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ENERGY METABOLISM DURING EMBRYONIC DEVELOPMENT AND LARVAL GROWTH OF AN ANTARCTIC SEA URCHIN

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Summary

Developmental energetics of an Antarctic sea urchin, *Sterechinus neumayeri*, were quantified to describe the physiological bases underlying ontogenetic changes in metabolic rate at extreme cold temperatures (–1.5 °C). Rates of development from a four-arm to a six-arm larval stage were not affected by food availability. The respiratory cost of development to the six-arm larval stage (day 60) was 14.0 mJ for fed larvae and 8.2 mJ for unfed larvae. We observed three phases of metabolic regulation during development. During embryogenesis (day 0–22), increasing metabolic rates were proportional to increases in cell numbers. During early larval development (day 22–47), the differences in respiratory rate between fed and unfed larvae were not accounted for by cell number, but by cell-specific metabolic rate (respiratory rate normalized to DNA content). Once an advanced larval stage had been reached (day 47–60), cell-specific respiratory rate and mitochondrial densities (citrate synthase activity normalized to DNA content) were more equivalent between fed and unfed larvae, suggesting that size-specific metabolic rates were determined at a level of physiological regulation that was independent of cell numbers or feeding history.

Key words: sea urchin, *Sterechinus neumayeri*, development, energetics, metabolic rate, respiration, mitochondrial density.

Introduction

Heusner (1991) described a central goal in comparative physiology to be an understanding of the differences between animals in metabolic intensity (i.e. metabolic rate per unit mass, or the mass coefficient a in $R=aM^b$, where R is rate of respiration, M is mass and b is the mass exponent). This question also applies to ontogenetic changes in metabolic rate during development where, with no increase in mass during embryogenesis, there are large increases in whole-organism metabolic rates. Understanding the physiological mechanisms that set metabolic intensities in embryos and larvae is important for describing the energy costs of development. Because respiration rates are not correlated with mass in embryos, metabolic rates respond to the energy requirements of development by mechanisms that are not understood.

For marine invertebrates, the importance of understanding the energy costs of embryonic and larval development has been primarily described in terms of the maternal allocation of energy to eggs (Vance, 1973). This approach produced many comparative studies of energy allocation, developmental mode and life-history strategies, particularly for echinoderms (Strathmann and Vedder, 1977; Turner and Lawrence, 1979; Lawrence et al., 1984; Strathmann, 1985; McClintock and Pearse, 1986; Emlet et al., 1987; McEdward and Chia, 1991; Jaeckle, 1995; Herrera et al., 1996). However, the maternal allocation of energy to an egg is only one component of developmental energetics. The other significant component is the actual energy metabolized by an embryo to reach a feeding larval stage. In contrast to the voluminous attention given to egg size and the energy invested in eggs, the energy required for development remains relatively undefined for any marine invertebrate.

In environments where food is scarce, the physiological basis of larval survival depends upon an egg's energy content and the metabolic rate at which those reserves are consumed during development. The seasonally restricted availability of food in the coastal margins of Antarctica has led to uncertainty regarding the relative importance of low food availability compared with low temperature in limiting developmental rates of polar marine invertebrates (Clarke, 1983, 1992). Many Antarctic echinoderms have extended developmental periods spanning several months to a year during which their larvae must survive in the water column (Pearse et al., 1991; Shilling and Manahan, 1994). For these larvae, the relationship between maternal investment in eggs and the cost of development to reach the feeding larval stage is further complicated by the asynchronous timing of reproduction relative to the austral-summer phytoplankton bloom (Pearse et al., 1991; Rivkin, 1991). Feeding larvae of many echinoderms appear in the water column several months before phytoplankton food sources are present, and thus the energy allocated to an egg must fuel early development and maintain metabolic activities until food becomes available (Shilling and Manahan, 1994).

In this study of the Antarctic sea urchin *Sterechinus neumayeri*, we focus on the developmental energetics of its embryos and feeding larvae to determine how energy metabolism is regulated during development. To understand the potential environmental impacts of low temperature and limited food, we assess the physiology of embryos and larvae of *S. neumayeri* at −1.5 °C and quantify the cell-specific effects of feeding on energy utilization, developmental rates and metabolic regulation.

Materials and methods

Animal culture and maintenance

Adult sea urchins, *Sterechinus neumayeri* (Meisner), were collected by SCUBA during austral spring from McMurdo Sound, Antarctica (off Cape Evans and the Delbridge Islands). Animals were maintained at constant temperature (−1.5 °C) in 1000 l running-seawater tanks in the Crary Laboratory Aquarium facility at McMurdo station until they were needed for spawning. Gametes were obtained by intracoelomic injections of 0.5 mol l^{-1} KCl. Eggs were pooled from several females for fertilization by sperm from one male. Once fertilization had been verified to be greater than 90 %, the fertilized eggs were transferred to four 200 l culture containers at a concentration of 7 eggs ml^{-1} (a density at which development remained normal). The culture temperature was maintained at −1.5 °C by immersing the 200 l culture containers in large aquarium tanks (1000 l) that had a high flow of ambient-temperature sea water pumped directly from McMurdo Sound. Temperature in the cultures was monitored daily and did not vary by more than ±0.2 °C during the culture period (October to December). Embryos and larvae were kept in suspension by mixing the cultures using vertical-plunging paddles (5–10 revs min^{-1}). Culture water was changed every 4 days by removing all individuals from containers and sieving onto 80 μm mesh screens. The culture container was then cleaned, fresh filtered sea water (FSW; 0.20 μm pore size) was added and, after enumeration for sampling, the embryos or larvae were replaced.

Once larvae were competent to feed following stomadeal breakthrough at day 22, the culture containers were assigned to the following treatments: two ‘unfed’ (FSW only); one ‘fed’ (receiving an equal mixture of the algae *Rhodomonas* sp. and *Dunaliella tertiolecta* at a final concentration of 15×10^3 cells ml^{-1} after every water change); and one ‘natural’ (after a water change, larvae were put into natural sea water filtered only through an 80 μm mesh to remove large zooplankton). On the basis of visual inspections, the algal cells added to the fed culture were never fully cleared between water changes. Daily mortality rates were below 0.5 % and not statistically different between fed and unfed cultures (analysis of variance of combined regressions). Morphometrics were determined by measuring the length of the left and right postoral arms and the total body length from the postoral arm tip to the dorsal apex of the larval body (McEdward, 1986). These measurements were made using a calibrated ocular micrometer under 10–20× magnification.

Energetics and metabolism

Elemental composition (as carbon and nitrogen) has long been used as an index of an organism’s caloric content (phytoplankton, Parsons et al., 1961; zooplankton, Platt et al., 1969), and the stoichiometry of this relationship has been defined quantitatively (Gnaiger and Bitterlich, 1984). In our study, body mass and energy content were estimated from elemental carbon and nitrogen content measured using a Carlo Erba NA1500 series II CN analyzer (after removing inorganic carbon by acid fuming). We have used the mass sum of elemental carbon and nitrogen as an index of body mass (CN_{mass}). Most of the organic mass in echinoderm tissues and eggs is composed of proteins and lipids (Lawrence and Guille, 1982; McClintock and Pearse, 1986; Jaeckle, 1995). The elemental sum of carbon and nitrogen should provide an index of these biochemical constituents during development and growth. We have restricted the use of these mass estimates to relative comparisons of the effect of feeding on larval physiology (i.e. fed compared with unfed) because elemental carbon and nitrogen contents only approximate total ash-free dry organic mass (Gnaiger and Bitterlich, 1984).

