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Biochemical Composition, Metabolism, and Amino Acid Transport in Planula–Larvae of the Soft Coral *Heteroxenia fuscescens*

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ABSTRACT We determined the monthly percentage of biochemical components in planulae of the soft coral *Heteroxenia fuscescens*, for a 3-year period, and evaluated the findings in relation to seasonal fluctuations in water temperature. We determined the biochemical profile and metabolic rate of aging planulae and examined the possible absorption of dissolved organic material (DOM) from the water by the planulae. Our study is the first to present a long-term biochemical profile of planulae. They contained an average of 2.2% ash, 51.5% lipid, 33.6% protein, and 1.3% carbohydrate. Calculation of the average energetic content of a planula revealed a value of 1.63 J planula^{-1}. Significant seasonal differences in planulae weight were noted between the summer and the other seasons. A significant decrease (41%) from the initial weight, 0.029 mg, took place in the planulae dry weight within 15 days. Significant decreases over time were also found in lipid (50%) and carbohydrate (83%) concentration but not in protein (20%). Metabolic rates of a planula was 0.06 μl O_2 planula^{-1} hr^{-1}. The study shows for the first time that a soft coral planulae can take up dissolved free amino acids from seawater. Even though each of the amino acids was initially present at equimolar concentrations, there was a much faster uptake for the neutral, nonpolar amino acids, than for polar and basic ones. The potential contribution to the metabolic demand of planulae, from the uptake of amino acids, is estimated to be 11%. It is suggested that this uptake does not appear to be due to energetic considerations, but may have a more significant impact on their nitrogen budget. *J. Exp. Zool. 287:401–412, 2000.*

In general, corals are regarded as having a nonfeeding larval stage (Crisp, '84). Schwarz et al. ('99) reported feeding behavior for planulae of *Fungia scutaria* that consisted of a mouth-opening response to the addition of ground animal tissue, as well as secretion of mucous strands that trapped particulate matter for ingestion. Nonfeeding larvae are considered to posses ample nutritive content, and as such, often called lecithotrophic (Crisp, '84; Jaeckle and Manahan, '89; Havenhand, '93). Studies of lecithotrophic marine invertebrate larvae have shown that larvae are capable of transporting dissolved organic material (DOM) from seawater (Jaeckle and Manahan, '89; Jaeckle, '94; Shilling et al., '96). However, to date no study has examined the possible uptake of DOM by coral planulae. Absorption of DOM by the planulae may offer them an additional energy resource, thus extending their longevity and competence periods. Several studies have shown that these periods are related to the energy content of the released larvae, and to the energy obtained during their planktonic phase (Richmond, '87, '88; Pechenik, '90; Qian et al., '90; Harms, '92; Jaeckle and Manahan, '92; Havenhand, '93).

Studies of competence and longevity of larvae in relation to their energetic content have emphasized the importance of stored energy for dispersal (Lucas et al., '79; Richmond, '87, '88; Jaeckle and Manahan, '89, '92; Pechenik, '90; Qian et al., '90; Harms, '92; Havenhand, '93; Jaeckle '94; Pechenik et al., '96). In a comparative study on several Red Sea soft coral planulae, a variability in order of magnitude among the energetic content of freshly released zooxanthellate planulae of *Xenia umbellata*: 0.012 ± 0.001 cal planula^{-1}, and the azooxanthellate *Parerythropodium fulvum fulvum*: 0.096 ± 0.002 cal planula^{-1} (Ben-David-Zaslow and Benayahu, '98) and *Heteroxenia fuscescens*: 0.58 ± 0.05 cal planula^{-1} was revealed (Ben-David-

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Zaslow and Benayahu, '96). These values indicate a low maternal energetic investment in zooxanthellate planulae compared to the azooxanthellates. The caloric content of the zooxanthellate planulae of *X. umbellata* initially increased, while in the azooxanthellate planulae of *H. fuscescens* and *P. f. fulvum* it steadily decreased with time (Ben-David-Zaslow and Benayahu, '96, '98, respectively). The caloric content of *X. umbellata* planulae started to decrease from day 35 of their release, at the same time that most of the planulae stopped being competent to metamorphose. Planulae of *H. fuscescens* have a shorter competency period (49 days) compared to *X. umbellata* (76 days) and *P. f. fulvum* (64 days). These results suggest that the symbiotic algae may significantly contribute to the energetic content of the planulae in the course of the first period of their life, but do not necessarily increase their competency.

In order to answer questions related to the significance of temporal changes, either seasonal and year to year, in the energetic content of planulae, and their relation to the dispersal capabilities of a given species, further physiological studies are required. Therefore, in this study we determined the monthly percentage of lipid, protein, and carbohydrate in the coral planulae for a 3-year period, and evaluated the findings in relation to seasonal fluctuations in the water temperature. We determined the biochemical profile and metabolic rate of aging planulae and examined the possible absorption of organic material from the water by the planula-larvae.

MATERIALS AND METHODS

Sampling

Sexually mature colonies of *Heteroxenia fuscescens* were sampled monthly from the coral reef across from the Marine Biology Laboratory (MBL) at Eilat (Red Sea) over a 3-year period (October 1994 to September 1997). The colonies were placed in containers with running seawater flowing at a rate of 2 liters min^{-1}. Prior to sunset the colonies were transferred to aerated aquaria and examined the following morning for the presence of released planulae. The obtained planulae were collected, washed in distilled water and frozen at –40°C. The frozen planulae (1 day old) were transported to Tel-Aviv for biochemical analyses (see below).

