature oöcytes. Females mate and extrude new eggs in late winter after molting; fertilized embryos develop for approximately one year before the larvae hatch (Stevens, 2006a; Stevens et al., 2008a). After hatching, females carry empty egg cases for one year until the next molt (Somerton and MacIntosh, 1985; Jensen and Armstrong, 1989).

Several marine invertebrate species exhibit a biennial spawning cycle, a life history thought to have evolved to facilitate maternal protection of eggs and juveniles from predators and adverse environmental conditions (Strathmann, 1985). In decapod crustaceans, females carry eggs attached to their pleopods located under the pleon. From an ecological point of view, this represents an advantage since eggs are ''protected'' from predation and other physical insults. However, maintenance of sufficient oxygen saturation within the clutch can be problematic (Fernández et al., 2000). Furthermore, oxygen requirements increase with the development of embryos. Eggs that are located in the middle of the clutch are more affected by hypoxia during development (Fernández et al., 2000; Baeza and Fernández, 2002). Therefore, oxygen provisioning to the developing embryos has to be resolved by mothers and is an important behavioral aspect of parental care that likely adds to the energetic cost of brooding.

In decapod crustaceans, the cost of brooding has been investigated among the brachyuran crabs. *Cancer setosus* (Molina, 1782) and *Homalapsis plana* (Milne-Edwards, 1834) show a direct relationship between active brooding behavior of females and oxygen supply to the inner eggs in the embryo mass (Baeza and Fernández, 2002; Fernández et al., 2003). Whereas several studies have focused on physiological and behavioral aspects of reproduction in

Brachyura (Baeza and Fernández, 2002; Brante et al., 2003; Fernández et al., 2003), to our knowledge there are no published studies of reproductive behavior and physiology in lithodids crabs.

Our goal was to assess the behavior and metabolic cost associated with brooding in a female lithodid crab, the blue king crab. Our aims were to determine if female blue king crab: 1) exhibit active brood care, and 2) if this behavior is affected by ambient water temperature. We hypothesized that brooding females would exhibit higher energetic expenditure than post-brooding females and that metabolic rates of brooding and post-brooding females would increase with temperature. Additionally, we measured: 3) metabolic rates of embryos and recently hatched larvae at three water temperatures, and 4) oxygen availability within the developing clutch of free-moving females. We hypothesized that oxygen availability would be greater at both top and bottom positions in the clutch than in the middle. We also examined: 5) the timing of larval hatching relative to position within the clutch, and we posited that larvae from the top or bottom position would hatch before those in the middle of the clutch.

Materials and Methods

Animal Collection

Females of *P. platypus* were captured by trawl in the eastern Bering Sea during October 2003. Crabs were held in tanks of re-circulating seawater aboard ship until returning to Dutch Harbor, Alaska. They were then packed in insulated coolers that were chilled with ice packs and transported to Kodiak, Alaska by air. Upon arrival at the laboratory, crabs were maintained in three 2500-L tanks with circulating seawater at either 6, 4 or 2°C. Water temperature was monitored continuously and maintained at the set-point (± 0.5°C) with either a heater, or chiller placed inside the tank. Crabs were fed an ad libitum diet comprised of squid (*Loligo* spp.) twice per week, and they did not receive food for 3 days prior to metabolic experiments. Experiments were completed between February and April 2006. The mean carapace length (CL) of females used for experiments was 131.1 ± 3.8 mm. Females were carrying embryos in the last stage of development (Stevens, 2006b). Approximately one month before hatching began, nine females ($n = 3$ for each temperature) were placed into individual 60-L aquaria within the chilled tanks to facilitate capture of larvae. Larvae hatched at night, and were removed each morning for counting and volumetric measuring; these results are reported separately (Stevens et al., 2008a), but were also used to determine appropriate sampling times (see below).

Oxygen Uptake of Brooding and Post-Brooding Females

Rates of oxygen consumption (MO_2) of each female was measured during two distinct 24- h time periods (light cycle was 10:14 h light:dark), initially when females were carrying eggs (brooding) and at a subsequent time following hatch (post-brooding). For determination of MO_2, crabs were placed into 31-L individual closed chambers that were submerged in a holding tank of 700-L seawater maintained at 6, 4 or 2°C and flushed with filtered seawater. During metabolic trials, chambers were sealed and seawater was mixed using a submersible pump to ensure homogeneous oxygen saturation within the chamber as described previously (Haukenes et al., 2009).

