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# OXYGEN UPTAKE BY EMBRYOS AND OVIGEROUS FEMALES OF TWO INTERTIDAL CRABS, *HETEROZIUS ROTUNDIFRONS* (BELLIIDAE) AND *CYCLOGRAPSUS LAVAUXI* (GRAPSIDAE): SCALING AND THE METABOLIC COSTS OF REPRODUCTION

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## Summary

***Heterozius rotundifrons*** **and** ***Cyclograpsus lavauxi*** **are crabs of similar size, whose intertidal habitats overlap. They differ in the number and size of their eggs. A 2 g ovigerous *H. rotundifrons* incubates 675 large yolky eggs (mean single-egg mass 269 μg; egg clutch 9.15 % of mass of female crab; increasing to 435 μg and 13.4 % at hatching). The egg clutch of a 2 g *C. lavauxi* is larger (15.4 % of crab mass increasing to 18.9 % at hatching) and contains more numerous (28 000), smaller (10.9 μg increasing to 20.3 μg) eggs. The longer development time of the larger eggs (194 days *versus* 56 days at 15 °C) results from a delayed increase in metabolic rate (diapause) and not metabolic scaling.**

**On the basis of the total mass of single eggs, the mass-specific metabolic rates of early embryonic stages of *H. rotundifrons* (0.72 μmol $g^{-1}$ $h^{-1}$ for the blastula stage at 15 °C) and *C. lavauxi* (1.13 μmol $g^{-1}$ $h^{-1}$) were similar to those of the adult female crabs (0.70 μmol $g^{-1}$ $h^{-1}$ for *H. rotundifrons* and 0.91 μmol $g^{-1}$ $h^{-1}$ for *C. lavauxi*) and increased 13- and 10-fold, respectively, by the time of hatching. Thus, early embryonic metabolic rates were much lower than expected from their mass, but the metabolic rates of pre-hatching embryos were consistent with the allometry of juveniles and adults. Possible interpretations of this apparently anomalous scaling of embryonic metabolic rates are discussed.**

**Mass-specific rates of oxygen consumption by ovigerous females (including the eggs) of both species were higher than for non-ovigerous crabs, in water and in air, and increased greatly during the development of the eggs. This difference was attributable mainly to the increasing metabolic rates of the attached embryos, but early ovigerous crabs (blastula stage) of both species also demonstrated a small elevation in metabolic rate by the crab itself, i.e. a metabolic cost of egg-bearing. In contrast, the elevation of the rate of oxygen consumption by late ovigerous females of *C. lavauxi* was less than predicted from the metabolic rate of eggs in a stirred respirometer. This suggests that, towards the end of development in *C. lavauxi*, the oxygen supply to the eggs *in situ* may be diffusion-limited by unstirred layers, an effect not observed for the larger eggs and more open egg clutch of *H. rotundifrons*.**

**The cost of development, in terms of total oxygen consumption of single eggs, from extrusion to hatching, was 3.34 μmol $O_2$ (approximately 1.5 J) for *H. rotundifrons* and 0.105 μmol $O_2$ (approximately 0.05 J) for *C. lavauxi*. This 30-fold ratio approximates the ratios of their initial masses and yolk contents but represents only approximately one-third of the initial energy contents of the eggs.**

Key words: crab, *Heterozius rotundifrons*, *Cyclograpsus lavauxi*, egg, embryo, development, rate of oxygen consumption, metabolic rate, reproductive cost, cost of development, scaling, allometry.

## Introduction

The developing eggs of intertidal crabs are typically carried externally for several months, attached to the abdominal pleopods. Thus, in addition to the costs of mating activities, the investment in reproduction by adult females includes both the provisioning of the eggs with yolk and brooding costs. With respect to the last two points, the New Zealand 'big-handed crab' *Heterozius rotundifrons* Milne Edwards (Belliidae) and the 'smooth shore crab' *Cyclograpsus lavauxi* Milne Edwards (Grapsidae) provide an interesting comparison. Ovigerous females of these species have a similar mass (approximately 2 g) and make rather similar investments in provisioning the eggs with nutrients. However, in *H. rotundifrons*, the brood consists of only a few hundred large yolky eggs (0.7–0.8 mm in diameter, 200–250 nl, when freshly laid) that take 6–9 months to develop and hatch in the field. In contrast, *C. lavauxi* carry

many thousands of very small eggs (0.25–0.3 mm in diameter, 10–12 nl) which hatch in approximately 2 months (McLay, 1988; Leelapiyanart, 1996). Their ranges overlap in the intertidal area, although they exhibit different zonation and habits. *H. rotundifrons* occupy the mid to lower intertidal zone and at low tide remain motionless, partially buried in sand, and *C. lavauxi* are typically found under boulders in the middle to upper intertidal zone and exhibit a modest aerobic scope for activity in air (Pellegrino, 1984; Innes et al., 1986).

The presence of the eggs could potentially raise the mass-specific metabolic rates of the ovigerous females and eggs above that of non-ovigerous crabs because of the higher metabolic rates of the adults, of the eggs or of both. For example, metabolic rate in the adult crab might be elevated by egg-grooming behaviour (Bauer, 1989), by ventilation of the eggs using pleopodal or branchial pumping (Wheatley, 1981) or by increased postural work. However, by separating the respiration rates of the embryos and the brooding females of the cladoceran *Daphnia magna*, Glazier (Glazier, 1991) found no detectable energetic cost of carrying a brood within the pouch. A similar approach for *Cancer pagurus* (Naylor et al., 1997) inferred a negative cost of egg-bearing; i.e. the ovigerous females were apparently hypometabolic.

The body masses of the egg-bearing females of these species are $10^5$–$10^6$ times greater than the masses of individual eggs, implying that the mass-specific metabolic rate of the egg clutch would be 10–20 times higher than that of the adult crab if the usual allometry were to apply (i.e. absolute metabolic rate$\propto$mass$^b$, where the mass exponent $b$ typically lies in the range 0.65–0.85; Smith and Kleiber, 1950; Zeuthen, 1953; Gould, 1966; Withers, 1992; Schmidt-Nielsen, 1997). Such a relationship has been demonstrated both intra- and interspecifically for juvenile and adult crabs, including *C. lavauxi* (Weymouth et al., 1944; Klein Breteler, 1975; Hawkins et al., 1982; Innes et al., 1986). McNamara et al. (McNamara et al., 1985) extended this allometry down to zoea in *Macrobrachium* spp., and the high metabolic rates recorded in late-stage eggs of *Carcinus maenas* (Wheatly, 1981) and *Cancer pagurus* (Naylor et al., 1997) also appear to fit this pattern. However, there are few studies that include earlier developmental stages in considerations of metabolic scaling in crustaceans. Thus, it is unclear for what proportion of their total incubation period these high rates would prevail. At the time of extrusion, the eggs/oocytes might be expected to have rather low metabolic rates similar to those of adult tissues, requiring an anomalous scaling of metabolic rate at some period of their development. An analysis of the time course of changes in rates of oxygen consumption during egg development would help to elucidate these issues and is also pertinent to considerations of mechanisms of delivery of oxygen to the embryos and to the utilisation of nutrient reserves by the embryos at different stages.

In this paper, we compare the resting oxygen requirements at 15 °C of ovigerous females of *H. rotundifrons* and *C. lavauxi*, carrying eggs at early and late stages, with those of non-ovigerous females, in both air and water. Oxygen consumption measurements were also made on eggs isolated from ovigerous crabs at successive developmental stages, permitting differences to be apportioned between the crabs and their brood. Integration of the oxygen consumption data over the whole period from oviposition to hatching provides an estimate of the total energetic cost of development of individual larvae, and these values are discussed in terms of the different brooding strategies exhibited by *H. rotundifrons* (few large eggs, long development) and *C. lavauxi* (many small eggs, short development).

## Materials and methods

### *Animals and maintenance*

Non-ovigerous and ovigerous females of *Heterozius rotundifrons* and *Cyclograpsus lavauxi* were collected from the intertidal zone at Kaikoura, New Zealand, and transported to the University of Canterbury, Christchurch, for maintenance in a recirculating seawater system (salinity 35 ‰, 15 °C, photoperiod 12 h:12 h L:D). Crabs with early-stage eggs were obtained during the spawning period in April for *H. rotundifrons* and in mid-November for *C. lavauxi*. Later-stage ovigerous crabs of the two species were collected simultaneously between November and January.

Schemes for staging development have been devised for both species by observation of the complete developmental sequence, from oviposition to hatching, in samples of eggs periodically removed from the pleopods of females held in the laboratory at constant temperature (15 °C), artificial tidal immersion:emersion (6.2 h:6.2 h) and photoperiod (12 h:12 h L:D) (Leelapiyanart, 1996). The main morphological changes are summarized in the Results section. For post-gastrula stages, the eye index (EI, the mean of the longest and shortest diameters of the lateral eyes; Helluy and Beltz, 1991) was also used as a staging criterion. The developmental stage of embryos is reported in days by microscopical comparison with this scheme. The approximate percentage of the original yolk remaining (± approximately 5 %) was estimated by summing the volumes of a number of regular solid bodies that approximated the dimensions of the yolk mass (Wear, 1974). Crabs were starved for 1 week prior to physiological measurements, which were carried out at 15 °C in daylight.

### *Oxygen consumption of ovigerous and non-ovigerous crabs*

Rates of oxygen consumption (absolute $\dot{M}_{O_2}$ in $\mu$mol h$^{-1}$, or mass-specific $\dot{M}_{O_2}$ in $\mu$mol g$^{-1}$ total mass h$^{-1}$) of individual crabs at rest in air were measured using constant-pressure differential respirometry (Davies, 1966). Surface water was blotted off each crab, which was placed in the respirometer and left for 5 h to settle and equilibrate to temperature. After verifying that $\dot{M}_{O_2}$ had stabilized, rates were calculated from the linear regression of volume changes recorded at 20 min intervals over 3 h.

