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Feeding by a "Nonfeeding" Larva: Uptake of Dissolved Amino Acids from Seawater by Licithotrophic larvae of the Gastropod Haliotis refescens

William Jaeckle, *Illinois Wesleyan University*

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Feeding by a "Nonfeeding" Larva: Uptake of Dissolved Amino Acids from Seawater by Licithotrophic larvae of the Gastropod *Haliotis refescens*

W. B. Jaeckle and D. T. Manahan

Abstract

Larvae of the red abalone (*Haliotis rufescens* Swainson) are functionally incapable of capturing particulate foods. The aim of this study was to determine whether these larvae could acquire energy from seawater in the form of dissolved organic material. Trochophore and veliger larvae were shown to acquire energy by transporting dissolved organic material from seawater. Both larval stages took up all classes of amino acids tested. The influx of radiolabeled alanine represented the net substrate flux, as determined by direct chemical measurement for both trochophore and veliger larvae. Although veliger larvae have a transport system to take up taurine from seawater, a net efflux was observed for this amino acid. The release of taurine occurred independently of the presence of either taurine or other amino acids in the medium. Transported alanine was used in both anabolic and catabolic pathways. The percent of ^{14}C-alanine in the trichloroacetic acid-insoluble fraction (macromolecules) of veliger larvae ranged from 21 to 56% of the total radioactivity in the larvae. No lipid biosynthesis was detected from ^{14}C-labeled alanine. Veliger larvae catabolized 15 to 19% of the total alanine taken up and released it as $^{14}CO_2$. The metabolic rate (oxygen consumption) and the rate of amino acid uptake were both determined for the same group of veliger larvae. The percent contribution that the uptake of amino acids, from a total concentration of 1.6 μM, made to the metabolic demand of abalone larvae ranged from 39 to 70%. Thus, these lecithotrophic larvae are not energetically independent of their environment, a result which differs from the current view of energy allocation to "nonfeeding" larvae.

Introduction

Lecithotrophic (nonfeeding) larvae of marine invertebrates cannot ingest particulate foods and are assumed to subsist entirely on endogeneous reserves (e.g. Thorson 1946, Chia 1974). This assumption, however, ignores the large amount of dissolved organic matter present in seawater. Dissolved organic material (DOM) is found in coastal marine environments at organic carbon concentrations ranging from 500 to 1 000 μg C l^{-1} (Williams 1975) and exceeds the amount of particulate organic carbon (Parsons 1975) by a factor of nearly 10. Recent developments in analytical techniques now provide evidence that the total concentration of DOM is even higher (ca. 300%) than previously reported (Sugimura and Suzuki 1988).

Previous accounts indicate that eggs and embryos, incapable of ingesting particulate foods, can transport a variety of organic compounds directly from seawater. Transport systems for amino acids (Epel 1972, Manahan 1983a), monosaccharides (Monroy and Tolis 1961), and nucleosides (Schneider and Whitten 1987) are activated following fertilization of eggs and oocytes from several species of marine invertebrates (e.g. the echinoderms *Strongylocentrotus purpuratus* and *Asterias forbesii* and the mollusk *Crassostrea gigas*). Older embryos and nonfeeding larval stages have also been reported to transport amino acids and monosaccharides from seawater. Fontaine and Chia (1968) demonstrated the appearance of radioactivity in brooded embryos of the ophiuroid *Amphipholis squamata* following exposure to radiolabeled glucose and glycine in seawater. Reish and Stephens (1969) showed that the nonfeeding trochophore stage of the annelid *Neanthes arenaceodentata* also accumulated ^{14}C-glycine from seawater.

The red abalone *Haliotis rufescens* Swainson is an archaeogastropod mollusk that releases a large number of primary oocytes which, following fertilization, develop into lecithotrophic larvae. The trochophore larvae hatch from the fertilization envelope within 24 h and develop into veliger larvae during the following day at a temperature of 15°

to 17 °C (Ebert and Houk 1984). Pelagic larvae of a related species, *H. tuberculata*, do not possess a metatrochal ciliary band or a complete digestive system (Crofts 1937) and, therefore, are physically incapable of capturing particles. Although organogenesis has not been described for *H. rufescens*, Crofts reported that the larvae of *H. tuberculata* cannot capture particles until metamorphosis has been completed. We report here that lecithotrophic larvae of the red abalone *H. rufescens* can feed by taking up dissolved free amino acids directly from seawater. At least for this specific fraction of the total DOM pool, the rates of uptake, when compared to the larval metabolic rate, are sufficient to supply a significant amount of energy to these larvae.