Dissolved oxygen concentration was measured each minute of the sample period (70-100 min) with a FOXY fiber optic oxygen sensor system (Ocean Optics Inc., U.S.A.). The sensor probe (tip size 300 μm) was inserted via cannula (diameter 1 mm) through the top of the chamber. Before initiation of the experiments, oxygen microsensors were calibrated to 0 (with a saturated solution of Na_2SO_3) and 100% air saturation (with aerated water from the holding tank). Both solutions (Na_2SO_3 and aerated water) were maintained at the same temperature of the experiments. Biological activity of the empty chambers was determined and considered negligible. Oxygen saturation was monitored continuously and was not allowed to fall below 80% of saturation during the metabolic trials. An oxygen depletion rate for each minute was calculated and a mean for the sampling period determined. Wet mass of females was recorded at the end of the experiment in order to generate estimates of oxygen consumption per unit of time and mass (μmol $O_2 \cdot min^{-1} \cdot g^{-1}$). Q_{10} values for females and stage I zoeae were calculated using the formula $Q_{10} = (p_2/p_1)^{10/(t2-t1)}$, where p_1 and p_2 were the values of oxygen consumption analyzed and $t_1 = 2$ and $t_2 = 6$ were the temperatures at which the parameter was measured.

Rates of Oxygen Consumption of Crab Embryos and Stage I Zoeae

To determine the effect of location in the clutch and temperature on rates of oxygen consumption of embryos, we defined three positions in the clutch as: 1) top, the first 5 mm of the embryo mass closest to the sternum; 2) middle, the center of the embryo mass; and 3) bottom, the first 5 mm of the embryo mass nearest the pleon. Thus, statistical treatments were 1) position in the clutch (top, middle and bottom), and 2) water temperature.

A small number of embryos (~ 100) was removed from each position of the clutch of each female and placed inside cylindrical individual micro-chambers of 11 ml each (2 cm diameter and 3.5 cm height). Thus, each female yielded 3 micro-chambers, each corresponding to the 3 positions that were sampled within the clutch. MO_2 of embryos from three positions in the clutch were measured simultaneously. Cylindrical micro-chambers were submersed in a holding tank at 6, 4 or 2°C (± 0.5°C) and sealed. A fiber optic sensor probe (FOXY, tip size 300 μm) was inserted through a cannula (diameter 1 mm) at the top of each micro-chamber. Oxygen sensors were calibrated as described above. Micro-chambers were continuously and gently inverted to avoid the formation of an oxygen gradient (Naylor et al., 1999). Rates of oxygen uptake were measured for one hour. All metabolic trials were preceded by a 1-hour measurement of background biological activity in the empty micro-chambers. Embryo wet mass was measured and recorded at the end of each metabolic trial. Results are expressed as μmol $O_2 \cdot min^{-1} \cdot g^{-1}$.

Estimates for MO_2 of crab zoea were measured in a similar fashion to how we estimated MO_2 of embryos. Briefly, the morning after hatching, approximately 20 stage I zoeae were collected from each female maintained in individual aquaria, and placed in the micro-chambers (as above). Stirred values were obtained due to the constant movement of the zoea. A prior experiment indicated that rates of oxygen consumption for stirred and unstirred zoeae were similar (Student's test, $P > 0.05$; data not shown).

Timing of Larval Hatching at Different Positions within Embryo Mass

To determine the sequence of larval hatching at the various positions (top, middle and bottom) within the embryo mass, the proportion of empty eggs at each position was estimated via microscopy weekly. Sub-samples of ~ 140 eggs were collected from each position within the embryo mass of the ovigerous females during the hatching period. Sub-samples were observed under a stereoscopic microscope and numbers of empty and live eggs were recorded, and the proportion of larvae (percentage) hatched at each position was calculated.

Oxygen Availability Within Embryo Mass

We measured oxygen availability at three positions within the clutch (top, middle, and bottom) of free-moving female crab using micro-sensors implanted in the embryo mass. Three micro-sensors (fiber optic sensor probe, FOXY, tip size 300 μm) of different lengths (one for each position) were inserted individually through cannula (diameter 1mm) and then fixed inside a 5 mm diameter piece of vinyl tubing. A single 7 mm hole was drilled through the 6th abdominal segment of each brooding female; tubing containing the micro-sensors was passed through the hole and glued to the pleon using cyanoacrilate, such that each micro-sensor was placed at different position in the embryo mass. The tips of micro-sensors extended ~ 4 mm beyond the cannula to ensure that they were in contact with the embryos within the embryo mass. Micro-sensors were calibrated (see description above) at 2 or 4°C before fixing them to the pleon of the female. The chamber holding the female was continuously flushed with saturated sea water. Females at 6°C were not used in this experiment because they were already hatching. After 2 h of acclimation, oxygen availability for each position in the embryo mass was digitally recorded each minute for 24 h.