The aquatic $\dot{M}_{O_2}$ of crabs at rest was measured using closed-system respirometry. Four 250 ml glass respirometers (three crabs and a seawater blank) were run simultaneously. Crabs

were allowed to settle for 5 h while aerated pasteurized sea water was recirculated from a common aerated reservoir, using a four-channel peristaltic pump. The chambers were then isolated, using the pump to maintain mixing, and $\dot{M}_{O_2}$ was calculated from the change in $P_{O_2}$ of seawater samples (1 ml) taken over a 3 h period, measured using a thermostatted oxygen electrode (Radiometer E5046 connected to Radiometer PHM 71 m), and corrected for any change in the blank.

### *Oxygen consumption of developing eggs*

The oxygen uptake of isolated eggs at different stages was measured using a glass respiration cell of volume 1 ml, thermostatted at 15 °C. An oxygen electrode (IL 1302 connected to Strathkelvin Instruments meter 761b) was fitted into the side of the chamber, and a known number of eggs (50 for *H. rotundifrons*; 1000–2000 for *C. lavauxi*) was introduced through a hole at the top, closed by a small glass stopper with needle overflow. Gentle mixing was achieved with a magnetic spinbar within the respiration cell. Eggs were detached from the crab approximately 1 h before measurements were made, and they were allowed to equilibrate to the temperature of the cell for 5 min. Their rate of oxygen uptake was calculated from the linear part of the $P_{O_2}$ change over time (12–84 min depending on stage), correcting for any change during a blank run.

### *Data analysis and correction for mass of the female crabs*

Mean values are given ± the standard error of the mean (S.E.M., $N$ is the number of observations), unless stated otherwise. The size ranges of the six groups of adult crabs (early and late ovigerous and non-ovigerous from each species) overlapped, but their mean masses differed. Thus, for statistical comparisons of $\dot{M}_{O_2}$ among these groups, individual values were adjusted to a standard body mass of 2 g (excluding the eggs), using mass exponents obtained by logarithmic regression (see Results). Common mass exponents were determined for each species by analysis of covariance (ANCOVA), after testing for parallelism. The 2 g-adjusted $\dot{M}_{O_2,2g}$ value was calculated for each crab as:

$$\dot{M}_{O_2,2g} = \dot{M}_{O_2,m} \times 2^b/m^b , \quad (1)$$

where $m$ is the mass of the crab (g), $\dot{M}_{O_2,m}$ (μmol $h^{-1}$) is the measured rate of oxygen uptake, $b$ is the common mass exponent in the relationship:

$$\dot{M}_{O_2,m} = am^b , \quad (2)$$

and $a$ is a constant.

To test whether the metabolic rates of ovigerous crabs were explained by the sum of the metabolic rates of non-ovigerous crabs and of their eggs (see Table 3 below), a Student's $t$-test was used to examine the null hypothesis $H_0$:

$$\bar{x}_1 - \bar{x}_2 - \bar{x}_3 = 0 , \quad (3)$$

where $\bar{x}_1$, $\bar{x}_2$ and $\bar{x}_3$ are the means of three independently determined variables, the $\dot{M}_{O_2}$ of ovigerous crabs, the $\dot{M}_{O_2}$ of non-ovigerous crabs and the $\dot{M}_{O_2}$ of the eggs, respectively, individual measurements first scaled to 2 g crab mass. The test statistic was:

$$|t'| = (\bar{x}_1 - \bar{x}_2 - \bar{x}_3)/(s_{\bar{x}_1-\bar{x}_2-\bar{x}_3}) < t_{\alpha,2,\text{d.f.}} . \quad (4)$$

The variance of $\bar{x}_1-\bar{x}_2-\bar{x}_3$ was obtained by summing the individual variances and the degrees of freedom (d.f.) estimate on the basis of (previously established) unequal variances of $\bar{x}_1$ and $\bar{x}_3$ (expression given by Zar, 1999; p. 129) and equal variances of $(\bar{x}_1-\bar{x}_3)$ and $\bar{x}_2$.

The cost of development for single eggs was estimated from the area under the $\dot{M}_{O_2}$/time curve, by linear interpolation, and a short extrapolation to the mean time of hatching. The $\dot{M}_{O_2}$ of cleavage stages was assumed to be similar to that of blastulae. Other means were compared by one-way analysis of variance (ANOVA) using Tukey's method for *post-hoc* comparisons and the least significant difference (LSD) method or $t$-tests for planned comparisons. Unless stated otherwise, a probability of 0.05 was accepted as statistically significant.

## Results

### *Morphological observations and properties of the eggs*

The mean fresh mass of ovigerous female *H. rotundifrons* (excluding the eggs) used for whole crab respirometry (see Fig. 4) was 1.98±0.55 g (mean ± S.D., range 1.01–3.00 g, $N$=25), and the initial mass of the egg clutch (blastula stage) in these crabs was 9.15±0.38 % (mean ± S.E.M., $N$=18) of the mass of the crab. A standardized 2 g crab therefore carried 675 eggs, each of mass 269 μg (Fig. 1A; Table 1). As in other decapods (Wear, 1974), the eggs increased in volume during development. By hatching, the mass of the egg clutch had increased to 13.4±1.36 % (mean ± S.E.M., $N$=7) of the mass of the crab, and the single-egg mass had increased to 435 μg. Ovigerous *C. lavauxi* of mean mass 1.82±0.91 g (mean ± S.D., range 1.12–5.10 g, $N$=16) carried egg clutches 70 % larger than those of *H. rotundifrons*, composed of eggs smaller by a factor of 25. Thus, at the blastula stage, the clutch was 15.4±0.19 % (mean ± S.E.M., $N$=7) of the mass of the crab, a 2 g crab carrying some 28 000 eggs, each of 10.9 μg (Fig. 1B; Table 2). At hatching, the relative size of the clutch had increased to 18.9±0.76 % (mean ± S.E.M., $N$=9), and the individual egg mass had doubled to 20.3 μg. The relative mass of the egg clutch was not significantly correlated with the mass of the crab in either species.

Newly extruded eggs of *H. rotundifrons* were prolate ellipsoids with dimensions of 712±8 μm×768±8 μm (mean ± S.E.M., five clutches; i.e. volume 204 nl), increasing to 874±12 μm×903±15 μm (360 nl) at hatching. For the smaller *C. lavauxi* eggs, the corresponding dimensions were 262± 6 μm×288±5 μm (10 nl), increasing to 325±9 μm×345±8 μm (19 nl). As development proceeded, the eggs of *H. rotundifrons* changed from orange, through red to dark brown (Figs 1, 2). *C. lavauxi* eggs changed from dark purple to light grey (Fig. 3). In air, the egg clutch of *H. rotundifrons* drained readily, whereas *C. lavauxi* eggs tended to retain water, possibly by capillarity in the finer interstitial spaces (Fig. 1).

Embryonic stages, specified in days, refer to the time taken

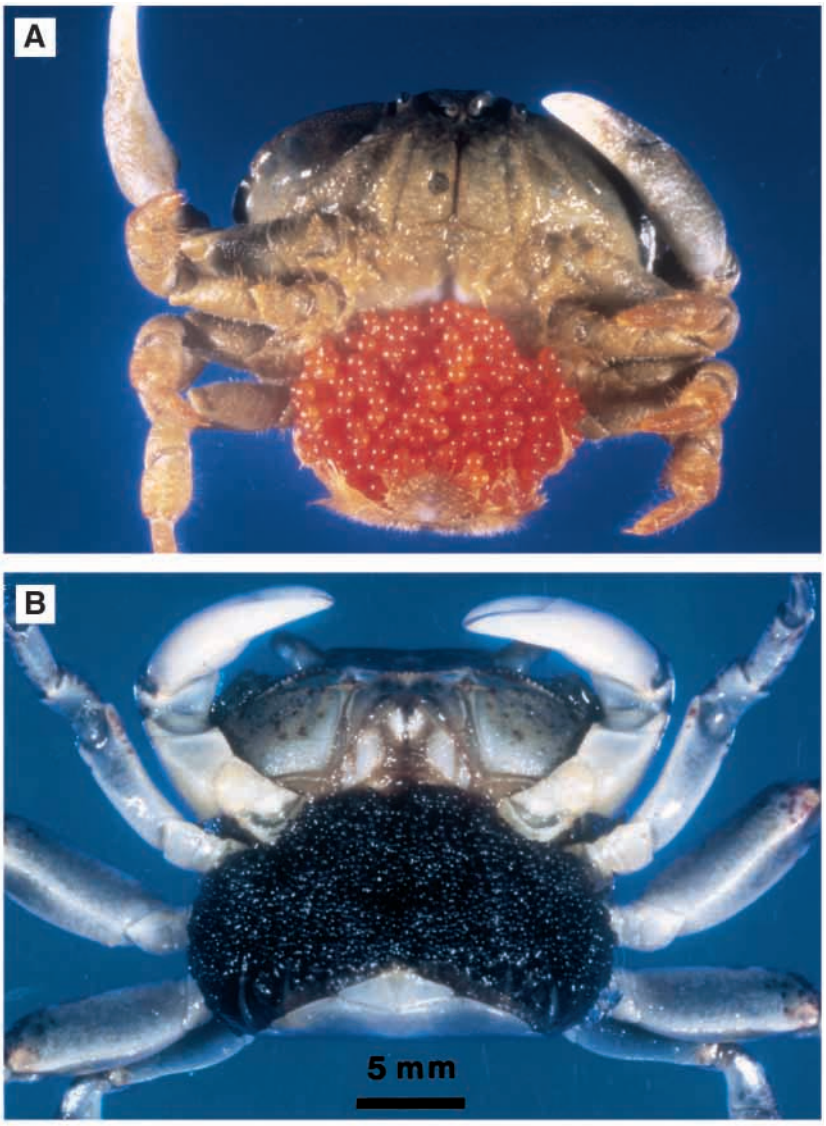


Fig. 1. Ovigerous female crabs with eggs at the blastula stage. (A) *Heterozius rotundifrons* (Belliidae). (B) *Cyclograpsus lavauxi* (Grapsidae). Scale bar, 5 mm.

to reach this morphological stage by eggs developing in the laboratory at 15 °C (see Materials and methods). The total time of development, from extrusion to hatching, at 15 °C, was 194±3 days (*N*=5 clutches) for *H. rotundifrons* and 56±1 days (*N*=5) for *C. lavauxi*. The main events of embryonic development and hatching in *H. rotundifrons* and *C. lavauxi* are summarized here for convenient reference.