Materials and methods

Larval culture

Fertilized oocytes of *Haliotis rufescens* Swainson were obtained from the "Ab Lab" (Port Hueneme, California) and cultured at 16° to 17 °C in filtered seawater (0.2 μm pore-size). The culture water was changed on a daily basis and replaced with freshly-filtered seawater. All experiments were conducted at 17 °C.

Under these culturing conditions, the larvae used in all experiments were xenic. We did attempt to obtain axenic suspensions of larvae by using direct removal of gametes from the gonads following the protocol of Langdon (1983). Although suspensions of active sperm were produced, in no instance were viable (fertilizable) oocytes collected from gravid females. Therefore, to check for the possible presence of adherent bacteria, veliger larvae were (i) stained with the DNA-specific fluorochrome 4',6-diamidino-2-phenylindole (DAPI, Porter and Feig 1980) and examined using epifluorescent microscopy, or (ii) larvae were fixed in 1% OsO_4, dehydrated in a series of ethyl alcohol washes, transferred to 100% hexamethyldisilazane (Nation 1983) and air-dried. The larvae were coated with gold and examined with an ISI scanning electron microscope (Model Super 2).

Net uptake of amino acids

A known number of abalone larvae was added to 50 or 100 ml of seawater that was first filtered (0.2 μm pore-size), and then autoclave-sterilized (hereafter referred to as "seawater") to produce concentrations of larvae ranging from 50 to 135 ml^{-1}. A mixture of 16 amino acids, (each amino acid prepared individually from crystalline powders, Sigma Chemical) representing acidic, basic and neutral functional groups, was added to the flask. Each substrate was initially present at a concentration of 100 to 250 n*M*. At every sampling interval, a 500 μl sample was removed and gently passed through a polycarbonate filter (0.2 μm pore-size) contained within a 13 mm filter housing (Nuclepore). In parallel, the amino acid mixture was added to a flask containing an identical volume of seawater, but no larvae. This flask served as a control for any changes in amino acid concentration attributable to surface adsorption in the vessel and uptake by bacteria present in the seawater. The change in the concentration for each of the 16 amino acids, in the presence or absence of abalone larvae, was measured using high-performance liquid chromatography (HPLC). The chromatographic procedures used are described elsewhere (Manahan 1989).

The concentrations of amino acids used in the net flux experiments were below the K_t, i.e., the substrate concentration where the transport rate is half the maximum (J_{max}). For veliger-stage larvae of *Haliotis rufescens*, the affinity (K_t) of the transport system for alanine, a representative neutral amino acid, is 23 μ*M* (Manahan et al. 1989). Therefore, the rate of depletion for each amino acid, present in the medium at 100 to 250 n*M* each, is nonlinear as a function of time, and first-order with respect to substrate concentration. To avoid inaccuracies associated with calculating the depletion rate by end-point analyses, the concentration of each of the 16 amino acids was natural log-transformed to produce a linear plot of the relationship between substrate concentration and time (see Fig. 1, upper graph). The rate of amino acid uptake by larvae was calculated from the first-order depletion constant "*k*", where *k* equals $(\ln [S_0]-[S_t])/t$ (see Segel 1976, p. 227). $[S_0]$ represents the substrate concentration at the start of the experiment and $[S_t]$ is the substrate concentration at the end of the experiment, time "*t*". For each amino acid, the values of $[S_0]$ and $[S_t]$ were calculated from the slope of a least-squares linear regression analysis using all data points. The rate of amino acid uptake (pmol $larva^{-1}$ h^{-1}) was calculated by multiplying the number of moles of each amino acid present at the beginning of the experiment by the depletion constant (*k*) for that amino acid, and dividing by the total number of larvae present in the experiment.