In months when the number of obtained planulae exceeded 200, they were placed in 500-ml PVC containers, filled with Millipore-filtered (0.2 μm) seawater (FSW), and transported alive to Tel-Aviv. Planulae were washed with FSW (0.2 μm) to remove organic debris, and later placed in batches of up to 50 planulae in Erlenmeyer flasks each containing 300 ml FSW (0.2 μm). The planulae were washed every day following the methodology given in Ben-David-Zaslow and Benayahu ('96, '98). They were kept for 15 days and their uptake rate of amino acids, oxygen consumption, and biochemical profile were determined (see below). The seasons were defined in this study according to the stratification of the water column at Eilat, following Lindell and Post ('95). "Winter" refers to December–March; "spring" to April and May; "summer" to June–September; and "autumn" to October and November. We considered an annual cycle to commence from October, when the thermocline starts to break, till September of the following year, when it is still in evidence. All analyses of temporal changes in biochemical composition of the planulae were performed according to these definitions.

Biochemical composition of Heteroxenia fuscescens *planulae*

The planulae were lyophilized, placed in a preweighed glass vial, and the vial and tissue were weighed. Batches of 40–50 planulae were analyzed for lipid and carbohydrate extraction; and 10 planulae were analyzed for protein. The number of planulae available varied throughout the year and dictated the experimental design. Ash, lipid, protein, and carbohydrate concentrations were expressed as percentages of dry planula weight. Percentage of ash was determined gravimetrically by placing pre-weighed planulae in a muffle furnace at 500°C for 4 hr (see Slattery and McClintock, '95). Lipid extraction was carried out three times with ethyl acetate (methods previously described by Ben-David-Zaslow and Benayahu, 1999). Protein was measured using Peterson's modification of the micro-Lowry spectrophotometric technique (Peterson, '77), using as a standard the protein bovine serum albumin (Sigma). Carbohydrate was measured using the phenol-sulfuric acid method of Dubois et al. ('56), using as a standard D-glucose (Sigma). Refractory material was estimated by subtracting the previously noted composition from 100%, and for the purposes of energetic quantification was assumed to represent insoluble proten (Slattery and McClintock, '95). Energetic composition of the planulae was calculated indirectly by multiplying appropriate coefficients by

each of the organic components: 9.46, 5.65, and 4.1 cal $\times$ g^{-1} for lipid, protein, and carbohydrate respectively (Fruton and Simmonds, '53). Total energy content of one planula was then calculated as the sum of the energy values attributed to each organic component. The same procedures were also carried out on planulae that had been cultured for 15 days. These were washed with autoclaved FSW (0.2 μm) to remove organic debris every 1–2 days and frozen in batches on day 3, 5, 8, and 15 for the biochemical analysis (three replicates were analyzed for each age).

Oxygen consumption of Heteroxenia fuscescens *planulae*

In August 1995 and 1998, planulae were transferred alive to Tel-Aviv in 500-ml PVC containers filled with 0.2 μm FSW, for respiration experiments. All glassware and seawater used in these experiments were autoclaved. Rate of oxygen consumption was measured for two and three batches respectively of 10 planulae each, derived from different colonies. Metabolic rate of planulae was measured with a MI-730 micro-oxygen electrode (Microelectrodes, Inc.), in a custom-built glass respirometer. The design chamber consisted of a water jacket and a chamber with a maximum volume of 5 ml. A narrow capillary tube (<1 mm diameter) through the seal allowed for compensation of pressure changes, while gas exchange was negligible. Water temperature within the respirometer was regulated by means of a water bath equipped with a circulation pump, circulating water through the water jacket in a constant temperature of 27°C similar to the ambient Red Sea temperature. Mixing within the chamber was achieved by means of a small (~8 mm) stirrer, placed near the electrode membrane and above a standard magnetic stirrer. An OM-4 Oxygen meter (Microelectrodes, Inc.) interfaced (AC-PC, Strawberry Tree Computers, Inc.) with an IBM-compatible PC allowed computer-logging of data, using Work Bench for Dos programming. Calibration procedures included both air-saturated seawater at 27°C to determine saturation voltage, and N_2 to remove oxygen for baseline determination. Oxygen levels were monitored on a computer screen to determine saturation and baseline levels. The background consumption of the electrode was measured for about 30 min before and after placing the planulae in the respiration chamber. The oxygen consumption rate (VO_2, μl planula^{-1} h^{-1}) was calculated using the following equation (Beiras and Pérez Camacho, '94):

$$VO_2 = (V/nt)\,(\Delta CO_2 - \Delta C_b)$$

where V = the volume of water, n = the number of planulae, t = the time interval, ΔCO_2 = the change in oxygen concentration at the experiments, and ΔC_b = the change in background oxygen consumption. Metabolic energy expenditure was calculated from the oxygen consumption using the conversion factor of 19.61 J ml^{-1} O_2 (Fruton and Simmonds, '53).

All respiration experiments were conducted in FSW, natural Red Sea water, run for about 2 hr. Calculations were made only for a decrease of 10–20% in oxygen levels, since a decrease of less than 10% is considered as falling within the possible error of the apparatus, while a decrease of above 20% could have negatively affected normal respiration rates.