In *H. rotundifrons* (Fig. 2A–E), cleavage commenced a few days after egg extrusion, passing from the morula to the blastula stage (>100 cells; Fig. 2A) at approximately 7 days. Cleavage occurred throughout the yolk, which thus comprised essentially 100 % of the egg volume up to the blastula stage. The blastula stage was relatively long (ending at approximately 56 days). Invagination and formation of the colourless tissue cap (gastrula stage) took place between days 57 and 105. At the end of this period, the yolk occupied approximately 85 % of the egg volume, the eyespots were just visible as paired thin crescents and faint chromatophores appeared (Fig. 2B). Between days 105 and 130, the chromatophores became more extensive, the eyes enlarged (EI 92 µm) and the yolk volume decreased to 80 % (Fig. 2C). At 130 days, the first irregular heart beats were discernible (Fig. 2C), and heart rate became rhythmic by day 150 (EI 150 µm; yolk 60 %). Subsequent development through naupliar and metanaupliar embryonic stages to the hatching zoea (see Wear, 1974) occurred with rapid yolk utilization. Between days 150 and 168 (yolk 33 %, EI 208 µm; Fig. 2D), the yolk was present as four lobes. By day 187 (yolk <10 %, EI 264 µm), only two small yolk lobes were observed, and at hatching (day 194, EI 285 µm; Fig. 2E), only traces of yolk remained.

The same stages were recognized in *C. lavauxi* embryos (Fig. 3A–E). Thus, the blastula stage (Fig. 3A) was reached after 5 days, gastrulation commenced at approximately 12 days and the eyespots and chromatophores became visible at day 33 (yolk 50 %; Fig. 3B). A heart beat was observed at approximately day 43 (yolk 25 %; EI 52 µm; Fig. 3C), and hatching occurred at day 56 (EI 85 µm). In summary, although development proceeded more rapidly in *C. lavauvi* than in *H.*

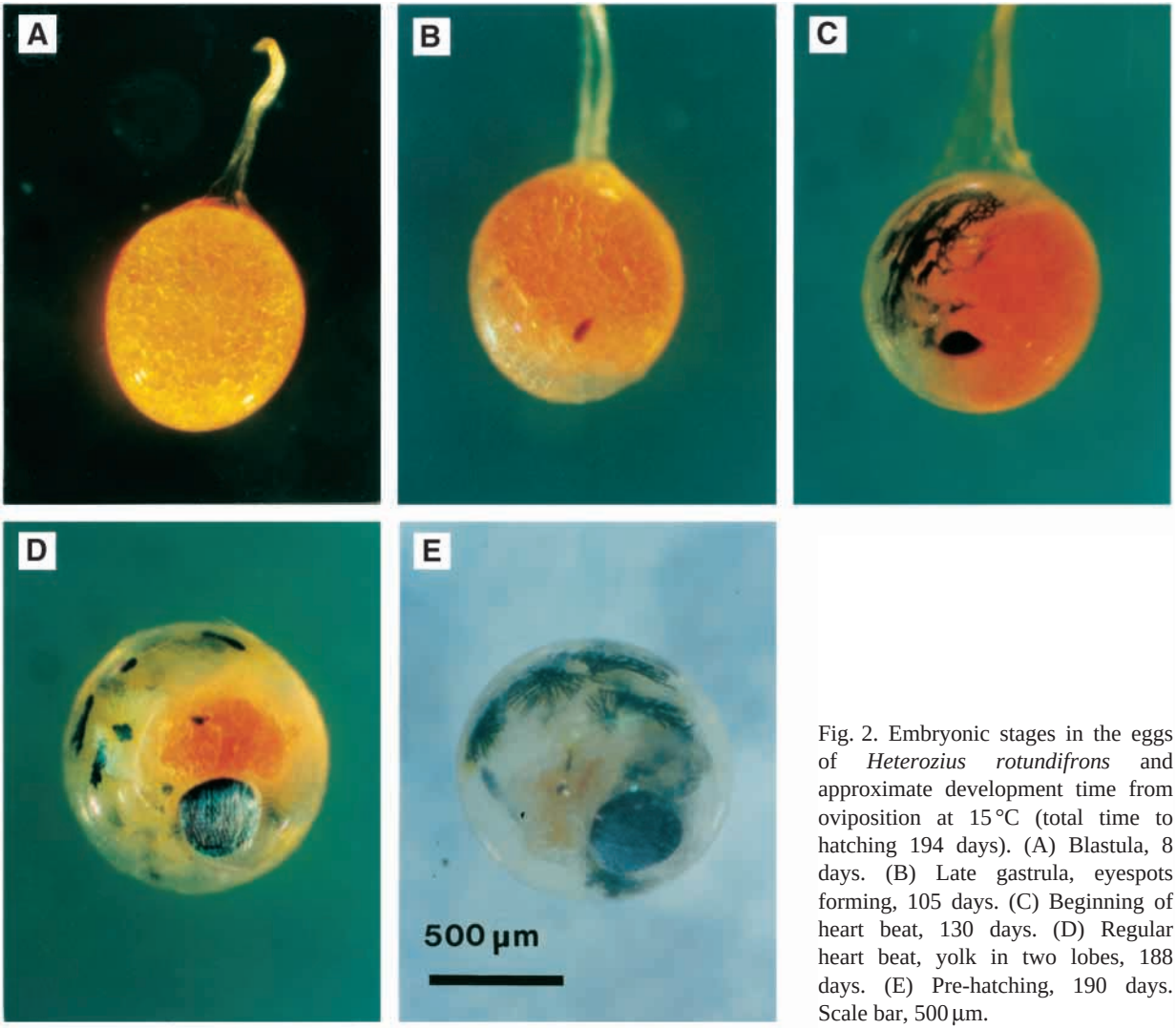


Fig. 2. Embryonic stages in the eggs of *Heterozius rotundifrons* and approximate development time from oviposition at 15 °C (total time to hatching 194 days). (A) Blastula, 8 days. (B) Late gastrula, eyespots forming, 105 days. (C) Beginning of heart beat, 130 days. (D) Regular heart beat, yolk in two lobes, 188 days. (E) Pre-hatching, 190 days. Scale bar, 500 µm.

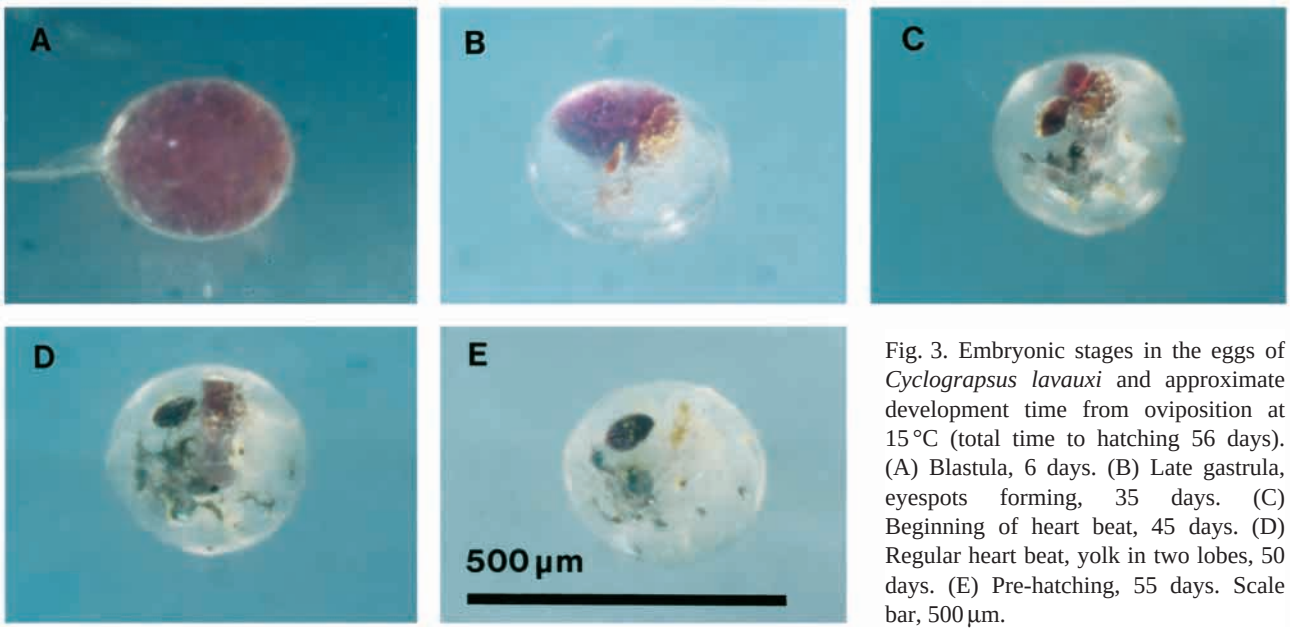


Fig. 3. Embryonic stages in the eggs of *Cyclograpsus lavauxi* and approximate development time from oviposition at 15 °C (total time to hatching 56 days). (A) Blastula, 6 days. (B) Late gastrula, eyespots forming, 35 days. (C) Beginning of heart beat, 45 days. (D) Regular heart beat, yolk in two lobes, 50 days. (E) Pre-hatching, 55 days. Scale bar, 500 µm.