To determine if the influx of ^{14}C-alanine represented the net substrate flux, a known amount of ^{14}C-alanine (168 or 170 μCi μmol^{-1}, New England Nuclear) was also added to the flask immediately prior to the beginning of an amino acid uptake experiment. The change in alanine concentration was then monitored by two independent analytical techniques. The change in total substrate concentration (both ^{12}C- and ^{14}C-alanine) was determined by HPLC and the change in ^{14}C-alanine was measured using isotope techniques. In order to detect the depletion of only ^{14}C-alanine, each sample was acidified for 24 h to volatilize any $^{14}CO_2$ in the sample before 5 ml of scintillation cocktail was added (Scinti-Verse, Fisher Scientific). All sample radioactivity was checked and corrected, if necessary, for quenching by the addition of a ^{14}C-toluene internal standard.

Taurine efflux and influx

Analysis of experiments designed to measure amino acid uptake by trochophore and veliger larvae revealed that the concentration of taurine in the medium continually increased during the course of each experiment. To determine

if the rate of taurine efflux from veliger larvae was influenced by the total concentration of free amino acids in seawater, the following experiments were conducted. Veliger larvae (3 d-old) were added to flasks containing either 500 n*M* taurine, 4 μM amino acids (16 different substrates at 250 n*M* per amino acid, see Fig. 1), or no additions (natural seawater). Samples were removed at 20 min time intervals, processed as described above, and the change in taurine concentration in the medium was determined by HPLC for each of the three experiments.

Influx of taurine was measured by exposing veliger larvae (3 d-old) to 997 n*M* total taurine. The total concentration of taurine initially present was comprised of both the ^{12}C-taurine in the seawater added with the larvae (552 n*M*), and the addition of ^{14}C-taurine (445 n*M*; 112.2 μCi μmol^{-1}, New England Nuclear). At 5 min intervals, a 500 μl sample was removed, layered onto 500 μl of silicone fluid (Versilube F-50, General Electric) in a 1.5 ml test tube, and centrifuged at 12 500 × *g* for 15 s in a Beckman Model "E" microfuge. To simultaneously measure taurine efflux, a 300 μl sample of the supernatant was retained for HPLC analysis. The remaining supernatant and oil were pipetted away and the larval pellet was obtained by cutting off the tip of the microfuge tube. The pellet of larvae was dissolved in 500 μl of tissue solubilizer (Scinti-Gest, Fisher Scientific) for 24 h, and the radioactivity was measured 24 h after the addition of 5 ml scintillation cocktail.

To examine the size of the free taurine pool in abalone larvae, a known number of veliger larvae (3 d-old) were placed in 75% ethanol (v/v). The free amino acids were extracted for 1 wk at −20 °C and the ethanol extract of the total free amino acid pool of the larvae was analyzed by HPLC (see net flux experiments above). To calculate the amount of taurine per larva, the molar amount of taurine in the extract was converted to moles per sample volume, and then divided by the total number of larvae in the sample.

Alanine influx and metabolism

To follow the metabolism of radiolabeled carbon from ^{14}C-U-alanine, a known number of larvae were placed in 10 ml of seawater (final volume) in a 20 ml vial. One μCi of ^{14}C-U-alanine, producing a substrate concentration of 595 n*M*, was added to the larval suspension, and the first samples were removed. At 10 min intervals, for a total time period of 1 h, two 500 μl samples of the larval suspension were removed and processed. To measure the rate of alanine transport, one sample was layered onto silicone fluid and the larval pellet was collected and treated as described above (see subsection "Taurine efflux and influx" above). The second sample of larvae was collected to determine the biochemical fate of the ^{14}C-label from transported alanine. These larvae were (i) gently washed twice with 10 ml of isothermal seawater, (ii) collected on filters with a 25 mm filtration unit (Millipore brand), and (iii) the filters and larvae were then immediately frozen on dry ice. The filters with the larvae were then freeze-dried for at least 3 h to remove any residual water. The larvae were removed from the filters and homogenized by ultrasonic disruption (Model VC 40 fitted with a microprobe, Sonics and Materials) in 1 ml of distilled water. All subsequent processing of tissue and biochemical fractionation steps that were used followed the procedures previously outlined (Jaeckle and Manahan 1989). The release of $^{14}CO_2$ by larvae was measured following the methodology of Manahan (1983 b).