Uptake of dissolved organic matter (DOM) from the water by Heteroxenia fuscescens *planulae*

In order to examine the possible uptake of amino acids from sea water by the planulae, an experiment was performed in August 1998. All glassware and seawater used in these experiments were autoclaved. Two replications of the experiment were conducted, for each 75 freshly released planulae of *Heteroxenia fuscescens* were washed three times in an autoclaved FSW (0.2 μm) to remove organic debris and bacteria, and then incubated in 50 ml of an equimolar mixture of seven neutral amino acids [Gly (glycine), Tyr (tyrosine), Met (methionine), Val (Valine), Phe (phenylalanine), Iso (isoleucine), Leu (leucine)], and 1 basic amino acid [Lys (lysine)], all from Sigma Chemical Co. Each amino acid was present at an initial concentration of 250 nM. The same amino acid composition was added to two flasks with no planulae to serve as a control for any changes that might have occurred due to surface absorption (Manahan et al., '89). A few drops of the water used to wash the planulae prior to the experiment were added to one of the control flasks to exclude the possibility that any changes observed in the amino acid concentration might be due to bacteria derived from the planulae surface. For each sampling, a 500-μl volume of the medium was removed and passed through a 0.2-μm Polycarbonate filter held in 25-mm housing (Gelman Sciences). Samples were taken hourly for the first 6 hr of the experiment and then an additional one after 24 hr. The seawater samples were frozen for 24 hr until determination of the amounts

of individual amino acids in the medium using high-performance liquid chromatography (HPLC) and fluorescence detection.

Fluorescence derivation of the amino acids was prepared following the technique of Lindroth and Mopper ('79), by mixing the samples with o-phthaldialdehyde reagent (OPA, Sigma) in a 1:1 ratio. Chromatography was carried out with a Waters Radial Pak, C_{18} Column (15 cm × 3.9 mm, particle size 5 μm). The buffers were controlled by a Waters 626 LC System with two Waters model 626 pumps. Mobile phases were composed of (A) 1:19:80 tetrahydrofuran : methanol : 0.05 M sodium acetate (pH = 6.3) and (B) HPLC grade methanol. Flow rate was 0.7 ml min^{-1} and the gradient program was 30% B for 2 min from initiation of the program, linear increase to 40% B in 8 min, 40% B for 2 min, increase to 50% B in 5 min, 50% B for 2 min, increase to 100% B in 6 min, 100% B for 5 min, followed by a return to initial conditions at the end of each 30-min run. The separated amino acids were detected with a Waters scanning fluorescence detector 474 using an excitation and emission wavelengths of 330 and 418 nm, respectively. The amino acids in the experimental samples were quantified by comparing their peak areas to those obtained with a standard containing the eight amino acids used in the study (see above).

Net uptake rates for each amino acid were determined from first-order depletion constants "k" (see Segel, '76, p 227; Jaeckle and Manahan, '89):

$$k = (\ln [S_0] - \ln [S_t])/t$$

where S_0 = the substrate concentration at the beginning of the experiment and S_t = the substrate concentration at the end of the experiment, and t = time. 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 $planula^{-1}$ hr^{-1}) was calculated by multiplying the number of moles of each amino acid presented at the beginning of the experiment by the total number of planulae present in the experiment.

RESULTS

Biochemical composition of Heteroxenia fuscescens *planulae*

Freshly released planulae

There was no significant difference in the biochemical composition of planulae of *Heteroxenia fuscescens* among years and seasons (one-way ANOVA, $P > 0.05$). In the course of the entire study period (1994–1997) planulae contained a monthly average percentage of 2.2 ± 0.1 ash, 51.5 ± 26.9 lipid, 33.6 ± 15.6 protein, and 1.3 ± 0.9 carbohydrate (n = 36 months, Table 1). Calculation of the average energetic content of a planula revealed a value of 1.63 ± 0.84 J $planula^{-1}$ (see Materials and Methods, and Table 1). Significant seasonal differences in planulae weight were noted only between the summer and the other seasons during the monitoring period, whereas no such differences were noted between other seasons (LSD-test, $P < 0.05$). In summer the planulae weighed on average 0.16 ± 0.1 mg (n = 12 months), whereas during the rest of the year they weighed less (fall: 0.044 ± 0.006 mg, n = 5 months; winter: 0.088 ± 0.05 mg, n = 8 months; spring: 0.033 ± 0.02 mg, n = 4 months).

Senescent planulae

Figure 1 presents the mean (± SD) dry weight and biochemical composition (lipid, protein and carbohydrate) of *H. fuscescens* planulae over time. A significant decrease (41%) from the initial weight, 0.029 ± 0.006 mg, took place in the planulae dry weight within 15 days (Fig. 1a, one-way ANOVA, $P < 0.05$). Significant differences over time were also found in the lipid and carbohydrate concentration but not in protein (Fig. 1b–d, one-way ANOVA, $P < 0.05$, $P < 0.001$, and $P > 0.05$, respectively). Lipid and carbohydrate levels on day 15 of the experiment were 0.018 ± 0.001 and 0.0007 ± 0.0002 mg respectively, which represent 50% and 83% reductions from their initial concentrations, respectively. Protein concentration declined by only 0.005 mg, corresponding to 20% of the protein concentration in freshly released planulae.

