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TAURINE METABOLISM IN LARVAE OF MARINE MOLLUSCS (BIVALVIA, GASTROPODA)

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Summary

Nonfeeding larvae of the gastropod *Haliotis rufescens* maintained a constant amount of taurine during embryonic and larval development and, since no *de novo* synthesis of taurine was observed in these larvae, the maternal endowment of taurine to the egg was sufficient for larval development to metamorphosis. In contrast, feeding larvae of the bivalve *Crassostrea gigas* increased their taurine content by a factor of 43 during growth to metamorphosis (from 86 to 311 μm, valve length). Taurine was not present in algae used to feed the larvae, suggesting that *de novo* synthesis of taurine by the larvae met their requirements. In unfed larvae, cysteic acid, cysteine sulfinic acid and hypotaurine were labeled from a [^{35}S]cysteine precursor, but taurine was not. Hyperosmotic treatment (from 33‰ to 44‰ salinity for up to 3 h) did not induce taurine synthesis in unfed larvae. However, larvae fed the alga *Isochrysis galbana* up-regulated their taurine synthesis from [^{35}S]cysteine by a factor of 11 (fed, 11.7±2.2 fmol taurine larva^{-1} h^{-1}; unfed controls, 1.08±0.33 fmol taurine larva^{-1} h^{-1}; means ± S.E.M.). Fed larvae also synthesized taurine from [^{35}S]methionine (18.4 fmol larva^{-1} h^{-1}). *I. galbana* contained 5 fmol cell^{-1} of cysteine and methionine (combined) and, based on known feeding rates, we calculated that there were sufficient taurine precursors in the algae to supply the taurine requirements of growing larvae. The lack of significant *de novo* taurine synthesis reported for adult bivalve molluscs has led to the conclusion that taurine is a dietary requirement. Our findings for larval forms differ in that there is significant *de novo* synthesis of taurine during development.

Key words: mollusc, larva, taurine, *Haliotis rufescens*, *Crassostrea gigas*.

Introduction

Taurine (2-aminoethane sulfonate), a nonprotein amino acid, is a small organic solute that can accumulate to high intracellular concentrations without perturbing macromolecules (Yancey *et al*. 1982). Although the functional roles of taurine are numerous and still being defined (Huxtable, 1992), its role in osmoregulation is well established. Marine invertebrates, most notably molluscs and arthropods, have high concentrations of taurine that regulate tissue osmolarity (Simpson *et al*. 1959; Lange, 1963; Allen and Garrett, 1971; Gilles, 1972; Bishop *et al*. 1983; Smith and Pierce, 1987).

Taurine synthesis from cysteine or methionine in mammalian systems is well-documented (Jacobsen and Smith, 1968; Huxtable, 1986). Studies by Allen and Garrett (1972) have shown that adults of the bivalve *Mya arenaria* have similar taurine biosynthetic pathways to mammals. Their studies, using ^{35}S-labeled methionine, revealed labeling of sulfur-containing intermediates in bivalves that were the same as those found in mammalian biosynthetic pathways. However, in adult molluscs, only small amounts of radioactivity from ^{35}S-labeled precursors appear in taurine (Allen and Awapara, 1960; van Thoai *et al*. 1963), indicating extremely slow rates of synthesis. Taurine synthesis rates in marine molluscs appear to be too slow to explain the high taurine levels found in tissues (Bishop *et al*. 1983), suggesting that adult molluscs acquire taurine *per se* from dietary sources. Diet, however, is an unlikely source of taurine for larval forms because the common algal species used as food by larval stages of marine invertebrates lack taurine (Brown, 1991; Flynn and Flynn, 1992; this study, see Fig. 3).

Larvae of the gastropod *Haliotis rufescens* are lecithotrophic (nonfeeding) during development and taurine is the major component in their free amino acid pools (Jaeckle and Manahan, 1989*a*). Taurine is also the major free amino acid in planktotrophic (feeding) larvae of the bivalve *Crassostrea gigas* (Manahan, 1989). Despite the importance of taurine to larval molluscs, little information is available regarding taurine

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requirements during development, sources of taurine for larvae (*de novo* synthesis, dietary source or uptake from sea water) or factors that regulate taurine synthesis. In this study, we investigated taurine metabolism during larval development of *Haliotis rufescens* and *Crassostrea gigas*, two species of molluscs with different life-history strategies.