To determine the biochemical integrity of the label in the free amino acid pools, veliger larvae (2 d-old) were exposed to radiolabeled alanine for 24 h and extracted with 75% ethanol (v/v). Individual amino acids were separated by two-dimensional thin-layer chromatography, TLC (Jones and Heathcote 1966), visualized with 0.2% ninhydrin (w/v) in 100% ethanol, and the radioactivity was detected with x-ray film (XOMAT AR, Eastman Kodak). The spatial correspondence between amino acid standards and the exposed autoradiograms was used to identify the amino acids that contained the ^{14}C-label.

Metabolic rates

Rates of oxygen consumption by larvae were measured on the same group of individuals used for the amino acid uptake experiments. Larvae were washed in seawater and placed in a precalibrated (345 μl) conical analyzer cup (2 ml total volume; Curtin Matheson). A Clark-type oxygen electrode (Model E5047, Radiometer Copenhagen) was placed down into the cup, excess seawater and air were discharged via a small purge hole melted through the cup, and 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 the change in the partial pressure of oxygen was monitored every minute for 22 min. During each experiment, the larval chamber and the electrode were immersed in a water bath at 17 °C ± 0.02 C° (Model RDL 20, Precision Instruments). At the completion of each experiment ($n \geq 3$), the larvae were removed from the chamber, counted (ca. 50 to 150 larvae), and the rate of change in the partial pressure of oxygen was calculated as mm Hg larva^{-1} h^{-1}. The depletion rate was converted to mol O_2 larva^{-1} h^{-1} by calibrating the electrode with the oxygen concentration in a 300 ml biological oxygen-demand bottle containing isothermal seawater (Winkler titration method, Parsons et al. 1984). Prior to each series of experiments with larvae, the oxygen consumption rate of the electrode in seawater was determined, and all rates of larval consumption were corrected for this measured value (always < 5% of experimental values).

Results

No bacteria were visible on the epithelium of veliger larvae of *Haliotis rufescens* following examination with (i) epifluorescence microscopy after DAPI staining, or (ii) scanning electron microscopy. Although bacteria were present on the

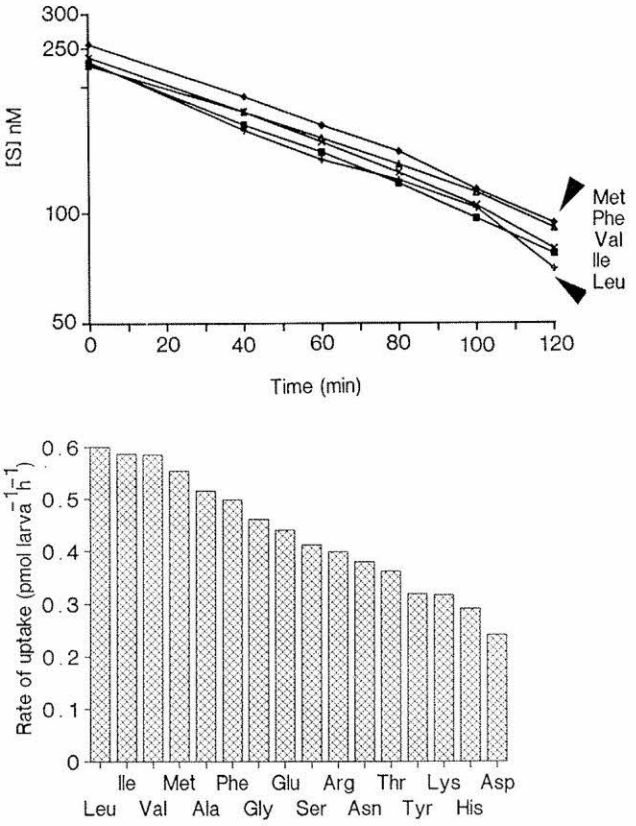

Fig. 1. *Haliotis rufescens.* Depletion of amino acids from medium in presence of 2 d-old veliger larvae (89 larvae ml^{-1}). Upper graph shows depletion from medium (100 ml) of 5 amino acids (other 11 not shown) to illustrate raw data used to calculate first-order depletion constant for each amino acid (see subsection "Net uptake of amino acids"). Rate of uptake for each amino acid was calculated, and is shown in lower graph. r^2 values of slopes used to determine uptake rates ranged from 0.91 to 0.99 for all amino acids. [*S*] refers to concentration of each amino acid. The following 16 amino acids were in the mixture: Ala (alanine), Arg (arginine), Asn (asparagine), Asp (aspartic acid), Glu (glutamic acid), Gly (glycine), His (histidine), Ile (isoleucine), Leu (leucine), Lys (lysine), Met (methionine), Phe (phenylalanine), Ser (serine), Thr (threonine), Tyr (tyrosine), Val (valine)

