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Growth and Energy Imbalance during Development of a Lecithotrophic Molluscan Larva (*Haliotis rufescens*)

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Abstract. Larvae of the gastropod *Haliotis rufescens* are classified as "nonfeeding" because they cannot capture particulate foods. However, for only 1 out of 5 independent cultures was a net decrease observed in dry organic weight during the complete period of larval development (5 to 7 days). In fact, there were net increases in dry organic weight from the oocyte (day 0) to the newly formed veliger larva (2-day-old). These weight increases during early development could be explained by increases in the amounts of specific biochemical components of the larvae, relative to oocytes. The metabolic rates of larvae were measured (oxygen consumption) and used to compare (i) the required energy for development with (ii) the energy supplied from the catabolism of biochemical reserves. This analysis revealed that the cost of development for larvae could not be explained by the rates of use of the energy stores initially present in the oocyte. Larvae, from two independent cultures, could only supply 25% or 71% of their energy requirements by the use of internal reserves. Larvae of *H. rufescens* cannot use particulate foods and, thus, this energy resource cannot be invoked. Estimates of the contribution that dissolved organic material in seawater could provide to larvae, showed that this pool of exogenous material could supply the missing energy. It is suggested that "nonfeeding" larvae can feed, but that their only available nutrients are in a dissolved form.

Introduction

The traditional separation of marine planktonic larvae into three trophic groupings (planktotrophy, facultative planktotrophy, and lecithotrophy) is based upon an ability, or lack of the ability, to concentrate and capture particulate foods from seawater (Thorson, 1946; Chia, 1974). However, this classification scheme does not consider an energy resource available to all soft-bodied invertebrate larvae, namely dissolved organic material (DOM) in seawater. By ignoring this resource, an implicit assumption has been made that planktotrophic (feeding) larvae are energetically dependent on the environment for nutrients, whereas lecithotrophic larvae are not. It follows then, that lecithotrophic (nonfeeding) larvae, using an energy source of fixed content (yolk), should continually decrease in organic weight during development. Growth, defined here as an increase in dry organic weight, can only occur in these nonfeeding forms following the development of distinct juvenile or adult feeding structures.

Changes in the biochemical composition have been well studied for embryos and larvae of marine invertebrates that produce planktotrophic larvae. There is general agreement in the literature that prefeeding embryos use internal energy stores (carbohydrate, lipid, and protein) to supply the energy requirements of early development (*e.g.*, Cognetti, 1982). Following the development of feeding structures, larvae deprived of particulate foods are also assumed to rely on an energy source of fixed content (yolk or accumulated reserves). This assumption is supported by the observed decreases in the biochemical components of starving larvae. For example, Millar and Scott (1967) reported that larvae of the bivalve *Ostrea*

edulis decreased both in dry organic weight and in all measured biochemical components during periods of starvation. Catabolism of the neutral lipid fraction provided the greatest proportion of liberated energy followed, in order, by protein and carbohydrate. Holland and Spencer (1973) also examined the changes in biochemical composition of starved *O. edulis* larvae. During starvation, the decrease in dry organic weight was explained by net decreases in lipid and protein, and again, the greatest amount of energy was made available through the catabolism of lipid. This pattern of energy use (lipid > protein > carbohydrate) was also seen during the cyprid stage of development (nonfeeding) in the barnacle *Balanus balanoides* (Lucas *et al.*, 1979). Mann and Gallager (1984, 1985) reported that the dry organic weight of larvae of the bivalves *Martesia cuneiformis, Teredo navalis,* and *Bankia gouldi* also decreased during starvation. For these larvae, the protein fraction, rather than the lipid fraction, served as the primary energy source during periods of nutrient deprivation. Studies have also been conducted examining net changes in organic weight and biochemical composition of echinoderms with nonfeeding development (Lawrence *et al.*, 1984; McClintock and Pearse, 1986). These authors have reported that, for a total of five species, there was little net change in the organic weight or energy content between eggs and juveniles. Lawrence *et al.* (1984) suggested that their results could be explained by the fact that either (i) the metabolic rates were too low to cause measurable depletion of energy reserves or (ii) the animals were gaining sufficient energy from dissolved organic material in seawater to offset the metabolic demands of development.