Table 1. *The masses of single eggs of* Heterozius rotundifrons *and* Cyclograpsus lavauxi *at different stages of their development and their rates of oxygen consumption in sea water at 15 °C*

| Development time at 15 °C (Days) | (%) | Morphological stage | Volume of yolk remaining[1] (%) | Mass of one egg[2] (μg) | Single-egg $\dot{M}_{O_2}$ (pmol egg$^{-1}$ h$^{-1}$) | Mass-specific $\dot{M}_{O_2}$ (μmol g$^{-1}$ h$^{-1}$) | *N* |
|---|---|---|---|---|---|---|---|
| *H. rotundifrons* (hatch at 194 days) | | | | | | | |
| 60 | 31 | Blastula–gastrula | ≈100 | 269±3 | 173±7 | 0.72±0.05 | 10 |
| 110 | 57 | Eyespots, chromatophores appear | 85 | 286±4 | 295±23 | 1.04±0.08 | 10 |
| 130 | 67 | Heart beat commences | 80 | 315±10 | 461±31 | 1.45±0.07 | 10 |
| 145 | 75 | Regular heart beat | 60 | 328±4 | 701±20 | 2.14±0.07 | 10 |
| 160 | 82 | Yolk as four large lobes | 50 | 362±2 | 1082±35 | 2.98±0.08 | 10 |
| 175 | 90 | Yolk as two small + two large lobes | 25 | 409±3 | 2170±46 | 5.31±0.13 | 10 |
| 190 | 98 | Pre-hatching, yolk as two small lobes | <5 | 435±9 | 3946±93 | 9.12±0.19 | 10 |
| *C. lavauxi* (hatch at 56 days) | | | | | | | |
| 10 | 18 | Blastula | ≈100 | 10.9±0.8 | 12.4±1.5 | 1.13±0.09 | 10 |
| 20 | 36 | Gastrula | 85 | 12.0±0.4 | 34.8±2.5 | 2.90±0.21 | 11 |
| 30 | 54 | Eyespots, chromatophores appear | 50 | 14.4±0.4 | 64.3±2.3 | 4.48±0.15 | 10 |
| 40 | 71 | Regular heart beat | 25 | 16.8±0.8 | 108.7±5.7 | 6.49±0.16 | 9 |
| 55 | 98 | Pre-hatching, yolk consumed | <5 | 20.3±1.0 | 215.5±6.2 | 10.83±0.63 | 10 |

Values are means ± S.E.M. *N* refers to the number of measurements on samples of 50 (*H. rotundifrons*) or 1000–2000 (*C. lavauxi*) eggs.

[1]A very approximate estimate of the volume of yolk remaining, as a percentage of the initial volume of the egg based on microscopic measurement. Note that the eggs increase in volume and total mass during development.

[2]Measured mass of the eggs, including yolk and membranes and, presumably, some extra-corporeal water.

*rotundifrons*, successive morphological stages appeared at approximately the same fraction of the total development time in the two species. A noticeable difference was that the yolk was consumed relatively earlier in *C. lavauxi* (Table 1; also compare Fig. 2B with Fig. 3B, and Fig. 2C with Fig. 3C, which represent similar embryonic stages).

### *Oxygen consumption of developing embryos and the cost of development*

The single-egg $\dot{M}_{O_2}$ values for *H. rotundifrons* and *C. lavauxi* are shown in Table 1. Between the blastulae and pre-hatching embryos, the total mass of the eggs less than doubled but their absolute $\dot{M}_{O_2}$ values increased by factors of 20 for *H. rotundifrons* and 14 for *C. lavauxi*. On a mass-specific basis, the increases are approximately 13-fold for *H. rotundifrons* and 10-fold for *C. lavauxi*. Despite the 25-fold difference between the two species in the individual masses of early eggs, their mass-specific $\dot{M}_{O_2}$ values are of similar magnitude (although they differ statistically, *t*-test; *H. rotundifrons*, 0.72±0.05 μmol g$^{-1}$ h$^{-1}$, *N*=10; *C. lavauxi*, 1.13±0.09 μmol g$^{-1}$ h$^{-1}$, *N*=10). The mass-specific metabolic rates of the early egg stages were also similar to the rates for non-ovigerous adult crabs in sea water (0.70±0.06 μmol g$^{-1}$ h$^{-1}$, *N*=18, for *H. rotundifrons*; 0.91±0.05 μmol g$^{-1}$ h$^{-1}$, *N*=15, for *C. lavauxi*; Fig. 4).

A plot of single-egg $\dot{M}_{O_2}$ against the development time observed in the laboratory (Fig. 5) shows that in the increase in the metabolic rate was steepest towards the end of incubation. In *H. rotundifrons*, there was a delay of approximately 100 days before any marked increase in metabolic rate. The area under these curves represents the total cost of development of each egg, from extrusion to hatching, in terms of oxygen consumed by the egg; values were 3.34 μmol $O_2$ for *H. rotundifrons* and 0.105 μmol $O_2$ for *C. lavauxi* (the latter value may be slightly overestimated because of higher rates *in vivo* than *in vitro* in the final stages, as discussed below).

### *Aerial and aquatic oxygen consumption of non-ovigerous and ovigerous crabs*

The total rates of oxygen consumption (μmol h$^{-1}$) for non-ovigerous *H. rotundifrons* and *C. lavauxi* and for ovigerous crabs carrying early (blastula) and late (pre-hatching) eggs, in water and in air, are shown as allometric equations in Table 2. There were no significant differences among the mass exponents (*b*) for these six treatment groups for either species (ANCOVA), which were therefore combined to generate common mass exponents (*H. rotundifrons* 0.65±0.11, d.f.=76) and *C. lavauxi* 0.94±0.05, d.f.=57). These values were significantly different from each other ($P$<0.000001). The mass exponent for *H. rotundifrons* was significantly less than 1.0 (negative allometry), but that for *C. lavauxi* was statistically similar to 1.0 (isometric scaling).

Increases in the overall mass-specific metabolic rates of ovigerous crabs are indicated by significant differences among the elevations of the regression lines ($\log_e a$; Table 2) and among the rates standardized to 2 g crab mass (Fig. 4) using the common mass exponents for each species. The $\dot{M}_{O_2}$ of non-ovigerous *H. rotundifrons* was similar in air and in sea water, and these rates were significantly lower (ANOVA, Tukey test) than those of non-ovigerous *C. lavauxi* in either medium. The $\dot{M}_{O_2}$ of non-ovigerous *C. lavauxi* in air was nearly twice the

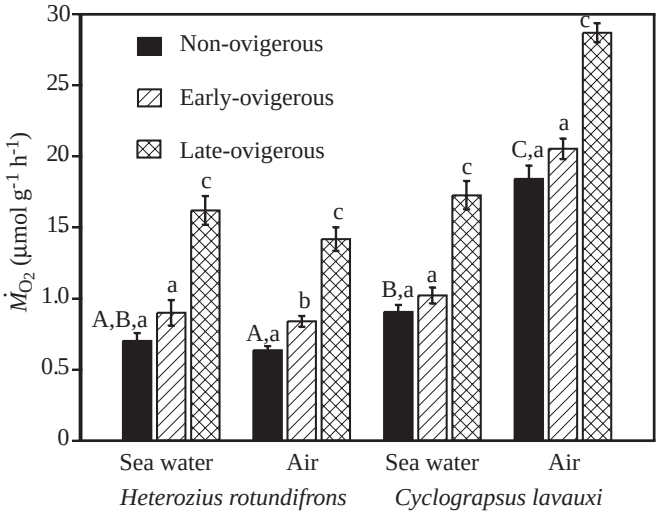


Fig. 4. Mass-specific rates of oxygen consumption $\dot{M}_{O_2}$ of non-ovigerous (filled columns), early-ovigerous (hatched columns; blastula stage) and late-ovigerous (cross-hatched columns; pre-hatching stage) crabs. Values for *Heterozius rotundifrons* and *Cyclograpsus lavauxi* in sea water and in air at 15 °C are shown. Individual values are adjusted to a total crab mass (excluding eggs) of 2 g using the common exponents in Table 2. Within the four species/medium groups, different lower case letters indicate significant differences among means for different stages of development. Among non-ovigerous crabs, means with different upper case letters are significantly different ($P$<0.05). Values are means ± S.E.M. ($N$ values are given in Table 3).

value in sea water ($P$<0.01, ANOVA, Tukey test). For both species, in air and in water, and carrying both early and late eggs, the mean mass-specific $\dot{M}_{O_2}$ of ovigerous crabs (including the eggs) was higher than the corresponding value for non-ovigerous females; differences were highly significant for late ovigerous crabs ($P$<0.001), but for early ovigerous crabs were significant ($P$<0.01) only for *H. rotundifrons* in air (ANOVA, LSD) (Fig. 4).