Carbon and nitrogen elemental compositions were additionally used to estimate the energy content of embryos and larvae. The protein content of these stages was measured using the Bradford assay with bovine serum albumin (BioRad) as the gravimetric standard (Leong and Manahan, 1999). We focus our energy estimates on protein and lipid composition using the following standard coefficients: protein mass is 17 % nitrogen and 53 % carbon, and lipid mass is 78 % carbon (Gnaiger and Bitterlich, 1984). We assume that 95 % of total organic carbon is accounted for by both protein and lipid carbon in echinoderms, given their negligible carbohydrate mass-composition. Protein mass was measured directly and used to estimate protein-carbon; lipid-carbon was then calculated by subtraction from total carbon (minus the protein-carbon) and used to estimate the lipid mass. Protein and lipid masses were then converted to energy equivalents using the combustion enthalpy coefficients of Gnaiger (1983): 24.0 kJ g^{-1} protein and 39.5 kJ g^{-1} lipid.

Respiration rates were measured in small (1 ml) biological oxygen-demand vials. These gas-tight vials were custom-designed for use with invertebrate embryos and larvae and yield accurate measurements of respiration rate at −1.5 °C (for a comparison of micro-respiration methods, see Marsh and Manahan, 1999). Embryos and larvae of *S. neumayeri* were placed in a series of vials (50–200 individuals per vial with 3–6 replicate vials per measurement) and incubated for 4–8 h at −1.5 °C; controls held no animals. The oxygen tension (P_{O_2}) in each vial was measured by injecting a sample into a micro-respiration chamber (75 μl sample volume) connected to a polarographic oxygen sensor maintained at −1.5 °C by a recirculating refrigeration bath. Temperature was continuously monitored using a thermometer in a replicate respiration chamber placed in series. The P_{O_2} (mmHg; 1 mmHg=0.1333 kPa) was calibrated to oxygen-saturated sea water (361 μmol l^{-1} at −1.5 °C and a salinity of 34 ‰), verified

by Winkler's direct chemical titrations of the oxygen content (Parsons et al., 1984). Respiration rates were converted to energy equivalents using a mean oxyenthalpic equivalent of 484.0 kJ mol^{-1} O_2 (Gnaiger, 1983). A cumulative energy cost of development was estimated by summing the total amount of energy consumed during each day of development. On days when a respiration rate was not measured, a linear mean rate was calculated from the preceding and succeeding days on which measurements were made.

Biochemistry

Citrate synthase (CS; E.C. 4.1.3.7) is an enzyme involved in regulating the activity of the Krebs cycle in mitochondria and has been used as a measure of aerobic metabolism and mitochondrial density (Hochachka and Somero, 1984). *In vitro* enzymatic catalytic rates for this enzyme were measured spectrophotometrically as the increase in absorbance at 412 nm caused by the production of mercaptide ions from Ellman's reagent (DTNB; Srere, 1969). Reaction conditions were optimized for maximum activity (CS_{opt}) at 4 °C in a 50 mmol l^{-1} imidazole buffer (pH 7.4). CS_{opt} rates are expressed as pmol citrate min^{-1} and are presented as both protein-specific activities (normalized to protein content) and as cellular activities (normalized to DNA content). The standard substrate concentrations used in the assay (330 μmol l^{-1} acetyl-coenzyme A and 330 μmol l^{-1} oxaloacetate) were verified to be non-limiting during the reactions. DNA content was measured directly in a fluorometer (DyNA Quant II; excitation wavelength 365 nm, emission wavelength 460 nm) in aqueous homogenates (50 mmol l^{-1} Tris-HCl, 2 mol l^{-1} NaCl, 20 mmol l^{-1} EDTA) using Hoechst's dye. Calf thymus DNA (Pharmacia) was used as a gravimetric standard. Direct counting of nuclei in early embryos stained with Hoechst dye under an epifluorescence microscope was used to check the estimated cellular DNA content.

Statistics

All statistical comparisons were performed using SigmaStat 2.0 software (Jandel Scientific) for the calculations. When assumptions of data normality were not supported, non-parametric tests were employed. When ratios of two measurements are presented, errors from each measurement were propagated to a standard error calculated by linear error propagation formulae (Caulcutt and Boddy, 1983).

Results

Morphometrics and mass

Total elemental carbon and nitrogen were measured during development and growth of *Sterechinus neumayeri* (Fig. 1). The ratio of elemental carbon to elemental nitrogen content generally reflects the biochemical composition of a tissue, with higher ratios indicative of greater lipid and carbohydrate levels (carbon-rich) and lower ratios indicative of greater protein levels (nitrogen-rich; Platt et al., 1969). Eggs of *S. neumayeri* had a C:N ratio of 5.0; during development, the C:N ratio

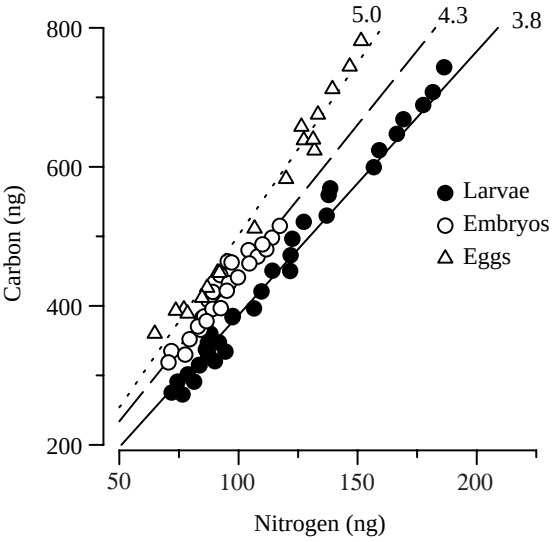

Fig. 1. The ratio of elemental carbon to nitrogen (C:N) in eggs, embryos and larvae of *Sterechinus neumayeri*. Each point represents a pooled sample of 100–500 individuals; the slope of a linear regression through each set of points was used to calculate the C:N ratio, which is presented next to each regression line. Fed and unfed larvae did not show any significant differences in elemental composition, and their values were pooled into one regression.

decreased to 4.3 in embryos and 3.8 in larvae (all ratios were significantly different; analysis of variance, ANOVA, $P<0.001$, Tukey's test $P<0.05$). This decrease in C:N ratio suggests that lipid reserves were catabolized during early development. The organic mass of eggs, embryos and larvae of *S. neumayeri* was estimated as the sum of elemental carbon and nitrogen contents (CN_{mass}; Fig. 2). During embryonic development from the egg to the feeding larval stage at day 22, there was no loss of body mass. Once larvae began to feed at day 22, their organic mass increased rapidly. Unfed larvae maintained their mass between days 22 and 34. At day 47, the formation of the third pair of larval arms was initiated in both fed and unfed larvae, and the mass of unfed larvae began to decline. Larvae that were cultured in unfiltered sea water (the 'natural' treatment) had a mass at day 50 that was equivalent to that of unfed larvae. The energy content of embryos and larvae was estimated from measured protein content and calculated lipid content (Fig. 2 inset). Beginning feeding on day 22 of development did not affect the subsequent rate at which a four-arm pluteus reached the six-arm stage, despite the large differences in nutritional state and body mass.

