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Ontogenic Changes in the Rates of Amino Acid Transport from Seawater by Marine Invertebrate Larvae (*Echinodermata, Echiura, Mollusca*)

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Abstract. Transport rates of amino acids were determined for larvae of different ages of the echiuran worm *Urechis caupo,* the gastropod *Haliotis rufescens,* the bivalve *Crassostrea gigas,* and the sea urchin *Strongylocentrotus purpuratus.* All larval forms showed an increase in the transport rate of amino acids during development. Trochophores of *U. caupo* increased their rate of net flux for each of 5 amino acids (100 n*M* each) by a factor of 1.6 and 2.2 during 1–3 days and 4–8 days, respectively, for two independent cultures. In *H. rufescens,* the maximum transport capacity (J_{max}) for alanine increased 3-fold during the 24 h required for the trochophore to develop into a veliger. In *C. gigas* veligers, there was a 9-fold increase in the maximum transport capacity for alanine during larval development from an 80 μm to a 300 μm larva. In sea urchins, the prism-stage larvae (2-day-old) had an alanine transport system with a K_t of 1.9 μM and a J_{max} of 8.1 pmol $larva^{-1}h^{-1}$. The kinetics of alanine transport in the pluteus-stage (4-day-old) were best described by two systems (System I: K_t = 1.0 μM with a J_{max} of 5.6 pmol $larva^{-1}h^{-1}$; System II: K_t = 132.0 μM with a J_{max} of 8.4 pmol $larva^{-1}h^{-1}$). In larvae of *C. gigas,* the relationship between the rate of alanine transport and body size was described by the equation, log J_{max} (pg $larva^{-1}h^{-1}$) = 1.6894(X) + (−0.5937), where X is the shell length in μm. It is illustrated that the allometric increase in respiration rates, during the growth of bivalve larvae, is matched by an ontogenic increase in amino acid transport capacity.

Introduction

Most of the literature concerning the uptake of dissolved organic material (DOM) from seawater by marine invertebrates has dealt with adult forms (Jorgenson, 1976; Stewart, 1979; Ferguson, 1982; Stephens, 1988). A smaller literature describing the uptake of DOM by larvae (Bass *et al.,* 1969; Reish and Stephens, 1969; Chia, 1972; Shick, 1975; Rice *et al.,* 1980; deBurgh and Burke, 1983; Manahan, 1983a) does exist. More recently, studies have been undertaken with bacteria-free (axenic) larvae that show that the animal is the agent responsible for the observed uptake rates of DOM (Manahan *et al.,* 1983; Davis and Stephens, 1984; Jaeckle and Manahan, in press).

There is a dramatic increase in the rate of amino acid transport following fertilization of eggs, such as those of the sea urchin (Epel, 1972; Allemand *et al.,* 1984), but information is scant on how the transport rates change in larvae during later development. However, in a qualitative way it has been demonstrated that the ability for uptake of dissolved amino acids is a continuous process and occurs throughout the entire life-history, at least for bivalves. Manahan (1983b) demonstrated that the activation of transport system(s) for glycine in eggs of *Crassostrea gigas* occurs 1 h after fertilization; in the veliger and pediveliger larvae, uptake occurs across the velum; and during metamorphosis, the developing gill buds are the primary sites of uptake (Manahan and Crisp, 1983).

The present work was undertaken to determine how the rates of amino acid transport change through ontog-

eny for a variety of marine invertebrate larval forms, representing three phyla. If the energetic contribution from DOM is to be quantitatively important throughout development, then, as larvae grow, they would have to increase their transport capacity to meet the increase in metabolic demand. Failure to do so would result in an increasingly smaller fraction of the larva's total energy needs (volume-dependent) being provided by transport processes (surface area-dependent). We report here that larvae increase their transport capacity as they increase in size during development and that, for bivalve larvae, the change in transport rates are proportional to the increase in metabolic rates, as measured by oxygen consumption.

Materials and Methods

Larval cultures

Adult sea urchins (*Strongylocentrotus purpuratus*) were purchased from Marinus Inc. (Long Beach, California) and adult echiuran worms (*Urechis caupo*) were purchased from Sea Life Supply Co. (Sand City, California). All sizes of bivalve larvae (*Crassostrea gigas*) were provided by Coast Oyster (Quilcene, Washington). Fertilized eggs of the gastropod *Haliotis rufescens* were provided by the Ab Lab (Port Hueneme, California).