TABLE 1. Biochemical composition (% dry weight $planula^{-1}$; mean ± SD) and the calculated proximate energetic composition (J $planula^{-1}$; mean ± SD) of a one-day-old planula of Heteroxenia fuscescens *in the course of the entire study period (October 1994 to September 1997)*

	Ash	Lipid	Protein	Carbohydrate	Refractory	Total
Biochemical composition	2.2 ± 0.01	51.5 ± 26.9	33.6 ± 15.6	1.3 ± 0.9	11.47 ± 9.3	
Calculated energetic content	—	1.32 ± 0.36	0.57 ± 0.25	0.0013 ± 0.0004	0.016 ± 0.007	1.63 ± 0.84

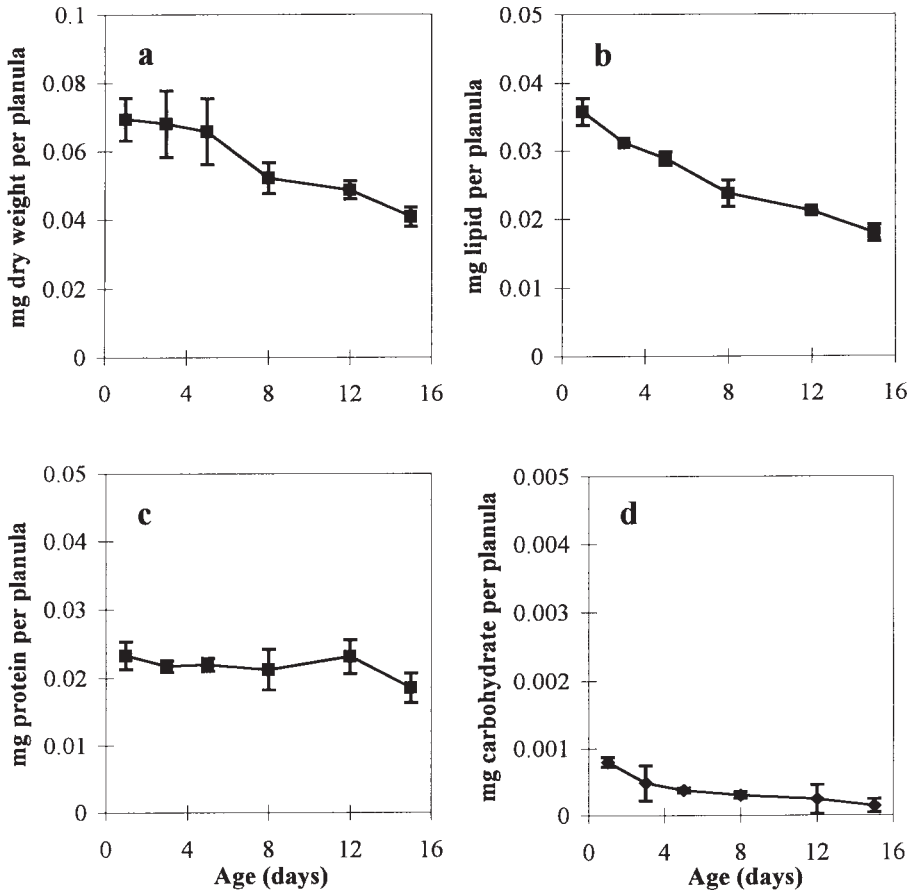

Fig. 1. Biochemical composition (mg dry weight; mean ± SD) of freshly released and senescent planulae of *Heteroxenia fuscescens.* 1–15-day-old planulae: (**a**) dry weight; (**b**) lipid concentration (n = 3 replications, 35 planulae each); (**c**) protein concentration (n = 3 replications, 10 planulae each); (**d**) carbohydrate concentration (n = 3 replications, 35 planulae each).

Oxygen consumption of Heteroxenia fuscescens *planulae*

Metabolic rate of an *H. fuscescens* planula expressed as oxygen consumption was 0.06 ± 0.02 µl O_2 planula^{-1} hr^{-1} (n = 5 replications, 10 planulae each). Metabolic energy expenditure, calculated from the oxygen consumption, was 0.029 ± 0.012 J planula^{-1} day^{-1}.

Uptake of dissolved organic matter (DOM) from the water by Heteroxenia fuscescens *planulae*

Reproductions of HPLC chromatograms were used to calculate uptake rates (Fig. 2). The amount of each of the eight different amino acids whose uptake from the media was tested, was found to decrease within 24 hr. Control experiments, incubating the same concentrations of amino acids in replicate flasks with no planulae, showed no detectable removal of amino acids from the water. However, removal of amino acids was detected after 24 hr in the control that contained a few drops of sea water in which planulae had been present (see Materials and Methods). These decreases were subtracted from the results obtained in the experiments on amino acid uptake. The planulae were shown to remove all the amino acids tested, both basic and neutral. The percentage of amino acids removed during the first 3 hr ranged from 3–9%, depending on the individual amino acid, and in the following 6 and 24 hr of the experiment, 8–15% and 35–62% removal were noted, respectively (Table 2).

Concentrations of all amino acids tested diminished with time (Fig. 3a). These data were used to calculate first-order depletion constants for each amino acid (see Materials and Methods), and individual rates of uptake were calculated (Fig. 3a–d). The uptake rate by 2-day-old planulae ranged between 8.8 ± 0.8 and 5.6 ± 0.5 pmol planula^{-1} hr^{-1} and the total uptake rate was 60.4 pmol planula^{-1} hr^{-1} (n = 2 replications, 75 planulae each). Although each amino acid was initially present at equimo-