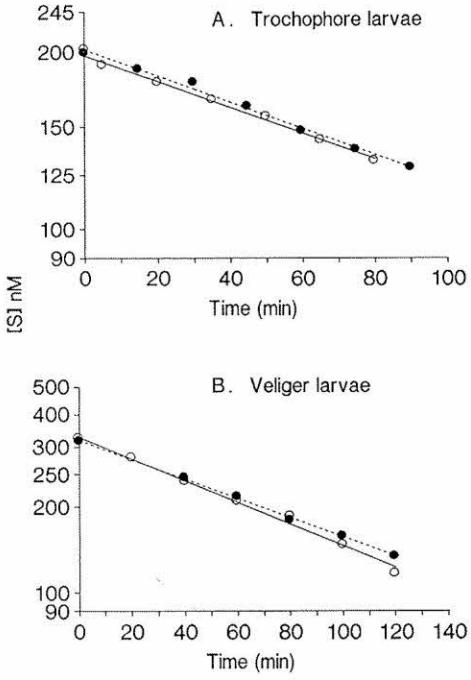

Fig. 2. *Haliotis rufescens.* Net flux of alanine in presence of (A) 1 d-old trochophore larvae (135 larvae ml^{-1}), and (B) 3 d-old veliger larvae (50 larvae ml^{-1}). Total concentration of alanine in medium was determined by HPLC (●) and isotope (○) techniques

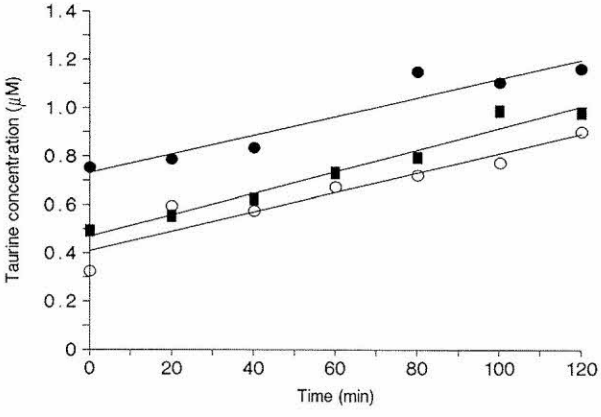

Fig. 3. *Haliotis rufescens.* Increase in concentration of taurine in seawater in presence of 3 d-old veliger larvae (89 ml^{-1}). Slopes for rates of taurine appearance in medium were not statistically different between the various treatments (Table 1). ●: Efflux in presence of taurine in the medium; ■: efflux in presence of mixture of 16 amino acids in the medium; ○: efflux of taurine in control seawater with no addition of amino acids. Amino acids used in the mixture were same as those listed in Fig. 1

larval shell, the number per larva was very small (<6 per larva).

Abalone larvae removed from seawater all the acidic, basic and neutral amino acids tested. The total rate of amino acid uptake by abalone veligers (2 d-old) was 6.97 pmol larva^{-1} h^{-1} (Fig. 1, lower graph). Control experiments showed no detectable decrease for any of the amino acids in flasks where larvae were not present. For both trochophores and veligers, the rate of alanine influx was equal to the net substrate flux, as evidenced by the fact that alanine depletion was identical when measured independently by both HPLC and isotope techniques (Fig. 2).

Veliger larvae of abalone continuously lost taurine to the environment during the time courses of all experiments (Fig. 3). This rate of efflux remained unchanged even when the larvae were placed into seawater containing either a mixture of 16 amino acids or taurine (Fig. 3, Table 1). In another set of experiments, the simultaneous rates were determined for the appearance of ^{14}C-taurine in larvae as well as the changes in taurine concentration in the medium. The rate of ^{14}C-taurine transport was 1.03 pmol larva^{-1} h^{-1} from an initial substrate concentration (both ^{12}C- and ^{14}C-taurine) of 997 n*M;* however, the simultaneous rate of efflux was much higher, at 7.9 pmol larva^{-1} h^{-1}. The amount of ethanol-extractable taurine in 3 d-old larvae was measured by HPLC and determined to be 469 pmol per larva.