Sea urchin gametes were collected from ripe adults, following injection with 0.5 *M* KCl, in 0.2 μm (pore-size, Nuclepore) filtered-seawater. Eggs and sperm were obtained from the gamete storage organs of *Urechis caupo* as previously described (Jaeckle and Manahan, 1989), except that they were not collected under aseptic conditions. Fresh 0.2 μm (pore-size) filtered-seawater was used for all cultures. All species were reared at 16–17°C, except *Crassostrea gigas* (20–21°C). During development, the water was changed either daily (mollusks), or every other day. Phytoplankton foods were not added to any of the cultures, except to the larvae of *C. gigas.*

To obtain larvae for experiments, suspensions were siphoned from the culture vessels onto mesh screens and washed with 0.2 μm (pore-size) filtered, autoclaved, seawater. The larvae were placed in a graduated cylinder and aliquots were removed for counting. A known volume of the larval suspension was then added to each experimental vial to give the required number of larvae ml^{-1}. Different larval stages, or ages, for each species were tested to determine changes in amino acid transport rates. These included: (i) the prism-stage (2-day-old) and the pluteus-stage (4-day-old) of *Strongylocentrotus purpuratus;* (ii) trochophores (1 to 8-day-old) of *Urechis caupo;* (iii) 1-day-old trochophores and 2-day-old veligers of *Haliotis rufescens;* and (iv) oyster veligers (*Crassostrea gigas*) of different sizes (80–300 μm shell length).

Kinetics of alanine transport

A known number of larvae (see figure legends) of either *Crassostrea gigas, Haliotis rufescens,* or *Strongylocentrotus purpuratus* were transferred into each of several (9–11) 20 ml vials, and sterile seawater was added until the final volume was 10 ml. One μCi of ^{14}C-alanine (168 or 170 μCi μmol^{-1}, New England Nuclear) was added to each vial. In addition, a known amount of cold carrier (^{12}C-alanine, Sigma Chemical Co.) was added to give the required substrate concentration in each vial. Depending on the species of animal (see Figs. 1, 4), these concentrations ranged from 0.5 to 500 μ*M*. A time course experiment of 6–8 min duration (*ca.* 1 sample per min) was then carried out with each vial to measure the rate of alanine transport into larvae at each substrate concentration. At approximately 1 min intervals, a 500 μl sample of the medium (containing larvae) was removed and placed on silicone oil (Versilube F-50, General Electric Co.) in a 1.5 ml centrifuge tube. The larvae were rapidly separated (*ca.* 5 s) from the medium by centrifugation at 12,500 × *g* (Beckman Model "E" microfuge). The radioactive supernatant and oil were aspirated away, the pellet collected by cutting off the bottom of the centrifuge tube, and the larvae immediately killed with 500 μl of tissue solubilizer (Scinti-Gest, Fisher Scientific).

The larvae were then digested for at least 24 h, 5 ml of scintillation cocktail was added (Scinti-Verse, Fisher Scientific), and the radioactivity was determined with an LKB model 1211 liquid scintillation counter. Sample radioactivity was corrected, if necessary, for quenching by the addition of a ^{14}C-toluene internal standard (New England Nuclear). A least-squares regression analysis was performed on each time course experiment to determine the rate of increase in radioactivity per larva with time. The slope (radioactivity as D.P.M. $larva^{-1}$ min^{-1}) of each time course experiment (*i.e.,* for each different substrate concentration) was corrected for the change in specific activity from the cold carrier. The rate of alanine transport, as a function of increasing substrate concentration, was calculated from these slopes and expressed as pmol $larva^{-1}$ h^{-1}. To determine the changes in the maximum transport capacity (J_{max}) for the veliger-stages of *Crassostrea gigas,* larvae of each size were exposed to 200 μ*M* alanine, and the rate of transport determined in a series of time course experiments as described above. Preliminary kinetic experiments showed that a substrate concentration of 200 μ*M* caused saturation of alanine transport into *C. gigas* larvae.

Net uptake of amino acids by Urechis caupo *larvae*

Trochophore larvae of *Urechis caupo,* at different ages, were exposed to an equimolar mixture of the amino acids Ile, Leu, Met, Phe, and Val (Sigma Chemical Co.),

each present at the start of the experiment at 100 n*M*. In parallel, the same amino acid mixture was added to an identical flask containing no larvae to serve as a control for any changes in substrate concentration that might have occurred due to surface adsorption. At each sampling time, a 500 μl volume of the medium was removed and passed through a 0.2 μm (pore-size) polycarbonate filter held in a 13 mm "Pop Top" filter housing (Nuclepore) to separate the seawater sample from the larvae. The seawater samples were then frozen until the amounts of individual amino acids in the medium were determined with high-performance liquid chromatography (HPLC) and fluorescence detection. Amino acids were derivatized with *o*-phthaldialdehyde (Lindroth and Mopper, 1979) and the fluorescent derivatives separated using a sodium acetate buffer system (Jones *et al.*, 1981) on an Ultrasphere ODS column (4.6 cm × 7.5 mm; 3 μm particle size). The eluent profile and HPLC equipment are described elsewhere (Manahan, 1989).