The only possible source of nutrients from the environment available to nonfeeding developmental stages of marine invertebrates would be in the form of DOM. Total carbon concentrations of DOM in coastal waters are at least ten times greater than those of organic carbon associated with particles (Parsons, 1975; Williams, 1975; MacKinnon, 1981; Sugimura and Suzuki, 1988). Despite the fact that only a small percentage (*ca.* 10%) of the total DOM has been chemically characterized (Williams, 1975), it is clear that biologically important compounds such as monosaccharides, amino acids, fatty acids, and nucleic acids are present in dissolved form in coastal marine environments (Testerman, 1972; Mopper *et al.*, 1980; Parkes and Taylor, 1983; Carlucci *et al.*, 1984; DeFlaun *et al.*, 1987).

There is evidence that planktotrophic larvae can take up specific compounds from the total pool of DOM (Reish and Stephens, 1969; Manahan, 1983; Manahan *et al.*, 1983; Davis and Stephens, 1984). However, the ability of nonfeeding larvae to transport specific fractions of DOM has been less extensively studied. Recently, it has been shown that nonfeeding trochophore and veliger larvae of the gastropod *Haliotis rufescens* are able to take up dissolved amino acids from seawater, and that transported alanine is rapidly used in specific anabolic and catabolic pathways in these veliger larvae (Jaeckle and Manahan, 1989).

In the present study, larvae of the gastropod *Haliotis rufescens* were cultured in natural seawater from the fertilized oocyte until they were competent to settle. The changes in dry organic weight, biochemical composition, and metabolism (oxygen consumption) were measured throughout the complete development of these larvae. An energy budget was constructed to assess the contribution of endogenous reserves to the energy demands of these larvae. This comparison revealed that larvae of *H. rufescens* are not in energy balance, suggesting that these "nonfeeding" larvae are obtaining energy from their environment, presumably in the form of dissolved organic material.

Materials and Methods

Culturing methods

Fertilized oocytes of *Haliotis rufescens* were obtained from the "Ab Lab" (Port Hueneme, California). The oocytes, embryos, and larvae were cultured in seawater at concentrations of approximately 5/ml at 16 to 17°C in 200-l culture vessels. It is known that the routine procedures (*e.g.*, sand filtering) used in marine laboratories to remove particles also affect the organic chemistry of seawater (Manahan and Stephens, 1983). To maintain the organic composition of seawater as close as possible to *in situ* conditions, all seawater used for larval culturing was passed only through a 0.2-μm (pore size, Nuclepore) polycarbonate filter. Assays for changes in the organic chemistry of seawater, caused by filtration procedures, were performed with high-performance liquid chromatography (procedures described elsewhere: Manahan, 1989). Filtered seawater samples were taken and analyzed for the concentrations of individual dissolved free amino acids. The total concentration of amino acids in the filtered seawater was always within the range reported for coastal marine environments (*i.e.*, 10 n*M* to 1 μM: Williams, 1975). Fresh seawater was collected daily, and used as soon as it had thermally equilibrated to the culture temperature. For each larval culture, following the formation of the definitive larval shell (day 2), the water was changed on a daily basis. At each sampling period, the larvae were gently sieved onto a 80-μm polyester mesh, and samples were retained for analysis of weight and biochemical composition. The culture vessel was then cleaned by a brief swabbing with a 5% sodium

hypochloride solution (bleach) followed by sequential washes of hot water, deionized water, and then filtered seawater.

Collection of oocytes and larvae

Samples of either oocytes, prior to fertilization, or larvae were placed in a graduated cylinder. The concentration of individuals was determined by counting several (usually 3 to 5) aliquots of the suspension until the coefficient of variation of the mean was less than 6%. Then, 9 samples of the suspension were removed and each sample placed in a separate 15-ml centrifuge tube. Following centrifugation, the pellet was washed with three times the sample volume using ammonium formate (3.4% w/v, isotonic with seawater). This washing procedure was then repeated twice. Replacement of seawater by ammonium formate is an important procedural step because ammonium formate is a volatile salt and there is no residual inorganic residue following drying. However, the ammonium formate solution must be passed through a 0.2-μm (pore size) filter immediately before use to avoid the carry-over of particulate material that will bias the measured weights. Six of the nine samples were placed in preashed (12 h at 500°C), preweighed aluminum boats, frozen, and retained for determinations of weights. The remaining samples were placed in 1.5-ml centrifuge tubes, frozen, and held at −20°C for biochemical analysis.

Weight determinations

Samples retained for determinations of organic weight were placed in an 80°C drying oven, and dried to constant weight (total dry weight). To determine the ash weight, organic material in each sample was combusted in a muffle furnace for 4 h at 450°C. Ashed samples were weighed, and 1-h reashing cycles repeated if the samples gained weight by hydration. Completely ashed materials do not adsorb water (Gnaiger and Bitterlich, 1984). This protocol avoids the inaccuracies associated with decomposition of $CaCO_3$ due to prolonged heating (Paine, 1971). All weight measurements were made with a Cahn Model 29 electrobalance (accurate to 0.1 μg). The amount of dry organic weight could then be calculated as the difference between the total dry weight and the ash weight. These values (dry organic and ash weight) were divided by the number of oocytes, or larvae, in each sample and the data expressed as μg material per individual.