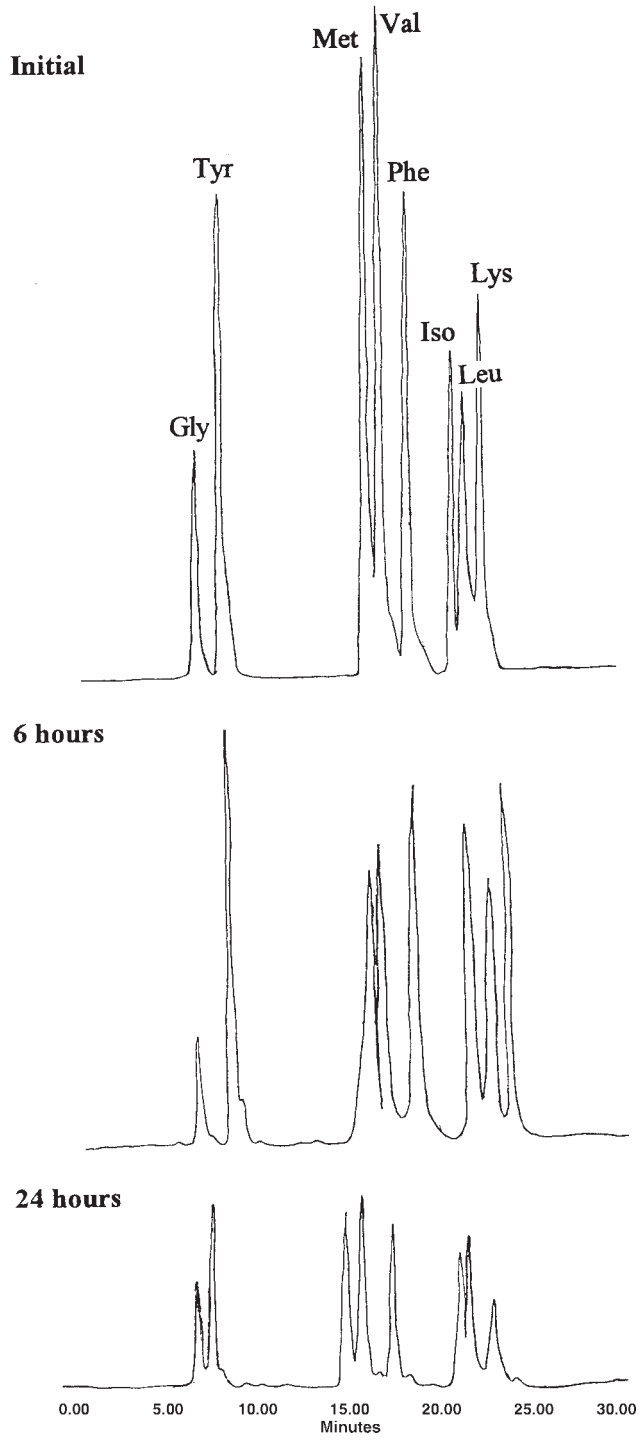

Fig. 2. HPLC chromatograms showing the sequential depletion of eight amino acids from seawater during the incubation of 2-day-old *Heteroxenia fuscescens* planulae. At t_0 each of the amino acids was present at concentration of 250 nM.

lar concentrations, (250 nM), there was a faster uptake for the neutral-nonpolar amino acids (Met, Val, Leu, Ile) than for polar (Gly) and basic (Lys) amino acids, a pattern which was found to be significant only after 24 hr of the experiment (one-way ANOVA, $P < 0.05$).

DISCUSSION

Only sparse information is available on biochemical components in marine invertebrate larvae in general and in coral planulae in particular. Most of the studies deal with lipid composition of the larvae tissue and with the relationship to growth and development (Kung and Ciereszko, '77; Suthers et al., '92; Arai et al., '93). McClintock and Pearse ('86) reported that dry tissue of eggs and juveniles of Antarctic echinoids and asteroids with lecithotrophic development, contained 33–53% protein and 36–59% lipid. Kung and Ciereszko ('77) found that lipids accounted for 75% of the dry weight of eggs of the gorgonian *Pseudopterogorgia americana*. Arai et al. ('93) reported that planulae of three species of stony corals from the genus *Acropora* and *Montipora* contained 60–70% lipid from dry weight. Prior to the present study, the only reference dealing with the biochemical profile of a coral planulae was that of Richmond ('87), reporting a biochemical composition of the stony coral *Pocillopora damicornis* of 70% lipid, 17% protein, and 13% carbohydrate. Our study is the first to present a long-term biochemical profile of soft coral planulae. In the tissues of the lecithotrophic planulae of *Heteroxenia fuscescens* the relative percentage of each biochemical component differ from the above values, yet lipid are the most common followed by protein and carbohydrate (Table 1).

Titlyanov et al. ('98) showed that lipid stores, yolk granules, and photosynthetic products in planulae of *Stylophora pistillata* contain insignificant quantities of nitrogen and phosphorus, which are necessary for metamorphosis. They suggested that one way in which planulae can gain these nutrients is by absorption of DOM. However, no study to date has examined the possible uptake by coral planulae of DOM, a resource known to be commonly utilized by other marine larvae (e.g., Davis and Stephens, '84; Jaeckle and Manahan, '89; Manahan, '90; Jaeckle, '94; see Table 3). Considering the large number of studies conducted on planulae during the last decade (Harrison and Wallace, '90; Benayahu, '97), and the absence of zooxanthellae in most planulae stages of broadcasting stony and soft corals (see Benayahu and Schleyer, '98), it is quite intriguing that DOM uptake by coral larvae has remained unexplored. The present study shows for the first time that planulae of *Heteroxenia fuscescens* can take up dissolved

TABLE 2. Uptake of amino acids by Heteroxenia fuscescens *planulae during 3, 6, and 24 hours*[1]

	Neutral									
	Nonpolar			Polar				Basic		
	3 hr	6 hr	24 hr	3 hr	6 hr	24 hr		3 hr	6 hr	24 hr
Phe	7	14	54	Gly 3	8	35	Lys	6	13	49
Met	6	15	62	Tyr 7	11	53				
Val	7	9	57							
Leu	9	15	60							
Ile	9	14	57							