The rate of disappearance from the medium was calculated for each individual amino acid using a first-order depletion constant "k" (see Segel, 1976, p. 227). The rate of substrate depletion with time (k) was calculated from the slope of the linear regression determined for each amino acid from ln-transformed substrate concentrations. The rate of uptake for each individual amino acid was calculated by multiplying the amount of each amino acid present at time 0 by its respective depletion constant (k). Dividing this rate by the total number of larvae in the experimental flask (see legend, Fig. 3), gave the rate of uptake as fmol larva^{-1} h^{-1} for each of the five amino acids.

Results

The effect of increasing substrate concentration on the rate of alanine transport by *Strongylocentrotus purpuratus* larvae is shown in Figure 1. The upper graph shows that alanine transport is saturable in prism-stage larvae at substrate concentrations as low as 20 μM. The experiment was repeated for sibling larvae, but two days later, when they had developed to the pluteus-stage (Fig. 1, lower graph). The rate of transport was not saturated in the pluteus-stage even when the concentration of alanine was increased to 500 μM. Figure 2 shows linear transformations (Eadie-Hofstee) of the kinetic data for both prism-stage and pluteus-stage larvae. Prism-stage larvae had a transport system with a K_t of 1.9 μM for alanine, and a J_{max} of 8.1 pmol larva^{-1} h^{-1} (Table I). However, the kinetics of alanine transport in pluteus-stage larvae were best described by two systems (Table I) having a combined effect of increasing the J_{max} to 12.3 pmol larva^{-1} h^{-1}. The first (System I), was a high-affinity system with a K_t of 1.0 μM and a J_{max} of 5.6 pmol larva^{-1} h^{-1}. The second (System II) was a low-affinity, but higher capacity, system with a K_t of 132.0 μM and a J_{max} of 8.4 pmol larva^{-1} h^{-1}. This series of experiments was repeated with a second culture of *S. purpuratus* larvae obtained from an independent spawning. A similar pattern was observed (data not shown) to that seen in Figures 1 and 2, with the appearance of a biphasic transport system in the pluteus-stage larvae.

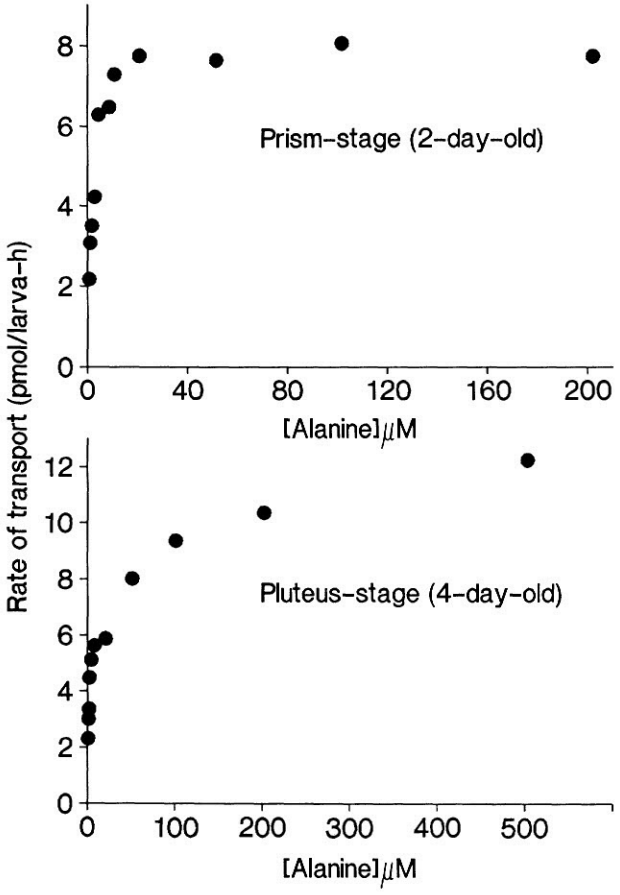

Figure 1. Ontogenic changes in the kinetics of alanine transport by larvae of the sea urchin *Strongylocentrotus purpuratus*. The upper graph shows the effect of increasing substrate concentration on the rate of alanine transport by prism-stage larvae (448 ml^{-1}). The lower graph was obtained for sibling larvae when they had developed to the pluteus-stage (408 ml^{-1}). Each data point represents a rate determined from a separate time course experiment. All values for r^2 were >0.93.