Biochemical composition

To remove any residual fluid, samples used for determination of biochemical composition were lyophilized for at least 8 h at a pressure of 0.2 mm Hg. The lyophilized samples were then sonicated in 1 ml of glass distilled water by ultrasonic disruption (Sonics and Materials Brand, Model VC 40 fitted with a microprobe). The homogenates were centrifuged for 10 min at 12,200 × g and then sonicated a second time. Care was taken throughout the sonication procedure not to heat the samples. To determine whether the observed changes in the dry organic weight of the oocytes and larvae were accounted for by corresponding changes in biochemical composition, the amount of carbohydrate, lipid, and protein was measured. The biochemical composition of the oocytes and larvae was determined using the fractionation scheme devised by Holland and Gabbott (1971), as modified by Mann and Gallager (1985), with two additional alterations: (i) homogenates were extracted in trichloroacetic acid (TCA) for 20 min at −10°C and (ii) the total protein was assayed using Coomassie Brilliant Blue G-250 (BioRad Laboratories) as a colorimetric reagent. The TCA-insoluble pellet was dissolved in 500 μl of 1.0 M NaOH by heating at 60°C for 30 min. The alkaline protein solution was then acidified with 300 μl of 1.67 M HCl, and 200 μl of the concentrated Coomassie Blue dye solution was then added. The absorbance of the samples (at 595 nm) was determined after 10 min, and no later than 60 min, following addition of the dye solution.

Rates of larval respiration

The metabolic rates of larvae, of different ages, were measured as the rates of oxygen consumption. Larvae were placed in a conical analyzer cup (Curtin Matheson; 2 ml total volume, precalibrated to 345 μl) with filtered, then autoclaved, seawater. A Clark-type oxygen electrode (Model E5057, Radiometer Copenhagen) was placed into the cup, and excess air and seawater were discharged through a small purge hole melted into the cup. The purge hole was sealed by the membrane o-ring. The electrode was connected to a blood-gas analyzer (Model PHM 73; Radiometer Copenhagen) and, following a 5-min equilibration period, the change in the partial pressure of oxygen (mm Hg) was monitored for 20 to 30 min. During all experiments, the respiration chamber and the electrode were immersed in a 17°C water bath (Model RDL 20; Precision Instruments; ±0.02°C). Prior to the experiments with larvae, the oxygen consumption rate of the electrode itself was determined under the same conditions used for experiments with larvae. At the end of each experiment, the larvae were removed from the respiration chamber and counted (50 to 150 larvae). The rate of change in the partial pressure of oxygen was corrected for any self-consumption by the electrode, and

Table I

Changes in the amount of dry organic weight between oocytes (day 0) and veliger larvae (day 5, 6 or 7), sampled at the end of the larval life span (competent to settle), during the complete development of Haliotis rufescens

Culture 1		Culture 2		Culture 3		Culture 4		Culture 5	
Age (day)		Age (day)		Age (day)		Age (day)		Age (day)	
0	1.36 ± 0.02	0	1.41 ± 0.06	0	1.65 ± 0.03	0	1.19 ± 0.04	0	1.39 ± 0.05
7	1.17 ± 0.05	6	1.42 ± 0.08	6	1.67 ± 0.03	6	1.20 ± 0.02	5	1.59 ± 0.09
					Percent difference between beginning and end of each culture				
	(−14.0%)		(+0.7%)		(+1.2%)		(+0.8%)		(+14.4%)

Cultures 1–5 refer to batches of larvae reared from gametes obtained from five separate spawings. All data are presented as the mean dry organic weight per individual (μg ± 1 standard error of the mean). The percent difference, given at the bottom of the table, represents the net change in dry organic weight for each culture.

calculated as mm Hg larva^{-1}h^{-1}. This depletion rate was converted to moles of oxygen consumed by calibrating the electrode relative to the amount of oxygen in isothermal seawater, as determined by the Winkler titration method (Parsons *et al.*, 1984).