### *Does the metabolic rate of the embryos account for the increased metabolic rate of ovigerous crabs?*

The total $\dot{M}_{O_2}$ of ovigerous crabs was compared with that of non-ovigerous females scaled to the same net mass (2 g) (Table 3). Although representing only 10–20 % of the total mass of ovigerous crabs, the brood of late-stage eggs consumed more oxygen than the adult crabs. For ovigerous *H. rotundifrons* carrying late-stage eggs, in water and in air, there was good agreement between the elevation of the rate of oxygen consumption associated with carrying the eggs and that of isolated eggs in the stirred egg respirometer. However, for late ovigerous *C. lavauxi*, the elevation of metabolic rate was only approximately half to three-quarters of that predicted from the egg respirometry (difference highly significant; *t*-tests; see Materials and methods and Table 3 for details). In contrast, the increase in the metabolic rate of early ovigerous crabs was several-fold greater than expected from the metabolic rate of the separated eggs (statistically significant for both species in air; not significant for *H. rotundifrons*, $P$=0.07, and *C. lavauxi* in water).

## Discussion

Rates of oxygen consumption by non-ovigerous females of the low-shore crab *H. rotundifrons* were similar in water and in air and were also similar to those of non-ovigerous *C. lavauxi* in water. However, the more terrestrial *C. lavauxi* exhibited a higher resting metabolic rate in air, which accords with the fact that *H. rotundifrons* are rather slow-moving crabs both in water and in air. At low tide, they are found partially buried in sand, and when disturbed in either medium they respond by freezing. In contrast, *C. lavauxi* forage in air and run when exposed at low tide (Innes et al., 1986; McLay, 1988; H. H. Taylor, unpublished observations).

The aquatic rate of oxygen consumption recorded here for *C. lavauxi* at 15 °C (0.91 μmol g$^{-1}$ h$^{-1}$) is similar to values reported previously for this crab at 10 °C (0.93 μmol g$^{-1}$ h$^{-1}$ for

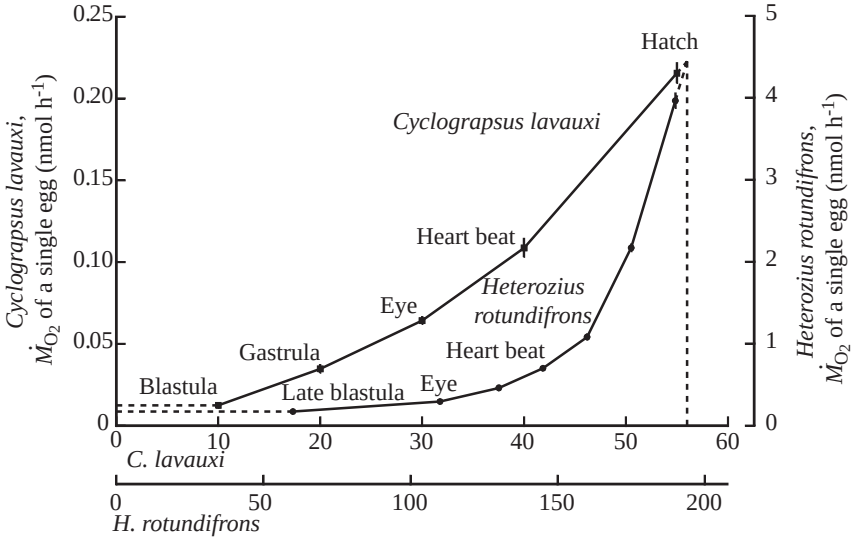


Fig. 5. Time course of the increase in the absolute rate of oxygen consumption ($\dot{M}_{O_2}$) by individual eggs of *Heterozius rotundifrons* and *Cyclograpsus lavauxi* at 15 °C. The vertical broken line indicates the mean time of hatching. The axes are scaled so that the times of hatching and the $\dot{M}_{O_2}$ at hatching coincide. The metabolic cost of development of one egg (the area under the curves) is 3.34 μmol $O_2$ for *H. rotundifrons* and 0.105 μmol $O_2$ for *C. lavauxi*. Values are means ± S.E.M. ($N$ values are given in Table 1).

Table 2. *Allometry of the total rates of oxygen consumption in terms of crab mass for non-ovigerous and ovigerous* Heterozius rotundifrons *and* Cyclograpsus lavauxi *in sea water, and in air, after 5 h of settling at 15 °C*

| Species | Medium | Egg stage | $\log_e a$ | $a$ | $b$ | $P$, $b$=0 ($P$, $b$=1) | $r^2$ | $N$ |
|---|---|---|---|---|---|---|---|---|
| *Heterozius rotundifrons* | Water | Non-ovigerous | –0.18±0.32 | 0.84[3] | 0.67±0.37 | 0.08 | 0.17 | 18 |
| | Water | Blastula | 0.11±0.28 | 1.11[3] | 0.65±0.38 | 0.11 | 0.11 | 17 |
| | Water | Pre-hatching | 0.96±0.14 | 2.60[4,***] | 0.42±0.24 | 0.14 | 0.38 | 7 |
| | **Common mass exponent (*b*) for aquatic crabs** | | | **0.59±0.21** | | 0.007 (0.056) | 0.18 | |
| | Air | Non-ovigerous | –0.11±0.16 | 0.90[3] | 0.48±0.17 | 0.01 | 0.39 | 15 |
| | Air | Blastula | 0.05±0.13 | 1.05[4,***] | 0.80±0.16 | 0.0004 | 0.70 | 13 |
| | Air | Pre-hatching | 0.51±0.23 | 1.67[5,***] | 0.89±0.33 | 0.04 | 0.55 | 8 |
| | **Common mass exponent (*b*) for aerial crabs** | | | **0.69±0.11** | | 0.0000 (0.007) | 0.55 | |
| | **All *H. rotundifrons* common mass exponent** | | | **0.65±0.11[1,***]** | | 0.0000 (0.003) | 0.31 | |
| *Cyclograpsus lavauxi* | Water | Non-ovigerous | 0.02±0.21 | 1.02[3] | 0.84±0.21 | 0.001 | 0.56 | 15 |
| | Water | Blastula | 0.09±0.08 | 1.10[4,***] | 1.26±0.21 | 0.0009 | 0.86 | 8 |
| | Water | Pre-hatching | 0.70±0.11 | 1.12[5,***] | 1.01±0.15 | 0.0003 | 0.87 | 9 |
| | **Common mass exponent (*b*) for aquatic crabs** | | | **0.99±0.11** | | 0.0000 (0.96) | 0.75 | |
| | Air | Non-ovigerous | 0.65±0.07 | 1.91[3] | 0.93±0.09 | 0.0000 | 0.93 | 11 |
| | Air | Blastula | 0.92±0.08 | 2.51[3] | 0.88±0.16 | 0.01 | 0.91 | 5 |
| | Air | Pre-hatching | 1.30±0.03 | 3.66[4,***] | 0.85±0.08 | 0.0000 | 0.92 | 11 |
| | **Common mass exponent (*b*) for aerial crabs** | | | **0.91±0.05** | | 0.0000 (0.10) | 0.93 | |
| | **All *C. lavauxi* common mass exponent** | | | **0.94±0.05[2,***]** | | 0.0000 (0.24) | 0.85 | |

$a$ and $b$ are the coefficients in the equation, $\dot{M}_{O_2}=aW^b$, obtained by least-squares linear regression of $\log_e\dot{M}_{O_2}$ on $\log_e W$, and are given with the standard errors of the estimates of $\log_e a$ and $b$. $W$ (crab mass) excludes the mass of the eggs of ovigerous crabs. $\dot{M}_{O_2}$ is in units of μmol $h^{-1}$; $W$ is in g.

Common mass exponents, $b$ (slopes in the logarithmic regression) were obtained by analysis of covariance after testing for parallelism. There were no significant differences among any of the $b$ values within each species.

**1,*****, **2,*****The difference between the two species in the common mass exponent, $b$, is highly significant ($P$<0.000001).

3,4,5Within each species/medium group, different number superscripts indicate significant differences ($P$<0.01) in elevation ($\ln\dot{M}_{O_2}$) among crabs with different egg stages or without eggs. *** indicates a significant difference ($P$<0.001) between ovigerous and non-ovigerous crabs. See Table 3 for mean $\dot{M}_{O_2}$ values adjusted to a common crab mass.

$P$ is the probability that $b$ is zero (or 1.0, obtained by testing $b$=0 in the regression of mass-specific $\log_e\dot{M}_{O_2}$ on $\log_e W$ ($t$-tests).

$r^2$ is the coefficient of determination.

a 2 g crab; Innes et al., 1986) and, hence, approximately 30 % lower than expected, assuming a $Q_{10}$ value of approximately 2. However, in the earlier study, the crabs were starved for only 1–2 days before respirometry, compared with more than 7 days here. Feeding of decapods, after starvation for approximately 1 week, has been associated with a greater than twofold elevation of metabolic rate, sustained for at least 2 days (e.g. *Crangon crangon*, Regnault, 1981; *Cancer pagurus*, Ansell, 1973; *Carcinus maenas*, Legeay and Massabuau, 1999; Legeay and Massabuau, 2000).

The aquatic metabolic rates at 15 °C of the two species of crab reported here are also lower than many values in the literature for decapods of similar mass (Table 4; Fig. 6). This must partly reflect the sedentary character of *H. rotundifrons* and *C. lavauxi* in water. In addition, as noted, the measurement conditions used here tended to minimise metabolic rates (non-feeding, more than 5 h settling, measurement during their least-active diurnal periods). Other studies do not always report fully the measurement or acclimation conditions but, clearly, many of the higher values in the literature (Table 4) were obtained from non-settled, recently fed animals (Weymouth, 1944), from animals placed in shaking respirometers (Hawkins et al., 1982) from tethered (McMahon et al., 1974) animals or they included periods of spontaneous activity (Ansell, 1973; Klein Breteler, 1975). Large differences in resting metabolic rates are also observed between seasons (Klein Breteler, 1975; Massabuau et al., 1984).