Table 1. *Haliotis rufescens*. Results of ANOVA comparing influence of amino acids in seawater on rate of taurine efflux from veliger larvae (Fig. 3). SS: sum of squares; MS: mean square; VR: variance ratio

Source of variation	SS	DF	MS	VR
Efflux in (i) control seawater and (ii) 750 n*M* taurine				
Combined regression	3 485.49	1	3 445.49	84.05***
Between slopes	1.26	1	1.26	0.03 NS
Between intercepts	3 174.31	1	3 174.31	76.54***
Residual	373.24	9	41.47	
Total	7 034.29	12		
Efflux in (i) control seawater and (ii) 4.0 μM amino acids				
Combined regression	4 037.50	1	4 037.50	141.67***
Between slopes	11.43	1	11.43	0.40 NS
Between intercepts	258.86	1	258.86	9.08**
Residual	284.79	10	28.50	
Total	4 592.79	13		

*** Significant at $P \leq 0.001$
** Significant at $P \leq 0.01$
NS not significant at $P > 0.05$

The radiolabeled carbon from ^{14}C-alanine was rapidly utilized, following transport, in both the anabolic and catabolic pathways of veliger larvae. Following an exposure to ^{14}C-U-alanine at a concentration of 595 n*M*, 3 d-old veliger larvae accumulated alanine at a rate of 0.477 pmol larva^{-1} h^{-1} (Table 2, Culture 1). The radioactivity in the larvae was partitioned solely between TCA-soluble material and TCA-insoluble material. No biosynthesis of chloroform-extractable material (total lipid) from alanine was detected. Table 2 shows that radioactivity per larva (3 d-old) was partitioned at a ratio of approximately 50:50 between TCA-soluble and TCA-insoluble fractions. Larvae from this same culture, 24 h later (now 4 d-old), transported alanine (595 n*M*) at a rate of 0.722 pmol larva^{-1} h^{-1}. Once again the amount of radioactivity in the TCA-soluble fraction, and the TCA-insoluble fraction, was nearly equal (Table 2) and there was no accumulation of label in the lipid fraction. Older veliger larvae (5 and 6 d-old), from another culture (No. 2), transported alanine at rates of 1.160 and 0.860 pmol larva^{-1} h^{-1}, respectively (Table 2). For these larvae, the majority of the radioactivity was recovered in the TCA-soluble fraction. For 5 d-old larvae, 0.870 pmol ^{14}C-alanine, or 75% of the total amount in tissue, was found in this fraction. Although the transport rate was lower for 6 d-old larvae, a similarly high percentage (79%) of radioactivity was found in the TCA-soluble fraction. Of the total alanine taken up (the sum of $^{14}CO_2$ and ^{14}C in the tissue), 15 to 19% was released as $^{14}CO_2$. Autoradiograms of the free amino acid pools (TLC-separated) revealed that radiolabeled alanine remained intact, but the carbon skeleton of alanine also served as a precursor for the synthesis of both glutamic and aspartic acid in 2 d-old veligers (data not shown).

The average metabolic rates of veliger larvae were 52.6 ± 2.1 and 68.0 ± 3.3 pmol O_2 larva^{-1} h^{-1} for 2 and 3 d-old larvae, respectively (Table 3).

Table 2. *Haliotis rufescens*. Rates of ^{14}C-alanine transport and metabolism by veliger larvae. All larvae used within each culture were siblings. Concentration of alanine was 595 n*M*. All rates are pmol alanine larva^{-1} h^{-1}. Percent of total alanine influx accounted for by CO_2 production was calculated in the following manner. CO_2 production rate was divided by 3 (3 carbon atoms per alanine molecule) to determine an equivalent rate for alanine, assuming complete catabolism. Percent of total uptake represented by production of $^{14}CO_2$ was calculated by dividing rate of alanine catabolism by sum of the rate of influx and the rate of CO_2 production

Age (d)	Influx	r^2	^{14}C in TCA-soluble material	^{14}C in TCA-insoluble material	$^{14}CO_2$ production	r^2
Culture 1						
3	0.477	0.98	0.209	0.268	0.109 (19% of total uptake)	0.92
4	0.722	0.99	0.346	0.376		
Culture 2						
5	1.160	0.98	0.870	0.290		
6	0.860	0.98	0.681	0.179	0.147 (15% of total uptake)	0.95