Brooding Behavior

Behavior of crabs was videotaped to later assess rates of ovigerous behavior, e.g., oxygen provisioning, of mothers. This experiment was conducted simultaneously with oxygen availability in the embryo mass. After attaching the micro-sensors to their pleon, females were placed individually into a 50-L aquarium with one transparent wall. The aquarium was immersed in a 700-L holding tank of seawater maintained at either 2 or 4°C, and was flushed continuously with aerated seawater during the 24 h experimental period. A total of 6 females ($n = 3$ per temperature) were videotaped over a 24 h period using a SVHS Panasonic AF-67740 video recorder and a Deep-Sea Multi-SeaCam 1060 underwater TV camera. Photoperiod was maintained at 10:14 h (light:dark); red light was used during the dark hours to enable video monitoring.

Percentage of time dedicated to flapping of the pleon and total occurrences of this event were assessed from analysis of the video tapes. This behavior was selected since it was directly related to an increase in oxygen saturation in the embryo mass (cf. brachyuran crabs, Baeza and Fernández, 2002). We also assessed the percentage of time females spent walking, although this is not a brooding-specific behavior.

Statistical Analyses

Since rates of oxygen consumption are related to body mass (Vernberg, 1983) a correlation analysis was used to test the independence between variables (Sokal and Rohlf, 1995). To test for differences in rates of oxygen consumption related to temperature and moment (day or night) in brooding or post-brooding females, we used one-way analysis of variance (ANOVAs). To assess whether rate of oxygen consumption of the females during and after brooding varied in each temperature we conducted a paired *t* test (Sokal and Rohlf, 1995). All data were evaluated for normality and homoscedasticity using Kolmogorov-Smirnov and Bartlett's test prior to the following statistical analyses. When differences were found, we conducted post hoc comparisons using Tukey's HSD test (Sokal and Rohlf, 1995). To estimate rates of oxygen consumption of brooding females, rates of oxygen consumption of their brooded embryos was subtracted. Embryo mass was calculated as the difference between the wet mass of brooding and post-brooding females. Thus, embryo mass was used to obtain the rate of oxygen consumption of the brood using the rate of oxygen consumption of embryos (previously described). We assumed the cost of brood care to be the difference in rate of oxygen consumption between brooding and post-brooding females (Baeza and Fernández, 2002).

The effect of position and temperature on the rate of oxygen consumption of embryos was tested using two-way nested ANOVA. The effect of temperature on the rate of oxygen consumption of zoeae I was tested using one-way ANOVA. Oxygen availability among different positions in the embryo mass of the brooding females was tested using repeated measures ANOVA. Differences in the oxygen availability between day and night in each position were tested using paired *t* test (Sokal and Rohlf, 1995).

To determine the phenology of larval hatching among the three positions of the clutch, differences were calculated for every observation (crab × date): top-middle, bottom-middle, and top-bottom. The mean value of each difference, standard deviation(s), and 95% confidence limit (CI, using $n = 40$, $t = 2.021$) were also calculated. Differences were considered significant at $\alpha = 0.05$ only if their confidence intervals excluded the value of 0.

Differences in the time spent in flapping the pleon or walking between females at 2 or 4°C and between day and night were compared using *t* test and paired *t* test, respectively. To determine if the attached optic fiber affected behavior, females were videotaped with and without micro-sensors. The percentage of time spent in flapping of the pleon and walking was compared between both conditions using a paired *t* test.

Results

Rates of Oxygen Consumption and Metabolic Cost of Brooding

Over the range in female *P. platypus* mass used in this study, there was no difference on rates of oxygen consumption (MO_2) attributed to mass. Hence, MO_2 of female was independent of body mass ($r^2 = 0.11$, $n = 9$, $P = 0.21$). MO_2 of brooding and post-brooding *P. platypus* during day increased significantly with temperature (ANOVAs, $P < 0.01$ in both cases) and was higher at 6°C than at 2°C (Tukey test, $P < 0.05$ in both cases, Fig. 1).