During the first 10 days of feeding (days 22–32), there were no differences in larval body length (Fig. 3A). Between day 32 and 47, fed larvae had a significantly higher rate of body length increase than unfed larvae (9.56 *versus* 5.63 μm day^{-1}; ANOVA, $P<0.001$). The body length of unfed larvae stopped increasing at approximately day 45 at 575 μm, while fed larvae continued to grow (to 666 μm at day 47; Fig. 3A). Body lengths of larvae in the 'natural' treatment were equivalent to those of the unfed larvae. The postoral arm lengths were also

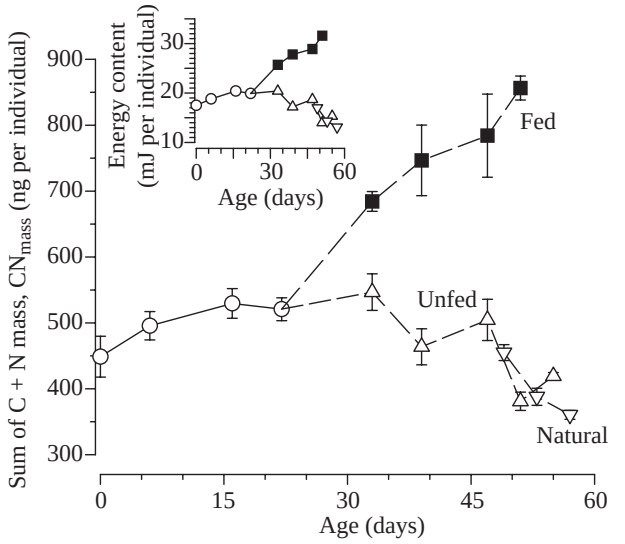

Fig. 2. Biomass of embryos and larvae of *Sterechinus neumayeri* calculated as the sum of elemental carbon and nitrogen. Embryos (○) were cultured for 22 days in filtered sea water. After day 22, larvae were divided into three experimental treatment groups: fed (■), high phytoplankton density; unfed (△), no phytoplankton present (filtered sea water only, 0.2 μm); and natural (▽), natural ambient phytoplankton present (coarsely filtered sea water, 80 μm). Values are plotted as means ±1 S.E.M. (*N*=6). The inset presents the biomass energy content of embryos and larvae estimated from protein content and calculated lipid composition (see text for further details).

measured for fed and unfed larvae and were equivalent up to day 40 (Fig. 3B). No difference was observed between left and right postoral arm lengths (left arm length data not shown). A comparison of the 'unfed' and 'natural' treatments (Figs 2, 3) indicates that there was no difference in size or energy content of larvae raised in filtered *versus* unfiltered, natural sea water. This comparison supports observations of very low phytoplankton concentrations in McMurdo Sound during austral spring (Rivkin, 1991).

DNA and protein content

During early development, the DNA content of embryos increased rapidly from fertilization to gastrulation (day 12; Fig. 4). At the hatching blastula stage of development (day 5), direct nuclear counts in embryos produced cell number estimates of 2171 and 2132 (mean 2152) with a DNA content of 3.20 and 2.68 ng individual^{-1} (mean 2.94 ng individual^{-1}). These measurements give a cellular DNA content of 1.37 pg cell^{-1}, which is close to the diploid value of 1.8 pg cell^{-1} for the sea urchin *Strongylocentrotus purpuratus* (Cameron et al., 1989). After gastrulation, cell division rates declined, and the change in DNA content slowed to a gradual rate of increase. Although DNA content was slightly higher in the fed larvae between day 22 and day 47, these differences were not statistically significant (paired *t*-test, $P>0.069$). At the formation of the third pair of larval arms (day 47), a higher rate of cellular proliferation occurred in fed larvae so that the DNA content doubled between day 47 and day 60 (paired *t*-test, $P<0.001$). Unfed larvae also initiated arm formation at the same time as did fed larvae (day 47), but with a smaller increase in cell number (DNA content; Fig. 4). Although cell number does not appear to be affected by feeding during early larval development (days 22–47), feeding does have a significant effect on larval cell number after day 47.

Protein was normalized to DNA content to provide a measure of the mean protein content per cell during development of *S. neumayeri* (Fig. 5). This ratio was higher in embryonic stages than in larval stages, but was equivalent in fed and unfed larvae (95 % confidence interval overlap for all samples). Despite the large differences in body size (Fig. 3A) and equivalent DNA levels in larvae until day 47 (Fig. 4), cellular protein content (cell size) was maintained at a constant ratio of between 10 and 15 pg protein pg^{-1} DNA. Because cell protein content and DNA content both remained relatively fixed during this period, the 45 % increase in energy content from day 22 to day 47 in fed larvae (from 19.9 to 28.9 mJ; Fig. 2 inset) did not result from a significant increase in cell number. After day 47, increases in larval energy content could have resulted from cell proliferation events that produced greater cell numbers in fed larvae while cell organic mass (protein content per unit DNA) remained unchanged (Fig. 5).

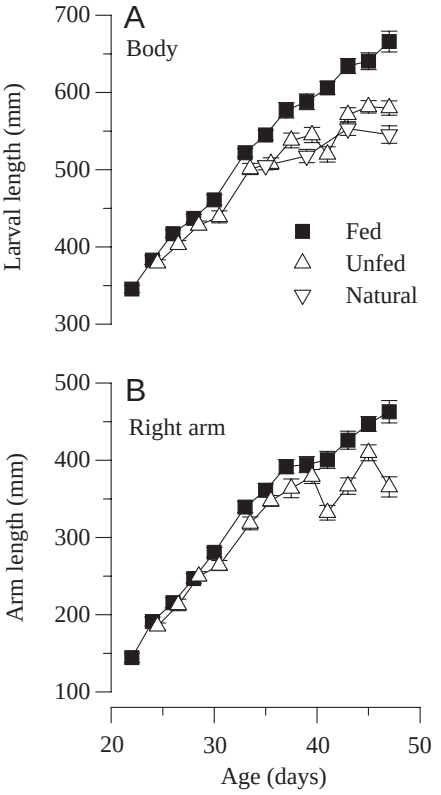

Fig. 3. Morphometrics of fed and unfed pluteus larvae of *Sterechinus neumayeri*. The total length of a larva (A) was measured from the postoral arm tip to the dorsal apex of the body. The lengths of both the right and left postoral arms were measured, but data for the right arm only (B) are presented (arm lengths were symmetrical). Values are plotted as means ±1 S.E.M. (*N*=50).

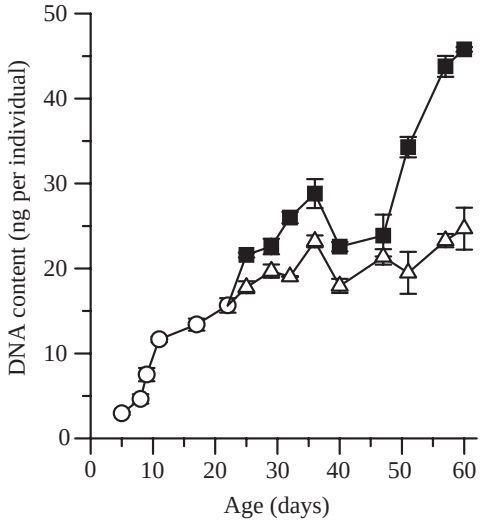

Fig. 4. DNA content of embryos (○) and fed (■) and unfed (△) larvae of *Sterechinus neumayeri*. Values are plotted as means ±1 S.E.M. (*N*=3).