Results

Changes in weight during the larval life span

Figure 1 shows the changes in dry organic weight, and ash weight, from an unfertilized oocyte (day 0) to a larva competent to settle (day 7). In this culture (Culture 1), and all others studied (see Table I, Cultures 1 to 5), there was a continual linear increase in ash weight during larval development. There was a statistically significant net increase in dry organic weight from the oocyte (day 0) to the newly formed veliger larva (day 2), as can be seen in Figure 1 (Variance ratio, VR = 25.0, $F_{0.001[1,9]}$ = 22.9). Similar increases during this period of development (day 0 to day 2) were seen in four other cultures. For Culture 1 (Fig. 1), there was a statistically significant net decrease from the oocyte, at 1.36 ± 0.02 μg, to the 7-day-old veliger at 1.17 ± 0.05 μg (VR = 18.3, $F_{0.01[1,8]}$ = 12.2). Of the 5 cultures, reared in an identical manner (Table I), this culture (Culture 1) was the only one that showed a net decrease in dry organic weight during the larval life span. For Cultures 2, 3, and 4 there was no statistically significant change in organic weight between the oocyte and the last larval stage sampled. Larvae from Culture 5 had a net increase in organic weight, from 1.39 ± 0.05 μg (day 0) to 1.59 ± 0.09 μg (day 5).

Changes in biochemical composition and energy content

The changes in the biochemical composition of larvae, for the entire lifespan of two independent cultures (Cultures 1 and 5), are presented in Figure 2. The corresponding weight and energy values, for each total lipid and protein fraction, are given in Table II. The amount of total carbohydrate was always below the level of detection (0.1 μg per sample using a glucose standard) and, thus, a value for carbohydrate could not be included in calculations of energy budgets. The increase in dry organic weight during the first two days of development of larvae from Culture 1 (see Fig. 1) was explained by the combined increases of both the total lipid and protein fractions (Table II, Culture 1, Day 0 to 2). For Culture 5, there was also a significant increase in the dry organic weight from the oocyte (1.39 ± 0.05 μg) to the 2-day-old veliger larva (1.81 ± 0.09 μg). Again, this initial growth could be ac-

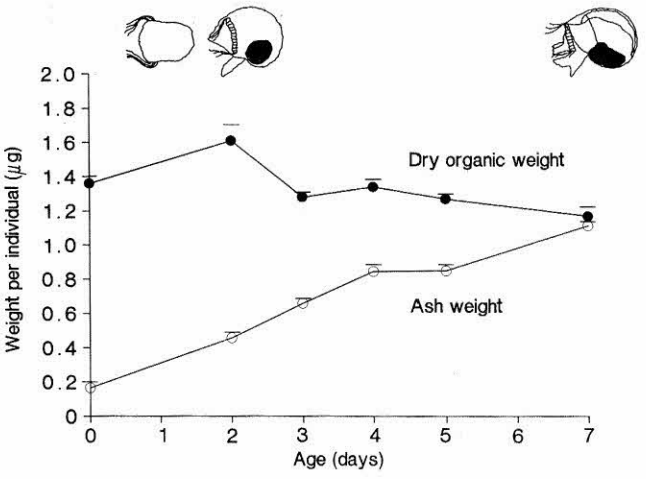


Figure 1. Change in the dry organic weight and ash weight (mean + 1 standard error of the mean) during the complete development of *Haliotis rufescens*, from the unfertilized oocyte (day 0) to a larva fully competent to settle (day 7). Diagrams of larval shape are redrawn from Leighton (1972).