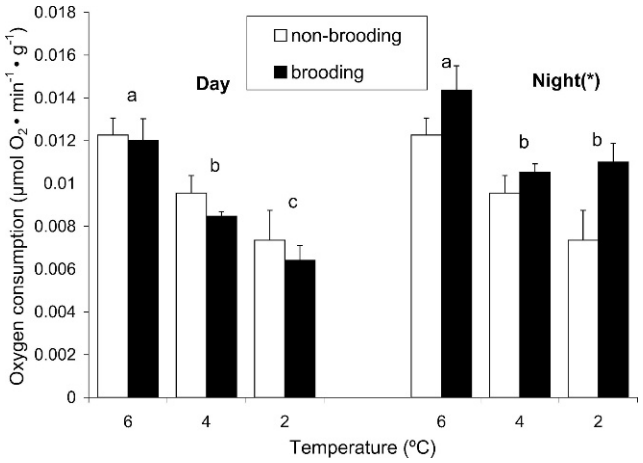

Fig. 1. *Paralithodes platypus*. Rates of oxygen consumption of brooding and non-brooding females during day and night at different water temperatures. Different letters indicate significant differences among temperatures. An * indicates significant differences between brooding and non-brooding females. Vertical lines indicate means ± SD. Oxygen consumption of brooding females was calculated by subtracting oxygen consumption of the brooded mass.

MO_2 of brooding and post-brooding females during the day were similar within each of the three analyzed temperatures (paired *t* test, $P = 0.20$, $P = 0.11$ and 0.22, for 6, 4 and 2°C, respectively (Fig. 1). Also, MO_2 of post-brooding females was similar between day and night (paired *t* test, $P = 0.09$, 0.06 and 0.09, for 6, 4 and 2°C, respectively; Fig. 1). Particularly, $Q_{10(6\text{-}2)}$ value for post-brooding females was about 3.5.

MO_2 of brooding females during night varied significantly with temperature (ANOVA, $F = 12.23$, $P = 0.007$). Mean MO_2 of brooding females at 6°C was significantly higher than MO_2 of brooding females at 4 or 2°C (Tukey test, $P < 0.02$ in both cases; Fig. 1), but the MO_2 of brooding females at 4 and 2°C did not significantly differ (Tukey test, $P = 0.78$). Moreover, MO_2 at night was always significantly higher in brooding females than in post-brooding females irrespective of temperature treatment (paired *t* test, $P = 0.01$, 0.03, 0.01, for 6, 4 and 2°C, respectively). On average, the increase in MO_2 of brooding females during night vs. day was 25% (Fig. 1).

Oxygen Consumption of Crab Embryos and Zoeae I, and Time of Hatching

Rates of oxygen consumption of crab embryos did not vary with temperature or position in the clutch (nested ANOVA, $F = 0.07$, $P = 0.94$ and $F = 0.30$, $P = 0.93$ for temperature and position, respectively), and averaged 0.060 ± 0.02 (µmol · g^{-1} · m^{-1}; Fig. 2). However, oxygen consumption rates of zoeae I differed among the three temperatures (ANOVA, $F = 6.26$, $P = 0.03$; Fig. 3). MO_2 of zoeae I at 6°C was significantly higher than at 2°C (Tukey test, $P = 0.04$), whereas MO_2 of zoeae I at 4°C did not differ significantly

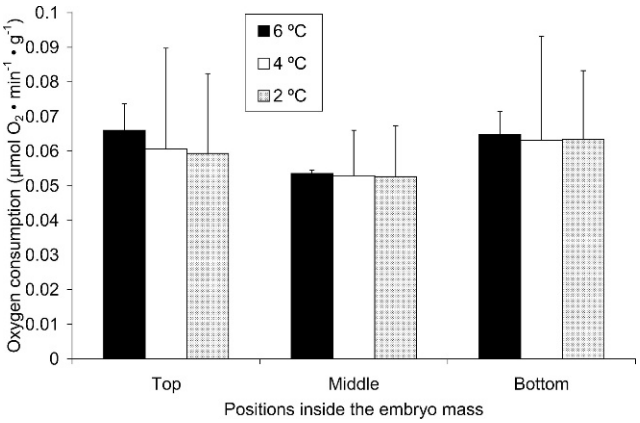

Fig. 2. *Paralithodes platypus*. Rates of oxygen consumption of crab embryos from different positions in the embryo mass at three temperatures. Vertical lines indicate mean values ± SD.

from those at either 2 or 6°C (Tukey test, $P > 0.05$ in both cases). $Q_{10(6\text{-}2)}$ value for stage I zoea was 2.6. Averaged across all temperature treatments the mean MO_2 of zoea was 4-fold higher than that for embryos.