Materials and methods

Chemicals

Amino acid standards, *o*-phthaldialdehyde (OPA), phenyl isothiocyanate (PITC) and ninhydrin were purchased from Sigma Chemical (St Louis, Missouri). Triethylamine was from Aldrich Chemical Co. (Milwaukee, Wisconsin). ^{35}S-labeled cysteine and methionine (specific activities approximately 44 TBq mmol^{-1}) were purchased from Du Pont/New England Nuclear Research Products (Wilmington, Delaware). The isotopic purities of both [^{35}S]cysteine (PITC-derivatized, see method below) and [^{35}S]methionine (OPA-derivatized, see below) were determined using high-performance liquid chromatography (HPLC) and fraction collecting, followed by liquid scintillation counting (Beckman, model LS 6000SC). The fractions collected at the elution times for hypotaurine and taurine (known from standards) contained radioactivity at background (50 cts min^{-1} or less) for both [^{35}S]cysteine and [^{35}S]methionine stocks.

Animals and algae

Fertilized eggs, trochophore larvae and veliger larvae of the gastropod *Haliotis rufescens* (red abalone) used for measuring taurine content were reared in flow-through culture containers. Sand-filtered sea water at varying ambient temperatures (range, 14–20 °C, winter to spring) was used for culturing. Radiolabeling experiments using gastropod larvae were carried out at 15 °C (ambient temperature for that particular culture) in filtered sea water (0.2 μm pore-size). Cultures from three independent spawnings were used for measuring changes in taurine content. Mass determinations (ash-free dry organic mass) of eggs and larvae of *H. rufescens* (culture 1) were made according to Jaeckle and Manahan (1989*b*).

Veliger larvae of the bivalve *Crassostrea gigas* (Pacific oyster) were reared in filtered sea water (0.2 μm pore-size, 24–25 °C) at 4–5 larvae ml^{-1}. Sizes (valve length, μm) of 50–60 individual larvae were measured for a given stage using an ocular micrometer. Microalgae (*Isochrysis galbana*) were added at approximately 45 000 cells ml^{-1} to feed the larvae, referred to as 'fed' in the experiments described below ('unfed' controls were not given algal food). All radiolabeling experiments using *C. gigas* were carried out at 24 °C.

Xenic cultures of *Dunaliella tertiolecta*, *Isochrysis galbana*, *Skeletonema costatum* and *Thalassiosira pseudonana* were harvested towards the end of the log phase of growth for determinations of free amino acid pools. Culture media was F/2 (Guillard and Ryther, 1962) with added silicate. Algal cultures were aerated, grown at 14–15 °C and continuously illuminated at 4.2×10^9–4.8×10^9 quanta cm^{-2} s^{-1}.

Amino acid analysis

Quantification of free amino acid pools and identification of ^{35}S-labeled amino acids

Measurements of free amino acid pools in algae and animals were made by extracting a known number of individuals into a 70:30 (v/v) ethanol:water solution. All water used was purified using a Nanopure water purification system (Barnstead/Thermolyne, Dubuque, Iowa). Material in the ethanol/water solution was ultrasonicated (Vibra Cell equipped with a 3 mm microprobe, Sonics and Material Inc., Danbury, Connecticut) for 3 s and then centrifuged (14 000 ***g***) for 15 min. Each sample was diluted prior to analysis of the OPA-derivatized amino acids by HPLC (protocol in Manahan *et al.* 1983). Radioactivity (^{35}S-label) in free amino acids, separated using HPLC, was measured either using an in-line radiochemical detector (Beckman, model 171, downstream of a Beckman 157 fluorescence detector) or by using a conventional fraction collector with subsequent liquid scintillation counting. A personal computer holding an E-Lab circuit board (OMS Tech, Miami, Florida) and software (OMS Tech) was used to acquire and integrate data from both detectors. Amino acid identification and quantification were based upon retention times and peak areas of standards.

Amino acid composition of algal proteins

Algal cells (*Isochrysis galbana*) were disrupted by ultrasonication. Proteins in the homogenate were then precipitated on ice for 20 min in a final concentration of 5 % trichloroacetic acid (TCA). Samples were centrifuged (14 000 ***g***, 15 min) to pellet the precipitate. The pellet was washed three times with HPLC-grade water (to remove free amino acids in the supernatant) and then resuspended by adding 200 μl of a 50:50 (v/v) formic acid:water solution. Samples of this solution (approximately 10 μg of protein) were dried in glass reaction tubes (6 mm×50 mm) using a Waters PicoTag Workstation (Waters Instruments, Milford, Massachusetts) and the protein was hydrolyzed under nitrogen by acid vapors (Tsugita *et al.* 1987). Amino acids were then derivatized with PITC for ultraviolet detection. A 20 μl injection volume was analyzed by HPLC using a PicoTag C-18 column (Waters Instruments, model 88131), heated to 40 °C with a column heater. Gradient elution of amino acids was performed with helium-sparged solvents (Bidlingmeyer *et al.* 1984).