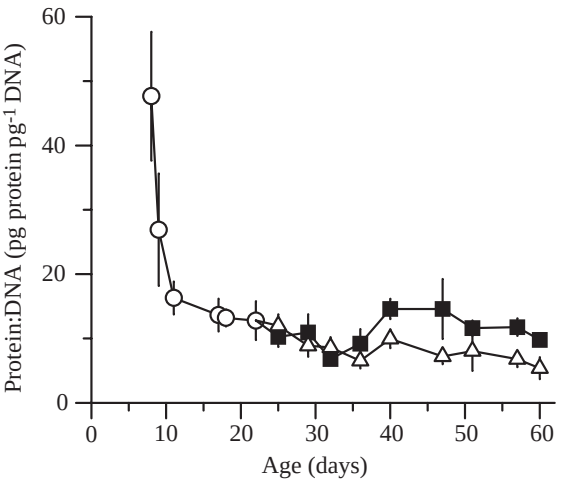

Fig. 5. Protein content normalized to DNA levels of embryos (○) and larvae of *Sterechinus neumayeri* from fed (■) and unfed (△) treatments. Values are plotted as means ±1 S.E.M. (*N*=3).

Respiration and energetics

Respiration rates increased steadily from fertilization to the four-arm pluteus (feeding) stage at day 22 (Fig. 6A). After the first week of feeding (day 32), fed larvae had higher respiration rates than unfed larvae, and this continued through the remainder of the study to day 60. Respiration rates of both fed and unfed larvae showed temporal variations correlated with developmental events. For example, there were parallel changes in respiration rates for both fed and unfed larvae after day 47 when the third pair of larval arms was forming. By day 60, respiration rates of fed larvae had more than doubled to 36 pmol O_2 h^{-1} individual^{-1} from the initial pre-feeding rate at day 22, and were almost three times higher than those of unfed larvae. Daily respiration rates were summed over the developmental period studied to estimate the total energy metabolized by fed and unfed larvae (Fig. 6B). In total, fed larvae expended 14.0 mJ during the first 60 days of development in comparison with the 8.2 mJ expended by unfed larvae (a 59 % difference). Between days 32 and 60, the rate of increase in total energy respired (cumulative) averaged 331±5 μJ day^{-1} for fed larvae and 150±2 μJ day^{-1} (means ± S.E.M., *N*=6) for unfed larva (Fig. 6B; mean rates were determined from the regression slopes of cumulative energy consumption over time between days 32 and 60; the slopes are significantly different when compared by ANOVA of regressions, $P<0.001$). In terms of energy equivalents, the metabolic rate of an unfed larva (Fig. 6B) would have utilized 0.86 % day^{-1} of the initial energy reserves of the egg.

In feeding larvae, increases in energy expenditure result not only from the capture, ingestion and assimilation of algal cells, but also from increases in the energy costs of growth and the maintenance of a greater biomass. To balance the energy costs associated with feeding against the energy gained from ingesting algae *ad libitum*, the differences in total respiration between fed and unfed larvae were compared with the differences in the energy contents of their corresponding mass. Using the stage just prior to feeding as the reference point (day 22), cumulative changes in energy expended in respiration and lost or gained in biomass between fed and unfed larvae were calculated during development to day 51. By day 51, fed larvae had expended 4.0 mJ more in oxygen consumption than unfed larvae ($\Delta R_{(F\text{-}U)}$=10.7–6.7 mJ, where R is the rate of oxygen consumption, F is fed and U is unfed), but had a net increase in energy content of 17.6 mJ over unfed larvae [+11.7 mJ increase in fed larvae, minus a −5.9 mJ decrease in the unfed larvae between days 22 and 51; $\Delta M_{(F\text{-}U)}$=11.7–(–5.9 mJ), where M is mass]. This difference equates to a 440 % return in body energy content gained for energy expended in feeding [$\Delta M_{(F\text{-}U)}/\Delta R_{(F\text{-}U)}$=(17.6/4.0)×100 %].

Cellular metabolism

To describe the potential physiological bases underlying developmental changes in respiration rates, relative to both cell mass and cell number, the activity of citrate synthase (CS_{opt}) was measured as a cellular marker of mitochondrial density. The significant correlation between rate of respiration and CS_{opt} in unfed larvae (unfed for >8 days) shows that changes in respiration rates were closely related to mitochondrial density (Fig. 7A; r^2=0.837 for data following 1 week of further development without food). For these unfed larvae, CS_{opt} per individual was a good index of metabolic rate (Fig. 7A). However, this relationship did not hold for the fed larvae (ANOVA of regression, slope not different from zero, $P<0.40$). Fed larvae had the same CS_{opt} activity as unfed larvae (approximately 20 pmol citrate min^{-1} $larva^{-1}$), despite having respiration rates that were more than twice as high. This comparison shows that mitochondrial activity was generally maintained at a low level relative to the maximum potential aerobic capacity in fed larvae.

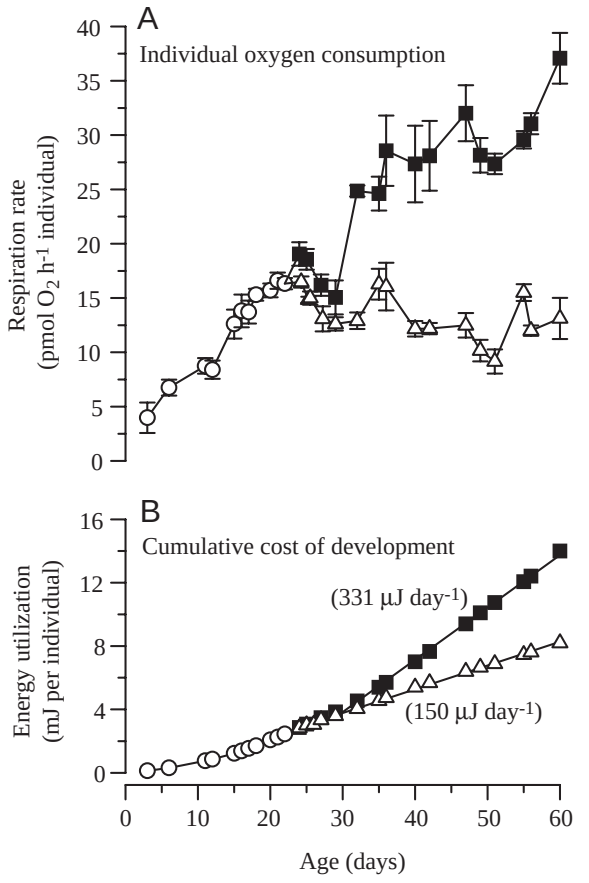

Fig. 6. Metabolic rates during development of embryos (○) and larvae of *Sterechinus neumayeri* from fed (■) and unfed (△) treatments. Individual respiration rates (A) are plotted as means ±1 S.E.M. (N=6). The cumulative energy cost of development (B) was estimated from the daily individual respiration rates. Between days 30 and 60, total energy consumption averaged 331 and 150 μJ day^{-1} individual^{-1} for fed and unfed larvae (estimated from the slope of linear regression functions; r^2=0.99 for each regression).