A gradient of timing of larval hatching was observed among positions in the embryo mass. The mean difference (± 95% CI) in percentage hatched from top to middle was 8.4 ± 4.0%, i.e., there is a 95% probability that the true difference lies between 4.4 and 12.3%, and it is therefore significantly different from 0 (Fig. 4A). Likewise the mean difference from bottom to middle was 12.5 ± 4.0%, i.e., lies between 8.5 and 16.5%, and is also significantly different from 0 (Fig. 4B). The mean difference from top to bottom was −4.1 ± 3.7%, i.e., lies between −7.8 and −0.5%, so is also significantly different from 0 (Fig. 4C). Therefore, hatching rates at the various positions within the clutch differed significantly in the order bottom > top > middle.

Oxygen Availability within the Embryo Mass

Oxygen availability within the embryo mass of brooding females was similar between 2 and 4°C (nested ANOVA, $F = 5.03$, $P = 0.06$), but varied among the different positions in the clutch (nested ANOVA, $F = 11.69$, $P < 0.001$; Fig. 5). The highest oxygen saturation was found at the top of the embryo mass and averaged 91 ± 4% over the 24 h period of measurement. Oxygen saturation averaged 79 ± 9% at the bottom of the embryo mass and 66 ± 9% in the middle (Fig. 5). Oxygen saturation within the embryo mass was similar between day and night (paired t test, $P = 0.23$, 0.38 and 0.20, for top, middle and bottom positions, respectively).

The 82% of the flapping of the pleon of the brooding females from video observations was coincident with a sudden increase in oxygen availability at the bottom of the embryo mass. The pattern of oxygen availability in the middle of the embryo mass fluctuated independent of pleonal flapping and thus was not significantly correlated with overt movements of the pleon (Fig. 6).

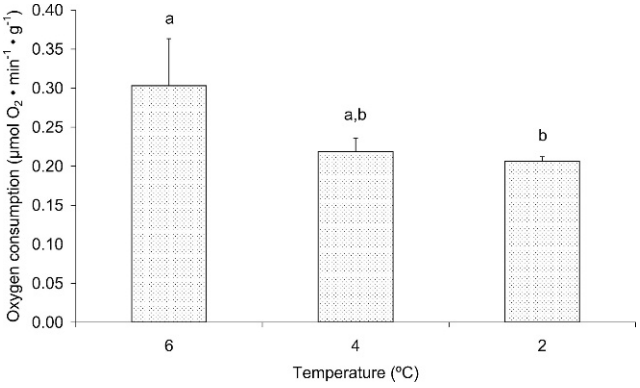

Fig. 3. *Paralithodes platypus*. Rates of oxygen consumption of zoea I at different temperatures. Different letters indicate significant differences. Vertical lines indicate mean values ± SD.