Taurine synthesis in larvae

Metabolism of sulfur-containing amino acids in gastropod larvae

Veliger larvae of *Haliotis rufescens* were incubated (600 larvae ml^{-1}) in 10 ml of sea water for 3 h with [^{35}S]cysteine (92.5 kBq ml^{-1}) and unlabeled cysteine (final concentration 10 μmol l^{-1}). Time-course experiments were carried out by periodically (7–9 time points) filtering samples of 300 larvae onto 8 μm (pore-size) polycarbonate filters (25 mm diameter). The larvae were washed three times with filtered sea water, then the free amino acid pools were extracted by immediately placing the filter and larvae into 70 % ethanol.

Metabolism of sulfur-containing amino acids in bivalve larvae

Unfed larvae. Larvae (*Crassostrea gigas*) in this treatment were held for 18–24 h without algal food prior to the experiments. These 'unfed' larvae were incubated at 600 larvae ml^{-1} in 10 ml of sea water with [^{35}S]cysteine (92.5 kBq ml^{-1}) and unlabeled cysteine at 10 μmol l^{-1}. Lower concentrations of larvae and cysteine were used for experiments requiring longer incubations (50 larvae ml^{-1} with 37 kBq ml^{-1} [^{35}S]cysteine and 500 nmol l^{-1} unlabeled cysteine in a 100 ml container). Depending on the experiment, the incubation times ranged from 3 to 26 h with 6–8 samples being taken during each experiment.

Hyperosmotic treatment. High-salinity sea water was made by evaporating normal-salinity sea water (33‰) to 44‰. Unfed larvae of *Crassostrea gigas* were incubated (600 larvae ml^{-1}) in 10 ml of high-salinity sea water for 3 h with [^{35}S]cysteine (92.5 kBq ml^{-1}) and unlabeled cysteine at 10 μmol l^{-1}.

Fed larvae. Bivalve larvae of *Crassostrea gigas* were cultured either with (treatment condition) or without (control) the algae *Isochrysis galbana* for 18–24 h prior to experiments. Fed or unfed larvae (50 larvae ml^{-1} in a 100 ml container) were incubated in parallel experiments for 26 h in sea water containing cysteine (37 kBq ml^{-1} [^{35}S]cysteine and 500 nmol l^{-1} unlabeled cysteine). For measuring rates of taurine synthesis, larvae were removed at each time point, filtered onto 8 μm (pore-size) polycarbonate filters (25 mm diameter), washed and counted to obtain the exact number of larvae being extracted into ethanol (numbers ranged from 122 to 548 larvae per sample). Radioactivity appearing in the taurine peak, separated using HPLC and collected with a fraction collector, was then measured with a liquid scintillation counter. Rates of [^{35}S]taurine synthesis were converted to mol larvae^{-1} h^{-1} using the specific activity of [^{35}S]cysteine in the medium (see Discussion). Fed larvae were also tested for their ability to synthesize taurine from [^{35}S]methionine.

Normal-phase, two-dimensional thin-layer chromatography (TLC) was used to confirm the identity of the ^{35}S-labeled products initially identified with reverse-phase HPLC as being hypotaurine and taurine. TLC analyses of ethanol extracts were carried out according to the procedures of Jones and Heathcote (1966) using cellulose TLC plates (plastic, 20 cm×20 cm, E. Merck, Darmstadt, Germany). Separated amino acids were visualized by spraying the TLC plates with ninhydrin (1 % w/v ninhydrin in ethanol) and then heating the plates for 30 min at 60 °C. The ^{35}S-labeled amino acids were identified by exposing the plate to X-ray film (for 24 h), then comparing the location of the spots on the film with the location of ninhydrin-derivatized standards on the TLC plates.

Results

Organic mass and taurine content during lecithotrophic larval development

A regression analysis (ANOVA) showed that the slope of the change in organic mass during development for the gastropod *Haliotis rufescens* (Fig. 1A) was not significantly different from zero ($P \geqslant 0.05$). Similarly, taurine content per individual did not decrease significantly during development from the egg (day 0) to the metamorphically competent larva at days 4, 5 and 8 (three different cultures reared at different