Absolute metabolic rates generally show negative allometry, in the case of decapod crustaceans scaling with mass to the power approximately 0.7–0.8 (i.e. –0.2 to –0.3 for mass-specific rates). This relationship applies intra- and inter-specifically to adult, juvenile and larval decapod crustaceans, ranging over more than six orders of magnitude of mass (Fig. 6; Weymouth, 1944; McNamara et al., 1985; Hawkins et al., 1982; Withers, 1992). Over the necessarily narrow mass range of adult females used in the present study, there were no significant differences in the mass exponents within each species, among non-ovigerous, early ovigerous and late ovigerous crabs, or between water and air, for either *H. rotundifrons* or *C. lavauxi*. However, there was a highly

Table 3. *Calculation of component of the total oxygen consumption by ovigerous crabs associated with carrying eggs and its comparison with the oxygen consumption of the separated eggs*

| Species | Medium | Egg stage | Mass of crab, excluding eggs (g) | Mass of eggs ($g^{-1}$ crab mass) | $\dot{M}_{O_2}$ for crabs of net 2 g ($\mu mol\ h^{-1}$) | | | Significance of difference between additional $\dot{M}_{O_2}$ of the eggs *in situ* and $\dot{M}_{O_2}$ of separated eggs[4] |
|---|---|---|---|---|---|---|---|---|
| | | | | | Total including eggs[1] | Additional due to the egg clutch *in situ*[2] | Separated eggs ($N$=10)[3] | |
| *Heterozius rotundifrons* | Water | Non-ovigerous ($N$=18) | 2.37±0.12 | | 1.41±0.11 | | | |
| | Water | Blastula ($N$=18) | 2.07±0.11 | 0.091±0.004 | 1.96±0.19 | 0.55±0.22 | 0.13±0.01 | NS (0.07) |
| | Water | Pre-hatch ($N$=7) | 1.74±0.21 | 0.134±0.014 | 3.69±0.27 | 2.28±0.29 | 2.45±0.05 | NS |
| | Air | Non-ovigerous ($N$=15) | 2.60±0.18 | | 1.23±0.06 | | | |
| | Air | Blastula ($N$=15) | 2.26±0.21 | 0.118±0.009 | 1.88±0.09 | 0.65±0.11 | 0.17±0.01 | *** |
| | Air | Pre-hatch ($N$=8) | 1.98±0.16 | 0.103±0.010 | 3.13±0.20 | 1.91±0.21 | 1.89±0.04 | NS |
| *Cyclograpsus lavauxi* | Water | Non-ovigerous ($N$=15) | 2.75±0.18 | | 1.81±0.10 | | | |
| | Water | Blastula ($N$=8) | 1.42±0.12 | 0.150±0.010 | 2.35±0.13 | 0.54±0.17 | 0.34±0.03 | NS |
| | Water | Pre-hatch ($N$=9) | 2.06±0.39 | 0.189±0.008 | 4.10±0.21 | 2.28±0.24 | 4.09±0.24 | *** |
| | Air | Non-ovigerous ($N$=11) | 2.05±0.37 | | 3.68±0.19 | | | |
| | Air | Blastula ($N$=5) | 1.53±0.24 | 0.147±0.015 | 4.72±0.20 | 1.03±0.27 | 0.33±0.03 | * |
| | Air | Pre hatch ($N$=11) | 1.31±0.14 | 0.199±0.010 | 6.88±0.171 | 3.20±0.25 | 4.30±0.25 | ** |

Values are means ± S.E.M. (adding variances to calculate standard error of differences between means; Zar, 1999). $N$ is the number of individual ovigerous and non-ovigerous crabs or the number of independent samples of eggs used in respirometry.

[1]Individual total $\dot{M}_{O_2}$ values were adjusted to a crab net mass of 2 g (i.e. excluding eggs) using the common mass exponent for each species (Table 2).

[2]The difference between the 2-g-adjusted $\dot{M}_{O_2}$ of ovigerous and non-ovigerous crabs.

[3]Individual estimates on samples of eggs in a stirred respirometer, adjusted to mean mass of eggs for 2 g crabs in each group.

[4]From a $t$-test of $H_0$:$\dot{M}_{O_2}$ (ovigerous crab) – $\dot{M}_{O_2}$ (non-ovigerous crab) – $\dot{M}_{O_2}$ (separated eggs)=0; all adjusted to a 2 g crab. $P$ reject $H_0$ is >0.05 not significant (NS), *$P$<0.05, **$P$<0.01, ***$P$<0.001, respectively. See Materials and methods for further details.

significant difference in their common mass exponents (0.65 for *H. rotundifrons* and 0.94 for *C. lavauxi*; Table 2). As these were heterogeneous groups, including both non-ovigerous and ovigerous crabs, the meaning of this difference is difficult to ascertain. Possibly, scaling of the size of the egg clutch is a factor. Innes et al. (Innes et al., 1986) reported exponents of 0.70 in water and 0.73 in air for non-ovigerous *C. lavauxi* at 10 °C.

When the metabolic rates of the embryos of *H. rotundifrons* and *C. lavauxi* are plotted against single-egg mass and compared with the general allometric relationship (Fig. 6), final-stage egg rates are consistent with larval, juvenile and adult rates, but the metabolic rates of the early egg stages are clearly much lower than predicted from their mass. The total mass of single eggs approximately doubled between cleavage and hatching, but the absolute metabolic rates of the eggs increased by factors of 20 for *H. rotundifrons* and 14 for *C. lavauxi*, giving slopes for the log-transformed plots of 6.16±0.25 (d.f.=6) and 4.24±0.55 (d.f.=4) respectively (+5.16 and +3.25 for mass-specific rates). Data calculated for *Carcinus maenas* embryos (Needham, 1933; Wheatley, 1981; using Wear, 1974, for volume and, hence, mass estimates) show a similar pattern (Fig. 6; absolute mass exponent, 3.86±0.63; d.f.=4).

What is the significance of the apparently anomalous scaling of metabolic rates observed during embryonic development?

Table 4. *Mass-specific rates of oxygen consumption at 15 °C, in water, of selected decapod crustaceans, related to fresh mass, feeding status, settling times and other conditions of measurement*

| Species | Starvation (days) | Settling time (h) | Notes | Fresh mass (g) | Rate of oxygen consumption ($\mu mol\ l^{-1}\ g^{-1}\ h^{-1}$) | Reference |
|---|---|---|---|---|---|---|
| *Macrobrachium olfersii* | 0 | 0.5 | Zoea | 0.000084 | 41.31 | McNamara et al. (1985) |
| *Macrobrachium heterochirus* | 0 | 0.5 | Zoea | 0.000116 | 38.79 | McNamara et al. (1985) |
| *Macrobrachium heterochirus* | 0 | 0.5 | Post-larva | 0.0066 | 18.94 | McNamara et al. (1985) |
| *Macrobrachium olfersii* | 0 | 0.5 | Post-larva | 0.0076 | 10.21 | McNamara et al. (1985) |
| *Carcinus maenas* | 0–7 | 0 | Juveniles, autumn, daily average | 0.06 | 2.732[1] | Klein Breteler (1975) |
| *Carcinus maenas* | 0–7 | 0 | Juveniles, spring, daily average | 0.06 | 6.118[1] | Klein Breteler (1975) |
| *Helice crassa* | 5 | 0.5 | Juveniles, shaken | 0.088 | 8.18 | Hawkins et al. (1982) |
| *Macrophthalmus hirtipes* | 5 | 0.5 | Juveniles, shaken | 0.155 | 5.37 | Hawkins et al. (1982) |
| *Crangon crangon* | 14 | 0 | | 0.332 | 3.19 | Regnault (1981) |
| *Crangon crangon* | 0 | 0 | | 0.332 | 7.34 | Regnault (1981) |
| *Cyclograpsus lavauxi* | <2 | 3–5 | | 0.5 | 2.84[1] | Innes et al. (1986) |
| *Carcinus maenas* | 0–7 | 0 | Juveniles, autumn, daily average | 0.6 | 3.519[1] | Klein Breteler (1975) |
| *Carcinus maenas* | 0–7 | 0 | Juveniles, spring, daily average | 0.6 | 5.030[1] | Klein Breteler (1975) |
| *Macrobrachium olfersii* | 0 | | | 1.074 | 2.98 | McNamara et al. (1985) |
| *Sesarma cinerea* | 1–7 | 0 | | 1.5 | 2.58 | Vernberg (1956) |
| *Cyclograpsus lavauxi* | <2 | 3–5 | | 2 | 1.86[1] | Innes et al. (1986) |
| *Pugettia producta* | 1 | 1 | Juveniles, multiple crabs | 2 | 6.6[1] | Weymouth et al. (1944) |
| *Uca pugilator* | 1–7 | 0 | | 2.3 | 2.37 | Vernberg (1956) |
| *Heterozius rotundifrons* | 7–14 | 5 | | 2.366 | 0.6312 | This study |
| *Cyclograpsus lavauxi* | 7–14 | 5 | | 2.747 | 0.868 | This study |
| *Macrophthalmus hirtipes* | 5 | 0.5 | Shaken | 2.77 | 2.24[1] | Hawkins et al. (1982) |
| *Helice crassa* | 5 | 0.5 | Shaken | 3.088 | 2.31[1] | Hawkins et al. (1982) |
| *Macrobrachium heterochirus* | 0 | | | 4.092 | 2.68 | McNamara et al. (1985) |
| *Cyclograpsus lavauxi* | <2 | 3–5 | | 5 | 1.43[1] | Innes et al. (1986) |
| *Uca minax* | 1–7 | 0 | | 6.6 | 1.49 | Vernberg (1956) |
| *Corystes cassivelaunus* | 1 | 24 | | 15.2 | 1.02 | Bridges and Brand (1980) |
| *Panopeus herbstii* | 1–7 | 0 | | 19.2 | 1.08 | Vernberg (1956) |
| *Pachygrapsus crassipes* | 1 | 2 | | 21 | 1.07 | Burnett and McMahon (1987) |
| *Galathea strigosa* | 1 | 24 | | 23.6 | 1.428 | Bridges and Brand (1980) |
| *Eurytium albidigitum* | 1 | 2 | | 27 | 0.65 | Burnett and McMahon (1987) |
| *Astacus leptodactylus* | 1.5 | 36 | Winter, daily average, cannulated | 40 | 0.94 | Massabuau et al. (1984) |
| *Astacus leptodactylus* | 1.5 | 36 | Summer, daily average, cannulated | 40 | 1.75 | Massabuau et al. (1984) |
| *Austropotomobius pallipes* | 1 | 12 | | 40 | 0.728 | Taylor and Wheatly (1980) |
| *Carcinus maenas* | ? | 0.5 | Tethered | 40 | 2.89 | Arudpragasam and Naylor, 1964 |
| *Orconectes virilis* | 3 | 72 | | 40 | 0.317 | McMahon et al. (1974) |
| *Orconectes virilis* | 3 | 0 | | 40 | 1.013 | McMahon et al. (1974) |
| *Ocypode albicans* | 1–7 | 0 | | 45.8 | 2.73 | Vernberg (1956) |
| *Carcinus maenas* | 7 | 24 | | 49 | 0.889 | Legeay and Massabuau (1999) |
| *Carcinus maenas* | 1 | 24 | | 49 | 1.592 | Legeay and Massabuau (1999) |
| *Callinectes sapidus* | 1–7 | 0 | | 142 | 1.33 | Vernberg (1956) |
| *Libinia dubia* | 1–7 | 0 | | 147 | 0.49 | Vernberg (1956) |
| *Menippe mercenaria* | 1–7 | 0 | | 163 | 0.595 | Vernberg (1956) |
| *Cancer pagurus* | >7 | >168 | Daily average | 200 | 0.9 | Ansell (1973) |
| *Cancer pagurus* | <1 | <24 | Daily average | 200 | 2.06 | Ansell (1973) |
| *Pugettia producta* | 1 | 1 | | 300 | 2.4[1] | Weymouth et al. (1944) |
| *Cancer pagurus* | 3 | 24 | | 414 | 0.77 | Naylor et al. (1997) |
| *Jasus edwardsii* | 1–7 | 48 | | 485 | 0.536 | Taylor and Waldron (1997) |