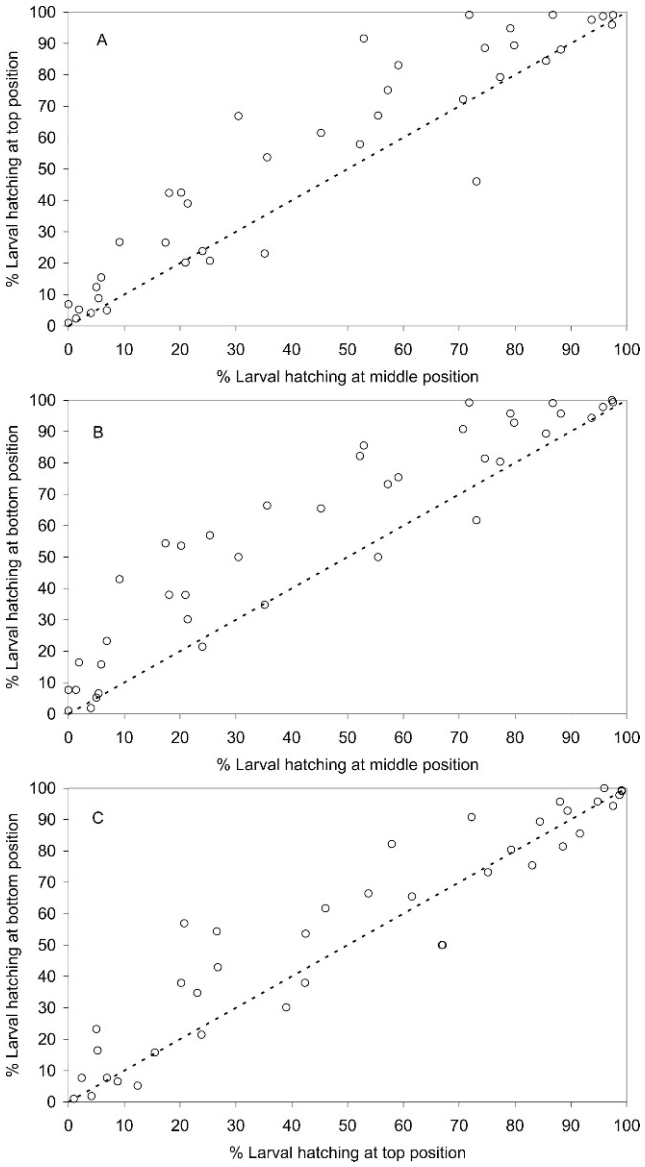

Fig. 4. *Paralithodes platypus*. Q-Q plot of larval hatching proportions between (A) middle and top, (B) middle and bottom, and (C) bottom and top positions of embryo mass during the larval hatching period. Dotted line represents the relation 1:1 which would indicate a similar larval hatching timing among positions.

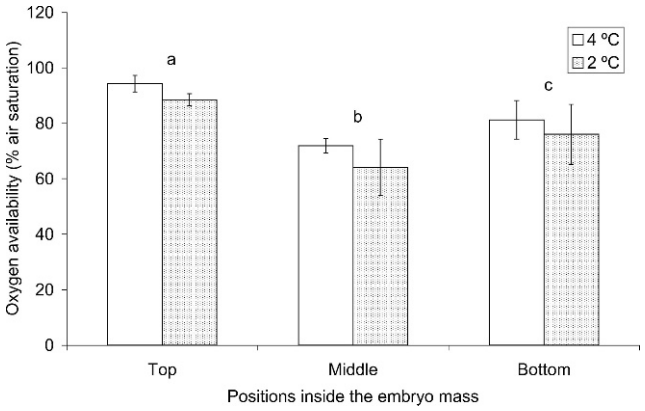

Fig. 5. *Paralithodes platypus*. Mean percentage of oxygen saturation at the top, middle and bottom position of the embryo mass at 2 and 4°C. Different letters indicate significant differences. Vertical lines indicate mean values ± SD.

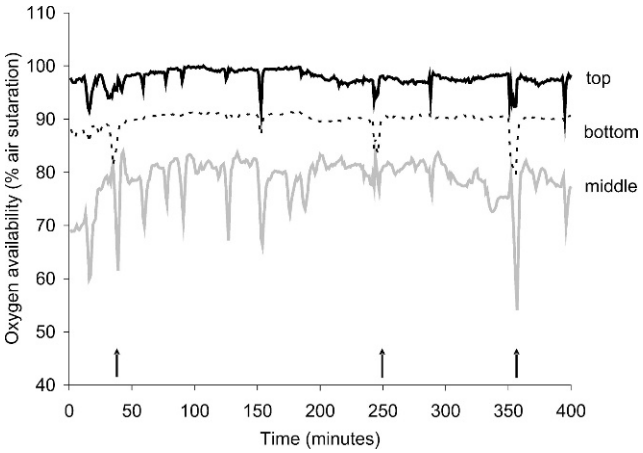

Fig. 6. *Paralithodes platypus*. Patterns of oxygen availability at the top, middle and bottom position of the embryo mass. Arrows represent time of observed abdominal flapping. These patterns are for one female, but results were consistent across female replicates. The information of oxygen availability (%) in the clutch for all females is presented in Figure 5.