During early development, there was a rapid increase in the protein-specific activity of CS_{opt} (Fig. 7B) that followed the increase in cell number during embryogenesis (Fig. 4). The protein-specific CS_{opt} activity did not show large differences between fed and unfed larvae (Fig. 7B). Although there was a twofold difference in respiration rates between fed and unfed larvae on day 47 (Fig. 6A), there was no difference in protein-specific CS_{opt} activities. After day 47, the specific activity of CS in fed larvae remained constant, indicating that the increase in cell number associated with the formation of the third pair of larval arms resulted in an equivalent increase in the total number of mitochondria (Fig. 7B,C). For unfed larvae, there was a large increase in protein-specific CS activity beginning at day 51. This increase could result from the selective breakdown by unfed larvae of proteins other than CS, resulting in a greater representation of CS in the total protein pool.

The pattern of decline in the cellular activity level of CS (i.e. CS_{opt} normalized to total DNA content, Fig. 7C) shows a continuous decrease in mitochondrial densities per cell during development. Although there were differences in the temporal changes of cell-specific CS activity during embryogenesis, there were no significant differences in the absolute values of these activities between fed and unfed larvae (two-way ANOVA comparing fed and unfed values after removing the effects of differences in developmental age; Fig. 7C). At day 60, both fed and unfed larvae had the same cellular CS_{opt} activity levels, suggesting that the number of mitochondria per cell during later development was independent of nutritional state and body mass.

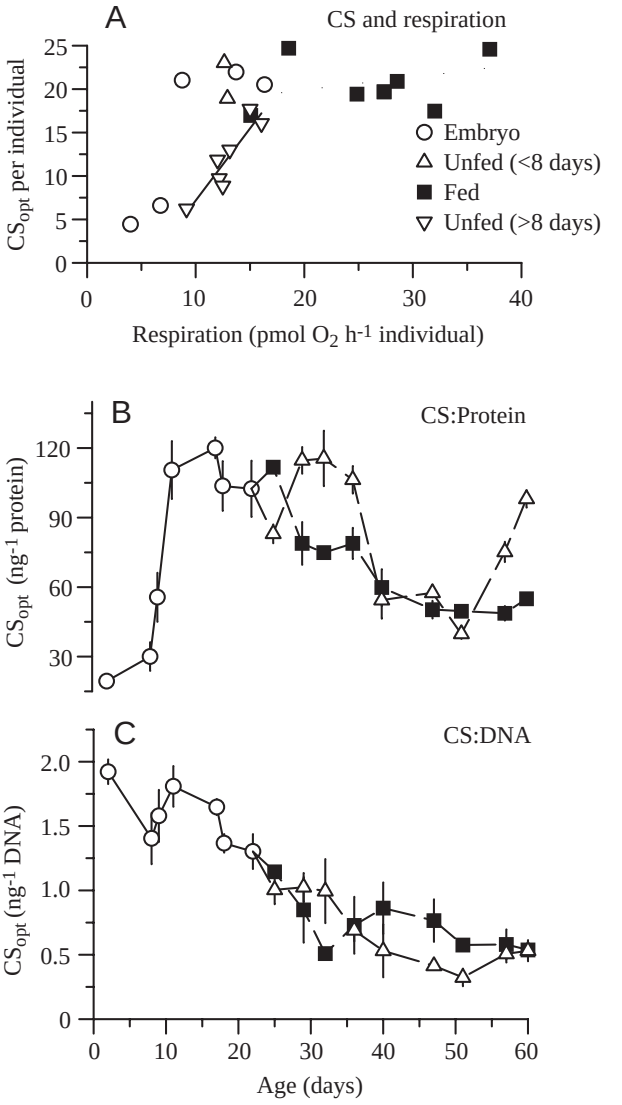

Fig. 7. Citrate synthase (CS) activity in embryos and larvae of *Sterechinus neumayeri* from fed and unfed treatments. (A) Optimized citrate synthase activities (CS_{opt}, pmol citrate min^{-1}) are plotted as a function of respiration rate. The regression functions presented used measurements between days 32 and 55 for fed (■) and unfed (▽; more than 8 days without food) larvae. CS_{opt} activities were normalized to protein content (B) and DNA content (C). For B and C, values are plotted as means ±1 S.E.M. (N=3).

Discussion

Metabolic processes during development

The relationship between respiration rate and mass is an important physiological parameter for evaluating rates of energy utilization. Fed larvae were bigger both morphologically and in mass than unfed larvae and had higher total oxygen consumption rates. A measure of the respiration rate per cell (per unit DNA; Fig. 8A) shows that, after the first week of feeding (day 32), cellular respiration rates were 29 % higher in fed than in unfed larvae. At the formation of the third pair of larval arms beginning on day 47, cellular respiration rates for fed larvae were 57 % higher than in unfed larvae (Fig. 8A; values calculated as described in Fig. 9 legend). Following this developmental point at day 47, the increase to day 60 in whole-organism respiration rate for fed larvae (24 % from day 47 to day 60; Fig. 6A) did not scale with the larger increase in cell number (48 % from day 47 to day 60; Fig. 4). The result was that, by day 60, cellular respiration rates in fed larvae were only 35 % greater than in unfed larvae (Fig. 8A; compared with 57 % on day 47). Normalizing respiration rate to elemental CN_{mass} (Fig. 8B) showed an increase in mass-specific respiration rates during embryogenesis until the feeding larval stage was reached (day 22). During larval development to day 47, there were large differences in cell-specific and mass-specific respiration rates between fed and unfed larvae. However, these differences decreased with subsequent development, indicating that growth in fed larvae after day 47 was paralleled by a concomitant decrease in respiration rate per unit size (either DNA or mass content).

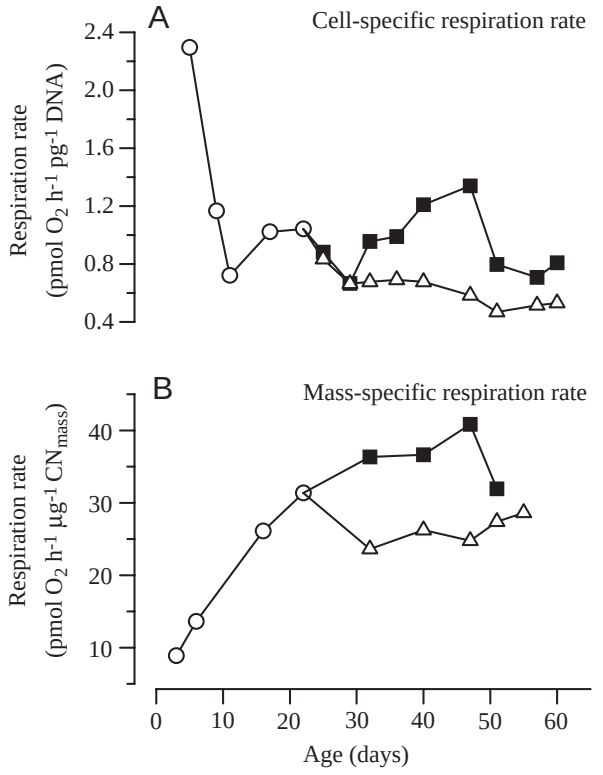

Fig. 8. Respiration rates during embryonic and larval development of *Sterechinus neumayeri* normalized to DNA content (A) and mass (B). Cell-specific respiration rates are presented as rates of oxygen consumption normalized to DNA content; mass-specific respiration rates are presented as rates of oxygen consumption normalized to biomass (as estimated by the sum of elemental carbon and nitrogen, CN_{mass}). Data are plotted for embryos (○) and larvae from fed (■) and unfed (△) treatments.