Only measurements made between 7 °C and 23 °C are included and are adjusted to 15 °C assuming $Q_{10}$=2. Dry mass and other mass bases have been converted to a wet mass basis using data given in the reference. In some cases, mean mass was estimated from ranges given in the reference.

[1]Calculated from allometric regression equations given in the reference.

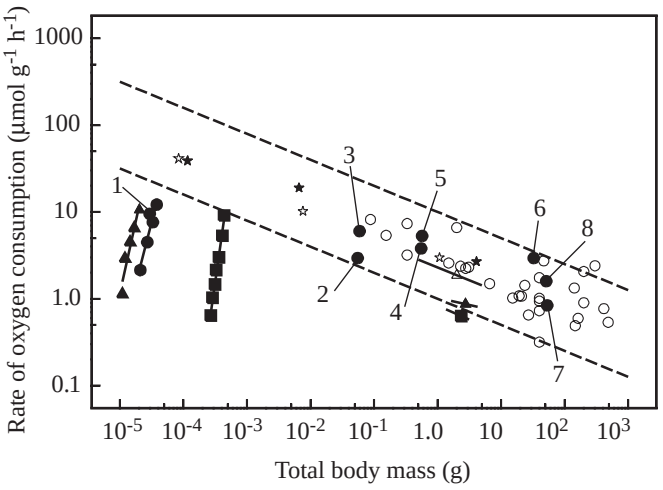


Fig. 6. Scaling of the mass-specific rates of oxygen consumption ($\mu mol\ g^{-1}\ h^{-1}$), in water at 15 °C, of developing crab eggs, with total body mass (g) compared with that of larval, juvenile and adult stages of selected decapod crustaceans. The regression lines for eggs (sequential stages, blastula to pre-hatching) of *Heterozius rotundifrons* (filled squares; $y=1.89x^{5.16}$) and of *Cyclograpsus lavauxi* (triangles; $y=2.06x^{3.25}$) and for unfed non-ovigerous adults of these species (corresponding symbols; $y=0.84x^{-0.33}$ and $y=1.02x^{-0.16}$ respectively), are taken from the present study. The regression line and open triangle (*C. lavauxi*) are taken from Innes et al. (Innes et al., 1986; recently fed, acclimated at 10 °C, adjusted to 15 °C; $y=1.64x^{-0.299}$). Filled and numbered circles, *Carcinus maenas*, blastula to pre-hatching, $y=6.85x^{2.89}$, calculated from Needham (Needham, 1933); 1, mean value for late-stage eggs from Wheatly (Wheatly, 1981); 2, 4, juveniles in autumn; 3, 5 juveniles in spring (Klein Breteler, 1975); 6, unsettled, tethered; 7, settled 24 h, starved; 8, settled 24 h, fed. Stars are mean values for zoea larvae, juveniles and adults of *Macrobrachium olfersi* (open symbols) and *M. heterochirus* (filled symbols) (McNamara et al., 1985). Details and sources of other data points (open circles) are given in Table 4. The dashed lines have a slope of −0.3 and delimit a 10-fold range in rate of oxygen consumption.

Conceivably, the total mass of the egg, which includes the external membranes and the yolk, is an inappropriate basis for allometric relationships. Dry mass (McNamara et al., 1985), shell-free dry mass (Hawkins et al., 1982), ash-free dry mass (Klein Breteler, 1975) and nitrogen content (Zeuthen, 1953) have also been used for normalization of decapod metabolic rates. However, basing embryonic scaling on similar attributes would also lead to anomalies. Although the total mass of crab eggs increases during development, primarily due to water uptake (N. Leelapiyanart and H. H. Taylor, unpublished data; Wear, 1974), and the embryonic primordia increase in size, the total dry mass of crab eggs decreases as yolk is metabolized (A. G. D. H. Seneviratna and H. H. Taylor, unpublished data; Pandian, 1970a; Pandian, 1970b; Biesiot and Perry, 1995).

Arguably, yolk-free mass more closely approximates the mass of respiring cytoplasm of the embryos. Rough microscopical estimates of the dimensions of the lobes of yolk (Table 2; Figs 2, 3) indicate that applying such a correction would improve conformity with conventional allometry for later-stage embryos. However, yolk-free mass is not a precisely definable variable and is not applicable to pre-gastrula stages. Total cleavage distributes the yolk uniformly among the blastomeres. Following gastrulation in other decapods, the yolk remains intracellular but is increasingly separated from the embryonic primordium, forming yolk ‘pyramids’ that extend centrally (Anderson, 1973). Fixation difficulties have limited the number of cytological studies of brachyuran eggs. Future consideration of the effective metabolic mass of the embryos must await better ultrastructural characterization of metabolically active cells.

Unicellular and multicellular animals lie on different allometric lines, displaying a threefold difference in metabolic rate, where their masses overlap (Withers, 1992). Thus, the anomalous allometry of the eggs might be interpreted as transitional between the lower metabolic rate of the unicellular zygote and that of the multicellular embryo. However, this interpretation is not supported by the observation that the major increase in metabolic rate occurred during organogenesis, not during cleavage. Another viewpoint on these data is provided by the observation that the mass-specific metabolic rates of the early embryonic stages of *H. rotundifrons* ($0.72\ \mu mol\ g^{-1}\ h^{-1}$) and *C. lavauxi* ($1.13\ \mu mol\ g^{-1}\ h^{-1}$) were similar to the metabolic rates of the adult female crabs ($0.70\ \mu mol\ g^{-1}\ h^{-1}$ for *H. rotundifrons* and $0.93\ \mu mol\ g^{-1}\ h^{-1}$ for *C. lavauxi*). It seems reasonable that the metabolic rates of the eggs on extrusion would depend on the metabolic rate of the ovarian tissue from which they originate. This might be further investigated by respirometry of ovarian tissue and eggs taken from crabs of different mass at maturity. As the fundamental bases of metabolic scaling are still unclear, particularly for ectotherms (for a discussion of current hypotheses, see Withers, 1992), further investigations into the ontogeny of metabolic rates would add a useful perspective to this central physiological phenomenon.

As noted above, non-ovigerous crabs and early eggs have similar mass-specific metabolic rates. However, the mass-specific metabolic rates of early ovigerous crabs (including the eggs) of both species were higher than for non-ovigerous females, in both air and water (Fig. 4; Table 3). As the stirred egg respirometer would be unlikely to underestimate the oxygen consumption of attached eggs, it appears that there is a metabolic cost to these crabs of carrying the eggs on the abdominal pleopods. It is unclear what this cost represents. In ovigerous *Carcinus maenas*, Wheatley (Wheatley, 1981) described ventilatory reversals, a modified emersion response and other behaviour patterns that appeared to be adapted to meet the increased oxygen demand of eggs incubated on the abdominal pleopods. We have not observed such types of behaviour in the two species studied here. Nevertheless, it is possible that the hyper-metabolism represented unobserved egg grooming, ventilatory movements of the pleopods, postural differences or generally elevated somatic metabolic rate. However, in *H. rotundifrons*, there is no difference between ovigerous and non-ovigerous crabs in their heart rate

or in the rate and direction of ventilation, even during hypoxia (Leelapiyanart, 1996).