[1]Numbers indicate the percentage of amino acid removal from the medium (initial concentration was 100%).

free amino acids from seawater (Figs. 2, 3). The concentrations of amino acids used in the experiments fall within values published for coastal marine environments, ranging from 10 to 1,500 nM (Mopper and Lindroth, '82; Jaeckle and Manahan, '89). Recent studies reported total concentration-values of dissolved free amino acids from various tropical waters associated with coral reefs such as Bermuda (100–212 nM; Ferrier, '91), Kenya (863–1076 nM; Schlichter and Liebezeit, '91) and

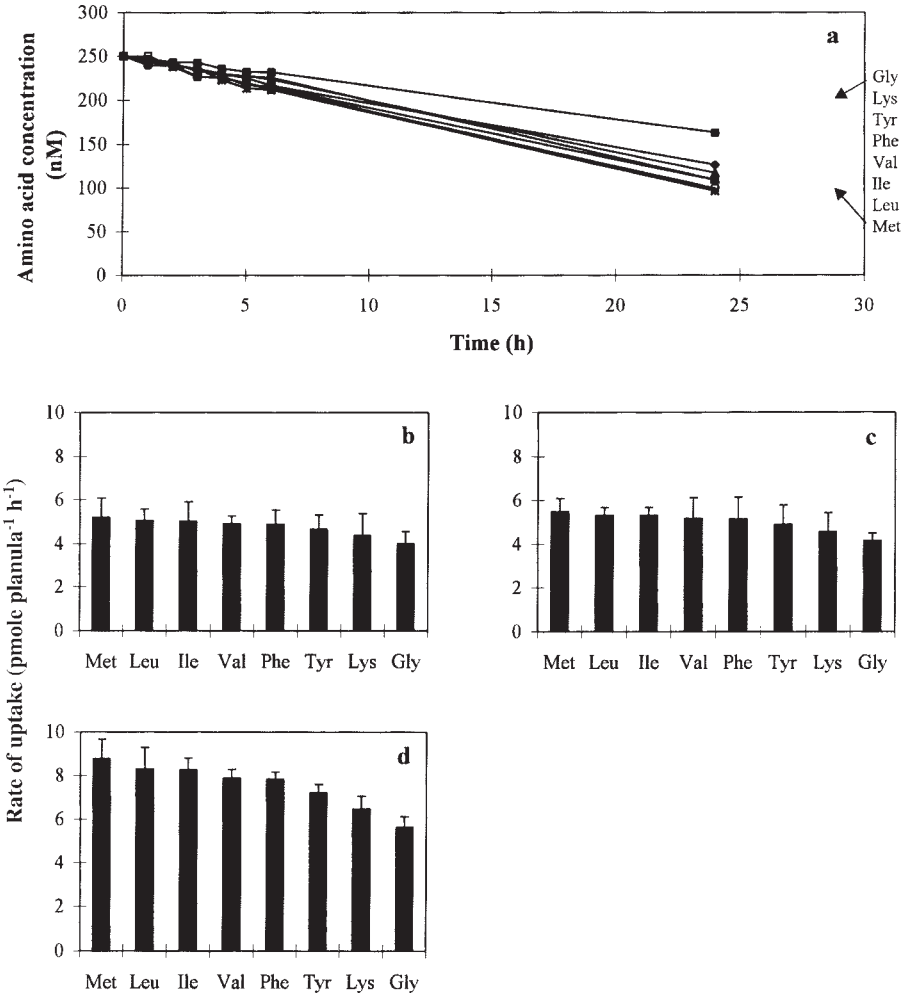

Fig. 3. Depletion of a mixture of eight amino acids from the medium by 2-day-old *Heteroxenia fuscescens* planulae (75 planulae in 50 ml). (**a**) Raw data of the amino acids depletion used to calculate first-order depletion constant for each amino acid assuming an initial concentration of 250 nM. (**b–d**) Uptake rate for amino acids after 3 (b), 6 (c) and 24 (d) hr. The r^2 values for the linear regressions of the ln-transformed rates were all >0.95.

TABLE 3. Summary of studies testing amino acid uptake in marine invertebrate larvae: (a) studies using HPLC technique; (b) studies using radioisotopic technique