The ontogenic changes in the net uptake rates of Ile, Leu, Met, Phe, and Val by trochophores of *Urechis caupo* are shown in Figure 3. These rates of uptake were determined with direct chemical measurements of the medium by HPLC and, hence, represent the net flux (*cf.* Johannes *et al.*, 1969). The individual flux rates for the five amino acids are plotted for larvae obtained from two independent spawnings (Fig. 3, upper and lower graph). Larvae from both cultures had an increase in the rate of net flux for each of the five amino acids. The summed

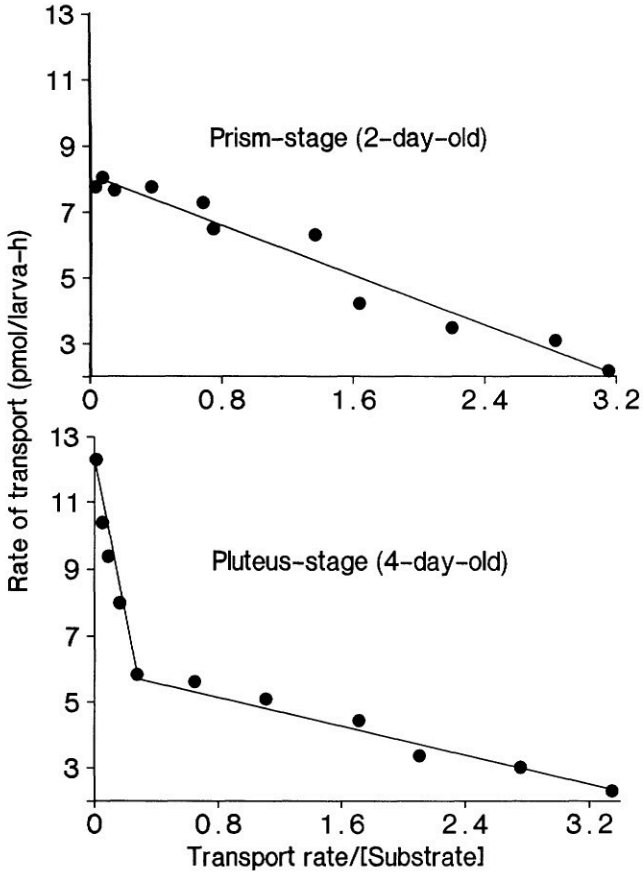

Figure 2. Eadie-Hofstee plots of the kinetic data (from Fig. 1) for alanine transport by two stages of sea urchin larvae (*Strongylocentrotus purpuratus*). The upper graph was obtained for the prism-stage larvae; the lower one for the pluteus-stage.

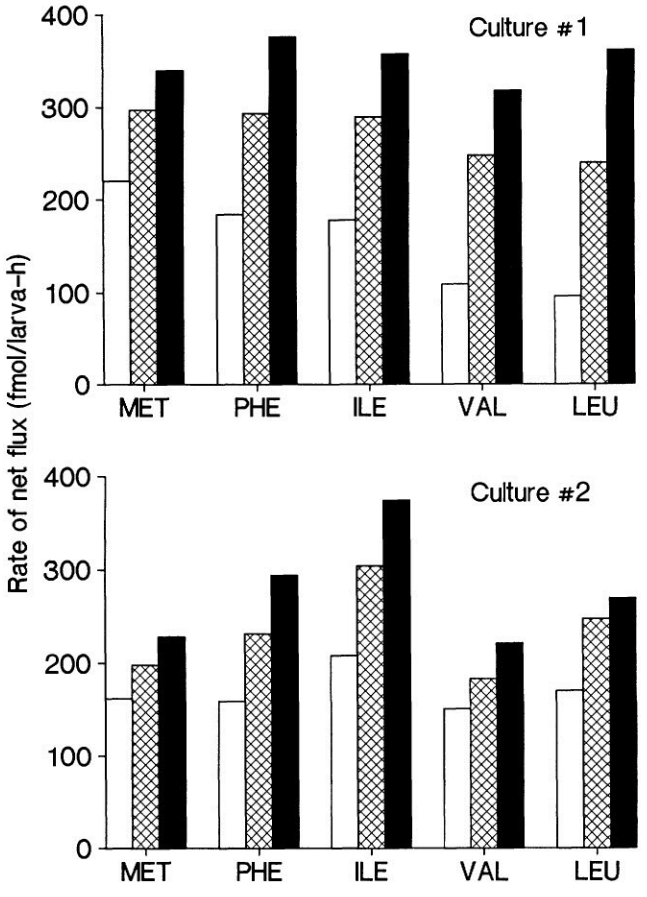

Figure 3. The change in the rate of net flux for five amino acids, as a function of age, for trochophore larvae of *Urechis caupo.* The upper and lower graphs were plotted using data from two cultures obtained from independent spawnings (nonsibling). Upper graph: the open bars, hatched bars, and solid bars represent experiments which were carried out with larvae of different ages (4-days, 6-days, and 8-days-old, respectively). Lower graph: open, hatched and solid bars represent 1-day-, 2-day-, and 3-day-old larvae, respectively. Each bar depicts a separate experiment where the depletion of substrates from the medium was determined with HPLC. Each substrate was present in a mixture at 100 n*M* each. The r^2 values for each of the ln-transformed linear regressions (n = 30) were all >0.95. The number of larvae ranged from 140 to 297 ml^{-1}, depending on the experiment.