Table 3. *Haliotis rufescens*. Comparison of percent of metabolic rate of larvae which could be accounted for by transport of dissolved free amino acids (1.6 μM: 100 n*M* each for the 16 individual amino acids). The 2 d-old and 3 d-old larvae were siblings. Metabolic rates, and rates of amino acid uptake, were determined for same batch of larvae but in a different experiment from that shown in Fig. 1. Percent contribution was calculated by dividing catabolic weight equivalent of oxygen consumption for protein by metabolic rate (see "Discussion" for details)

Age (d)	Metabolic rate (pmol O_2 larva^{-1} h^{-1})	Amino acid uptake rate (pmol larva^{-1} h^{-1})	Oxygen equivalents [pg protein (pmol O_2)]	% contribution
2	52.6	6.89	811 (36.9)	70%
3	68.0	4.93	580 (26.4)	39%

Discussion

Trochophore and veliger larvae of *Haliotis rufescens*, previously considered to be "nonfeeding", are not energetically independent of their environment, because they can take up dissolved free amino acids from seawater (Fig. 1). The concentrations of amino acids used in the experiments presented here fall within values published for coastal marine environments (ranging from 10 to 1 500 n*M*) (Christensen and Blackburn 1980, Mopper and Lindroth 1982, Carlucci et al. 1984, Fuhrman and Bell 1985, Laanbroek et al. 1985). Thus, the structural inability of soft-bodied invertebrate larvae to clear particles from seawater does not exclude the possibility that they can gain energy during development. Although the

tissues responsible for amino acid transport in these larvae were not identified, the velum (veliger-stage) represents the most likely site for uptake. Manahan and Crisp (1983) demonstrated with autoradiography that the larval velum was the primary site of glycine transport in three species of bivalves (*Crassostrea gigas, Ostrea edulis,* and *Mytilus edulis*). The influx of radiolabeled alanine equaled the net substrate flux for both trochophore and veliger larvae (Fig. 2). This result eliminates the possibility that exchange diffusion is acting as the mechanism of alanine transport (cf. Johannes et al. 1969). The appearance of ^{14}C-labeled alanine in larval tissues, following exposure to ^{14}C-alanine, therefore, represents a net energy gain to the larvae.

The larvae used in these experiments were xenic, and bacteria may have been present on the larvae. Sterile oocytes, eggs and sperm have been obtained by aseptic removal, or collection, from certain marine invertebrates (e.g. Langdon 1983, Manahan et al. 1983). Our attempts to collect fertilizable oocytes directly from the gonads of ripe abalone have been unsuccessful and, therefore, we could not produce axenic suspensions of larvae. Yet, direct examination of veliger larvae revealed that no bacteria were adhering to the epithelium and very few bacteria were present on the larval shell. Further, previous reports have shown that there were no differences in the rates of amino acid uptake between axenic and xenic larvae of the sand dollar *Dendraster excentricus* (Davis and Stephens 1984) and the echiuran worm *Urechis caupo* (Jaeckle and Manahan 1989). Thus, during the relatively short time-courses of our experiments, the contribution of bacterial uptake to the measured rates of amino acid uptake by larvae was probably low.

Although abalone veligers have a transport system for taurine, the net flux of taurine for both veligers (Fig. 3) and trochophores (data not shown) was out of the larvae. Taurine is lost continually from veliger larvae, and the rate of efflux was independent of environmental concentrations of either taurine or other amino acids (Fig. 3, Table 1). The taurine concentrations at the start of each experiment (see y-intercepts, Fig. 3) were different for each of the three treatments (Table 1; see ANOVA for "Between intercepts"). This result was expected, because the magnitude of the initial taurine concentration is dependent on the time elapsed between the introduction of the larvae to the flask and removal of the first sample. Similar results on taurine efflux have been found for axenic and xenic bivalve larvae (*Crassostrea gigas*; Manahan 1989). No efflux of the major component (glycine) of the free amino acid pool was observed when similar experiments were conducted on trochophore larvae of the echiuran worm *Urechis caupo* (Jaeckle and Manahan 1989). Thus, the observed efflux of taurine from larvae of *Haliotis rufescens* was unlikely to have been caused by some experimental artifact (e.g. damage to larval tissues). These observations with larval mollusks contrast with those reported for adults. Wright and Secomb (1984) propose that the taurine transport system in adult bivalves (*Mytilus californianus*) provides a mechanism to reaccumulate taurine that is lost from the ctenedia by passive diffusion. However, a different situation seems to exist for larvae. At the measured rate of efflux (7.9 pmol larva^{-1} h^{-1}), a larva would lose 40% of its taurine pool (469 pmol larva^{-1}) per day.