Species	Larval type	Uptake rate (units)	Amino acids	Reference
(a)				
Echiura				
Urechis caupo	Trochopore	Summed net flux: 787–1751 fmol larva^{-1} hr^{-1}	Ile, Leu, Met, Phe, Val	Manahan et al., '89
Mollusca Gastropoda				
Haliotis rufescens	Veliger	0.27–0.6 pmol larva^{-1} hr^{-1} combined rate: 6.97 pmol Larva^{-1} hr^{-1}	Phe, Met, Val, Ala, Leu, Ile, Gly, Ser, Arg, Asn, Thr, Tyr, Asp, Glu, His, Lys	Jaeckle and Manahan, '89
Bivalvia				
Crassostrea giga	Veliger	5–70 fmol larva^{-1} hr^{-1}	Phe, Met, Val, Ala, Leu, Ile, Gly, Ser, Arg, Asn, Thr, Tyr, Asp, Glu, His, Lys	Manahan, '89
Echinodermata				
Dendraster excentricus		0.08–3.66 10^{-13} Larva^{-1} hr^{-1} combined rate: 2.04 pmol larva^{-1} hr^{-1}	Phe, Met, Ala, Leu, Ile, Ser, Arg, Asn, Tyr, Asp, Glu, His, Lys, Tau	David and Stephens, '84
(b)				
Porifera				
Tedania ignis	Parenchymula	2.73 ± 0.6 pmol larva^{-1} hr^{-1}	Ala	Jaeckle, '95
Mollusca Gastropoda				
Haliotis rufescens	Veliger Trochophore	0.477 pmol larva^{-1} hr^{-1}	Ala Ala	Jaeckle and Manahan, '89
Haliotis rufescens	Trochophore Veliger	J_{max} = 22.7 pmol larva^{-1} hr^{-1} J_{max} = 71.1 pmol larva^{-1} hr^{-1}	Ala	Manahan et al., '89
Haliotis rufescens	Veliger Plantigrade	J_{max} = 61.2 pmol larva^{-1} hr^{-1} J_{max} = 182 pmol larva^{-1} hr^{-1}	Ala	Shilling et al., '96
Bivalvia				
Crassostrea gigas *Ostrea edulis* *Mytilus edulis* *Pecten maximus*	Pediveliger Newly settled	Autoradiographic shoed uptake and incorporation	[^{3}H]Gly	Manahan and Crisp, '83
Crassostrea giga	Veliger	Increase during larval development	Ala	Manahan et al., '89
Crassostrea giga	Veliger	J_{max} = 4.6 and 2.2 pmol larva^{-1} hr^{-1}	Ala, Leu	Manahan, '89
Echinodermata Echinoidea				
Strongylocentrotus purpuratus	Prime-stage Pluteus-stage	J_{max} = 8.1 pmol larva^{-1} hr^{-1} J_{max} = 12.3 pmol larva^{-1} hr^{-1}	Ala	Manahan et al., '89
Paracentrotus lividus	Fertilized eggs (60 min)	J_{max} = ~75, ~100 and 1.5 pmol μg^{-1} hr^{-1}	Ala, Val	Allemand, et al. '85
Asteroidea				
Odontaster validus	Bipinnaria-stage	J_{max} = 6.51 pmol μg^{-1} hr^{-1}	Ala	Shilling and Manahan, '94
Asterina miniata	Bipinnaria-stage	J_{max} = 17.15 pmol μg^{-1} hr^{-1}	Ala	Manahan, '94
Acanthaster planci	Gastrula (24 hr) Bipinnaria (96 hr) Brachiolaria (192 hr)	J_{max} = 15.5, 11.0, and pmol μg^{-1} hr^{-1} J_{max} = 30.2, 31.3, and 3.8 pmol μg^{-1} hr^{-1} J_{max} = 35.0, 24.6, and 3.7 pmol μg^{-1} hr^{-1}	Ala, Leu, His	Hoegh-Guldberg, '94
Bryozoa				
Bugula nertina		0.366 ± 0.015 pmol larva^{-1} hr^{-1}	Ala	Jaeckle, '94

the Great Barrier Reef (0–170 nM; Hoegh-Guldberg and Welborn, '92, in Hoegh-Guldberg, '94). In the current study the planulae utilize all the available amino acids both basic and neutral. The percentage of amino acid depletion over 3 hr ranged from 3–9%, depending on the type of the amino acid. During 6 and 24 hr depletion ranged from 8–15% vs. 35–62%, respectively (Table 2). These results illustrate that even though each of the amino acids was initially present at equimolar concentrations, there was a much faster uptake for the neutral, nonpolar Met, Val, Leu, and Ile than for polar (Gly) and basic (Lys) amino acids. The ability to transport acidic, basic, and neutral amino acids or only neutral ones has been reported in other studies dealing with such uptake by marine invertebrate larvae (see Manahan, '90; Table 3). Manahan ('89) concluded that no inherent differences exist in transport ability of specific amino acid classes among invertebrate groups, and that the biological significance of these qualitative differences is still unknown.

The rate of amino acids uptake by 2-day-old planulae ranged between 8.8 ± 0.8 and 5.6 ± 0.5 pmol planula^{-1} hr^{-1} in a mixture of eight amino acids used in our study. Measuring the simultaneous uptake of multiple substrates from mixtures provides useful qualitative data on uptake rates. However, data obtained in this way may be inaccurate in determining the quantitative relationship between transport rates for different substrates (Manahan, '89). In Manahan's ('89) study, measurements of amino acids utilization from a mixture with HPLC reported a preferential uptake of leucine over alanine. The reverse was seen, however, when the kinetics of transport for these two substrates were studied independently, and alanine uptake rate was shown to be higher than that of leucine. In contrast, Jaeckle and Manahan ('89) found for trochophores and veligers of *Haliotis rufescens* that the rate of alanine influx was equal to the net substrate flux, as evidenced by alanine depletion that was identical when measured independently in a mixture by HPLC or individually by isotope techniques. Competition experiments using a wide range of amino acids have been carried out to study the specificity of amino acid transport in unfertilized and fertilized eggs of sea urchins (Allemand et al., '85). In unfertilized eggs, valine uptake was reduced in the presence of amino acids with branches or a ring such as Leu, Phe, and Val, which characterized the L system. Linear amino acids (Ala, Gly, Ser), which are specific to A and ASC systems, did not interfere with valine uptake (for details see Allemand et al., '85). Competition experiments in fertilized eggs showed that all the amino acids tested (Gly, Ala, Ser, Val, Phe, Leu) compete with alanine for transport. It is important to simulate the concentrations and mixtures of individual amino acids that occur in the natural environment of the organism in question in order to determine ecologically realistic uptake rates. Considering the poor state of knowledge about larval distributions in the sea (Young and Chia, '81), the possible amino acid concentrations that a coral planula might encounter in its microhabitat can at this stage only be speculated about.