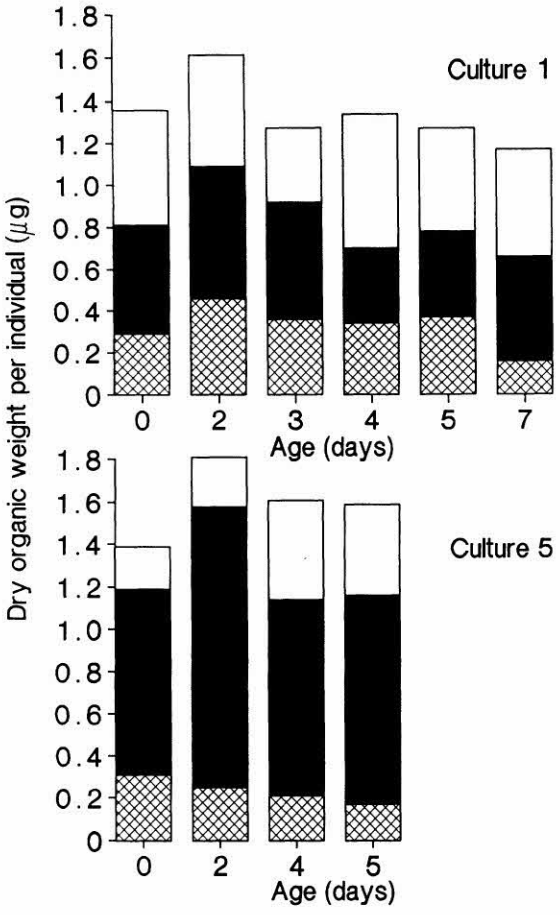


Figure 2. Changes in the biochemical composition during the complete development of *Haliotis rufescens*, from the unfertilized oocyte (day 0) to a larva fully competent to settle. Larvae in Culture 1 took 7 days to reach competence; those in Culture 5 took 5 days. For each sample the dry organic weight is represented by the sum of the weights of protein, total lipid, and the uncharacterized fraction (remainder). The cross-hatched portions of the bars represent protein; the solid portion represents total lipid; the open portions represent the remainder fraction.

counted for by the net changes in lipid and protein. There was a slight decrease in protein of −0.06 μg (day 0 to 2), and a large increase in total lipid of +0.45 μg during the same time period (Table II, Culture 5, Day 0 to 2). Following the formation of the veliger larva (day 2), the changes in protein for larvae from both Cultures 1 and 5 were qualitatively similar. There was a 65% decrease (0.30 μg) in protein content of larvae from Culture 1 (Day 2 to 7) and a 32% decrease in the amount of protein in larvae from Culture 5 (Day 2 to 5).

For lipid, the net changes were similar between Cultures 1 and 5 (day 2 to 5; day 2 to 7, respectively), following the net increases in total lipid observed during the period of day 0 to day 2 for both cultures (Table II). Larvae from Culture 1 had a decrease of 0.13 μg of lipid (21%) from day 2 to day 7. Those in Culture 5 also had a decrease in lipid content of 0.34 μg (26%) from day 2 to day 5.

The energy equivalents of the changes in weight for lipid and protein were calculated using the combustion enthalpy values for each fraction (lipid = 39.5 kJ/g, protein = 24.0 kJ/g; Gnaiger, 1983). As observed by other workers (*e.g.*, Holland and Gabbott, 1971), the sum of the weights of the measured biochemical components per individual (Fig. 2, filled bars), did not equal the dry organic weight. The "remainder" fraction (see Fig. 2, open bars), is defined as the difference between the dry organic weight and the sum of the measured biochemical components. To account for the energy represented by this material, a value of 27.0 kJ/g was given for the enthalpy of combustion of this uncharacterized fraction. This value is equal to the average of the combustion enthalpies of carbohydrate (17.5 kJ/g), lipid, and protein. In the absence of biochemical information on what comprises the remainder fraction, an average value for enthalpy of combustion was taken to be representative of the low molecular weight compounds that probably make up the majority of the remainder fraction. In Culture 1, the energy equivalent of the uncharacterized material (see "remainder," Table II) decreased from 14.85 mJ, at day 0, to 13.77 mJ by day 7. Larvae in Culture 5 had 5.40 mJ, at day 0, and increased to 11.61 mJ per larva by day 5.

The metabolic rates of larvae

The rates of oxygen consumption by veliger larvae of *Haliotis rufescens* are presented in Figure 3. The data presented in this figure are based upon the results of 47 independent determinations, obtained with larvae from 4 different cultures. The inset in Figure 3 shows the mean respiratory rate per larva, at each day of development examined. The average respiratory rate ranged from a low of 58 ± 7.2, for a 2-day-old larva, to a high of 93 ± 13 pmol O_2 $larva^{-1}$ h^{-1} for a 3-day-old larva.

Discussion

Larvae of *Haliotis rufescens* are presumed to be nonfeeding because they cannot capture particulate foods. Thus, a net loss of the organic weight contained in the oocyte would be expected during larval development. However, for four out of five cultures of larvae studied, there was a net increase in dry organic weight during early development from an oocyte (day 0) to a newly formed veliger larva at day 2 (*e.g.*, Fig. 1, day 0 to 2). These increases in dry organic weight were explained by changes in the amounts of protein and lipid during the same time period (Table II). Similarly, in only one out

Table II

Changes in dry organic weight and energy content during the complete larval development of Haliotis rufescens

Culture 1

Weight of each biochemical fraction

Age (day)	0	2	3	4	5	7
Dry organic weight						
Mean ± 1 SE	1.36 ± 0.02	1.61 ± 0.09	1.28 ± 0.01	1.34 ± 0.04	1.27 ± 0.03	1.17 ± 0.05
Protein						
Mean ± 1 SE	0.29 ± 0.00	0.46 ± 0.01	0.36 ± 0.01	0.34 ± 0.01	0.37 ± 0.03	0.16 ± 0.01
Total lipid						
Mean ± 1 SE	0.52 ± 0.02	0.63 ± 0.03	0.56 ± 0.07	0.36 ± 0.05	0.41 ± 0.02	0.50 ± 0.03