rates of net flux were 787 fmol $larva^{-1}$ h^{-1} for 4-day-old larvae (Fig. 3, upper graph, sum of open bars) and 1751 fmol $larva^{-1}$ h^{-1} for 8-day-old larvae (sum of solid bars). Corresponding values for the second culture (Fig. 3, lower graph) were 850 fmol (1-day-old larvae) and 1386 fmol $larva^{-1}$ h^{-1} for 3-day-old larvae.

Figure 4 (upper graph) illustrates that there was a substantial increase in the transport capacity (J_{max}) for alanine during development of *Haliotis rufescens,* from the trochophore (J_{max} = 22.7 pmol $larva^{-1}$ h^{-1}) to the veliger-stage (J_{max} = 71.1 pmol $larva^{-1}$ h^{-1}). Again, these were sibling larvae for which the same kinetic experiment was repeated 1 day later, by which time the trochophores had developed into veligers. This increase in both J_{max} and K_t (see Fig. 4, lower graph) for veligers of *H. rufescens* was observed for three other cultures of larvae obtained from independently spawned batches of eggs and sperm. Larvae of *Haliotis* spp. are nonfeeding through out their entire lifespan (Crofts, 1937) and, hence, there is no major increase in biomass until after metamorphosis. There-

Table I

The appearance of biphasic kinetics for alanine transport during the development of Strongylocentrotus purpuratus *larvae*

Stage	K_t (μM)	J_{max} (pmol $larva^{-1}$ h^{-1})
Prism-stage (2-day-old)	1.9	8.1
Pluteus-stage (4-day-old)	1.0	5.6 (System I)
	132.0	8.4 (System II)

Curve fitting was calculated by microcomputer using "PC-nonlin" software.

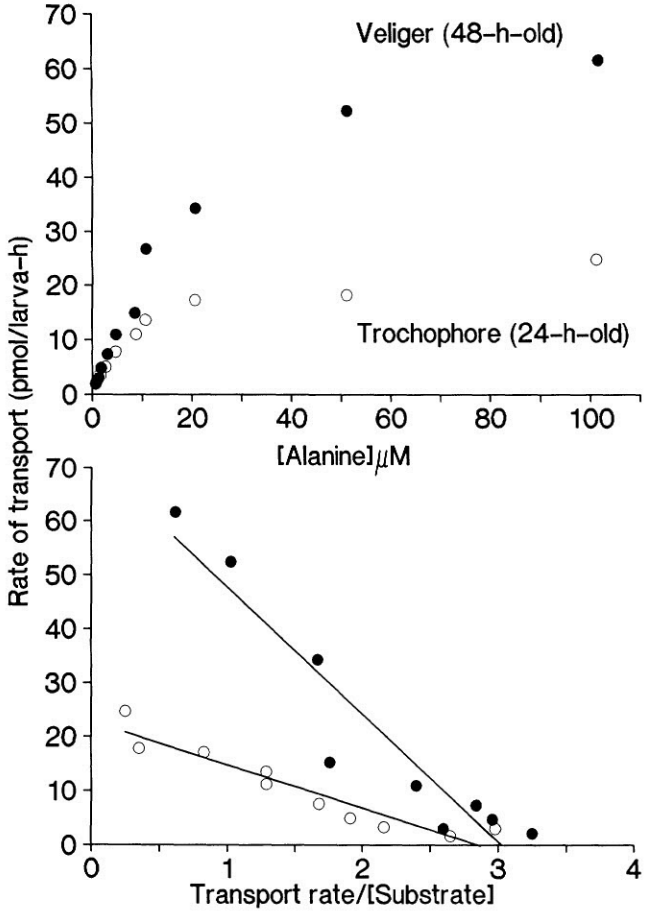

Figure 4. Upper graph (A): a comparison of the effect of increasing substrate concentration on the transport rate of alanine by two larval stages of *Haliotis rufescens*. Each point represents the rate determined from a time course experiment at that particular substrate concentration (all values for r^2 were >0.91). Lower graph (B): Eadie-Hofstee plots of the raw data from the upper graph. Trochophores (210 larvae ml^{-1}) had a K_t of 8.0 μM and a J_{max} of 22.8 pmol $larva^{-1}h^{-1}$; veligers (274 ml^{-1}) had a K_t of 22.7 μM and a J_{max} of 71.1 pmol $larva^{-1}h^{-1}$.

fore, further experiments to investigate ontogenic changes in transport rate as a function of size were carried out with feeding larvae of the bivalve *Crassostrea gigas*. Veligers of this species undergo substantial increase in size during growth when fed with cultures of phytoplankton (Helm and Millican, 1977).