The increase in respiration rate in fed larvae from day 30 to day 47 occurred without a concomitant increase in either cell number (Fig. 4) or protein-specific activity of citrate synthase (Fig. 7B). This suggests that larvae had a fixed mitochondrial density that did not vary as a consequence of nutritional state during this period. Instead, the aerobic activity of mitochondria was maintained at a relatively low fraction of the maximum capacity, and increased demands for respiration in actively feeding larvae were probably met by an increase in the physiological activity of the existing complement of enzymes within the mitochondria. This is evident in the increase in cell-specific respiration rate of fed larvae (Fig. 8A) in the absence of a parallel change in the cellular content of CS (pg^{-1} DNA; Fig. 7C). In contrast, unfed larvae maintained steady cell-specific and mass-specific respiration rates (Fig. 8A,B) indicative of a constant level of maintenance metabolism in the absence of feeding. After day 47, the cell-specific respiration rates in fed larvae declined rapidly, and by day 60, fed larvae were more physiologically similar to unfed larvae when metabolic rate was scaled to an index of organismal size (DNA). The DNA content of fed and unfed larvae did not differ until the onset of formation of the third pair of larval arms at day 47 (Fig. 4). Despite the physiological differences that existed at day 47 (e.g. respiration rate; Fig. 6A), it appears that cell numbers were equivalent up to this stage of development. In terms of magnitude, protein content normalized to DNA content (Fig. 5) was similar in fed and unfed larvae, suggesting that, even with the difference in mass (Fig. 2), cellular protein content (i.e. cell size) was relatively stable. Both cell size and cell number were similar between day 22 and day 47 for both fed and unfed larvae.

During embryonic development (days 3–22), a 3.6-fold increase in mass-specific respiration rate (Fig. 8B) can be readily explained by increasing cell numbers, with a 4.1-fold increase in respiration rate (Fig. 6) and a 5.6-fold increase in DNA content (Fig. 4). In contrast, during early larval development (from the four-arm to the beginning of the six-arm pluteus stage; days 22–47), increases in cell number in fed larvae (1.5-fold higher DNA content; Fig. 4) did not parallel the increases in metabolic rate (2.3-fold higher respiration rate; Fig. 6). During this period from day 22 to day 47, differences in respiration rates between fed and unfed larvae gradually increased to more than 60 % of the values in the fed treatment (Fig. 9). Here, instead of cell number, differences in cell-specific respiration rates could explain most of the observed differences in total respiration rate (97 %) between fed and unfed larvae (inset Fig. 9; regression slope not significantly different from unity; slope=1.14±0.11; mean ± S.E.M., r^2=0.965). Thus, the difference in metabolic intensity between

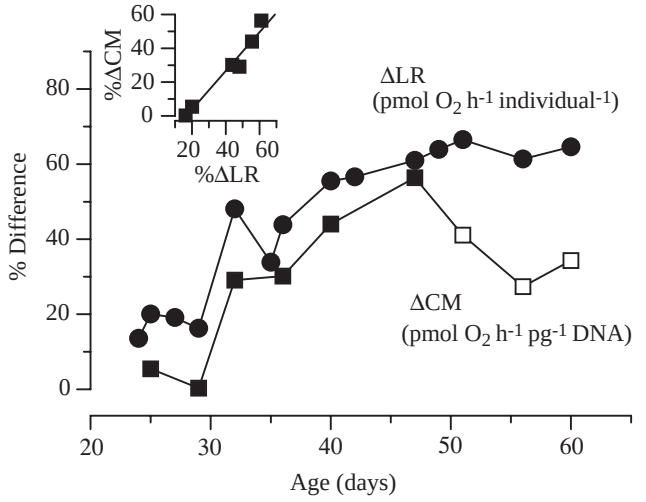

Fig. 9. Differences in respiration rates between fed and unfed larvae of *Sterechinus neumayeri*. Respiration rates are taken from Fig. 6 (per individual) and Fig. 8A (per unit DNA) to illustrate the percentage change in whole-organism respiration rates that can be explained by metabolic rate per cell. Differences in rates between fed and unfed larvae are plotted as a percentage of the corresponding value in fed larvae, i.e. [(fed−unfed)/fed]×100. A linear regression of the difference between larval (ΔLR) and cellular (ΔCM) respiration rates to day 47 of development is plotted in the inset (slope=1.14±0.11; mean ± S.E.M., r^2=0.965, P<0.001). In the main graph, there are fewer cellular respiration data points because DNA content was measured less frequently than respiration. Filled symbols in the main graph represent points used for the regression in the inset.

fed and unfed larvae up to day 47 could be accounted for almost entirely by differences in their cell-specific metabolic rates.

Once the six-arm larval stage had been initiated (day 47), differences in cell-specific metabolic rates were no longer tightly correlated with the differences in total respiration rate between fed and unfed larvae (Fig. 9). Similarly, the changes in cell number in fed larvae could not account for changes in metabolic intensity (twofold increase in DNA content with only a 1.2-fold increase in respiration rate; days 51–60). It is likely that this physiological pattern (days 47–60; Figs 8A,B, 9) results from the appearance of a mass-specific metabolic rate that is less dependent on cell numbers and nutritional state (fed or unfed). At this point in development, it appears that both fed and unfed larvae possess a similar, species-specific metabolic intensity per unit size (Fig. 8) such that they would share a near-equivalent mass coefficient [i.e the variable a in the equation $R=aM^b$, where R is respiration rate, M is mass and b is the mass exponent]. At some point during development, a transition occurs in metabolic regulation from the early stages in embryogenesis, where a few large cells account for metabolic rate, to the later stages in larval development, where many smaller cells have tissue-specific metabolic activities.

To summarize, there are three phases of metabolic regulation during development in *Sterechinus neumayeri*. First, in embryos, increases in respiration rates can be accounted for solely by increases in the total number of cells. Second, in early larvae, respiration rates are determined not by cell numbers or mitochondrial densities per larva, but by changes in cell-specific biochemical activities. Third, in older larvae, size-specific respiration rates decline at the level of whole-organism physiology. The mechanisms underlying these changes in size-specific metabolic rate are unknown, as for animals in general (see Heusner, 1991). In developmental stages, it is likely that increases in tissue differentiation result in more cell types with low metabolic activities that would lower size-specific physiological rates.

Energy utilization during development

An underlying premise to discussions of maternal energy investment in eggs is that the allocation of reserves is linked both to the energy requirements of development and to the size at metamorphosis required for subsequent survival. Thus, egg size is considered to be under strong selective pressures and highly relevant for evaluating developmental modes in terms of life-history strategies. A critical energetic quantity that affects developmental mode is the amount of energy an embryo will require to reach larval metamorphosis. Once the energy requirements of a species for development are defined, then the amount of maternal energy invested in an egg can be fully evaluated (i.e. the '***s***' parameter for egg energy allocation in the model of Vance, 1973). Although some studies have measured various components of embryonic and larval energetics (Zeuthen, 1947; Pechenik and Eyster, 1989; Rodriguez et al., 1990; Shilling and Manahan, 1994; Hoegh-Guldberg and Manahan, 1995; Shilling et al., 1996), only a few have attempted to provide complete estimates of the total energy costs of development (Jaeckle and Manahan, 1989; Hoegh-Guldberg, 1994; Hoegh-Guldberg and Emlet, 1997). Even these estimates are still questionable because of the difficulty of making accurate micro-respiration measurements (Hoegh-Guldberg and Manahan, 1995; Shilling et al., 1996; Marsh and Manahan, 1999).