Following transport by veliger larvae, radioactivity derived from ^{14}C-alanine was readily utilized in larval metabolism. All radioactivity in the larvae, from two independent cultures, was recovered in either TCA-soluble and TCA-insoluble fractions; no lipid synthesis from ^{14}C-alanine was detected during the experimental time period (Table 2). The amount of radioactivity in the TCA-soluble fraction changed in proportion to the changes in the rate of alanine transport for each of the two cultures. For example, in Culture 1 (Table 2), the change in the influx rate of alanine increased by 1.5 (0.722 vs 0.477 pmol larva^{-1} h^{-1}) between 2 and 3 d-old larvae; the amount of radioactivity in the TCA-soluble fraction increased by a similar value of 1.7 (0.346 vs 0.209 pmol larva^{-1} h^{-1}). Radiolabeled alanine is also utilized in energy-releasing pathways. The amount of $^{14}CO_2$ released, expressed as a percentage of the total alanine transported, was very similar at 19 and 15% for 3 d and 6 d-old larvae, respectively (Table 2).

The energetic contribution provided to abalone larvae by the transport of free amino acids can be estimated by comparing the rate of substrate uptake with the measured metabolic rate. For 2 d-old larvae, the rate of amino acid uptake from a total substrate concentration of 1.6 μM, was 6.89 pmol larva^{-1} h^{-1}. This value was obtained in a separate experiment from that shown in Fig. 1. The measured rate of oxygen consumption for the same culture of individuals used to determine rates of amino acid uptake was 53 pmol O_2 larva^{-1} h^{-1}. The catabolic weight equivalent of oxygen consumption for protein is 21.95 g protein mol^{-1} O_2 (Gnaiger 1983). The conversion of the amount of amino acid taken up to an equivalent weight of protein is as follows: a larva took up 6.89 pmol of amino acid in 1 h; the average molecular weight of the amino acids used in our experiments (see Fig. 1) was 140 g mol^{-1}; the rate of amino acid uptake is, then, equal to 965 pg amino acid; free amino acids contain 16% more chemically bound water relative to protein (Gnaiger and Bitterlich 1984); thus, an uptake rate of 965 pg amino acid is equivalent to 811 pg protein. The catabolic weight equivalent of 811 pg protein is equal to 37 pmol O_2. The potential contribution to the metabolic demand of larvae, from the uptake of amino acids, is estimated to be 70% (i.e., the ratio of 37:53; Table 3). Similar calculations revealed that 3 d-old larvae could support 39% of their measured metabolic rate by the observed rate of amino acid uptake (4.93 pmol larva^{-1} h^{-1}; Table 3). The comparison of the energy contribution provided through the transport of free amino acids from seawater by larvae is independent of any metabolic coupling between these two physiological processes. It is used here simply to provide a means of estimating the potential contribution of dissolved free amino acids to the energy requirements of abalone larvae.

These estimates, of the contribution to nonfeeding larvae provided by the uptake of free amino acids, are supported by studies of the energy cost for direct development in echinoderms (Turner and Rutherford 1976, Lawrence et al. 1984, McClintock and Pearse 1986). These authors reported

little change in total biomass and energy content between the egg and the juvenile. The fact that lecithotrophic larvae can take up DOM from seawater may be an explanation for these observations.

Nonfeeding abalone larvae do feed; however, the resource that is utilized is DOM. Even though the percent of the total DOM pool represented by dissolved amino acids is low (approximately 1%; Williams 1975) the potential energy gain to the larvae from these substrates is high. For abalone larvae, amino acid uptake could account for 70 and 39% of the metabolic rate of 2 and 3 d-old veliger larvae, respectively. The assumption that "nonfeeding" larvae are energetically isolated from their environment seems incorrect. If the advantages and disadvantages of lecithotrophy are to be fully understood in invertebrate life-history strategies, the trophic role of dissolved organic material should be considered.

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