Preliminary experiments showed that the rate of alanine transport by veliger larvae of *Crassostrea gigas* could be saturated at concentrations of 200 μM. This was the substrate concentration chosen for further experiments with bivalve larvae. Larvae representing seven different sizes, ranging from 80 μm to 300 μm in shell length, were chosen and their transport rates for alanine measured at 200 μM. Their respective rates of transport are shown in Figure 5 (upper graph), where each data point represents the rate obtained from a separate time course experiment (all r^2 values >0.95). The upper graph (Fig. 5) shows that there is a continuous increase in the value for J_{max} during larval development from an 80 μm larva to a 300 μm larva. The least-squares regression of the increase in the log-rate of alanine transport, as a function of increasing body size (log-shell length), is described by the allometric equation: Rate of transport (log J_{max}, pg $larva^{-1}$ h^{-1}) = 1.6894(X) + (−0.5937), where X = shell length. The units for alanine transport in Figure 5 are presented as pg alanine $larva^{-1}$ h^{-1} so as to permit easy

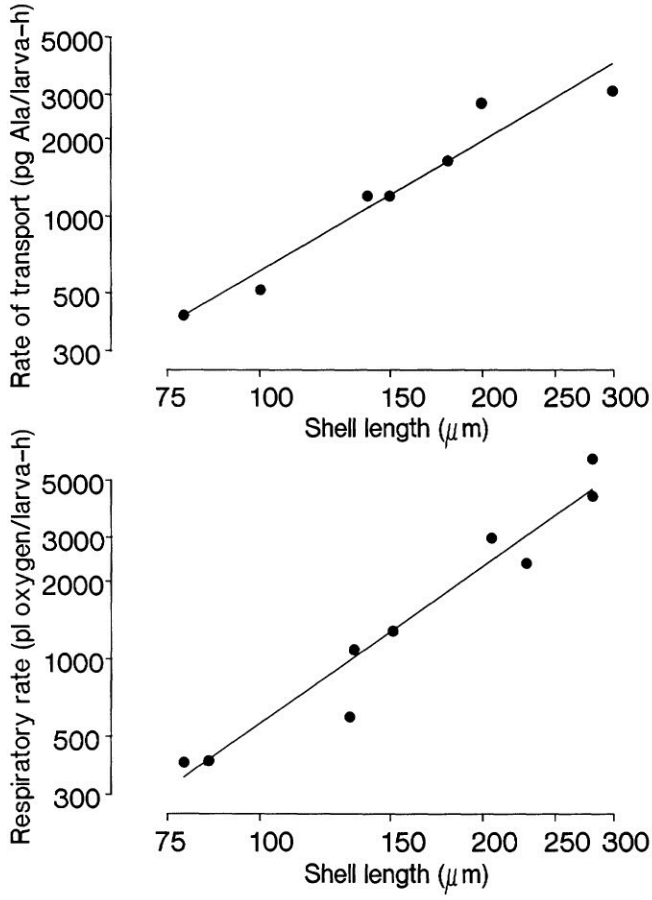

Figure 5. Upper graph (A): the logarithmic scaling of ontogenic changes in the maximum transport capacity (J_{max}) for alanine as a function of size for veliger-stage larvae of *Crassostrea gigas*. Each point represents the rate determined from a time course experiment for that particular size of larva (all values for r^2 were >0.95). The concentration of larvae used ranged from 150–400/ml, depending on size. Lower graph (B): the logarithmic scaling of respiration rate as a function of size for veliger-stage larvae of *C. gigas* (redrawn from Gerdes, 1983).

ANOVA: comparison of regressions for maximum transport capacity (J_{max}) and respiratory rate, as a function of larval size.

Source	Sum sq	d.f.	M. sq.	VR	$F_{.05}$
Combined regression	2.361	1	2.361	234.019	4.67
Between slopes	0.017	1	0.017	1.716	
Between constants	0.003	1	0.003	0.327	
Residual	0.131	13	0.010		
Total	2.512	16			

comparison to the respiration rates of similar sized larvae (1 pg protein requires 1 pl O_2 for complete combustion—Stephens, 1963). Gerdes (1983) gives data on the changes that occur in respiration rates during growth of *C. gigas* larvae. These raw data (taken from his Table III) were plotted on Figure 5 (lower graph). The least-squares regression of the increase in the rate of respiration is described by the equation: log-rate of respiration (pl O_2 $larva^{-1}$ h^{-1} = 2.0344(X) + (−1.3163). A comparison of the slope in Figure 5A, with that of Figure 5B, illustrates the close correspondence between (i) the ontogenic increase in J_{max} for alanine transport (slope = 1.7) and (ii) the increase in respiration rates for larvae of the same species (slope = 2.0). These two slopes are not statistically different (VR = 1.7, $F_{.05[1,13]}$ = 4.67, see legend to Fig. 5A, B).