Energy content of each biochemical fraction

Protein (mJ)	6.96	11.04	8.64	8.16	8.88	3.84
Total lipid (mJ)	20.54	24.89	22.12	14.22	16.20	19.75
Remainder (mJ)	14.85	14.04	9.72	17.29	13.23	13.77

Culture 5

Weight of each biochemical fraction

Age (day)	0	2	4	5
Dry organic weight				
Mean ± 1 SE	1.39 ± 0.05	1.81 ± 0.09	1.61 ± 0.08	1.59 ± 0.09
Protein				
Mean ± 1 SE	0.31 ± 0.00	0.25 ± 0.01	0.21 ± 0.01	0.17 ± 0.00
Total lipid				
Mean ± 1 SE	0.88 ± 0.09	1.33 ± 0.05	0.93 ± 0.04	0.99 ± 0.05

Energy content of each biochemical fraction

Protein (mJ)	7.44	6.00	5.04	4.08
Total lipid (mJ)	34.76	52.54	6.74	39.11
Remainder (mJ)	5.40	6.21	12.69	11.61

Larvae in Cultures 1 and 5 were reared from gametes obtained from separate spawnings. The weights of each protein and total lipid fractions were converted to an equivalent energy by multiplying each weight fraction by its value for enthalpy of combustion (total lipid = 39.5 kJ/g; protein = 24.0 kJ/g). The "remainder" fraction was calculated as the difference between the dry organic weight and the sum of the measured biochemical fractions. The energy value for this uncharacterized fraction was taken to be the average (27.0 kJ/g) of the combustion enthalpies for carbohydrate (17.5 kJ/g), lipid, and protein. All weight values are presented as the mean (μg) ± 1 standard error of the mean.

of five independent cultures of larvae was there a statistically significant decrease in dry organic weight from the oocyte to a competent larva (Culture 1, Fig. 1 and Table I). For the other four cultures (Table I), there was either no significant net change (Cultures 2, 3 and 4), or an increase in dry organic weight (Culture 5).

The net changes in dry organic weight, from the oocyte to a larva that is competent to settle, ranged from a decrease of 14% (Culture 1) to an increase of 14% (Culture 5) (Table I). An energy budget was constructed for each of these cultures because they represented the maximum range of weight changes observed. The amount of energy required by larvae in Cultures 1 and 5 was calculated from their respiratory rates (Fig. 3). The total rate of oxygen consumed by larvae was calculated for their entire life span. Where no measurements were made for a particular day, a value was estimated based on the rate measured for the nearest day. The energy equivalent to the rate of oxygen consumption was then compared to the available energy released by the catabolism of internal biochemical reserves (Table II). This comparison is given in Table III. For both cultures, the energy available from the net changes in lipid, protein, and the "remainder" fractions were always insufficient to account for the energy requirements of larval development. For Culture 1, there was a net decrease in all biochemical components

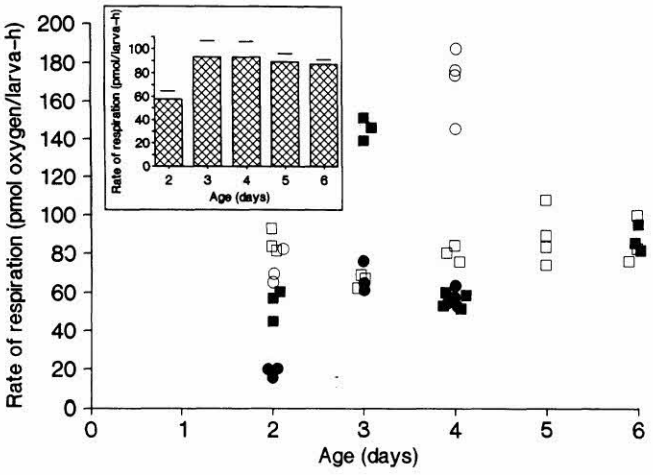


Figure 3. Metabolic rates of veliger larvae of *Haliotis rufescens*. Data are presented from four cultures, obtained from independent spawnings. The respiratory rates of larvae from Culture 1 are depicted as solid rectangles; larvae from Culture 5 are shown as solid circles. Other symbols represent larvae whose rates of respiration were not discussed in the text. The inset shows the mean respiratory rate (+1 standard error of the mean) averaged for all larvae for each day.