Brooding Behavior

The percentage of time that brooding females were engaged in flapping of the pleon behavior was 30% higher during the night than during the day (*t* test, $P = 0.03$, Table 1). However, the percentage of time that brooding females spent walking was similar between day and night (Table 1). Temperature did not influence the percentage of time animals flapped their pleons or walked; durations of these behaviors were similar between brooding females at both 2 and 4°C during day (*t* test, $P = 0.44$ and 0.49 for flapping of the pleon and walking, respectively) and night (*t* test, $P = 0.48$ and 0.12 for flapping the pleon and walking, respectively). Neither brooding nor walking behavior of females was affected by the optic fiber microsensors. The mean percentage time spent flapping the pleon or walking was similar between females with or without optic fibers sensors in the embryo mass ($P = 0.27$ and 0.3, respectively, Table 1).

Discussion

The results presented here represent the first physiological measurements made on a lithodid in the laboratory. We were able to determine: 1) rates of oxygen consumption of brooding and post-brooding females, embryos and zoeae I, and the effects of temperature on these parameters; 2) the cost of brooding for females; and 3) differences in oxygen available to embryos and timing of larval hatching based on position within the clutch.

The MO_2 of post-brooding female *P. platypus* at 6°C is comparable to values reported for other cold-water crabs found in Alaskan and crabs and squat lobster from sub-Antarctic waters (Table 2). The MO_2 of *P. platypus* is also similar to the metabolic rate of a brachyuran crab *C. setosus* in the southern limit of distribution (Table 2).

The MO_2 of *P. platypus* increased significantly with temperature, almost doubling between 2 and 6°C, resulting in $Q_{10(6\text{-}2)}$ of 3.5 for post-brooding females in the current study. Because MO_2 increases with temperature and oxygen solubility decreases with increased temperature, maintenance costs should be higher for animals that inhabit warmer waters. Although some crustaceans are thermally insensitive over a wide temperature range (see Vernberg, 1983 and references therein), most crustacean species exhibit increased oxygen consumption with increased temperature. This tendency was found in *C. setosus*, where an increase of sea-water between 10 and 18°C translates to a 3-fold increase in MO_2 (Brante et al., 2003). The sub-Antarctic *Munida gregaria* (Fabricius, 1793) doubles its MO_2 between 6 and 18°C in laboratory experiments (Avalos et al., 2006).

Female *P. platypus* carry eggs attached to their pleopods and their care has an associated metabolic cost. This cost is most evident during night as a 24% increase in metabolic rate compared to post-brooding females. Although this difference is significant, it is low compared to the cost of brooding in *C. setosus*, in which the metabolic rate of brooding females was 2-fold higher than that of post-brooding females (Brante et al., 2003). Flapping of the pleon by brooding females of *C. pagurus* Linnaeus, 1758 also occurs during night (Naylor et al., 1997), similar to our observations of brooding *P. platypus*. Since brooding and post-brooding females did not differ in the amount of time spent walking either in day or night, we attribute the extra energetic expenditure to the cost of maintenance of their brood. Thus, the increased metabolic cost we observed in brooding females at night likely results from increased flapping of the pleon since this behavior increased at night. In addition to flapping the pleon, other brooding behaviors could contribute to the increased metabolic demand of brooding females as compared to non-brooders such as initiating and maintaining a standing position and pereiopod and chela probing; these behaviors were not quantified in the current study. Similarly, we were unable to test the relationship between the metabolic cost of embryo ventilation and temperature – known to decrease in *C. setosus* with decreasing temperature (Brante et al., 2003) –

Table 1. *Paralithodes platypus*. Mean percentage of time that each behavior was performed by brooding females compared by paired *t* test: (A) during day or night, and (B) with or without attached optic fibers in the embryo mass. Data are expressed as mean ± SD of a sample of 6 (A), and 3 animals (B).

	A			B		
	Day	Night	*P*	With sensors	Without sensors	*P*
Abdominal flapping	5.23 ± 2.42	6.79 ± 1.55	0.03	6.68 ± 1.90	7.90 ± 2.03	0.27
Walking	27.39 ±13.37	23.32 ± 10.80	0.17	28.03 ± 19.20	21.71 ± 7.44	0.30

because of the narrow temperature range we used in the current study.