Discussion

The present work was undertaken to describe how the rates of amino acid transport change during larval development. Changes were determined for trochophores of the echiuran worm (*Urechis caupo*), trochophores and veligers of the gastropod (*Haliotis rufescens*), veligers of the bivalve (*Crassostrea gigas*), and prism-stage and pluteus-stage larvae of the sea urchin (*Strongylocentrotus purpuratus*). Where J_{max} was determined, all larval forms studied showed an increase in transport capacity for amino acids as development proceeded. Obviously the surface area of larvae increases during development from the simple shape of an egg to a more complex larval form (McEdward, 1984) with extensive arms (*e.g.*, echinoderm pluteus), or large organs used for swimming (*e.g.*, molluskan velum). The simplest interpretation for the observed increase in the J_{max} for alanine transport is that, as larvae increase in surface area, the number of transport sites increases.

For bivalve larvae, the value for J_{max} increased by a factor of 9-fold as the veliger larvae of *Crassostrea gigas* increased in size from 80 μm to 300 μm. A change in the value for J_{max} can be considered as a measure of the change in the number of sites for amino acid transport. The metabolic rates for growing larvae of marine invertebrates are known to increase in direct proportion to increasing weight, with weight exponents of about 1.0 (Zeuthen, 1947; Gerdes, 1983; Sprung, 1984). Thus, unless the transport capacity for dissolved organic substrates is increased during larval growth, the relative input that the uptake of DOM would contribute to the energy needs of a larva would decrease as development proceeds. Figure 5 presents a comparison of (i) the increase in respiration rates with (ii) the increase in the transport capacity for alanine. The slopes of both the lines for alanine transport and respiratory rate are statistically similar at 1.7 and 2.0, respectively (see ANOVA in legend to Fig. 5). Thus, growing larvae scale these two processes in direct proportion. An estimate of how larvae scale (i) the absolute amount of alanine transported to, (ii) their corresponding metabolic demands, can be determined as follows. The data for the respiration rate of larvae (Fig. 5, lower graph) were taken from Gerdes' (1983) experiments, which were conducted at 25°C. From the regression line given in Figure 5 (lower graph), a 150 μm larva would have a respiration rate of 1291 pl O_2 $larva^{-1}$ h^{-1} [log rate at 25°C = 2.0344(X) + (−1.3163), where X is shell length]. A temperature of 20°C was used during the measurements of alanine transport by larvae of *C. gigas* (Fig. 5, upper graph). Correcting the metabolic rate for temperature by assuming a Q_{10} of 2, a 150 μm larvae would have a respiration rate of 913 pl O_2 $larva^{-1}$ h^{-1} [log rate at 20°C = 2.0344(X) + (−1.4669)]. From the regression (Fig. 5, upper graph) for the rate of alanine transport [log rate = 1.6894(X) + (−0.5937)], a 150 μm larvae would transport 1210 pg Ala $larva^{-1}$ h^{-1}. Could this rate of alanine transport account for the metabolic rate, as measured by oxygen consumption? Usually, a 1:1 ratio for grams of amino acid (protein) and liters of oxygen is taken for these calculations (see above). However, the correct stoichiometry for the complete oxidation of alanine to ammonia requires 3 mol O_2 per mol of alanine: 1 mol Ala (89 g) requires 3 mol O_2 (at 22.4 l mol^{-1}) = 89/67.2. This gives a ratio of 1.32 g Ala = 1 l O_2 (Gnaiger, 1983). Thus, a 150 μm larva with a transport capacity of 1210 pg Ala $larva^{-1}$ h^{-1} could balance a metabolic rate of 917 pl O_2 $larva^{-1}$ h^{-1}, which is close to the respiratory rate for a larva of this size (913 pl O_2 $larva^{-1}$ h^{-1}). A larva with a shell length of 150 μm was chosen for these calculation because it was in the middle of the size range studied. Similar calculations revealed that an 80 μm larva could account for 117% of its respiratory rate, with the value decreasing to 79% for a 300 μm larva. Larvae in nature are unlikely to be able to utilize 100% of their J_{max} capacity, nonetheless, the above calculations show that the J_{max} value increases during larval growth so that sufficient substrate can be supplied to the larva in order to help meet the increasing demand for energy as growth proceeds.