and the sum of the equivalent energies (−4.99 mJ) equals the amount of energy available to the larva from its reserves. In this case, 71% of the metabolic needs (+6.99 mJ) could be accounted for by the energy available from internal reserves (*i.e.*, 4.99/6.99 = 0.71, Table III). For Culture 5, there was a net increase in weight during development for both the lipid and remainder fractions (Table II). Thus, these increases represent an energy requirement for the larva. In these larvae, the amount of required energy was (i) the sum of the energy equivalent of the total amount of oxygen consumed over 5 days (+2.93 mJ) and (ii) the energy equivalent to the net increases in the biochemical components (+10.56 mJ). The amount of energy available from the net decrease in protein (−3.36 mJ) represented only 25% of the total energy required during the 5 days of development (*i.e.*, 3.36/13.49 = 0.25, Table III).

In general, larvae of *Haliotis rufescens* do not show a decrease in dry organic weight and energy content (Fig. 1, Tables I, II) during development. This finding is surprising in view of the fact that these larvae cannot use particulate foods and were cultured in natural seawater that had been passed only through a 0.2-μm (pore size) filter. Yet our results support previous findings on the net biochemical and energy changes that occurred in invertebrates unable to feed during development. When the energy content was compared between eggs, and later developmental stages, for six species of echinoderms collected from temperate and antarctic environments (Turner and Rutherford, 1976; Lawrence *et al.*, 1984; McClintock and Pearse, 1986), the results showed little net change in energy content between eggs and juveniles. Also, in a study of the energetics of embryonic development in the teleost *Sebastes schlegeli*, Boehlert *et al.* (1986) reported that the energy provided by the catabolism of endogenous reserves represented only 55% of the metabolic demand (oxygen consumption). In this latter case, the source of exogenous energy was presumed to be provided as dissolved organic compounds in ovarian fluid.

A possible alternative explanation for the observed imbalance between the rate of use of energy reserves and the measured metabolic rates of larvae, is that the latter were affected by some experimental artifact and were too high during our experiments. The average respiratory rate of all larvae of *Haliotis rufescens* measured (from Fig. 3) was 84 pmol O_2 larva^{-1} h^{-1}. The dry organic weight, averaged for all veliger larvae (Cultures 1 and 5, Table II) was 1.5 μg. Thus, the average metabolic rate was 56 μmol O_2 g^{-1} h^{-1} at a temperature of 17°C. This value is at the lower end of the metabolic rates of marine invertebrate larvae (see Crisp, 1976) and is lower than that reported for a feeding larvae of the gastropod *Ilyanassa obselotus* (165 μmol O_2 g^{-1} h^{-1}, recalculated from Pechenik, 1980, assuming a Q_{10} of 2). Hence, the possibility that our reported values are artificially high is not viable. However, these analyses do suggest that within the Gastropoda, lecithotrophic larvae may have a lower metabolic rate than planktotrophic veligers. This difference may be due to the larger size of the velum of planktotrophic larvae when compared to lecithotrophic forms (Fretter and Graham, 1962). This suggestion is also supported by the work of Erickson (1984), who showed that differences in the respiratory rates of veliger larvae of the gastropod *Strombus gigas* corresponded with the extent of velar lobe expansion.

The calculated values given in Table III show that the energy reserves, initially present in the oocyte, are not being used by these larvae to supply their metabolic needs. However, this does not mean that there is insufficient energy in the oocyte to meet the metabolic demands of larval development. The energy content per oocyte (day 0) from Culture 1 was 42.35 mJ and 47.60 mJ for Culture 5. The amount of energy equivalent to the total number of moles of oxygen consumed (Fig. 3) during the complete development of larvae from these two cultures was 6.99 mJ and 2.93 mJ per larva for Cultures 1 and 5, respectively (Table III). Therefore, the amount of energy initially present in the oocyte exceeded the metabolic demand by 6-fold (*i.e.*, 42.35/6.99 = 6) for Culture 1 and 16-fold (*i.e.*, 47.60/2.93 = 16) for Culture 5. Even though there is more than enough energy contained in an oocyte to meet the metabolic demand, the

Table III

An energy budget for the complete larval development of Haliotis rufescens

Symbols used: "+" = required energy, and "−" = available energy.