MO_2 of zoea I of *P. platypus* averaged 4-fold higher than that of embryos just prior to hatching. This result is consistent with studies of *C. pagurus* and *Euphasia superba* Dana, 1852 both of which exhibit an abrupt increase in oxygen uptake following metamorphosis of eggs to zoea (Quetin and Ross, 1989; Naylor et al., 1999). Zoea of *P. platypus* are planktotrophic (Stevens et al., 2008b) that hatch from small eggs without a yolk reserve and depend on planktonic prey for energy (Rabalais, 1991). In fact, the ability to capture prey in the first stage zoea of Alaskan king crabs declines dramatically after 84 h without food (Paul and Paul, 1980). Likely, the increased MO_2 observed during the zoea I stage facilitates the rapid swimming necessary for prey capture by zoea. An alternative or even complementary explanation for increased rates of oxygen uptake is that after shedding the egg membrane oxygen diffusion distances are greatly reduced thus enabling greater rates of uptake (Naylor et al., 1999). The MO_2 of zoea I of *P. platypus* increased with temperature, as expected, and the $Q_{10(6\text{-}2)}$ of 2.6 is similar to that for zoea I of another cold water king crab, *Paralomis granulosa* White, 1846 (Thatje et al., 2003), with a $Q_{10(15\text{-}1)}$ of 2.1.

Metabolic rates of embryos of *P. platypus* sampled from different positions in the clutch were similar despite the fact that oxygen saturation differed with position. In contrast, results from brachyuran crab species indicate fairly homogeneous concentrations of oxygen throughout the embryo mass at least during the later stages of embryogenesis (Baeza and Fernández, 2002). It is known that among reproductive survival strategies hatching is a key aspect to consider (Stevens, 2006a). Thus, differences in oxygen concentration within the clutch of *P. platypus* appear to have developmental consequences with respect to timing of hatching; larvae from the periphery hatched prior to larvae from the middle of the embryo mass. This spatial-temporal pattern of hatching is probably related to the timing at which embryos attain a threshold maturity and is probably a function of metabolic rates.

Even though the limitation of oxygen diffusion within the clutch is ameliorated by pleonal flapping of females (Baeza and Fernández, 2002; this study), the oxygen needed to coordinate embryo maturation appears sufficient for brachyurans but not lithodids. Thus, crabs of the genus *Chionoecetes* exhibit a relatively synchronous hatching within a few days [11 days for *Ch. opilio* Fabricius, 1788 (Webb et al., 2007) and 9.4 days for *Ch. bairdi* Rathbun, 1924 (Stevens, 2003)]. However, this process takes on average 30 or more days for lithodids crabs (~ 37 days for *Paralomis granulosa* and *Lithodes santolla* (Molina, 1782) (Thatje et al., 2003), 32 days for *Paralithodes camtschaticus* (Tilesius, 1815) (Stevens and Swiney, 2007) and 29 days for *P. platypus* (Stevens, 2006a)).

Since each of these high latitude species display extended embryonic development (Stevens, 2003; Thatje et al., 2003; Webb et al., 2007), the differences in the duration of hatching could be due to genetic differences among species, environmental cues (Stevens, 2003) or alternate strategies used by animals to increase larval survival (Stevens, 2006a). Specifically, *P. platypus* has a protracted and asynchronous hatching period ranging from 28 to 34 days (Stevens, 2006a), and it could be considered as a possible bet hedging strategy (Stevens, 2006a and references therein). Species that utilize this strategy could obtain greater mean fitness by producing fewer offspring per unit of time rather than by maximizing the number of offspring with highly variable survival (Philippi and Seger, 1989). This strategy may not only be determined genetically, environmental factors such as oxygen availability within the clutch and temperature could have an important influence. Possibly, the oxygen gradient that we observed in these large clutches could be a mechanism to delay hatching of larvae from the middle of the clutch, and extend the hatching periods for these large decapods.

Table 2. Rates of oxygen consumption (MO_2) found in the current study for non-brooding female *Paralithodes platypus* as well as in other cold water crabs or squat lobsters from the literature.

Specie	Mean MO_2 ($\mu mol \cdot g^{-1} \cdot m^{-1}$)	Water temperature (°C)	References
Paralithodes platypus	0.012 ± 0.001	6	Current study
Lithodes santolla (Molina, 1782)	~ 0.017	8	Comoglio et al., 2005
Paralomis granulosa (Jacquinot, 1847)	~ 0.013	8	Comoglio et al., 2008
Chionoecetes opilio (Fabricius, 1788)	~ 0.013	8	Foyle et al., 1989
Chionoecetes bairdi Rathbun, 1924	~ 0.011	5	Paul and Fuji, 1989
Chionoecetes bairdi	~ 0.021	8	Haukenes et al., 2009
Cancer setosus	~ 0.01	10	Brante et al., 2003
Munida gregaria (Fabricius, 1793)	0.011 ± 0.004	8	Romero et al., 2006

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