The greatly elevated rates of oxygen consumption by late stage ovigerous crabs were clearly attributable to the high metabolic rates of the pre-larvae. Although representing only 13 % of the total mass of ovigerous *H. rotundifrons* and 19 % of that of *C. lavauxi*, the rate of oxygen consumption of the egg clutch equalled, or exceeded, that of the parent crab (Table 3). For *C. lavauxi*, the discrepancy between *in vitro* and *in situ* respirometry of the eggs was in the opposite direction to that of the early stages; i.e. the metabolic rate of the isolated eggs in the stirred respirometer was considerably greater than the difference between the metabolic rates of ovigerous and non-ovigerous crabs (Table 3). Possible explanations are that disturbance during their removal from the pleopods raised the metabolic rate of the pre-larva or, more likely, that diffusion limitations within the egg clutch reduced egg $\dot{M}_{O_2}$ *in situ*. Another possibility is that late-stage ovigerous crabs are hypometabolic, as inferred for ovigerous *Cancer pagurus* (Naylor et al., 1997). However, uncertainty concerning the degree of diffusion limitation within the egg mass prevents a single unequivocal explanation in any of these species. Indeed, diffusion limitation within the egg clutch would allow the possibility that the metabolic rate of *H. rotundifrons*, or of *C. lavauxi*, carrying late-stage embryos was, in fact, elevated, as in early ovigerous crabs.

Restricted oxygen diffusion into the egg mass of *Cancer pagurus* was demonstrated by measurements of $P_{O_2}$ within the eggs *in situ* and from respirometry of stirred and unstirred eggs (Naylor et al., 1997). Such localised hypoxia within the egg clutch could differentially affect the rate of development of the eggs. Experimental hypoxia has been observed to delay development in crustacean eggs (Lutz et al., 1992; Lutz et al., 1994; Andrew, 1993) and agitated, isolated eggs of the crabs *Carcinus maenas*, *Liocarcinus holsatus* and *Necora puber* developed faster, with a higher survival rate, than their siblings attached to the pleopods (Hartnoll and Paul, 1982; Choy, 1991). As *C. lavauxi* releases all its larvae on a single tide (Leelapiyanart, 1996), the mechanisms ensuring synchrony of development throughout the clutch deserve further investigation.

There was good agreement between the *in vitro* and *in situ* estimates of oxygen consumption for the late-stage eggs of *H. rotundifrons* (Table 3). The eggs drain more easily in air and the clutch is smaller with wider spaces between the eggs (Fig. 1A), thus reducing unstirred layer effects in both air and water. In view of the very long incubation times of *H. rotundifrons* eggs (6–9 months in the field), the uncertainties concerning oxygen diffusion referred to above, and the crabs' habit of burying in the substratum, further investigation of the effects of hypoxia on development rate is desirable. Indeed, some asynchrony in development is evident from larval release spanning several days in this species (Leelapiyanart, 1996).

Within related crustacean taxa, a direct relationship has been observed between egg size and the duration of embryonic development (Wear, 1974; Steele and Steele, 1975). The two species studied here conform to this empirical rule. Steele and Steele (Steele and Steele, 1975) concluded that this is a consequence of the negative allometry of metabolic rate (if yolk content and metabolic rate both scaled isometrically, the nutrient reserves of small and large eggs would last equal times). The present study does not support this explanation. Despite their size differences, the mass-specific metabolic rates for both early- and late-stage embryos were similar for *H. rotundifrons* and *C. lavauxi* (Figs 5, 6), and yet development times at 15 °C differed by a factor of 3.5. The principal dissimilarity between these species was in the time course of the increase in metabolic rate. In *H. rotundifrons*, the increases in metabolic rate and organogenesis were delayed by several months, corresponding to a period of egg diapause. Correspondingly, there was general agreement between the time courses of their rates of oxygen consumption (Fig. 5) and of the visible rates of disappearance of the yolk (Table 1). A similar egg diapause at constant temperature has been reported in other decapods (Wear, 1974; Helluy and Beltz, 1991).

Single eggs of *H. rotundifrons* consumed a total of 3.34 μmol of oxygen between oviposition and hatching, and those of *C. lavauxi* consumed 0.105 μmol, corresponding to the release of approximately 1.5 J and 0.05 J of energy, respectively (energy equivalent of oxygen uptake from Schmidt-Nielsen, 1997). This 30-fold ratio in the energetic cost of development is in approximate proportion to their initial total masses (270 μg for *H. rotundifrons* and 11 μg for *C. lavauxi*). Assuming the dry mass of the eggs was approximately half of the total mass and that the yolk contains approximately equal proportions of lipid and protein, as in other decapod eggs (Pandian, 1970a; Pandian, 1970b; Adiyodi, 1988; Biesiot and Perry, 1995), complete catabolism of the yolk would have consumed approximately 8.9 μmol of oxygen for *H. rotundifrons* and 0.36 μmol for *C. lavauxi* (Schmidt-Nielsen, 1997). The difference presumably represents the energy content of the pre-larval tissues because relatively little visible yolk remains (Figs 2, 3). Analyses and calorimetry of other decapod eggs similarly indicate that the greater proportion of the initial energy content and dry mass of the egg remain at hatching and that development depends mainly on the metabolism of lipid (Pandian, 1970a; Pandian, 1970b; Biesiot and Perry, 1995).

Starvation survival times by zoea (Diesel and Schuh, 1998) and lipid/protein ratios (Shakuntala and Reddy, 1982) indicate that larger eggs often produce larvae with a greater endotrophic potential than smaller eggs. It is unlikely that such differences are significant factors for larval survival in *H. rotundifrons* and *C. lavauxi*. The respirometry and microscopical observations reported here suggest that, although the smaller eggs initially use up their reserves more quickly, the relative energy contents at the time of hatching are similar and that negligible free yolk remains in the zoea of either species.

In interspecific comparisons, the masses of the egg clutches carried by crabs scale isometrically at approximately 10 % of the dry body mass of the crab, a value apparently determined

by space constraints for vitellogenesis in the rigid cephalothorax (Hines, 1992). Exceptions that tend to support this rule are the very soft-bodied pinnotherids, which have egg clutches up to 97 % of dry body mass (Hines, 1992), and semi-terrestrial grapsids that show maternal care of larvae and have extremely small clutches (Diesel, 1989; Diesel and Horst, 1995). Thus, for most crabs, the energetic investment in the egg clutch is rather constant, and selection pressure acts on the trade-off between egg size and egg number. It is unclear how differences in egg size relate to the survival of the larval stages of the two species studied here. Larger eggs produce larger larvae that are considered to be better able to survive variable conditions such as predation, starvation, water currents, salinity and desiccation (Steele and Steele, 1975; Shakuntala and Reddy, 1982; Schuh and Diesel, 1995; Diesel and Horst, 1995; Pollock, 1997; Hancock et al., 1998; Diesel and Schuh, 1998). The high-shore crab *C. lavauxi* has small eggs and yet it is exposed to a much greater range of environmental stress, as a result of freshwater run-off, desiccation, temperature and predation, than is *H. rotundifrons*. However, these factors act on the eggs, not on the larvae. Clearly, the factors influencing egg fitness are different from those influencing larval fitness. Wear (Wear, 1974) has demonstrated experimentally that smaller eggs are better able to survive high temperatures than larger yolky eggs, although the mechanism is unclear.

*C. lavauxi* releases the whole larval brood simultaneously, although release is not synchronised among crabs and takes place at both diurnal and nocturnal high tides (Leelapiyanart, 1996). There are five zoeal stages (Wear and Fielder, 1985), but there is little other information concerning planktonic development and settlement. The egg clutch consists of many small eggs (approximately 28 000 in a 2 g crab), which presumably offsets initial predation and mortality during larval dispersal. Larval numbers are maximised by a relatively high energetic investment in the brood (15.4 % of crab wet mass, equivalent to 22 % of dry mass; Innes et al., 1986), corresponding to the highest values recorded in non-pinnotherid crabs (Hines, 1992).

In contrast, *H. rotundifrons* incubates a small number of larger eggs (approximately 675 for a 2 g crab). As discussed above, larger eggs are often associated with extended development times, but this extension is primarily dictated not by size but by the egg diapause, presumably a device for ensuring appropriate seasonal timing of adult and larval phases. The larger eggs are evidently associated with greater larval fitness, permitting the lower egg numbers and lower maternal investment in the clutch (9.1 % of crab wet mass, approximately 13.5 % of dry mass), but the specific mechanisms are unknown for *H. rotundifrons*. As noted above, larger larvae are presumed to be better able to survive predation and other adverse conditions. In addition, *H. rotundifrons* releases its eggs on several successive twilight tides, which avoids ‘putting all its eggs in one basket’ and coincides with minimum planktivore activity (Christy, 1986). Among xanthid crabs (which are closely related to the Belliidae), there is a tendency for larger eggs to be associated with abbreviated development, a reduction in or absence of planktonic zoea and, in some cases, maternal care of megalopae (Wear, 1967; Wear, 1968; Greenwood and Fielder, 1984; Wear and Fielder, 1985). *H. rotundifrons*, with only two short zoeal stages (Wear, 1968), has proceeded some distance in this evolutionary direction, but many other aspects of the life history of the larvae are presently unknown.

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