Culture 1		Culture 5	
Available energy (decreases in biochemical content)		Available energy (decreases in biochemical content)	
Protein (mJ)	−3.12	Protein (mJ)	−3.36
Lipid (mJ)	−0.79	Lipid (mJ)	+4.35
Remainder (mJ)	−1.08	Remainder (mJ)	+6.21
Sum of available energy	−4.99	Sum of available energy	−3.36
Required energy (metabolic rate over 7 days)		Required energy (metabolic rate over 5 days)	
13.27 nmol O_2/larva =	+6.99 mJ	5.56 nmol $O/_2$/larva =	+2.93 mJ
(increases in biochemical content)		(increases in biochemical content)	
None	0.00 mJ	Lipid and remainder	+10.56 mJ
Sum of required energy	+6.99	Sum of required energy	+13.49 mJ
Energy balance (available/required)		Energy balance (available/required)	
[(−4.99)/(+6.99)] = 71%*		[(−3.36)/(+13.49)] = 25%*	

*Contribution from energy stores to metabolic demand

Larvae in Cultures 1 and 5 were reared from gametes obtained from separate spawnings. The net change in total energy per individual, over the course of development, was taken from the data given in Table II (Culture 1, day 0–7; Culture 5, day 0–5). The metabolic rates of the larvae for each culture were calculated from the data provided in Figure 3. To relate the energy demand of development to the energy made available from the catabolism of internal stores, the total amount of oxygen consumed was converted to an energy equivalent. The energy reserve having the largest net decrease throughout development was protein and, therefore, the oxyenthalpic equivalent of this reserve was used (protein 527 kJ/mol O_2, from Gnaiger, 1983).

conclusions drawn from the energy budgets given in Table III are that larvae do not fully use this source of energy. Similar conclusions have been made by Lawrence *et al.* (1984) who reported that there was little change in energy content, between the egg and the juvenile, for the echinoid *Abatus cordatus* and the asteroid *Anasterias perreiri*. These authors suggested that the function of the large energy content of these eggs, 15.5 J/egg and 39.6 J/egg, respectively, was to produce a large juvenile rather than supply a large amount of energy for development. Also, Emlet *et al.* (1987) reported that, in general, juvenile asteroids that develop from nonfeeding larvae are larger than juveniles produced from feeding larvae. Thus, for development of *Haliotis rufescens*, the adaptive significance of the large amount of energy initially present in the oocyte may be related to juvenile survivorship, rather than energy metabolism during larval development.

If larvae of *Haliotis rufescens* are not using their internal reserves to fully meet the energy demands of development, there must be an input of material from the environment. Veliger larvae of *Haliotis* spp. cannot ingest particulate foods and, therefore, the most likely source of nutrients for these larvae would be in a dissolved form. Jaeckle and Manahan (1989) showed that veliger larvae can take up dissolved free amino acids from seawater, and that the rate of amino acid uptake, from a concentration of 1.6 μM, was sufficient to supply 55% of the metabolic demand of the larvae.

Larvae from Culture 5 had the higher energy imbalance, where only 25% of the metabolic cost of development could be accounted for by the use of energy stores. Could the rate of DOM uptake account for the missing energy observed for these larvae? The seawater used to culture the larvae was natural and, thus, approximated *in situ* concentrations of DOM. The calculations given below suggest that DOM could supply this missing energy. The average rate of amino acid uptake from a concentration of 1.6 μM (Jaeckle and Manahan, 1989) was 5.9 pmol amino acid larva^{-1} h^{-1}. Based on the average molecular weight of the amino acids used (140 g/mol), this equaled 826 pg larva^{-1} h^{-1}, or 99 ng larva^{-1} (5-days)$^{-1}$. Giving DOM a value for combustion enthalpy based on the average of carbohydrate, protein, and lipid (27.0 kJ/g, see Table II), 99 ng of DOM would equal 2.7 mJ/larva over a 5-day period. From Table III, it can be seen that an additional 10.13 mJ (13.49–3.36 = 10.13) was needed to balance the required energy. Thus, dis-

solved amino acids could contribute 27% of the missing energy (2.7/10.13 = 0.27). Dissolved amino acids represent less than 1% of the total pool of DOM in seawater (Williams, 1975). Thus, if only 4% of the total pool of DOM was used by larvae, at rates similar to those observed for the amino acid fraction, then 100% of the missing energy would be supplied from DOM. The requirement that 4% of the DOM has to be used is not an unreasonable estimate, given the wide variety of biologically available organic compounds that make up the pool of DOM in seawater.

The increase in both organic weight and specific biochemical components in these larvae suggests the question: are lecithotrophic invertebrate larvae really "nonfeeding"? Larvae of *Haliotis rufescens* can take up and metabolize dissolved free amino acids from seawater (Jaeckle and Manahan, 1989) and increase in organic weight (this study). This suggests that these "nonfeeding" larvae are feeding. However, the source of exogenous food exists in a dissolved form.

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