Our attempts to obtain accurate measurements of K_t for veliger-stage larvae proved to be difficult. Presumably, the constant experimental agitation required for reproducible sampling caused the veligers to retract their velum, the primary organ responsible for amino acid transport (Manahan and Crisp, 1983). Figure 4 shows that there is approximately a 3-fold increase in the J_{max} value for a veliger of *Haliotis rufescens*, when compared to a trochophore. However, the K_t also increased (*i.e.*, lower affinity) 3-fold, assuming a single-affinity transport system. The Eadie-Hofstee plot of the data for veligers of

H. rufescens (Fig. 4, lower graph) suggests the possibility that there may be a biphasic transport system (*cf.* the pluteus-stage larvae in Fig. 2, lower graph), but the variance in the data for the veliger-stage of *H. rufescens* did not permit detailed analysis. Only with sea urchin larvae could changes in the affinity (K_t) of the transport system be reliably determined (Figs. 1, 2). Both prism-stage and pluteus-stage larvae of *Strongylocentrotus purpuratus* have a high affinity transport system with a K_t for alanine of 1–2 μM. Yet, at the pluteus-stage a low affinity transport system appears (Fig. 2), with a K_t of 132 μM (Table I), in addition to the presence of a high affinity system (K_t = 1 μM). The higher affinity, lower capacity, system of the pluteus-stage (Table I) had a K_t of 1.0 μM and a J_{max} of 5.6 pmol Ala larva^{-1} h^{-1}. From a substrate concentration of 500 nM, this system could transport 166 pg Ala larva^{-1} h^{-1}, equivalent (see above) to 126 pl O_2 larva^{-1} h^{-1}, or 36% of the metabolic rate (Scholander *et al.*, 1952) of developing *S. purpuratus* larvae.

Why would echinoderm larvae have a K_t for alanine of 132 μM when the natural environmental concentrations in seawater (Carlucci *et al.*, 1984; Henrichs and Williams, 1985) are thought to be approximately 100–1000 times lower? It is unlikely that, during the short time course experiments (6–8 min) used to construct the kinetic plots (Figs. 1, 2) for pluteus-stage larvae of *Strongylocentrotus purpuratus,* any substantial uptake occurred via the digestive system. In pluteus-stage larvae of another echinoid (*Dendraster excentricus*), the primary sites of uptake for a mixture of ^{3}H-amino acids were determined by autoradiography to be the epidermis and the ciliary bands (deBurgh and Burke, 1983). Very little radioactivity was localized in the esophagus and stomach. deBurgh and Burke (1983) did not see any ontogenic change in the K_t for amino acid transport in *D. excentricus,* although they emphasized that their values should be interpreted as relative, rather than absolute, because they used a mixture of amino acids and not individual substrates. For bivalve larvae, Manahan and Crisp (1983) reported similar observations with respect to the lack of uptake of ^{3}H-glycine by the digestive system of bivalve larvae (*Crassostrea gigas* and *Ostrea edulis*).

Given the natural variability in oceanic primary production rates, it is not surprising that there should be spatial and temporal variations (Mopper and Lindroth, 1982) in the amounts of DOM in different environments. However, these variations can be surprisingly large, even over relatively small scales. Smith (1986) found that the concentrations of dissolved organic nitrogen differed by a factor of 1000 in seawater samples taken just 2 m apart. On even smaller scales, multiphasic transport kinetics have been observed in isolated strains of a marine bacterium, where the K_t values for dissolved glucose range from as low as 3 nM to a high of 4 mM (Nissen *et al.*, 1984). These authors interpret their data as indicating that "microzones" of very high nutrient concentrations exist in small volumes that are relevant to bacteria, but are beyond the current technical limits of direct chemical determinations (Mopper and Dawson, 1986). A possible interpretation for the appearance of biphasic kinetics during the development of sea urchin larvae is that these larvae may occasionally experience much higher concentrations of amino acids in the microenvironments in which they live. The presence of a second, higher capacity, system for alanine transport (K_t = 132 μM; J_{max} = 8.4 pmol larva^{-1} h^{-1}) would permit a higher rate of transport at any substrate concentration exceeding 260 μM, than could be achieved with the lower capacity system (K_t = 1.0 μM; J_{max} = 5.6 pmol larva^{-1} h^{-1}). However, the concept that microzones containing enhanced nutrient concentrations exist in the water column is highly controversial (Jackson, 1980; Williams and Muir, 1981). Current data on the distribution of larvae in the field (Young and Chia, 1987) together with information on the composition of DOM in seawater (Wangersky, 1978), and its microspatial heterogeneity, are still too scant to permit generalizations to be made concerning ambient concentrations that might be relevant to the larvae of marine invertebrates.

At least in the case of bivalve larvae, their transport capacity for amino acids is scaled to match their increasing metabolic demands throughout development. The percent of this transport capacity that is actually utilized will depend on the ambient concentrations of amino acids in the immediate environment of the larva.

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