The energetics of development were first described by Tangl (1903) as the amount of energy respired during embryogenesis, which he called '*Entwicklungsarbeit*' or 'the work of development'. An early review of embryonic energetics (Needham, 1931) concluded that most metazoans (although data were primarily from chordates) respired 40–50 % of the egg's energy content to develop to a feeding stage (for reviews, see Calow, 1977; Wieser, 1994). For *Sterechinus neumayeri*, the initial energy content of an egg was 17.5 mJ (Fig. 2). The total energy cost of embryogenesis was 2.45 mJ during the 22 days from fertilization to the formation of a feeding larval stage (Fig. 6B). Thus, assuming no uptake of dissolved organic material by embryonic stages, only 14 % of the initial maternal allocation of energy to the egg was required to reach the feeding larval stage. This low fraction of energy consumption during embryogenesis is noteworthy because most of the egg's energy resources are available for further development in the absence of particulate food (four- to six-arm pluteus stage).

Respiration rates of unfed larvae provide an accurate measure

of the costs of development and maintenance metabolism. The first 60 days of development for unfed larvae required 8.2 mJ per individual to reach the six-arm pluteus stage (Fig. 6B). At this point, 57 % of the egg's initial energy content (17.5 mJ) should have been metabolized, assuming no input of exogenous energy. However, our measurements of the remaining energy content after 55 days without food showed that larvae still had 15.3 mJ per individual, a decline of only 13 % from the egg's initial energy content (compared with a predicted value of 57 %). This observation of mass maintenance (Fig. 2) above predicted respiratory losses has been documented previously for other developing marine invertebrates (gastropods, Jaeckle and Manahan, 1989; Shilling et al., 1996; Antarctic echinoderms, Shilling and Manahan, 1994). The lower C:N ratio in embryos than eggs (Fig. 1) suggests that the lipid reserves of the eggs were utilized during early development. However, despite this catabolism, the energy imbalance from the observation of mass stasis in unfed larvae reared in filtered sea water (Fig. 2) can best be explained from the acquisition of exogenous resources, such as dissolved organic material (Manahan, 1990). This uptake would significantly increase the metabolic life-span of a larva in the absence of particulate food.

For larvae of *S. neumayeri*, feeding on phytoplankton was not required for normal development from a four-arm pluteus to a six-arm stage. At day 55, an unfed larva had an energy content of 14.1 mJ (Fig. 2) and a respiration rate of 150 μJ day^{-1} (Fig. 6B). How long past day 55 could a larva survive without particulate food, with no input of dissolved organic material and no subsequent down-regulation of metabolism? Assuming that a larva is viable following a 50 % reduction in the starting mass of its egg (i.e., Needham's, 1931, 'work of development' value of 50 %), then this value for *S. neumayeri* would be 8.8 mJ (egg 17.5 mJ). At the measured respiration rate (0.15 mJ day^{-1}), it would take 35 days to utilize 5.3 mJ, which would deplete the energy content to the 50 % value of 8.8 mJ [(14.1–8.8)/0.15=35]. Thus, larvae of *S. neumayeri* could potentially survive for at least 90 days (35+55) without food, which would bring them near to metamorphosis (115 days, Bosch et al., 1987). These calculations reveal how little exogenous nutrition the larvae of *S. neumayeri* would need to reach metamorphosis and suggest a physiological basis for surviving any asynchronous timing between reproduction and phytoplankton availability around Antarctica (Pearse et al., 1991; Stanwell-Smith and Peck, 1998).

When the four-arm pluteus larval stage began feeding *ad libitum* at day 22, the metabolic costs to day 60 increased from 8.2 mJ (unfed larvae) to 14.0 mJ per individual (Fig. 6B). Feeding resulted in a 71 % increase in energy metabolism that represents energy expended in feeding plus the energy costs associated with growth and the subsequent increased maintenance metabolism. Despite this 71 % increase in energy output, the biomass gained from feeding exceeded the metabolic investment in obtaining food (capture, digestion, assimilation, etc.), returning 440 % in energy content for the energy expended in feeding, inclusive of the increased costs of growth and maintenance.

Physiological implications for polar larvae

The lack of a particulate food resource does not appear to pose a 'starvation' stress on the early larvae of *S. neumayeri*. Low seawater temperatures in the coastal margins of Antarctica result in low metabolic rates, and we have shown in the present study that feeding larval stages of *S. neumayeri* are not dependent on the availability of phytoplankton food to complete early development to day 60. In addition, a large potential exists in these embryos and larvae for the uptake of dissolved organic material that could further reduce the requirements for particulate food resources. Although the availability of particulate food does affect metabolic rates during the four-arm larval stage of development, at the six-arm larval stage, size-specific metabolic rates between fed and unfed larvae are more equivalent. We conclude that slow developmental rates in *S. neumayeri* are primarily driven by low temperature, but that the corresponding low metabolic rates allow the energy content of the mid-sized eggs of this species to provide a large portion of the energy needs for development to a late planktotrophic larval stage.

The metabolic relationship between energy utilization and mass of a larva is part of the general problem of understanding the physiological processes that set metabolic intensity (Heusner, 1991). Whether these physiological processes include mechanisms that are more efficient at cold temperatures and would increase a larva's metabolic life span are not understood at present. Describing what sets metabolic intensity in Antarctic larvae will require further studies of changes in macromolecular synthesis associated with growth and maintenance. This work will also require a better understanding of the impact of cold polar temperatures in altering the energetic cost to the organism of specific enzymatic processes (e.g. the Na^+ pump; Leong and Manahan, 1999).

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References

Bosch, I., Beauchamp, K. A., Steele, M. E. and Pearse, J. S. (1987). Development, metamorphosis and seasonal abundance of embryos and larvae of the Antarctic sea urchin *Sterechinus neumayeri*. *Biol. Bull.* **173**, 126–135.

Calow, P. (1977). Conversion efficiencies in heterotrophic organisms. *Biol. Rev.* **52**, 385–409.

Cameron, R. A., Britten, R. J. and Davidson, E. H. (1989). Expression of two actin genes during larval development in the sea urchin *Strongylocentrotus purpuratus*. *Mol. Reprod. Develop.* **1**, 149–155.

Caulcutt, R. and Boddy, R. (1983). *Statistics for Analytical Chemists*, pp. 22–28. New York: Chapman & Hall Ltd.

Clarke, A. (1983). Life in cold water: the physiological ecology of polar marine ectotherms. *Oceanogr. Mar. Biol. Annu. Rev.* **21**, 341–453.

Clarke, A. (1992). Reproduction in the cold: Thorson revisited. *Invert. Reprod. Dev.* **22**, 175–184.

Emlet, R. B., McEdward, L. R. and Strathmann, R. R. (1987). Echinoderm larval ecology viewed from the egg. In *Echinoderm Studies*, vol. 2 (ed. M. Jangoux and J. M. Lawrence), pp. 55–136. Rotterdam: A. A. Balkema.