Several studies have shown that longevity and competence periods are related to the energy content of the released larvae and to the energy obtained during their planktonic phase (Richmond, '87, '88; Pechenik, '90; Qian et al., '90; Harms, '92; Jaeckle and Manahan, '92; Havenhand, '93). These energetic considerations are mostly suitable for species with lecithotrophic larvae competent to metamorphose at release from the parent, or soon after (Pechenik, '90). It has been concluded that knowledge of the initial amount of energy contained within a larva, the rate at which energy is expended under natural conditions, and the amount of energy that must be held in reserve to permit successful metamorphosis, allows estimation of the length of time that metamorphosis can be delayed before the ability to metamorphose successfully is compromised (Lucas et al., '79; Richmond, '87, '88; Pechenik, '90; Jaeckle, '94). For example, cyprid larvae have the ability to swim for about four weeks at 10°C without utilizing reserves required for their metamorphosis, but the survival of larvae triggered to metamorphose beyond this period declined dramatically (Lucas et al., '79). Jaeckle ('94) found that larvae of the bryozoan *Bugula neritina* are generally capable of metamorphosis within 2 hr of release, and that DOM transport provides the potential to extend their competence period by serving as an alternative source of energy. In a previous study it has been noted that 2-day-old planulae of *H. fuscescens* possessed an average of 0.5 ± 0.05 cal planula^{-1}, and that their energy expenditure, during the next four days was minimal: 2.5×10^{-3} cal planula^{-1} day^{-1}, i.e., 0.52 µl O_2 planula^{-1} day^{-1} (Ben-David-Zaslow and Benayahu, '96). In the present study we calculated from the biochemical profile of the planulae an average of 0.4 ± 0.2 cal planula^{-1} (Table 1, converted from joules to calories) and an energy expenditure, calculated from the oxy-

gen consumption, of $7 \times 10^{-3} \pm 2 \times 10^{-3}$ cal planula^{-1} day^{-1}. In his study on stony corals, Richmond ('88) measured a caloric content of 0.74 cal planula^{-1} and energy expenditure of 5.06×10^{-3} cal planula^{-1} day^{-1} for *Pocillopora damicornis* planulae and 0.29 cal planula^{-1} and 2.93×10^{-3} cal planula^{-1} day^{-1} for *Acropora tenuis*. If one uses Richmond's ('88) equation predicting the competence period of planulae from energy content and rate of expenditure, a competence period of 155 days was obtained for *H. fuscescens* planulae for the results of the bomb calorimeter (Ben-David-Zaslow and Benayahu, '96) and of 37 days for the results obtained in this study. A value of 49 days competence period was recorded for planulae of *H. fuscescens* (Ben-David-Zaslow and Benayahu, '96). The different results achieved from the calculation and from the experimental value may be due to the method being used, and to changes in the energy expenditure measured with planulae age.

Absorption of DOM by the planulae might contribute to their energy budget and thus extend their longevity and competence periods. The energetic contribution to *Heteroxenia fuscescens* planulae provided by the transport of free amino acids can be estimated by comparing the rate of substrate uptake with the measured metabolic rate. The total uptake rate of amino acids from the seawater by the planulae was 60.4 pmol planula^{-1} h^{-1}. The measured rate of oxygen consumption for 2-day-old planulae was 0.06 μl O_2 planula^{-1} h^{-1}. The catabolic weight equivalent of oxygen consumption for protein is 1.04 g protein ml^{-1} O_2 (Fruton and Simmonds, '53). The average molecular weight of the amino acids mixture used in this experiment was 137 g mol^{-1}. The rate of amino acid uptake is therefore equal to 8280 pg amino acids at the experimental concentration. Free amino acids contain 16% more chemically bound water relative to protein (Jaeckle and Manahan, '89); thus, an uptake rate of 8280 pg amino acids is equivalent to 7140 pg protein that equals 7×10^{-3} μl O_2. The potential contribution to the metabolic demand of planulae, from the uptake of amino acids, is estimated to be 11%. Hoegh-Guldberg et al. ('97), working on larvae of the crown of thorns starfish *Acanthaster planci* larvae in the Great Barrier Reef, found that amino acid concentration in the ambient water did not exceed 70 nM. They suggested that their uptake by the larvae does not appear to be due to energetic considerations, but may have a more significant impact on the nitrogen budget. In a field study it was found that uptake of dissolved amino acids by colonies of *Pocillopora damicornis* can contribute only about 11.3 % of the nitrogen demand. This contribution, however, may be an important source of nitrogen when other sources such as ammonium are scarce or during periods when high concentrations of dissolved amino acids become sporadically (Hoegh-Guldberg and Williamson, '99). Measuring the biochemical composition of senescent planulae of *H. fuscescens* revealed a decrease in lipid and carbohydrate concentrations, but not in protein (Fig. 1). Hence, these results imply that planulae may use DOM as a source for nitrogen.

Alcyonacean soft corals constitute a dominant group of organisms on the northern Red Sea reefs (Benayahu and Loya, '77, '81). Given the prevailing current regime in the Gulf of Eilat (Genin and Paldor, '98), larvae are advected from the southern Red Sea reefs to the Eilat within a period of a few weeks. The findings of the present study support the previous results (Ben-David-Zaslow and Benayahu, '96; '98), which indicate that soft coral planulae derived from the reefs of the southern Red Sea will be competent on arrival at the Eilat reefs and may recruit there.

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