Gnaiger, E. (1983). Calculation of energetic and biochemical equivalents of respiratory oxygen consumption. In *Polarographic Oxygen Sensors* (ed. E. Gnaiger and H. Forstner), pp. 339–345. New York: Springer-Verlag.

Gnaiger, E. and Bitterlich, B. (1984). Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept. *Oecologia* **62**, 289–298.

Herrera, J. C., McWeeney, S. K. and McEdward, L. R. (1996). Diversity of energetic strategies among echinoid larvae and the transition from feeding to nonfeeding development. *Oceanologica Acta* **19**, 313–321.

Heusner, A. A. (1991). Size and power in mammals. *J. Exp. Biol.* **160**, 25–54.

Hochachka, P. W. and Somero, G. S. (1984). *Biochemical Adaptation*. Princeton, NJ: Princeton University Press. 537pp.

Hoegh-Guldberg, O. (1994). Uptake of dissolved organic matter by larval stage of the crown-of-thorns starfish *Acanthaster planci*. *Mar. Biol.* **120**, 55–63.

Hoegh-Guldberg, O. and Emlet, R. B. (1997). Energy use during the development of a lecithotrophic and a planktotrophic echinoid. *Biol. Bull.* **192**, 27–40.

Hoegh-Guldberg, O. and Manahan, D. T. (1995). Coulometric measurement of oxygen consumption during development of marine invertebrate embryos and larvae. *J. Exp. Biol.* **198**, 19–30.

Jaeckle, W. B. (1995). Variation in the size, energy content and biochemical composition of invertebrate eggs: Correlates to the mode of larval development. In *Marine Invertebrate Larvae* (ed. L. McEdward), pp. 49–78. New York: CRC Press.

Jaeckle, W. B. and Manahan, D. T. (1989). Growth and energy imbalance during the development of a lecithotrophic molluscan larva (*Haliotis rufescens*). *Biol. Bull.* **177**, 237–246.

Lawrence, J. M. and Guille, A. (1982). Organic composition of tropical, polar and temperate-water echinoderms. *Comp. Biochem. Physiol.* **72**B, 283–287.

Lawrence, J. M., McClintock, J. B. and Guille, A. (1984). Organic and caloric content of eggs of brooding asteroids and an echinoid (Echinodermata) from Kerguelen (South Indian Ocean). *Int. J. Invert. Reprod. Dev.* **7**, 249–257.

Leong, P. K. K. and Manahan, D. T. (1999). Na^+/K^+-ATPase activity during early development and growth of an Antarctic sea urchin. *J. Exp. Biol.* **202**, 2051–2058.

Manahan, D. T. (1990). Adaptations by invertebrate larvae for nutrient acquisition from seawater. *Am. Zool.* **30**, 147–160.

Marsh, A. G. and Manahan, D. T. (1999). A method for accurate measurements of the respiration rates of marine invertebrate embryos and larvae. *Mar. Ecol. Prog. Ser.* (in press).

McClintock, J. B. and Pearse, J. S. (1986). Organic and energetic content of eggs and juveniles of Antarctic echinoids and asteroids with lecithotrophic development. *Comp. Biochem. Physiol.* **85**A, 341–345.

McEdward, L. R. (1986). Comparative morphometrics of echinoderm larvae. I. Some relationships between egg size and initial larval form in echinoids. *J. Exp. Mar. Biol. Ecol.* **96**, 251–265.

McEdward, R. R. and Chia, F. (1991). Size and energy content of eggs from echinoderms with pelagic lecithotrophic development. *J. Exp. Mar. Biol. Ecol.* **147**, 95–102.

Needham, J. (1931). *Chemical Embryology*, vol. 2, pp. 946–987. London: Cambridge University Press.

Parsons, T. R., Maita, Y. and Lalli, C. A. (1984). *A Manual of Chemical and Biological Methods for Seawater Analysis.* Oxford: Pergamon Press. 173pp.

Parsons, T. R., Stephens, K. and Strickland, J. D. (1961). On the chemical composition of eleven species of marine phytoplankton. *J. Fish. Res. Bd Can.* **18**, 1001–1016.

Pearse, J. S., McClintock, J. B. and Bosch, I. (1991). Reproduction of Antarctic benthic marine invertebrates: Tempos, modes and timing. *Am. Zool.* **31**, 65–80.

Pechenik, J. A. and Eyster, L. S. (1989). Influence of delayed metamorphosis on the growth and metabolism of young *Crepidula fornicata* (Gastropoda) juveniles. *Biol. Bull.* **169**, 417–430.

Platt, T., Brawn, V. M. and Irwin, B. (1969). Caloric and carbon equivalents of zooplankton biomass. *J. Fish. Res. Bd Can.* **26**, 2345–2349.

Rivkin, R. R. (1991). Seasonal patterns of planktonic production in McMurdo Sound, Antarctica. *Am. Zool.* **31**, 65–80.

Rodriguez, J. L., Sedano, F. J., Garcia-Martin, L. O., Pérez-Camacho, A. and Sánchez, J. L. (1990). Energy metabolism of newly settled *Ostrea edulis* spat during metamorphosis. *Mar. Biol.* **106**, 109–111.

Shilling, F. M., Hoegh-Guldberg, O. and Manahan, D. T. (1996). Sources of energy for increased metabolic demand during metamorphosis of the abalone *Haliotis rufescens* (Mollusca). *Biol. Bull.* **191**, 402–412.

Shilling, F. M. and Manahan, D. T. (1994). Energy metabolism and amino acid transport during early development of Antarctic and temperate echinoderms. *Biol. Bull.* **187**, 398–407.

Srere, P. A. (1969). Citrate synthase. In *Methods in Enzymology*, vol. 13 (ed. J. M. Lowenstein), pp. 3–11. New York: Academic Press.

Stanwell-Smith, D. and Peck, L. S. (1998). Temperature and embryonic development in relation to spawning and field occurrence of larvae of three Antarctic echinoderms. *Biol. Bull.* **194**, 44–52.

Strathmann, R. R. (1985). Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. *Annu. Rev. Ecol. Syst.* **16**, 339–361.

Strathmann, R. R. and Vedder, K. (1977). Size and organic content of eggs of echinoderms and other invertebrates as related to developmental strategies and egg eating. *Mar. Biol.* **39**, 305–309.

Tangl, F. (1903). Beiträge zur Energetik der Ontogenese. Zweite Mitteilung. Über den Verbauch an chemischer Energie während der Entwicklung von Bacterienkulturen. *Pflügers Arch.* **98**, 475–489.

Turner, R. L. and Lawrence, J. M. (1979). Volume and composition of echinoderm eggs: implications for the use of egg size in life-history models. In *Reproductive Ecology of Marine Invertebrates* (ed. S. E. Stancyk), pp. 25–40. Columbia: University of South Carolina Press.

Vance, R. R. (1973). On reproductive strategies in marine benthic invertebrates. *Am. Nat.* **107**, 339–361.

Wieser, W. (1994). Cost of growth in cells and organisms: General rules and comparative aspects. *Biol. Rev.* **68**, 1–33.

Zeuthen, E. (1947). Body size and metabolic rate in the animal kingdom with special regard to the marine micro-fauna. *C.r. Trav. Lab. Carlsberg Ser. Chim.* **26**, 17–161.