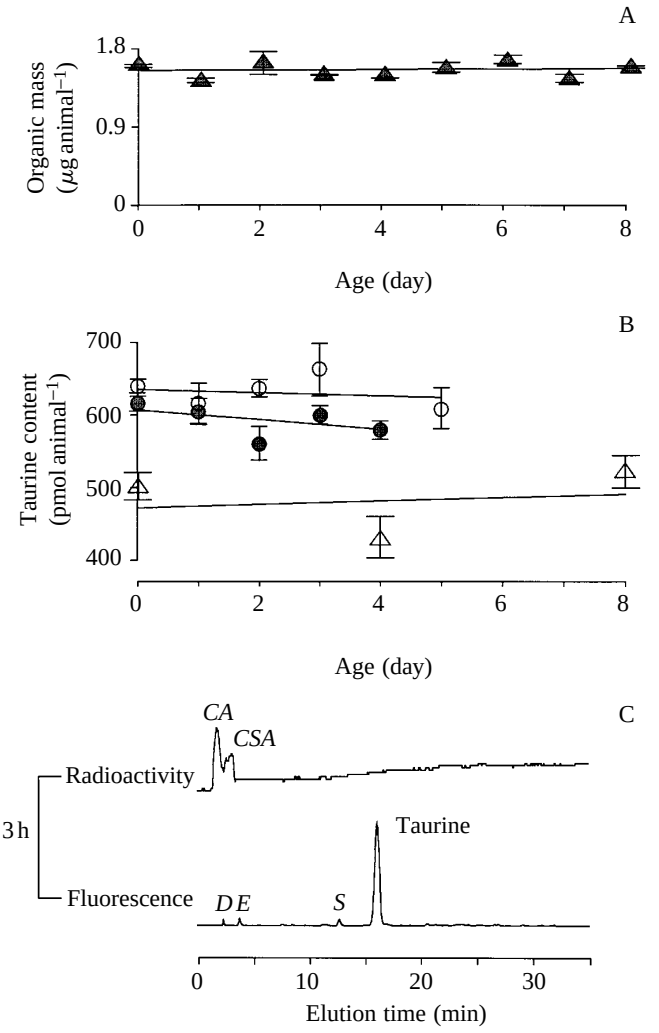

Fig. 1. (A) Ash-free dry organic mass (mean ± 1 S.E.M., N=4–6 for each mean) of the gastropod *Haliotis rufescens* through larval development (day 0=egg) to metamorphosis. The line is a fitted least-squares regression through all the mass data points (N=47) and the slope is not significantly different from zero (variance ratio=0.49, $P \geqslant 0.05$). (B) Taurine content (mean ± S.E.M., N=3 for each mean) measured with HPLC for three cultures (gametes from separate spawnings: ●, ○, △) of *H. rufescens* through development to metamorphosis. Different developmental times for the cultures to reach metamorphosis were a result of culturing at varying ambient seawater temperatures (range 14–20 °C). The slopes of the lines are not significantly different from zero, $P \geqslant 0.05$ (●, variance ratio=0.13; ○, variance ratio=0.19; △, variance ratio=0.04). (C) HPLC chromatograms of free amino acids extracted from 5-day-old veliger larvae of *H. rufescens* after incubation in sea water containing [^{35}S]cysteine. The upper chromatogram shows amino acids containing ^{35}S, the lower chromatogram shows amino acids in the free amino acid pool. *CA*, cysteic acid; *CSA*, cysteine sulfinic acid; *D*, aspartic acid; *E*, glutamic acid; *S*, serine.

ambient temperatures) (Fig. 1B; variance ratios 0.04–0.19 for three independent cultures, see legend for statistics).

Taurine synthesis by gastropod larvae

No measurable radioactivity appeared as taurine in 5-day-old veliger larvae of *Haliotis rufescens* after exposure to [^{35}S]cysteine (Fig. 1C), even though larvae synthesized metabolic intermediates for taurine synthesis (cysteic acid and cysteine sulfinic acid). Similar results showing no taurine synthesis were observed for seven other samples of larvae taken between 15 min and 3 h during this experiment.

The peaks present in the upper chromatogram (tracing from radiochemical detector) of Fig. 1C represent radioactivity (^{35}S) in amino acids synthesized from the precursor ([^{35}S]cysteine) and, therefore, can only be sulfur-containing amino acids. Peaks in the lower chromatogram (tracing from fluorescence detector) show the relative abundance of amino acids in the free amino acid pool (note that cysteic acid and cysteine sulfinic acid were not detectable; aspartic acid and glutamic acid were detectable). There was no co-elution of cysteic acid, cysteine sulfinic acid, aspartic acid and glutamic acid, since they had retention times (min) under the HPLC conditions used of 1.80, 2.20, 2.47 and 3.40, respectively. The fact that the detectors were placed in series resulted in the radiochemical peaks being shifted to the right relative to fluorescence detection. The time offset in radiochemical detection relative to fluorescence was 40 s, determined using ^{14}C-labeled and non-radiolabeled taurine standards. We used this time offset, together with the retention times of standards for cysteic acid, cysteine sulfinic acid and hypotaurine, to identify the ^{35}S-labeled amino acids eluting as radiochemical peaks.

Taurine content during planktotrophic larval development

Taurine content per larva of the bivalve *Crassostrea gigas* increased from 11.9 pmol for 86 μm larvae to 516 pmol in 311 μm larvae, an increase of 43-fold during the larval lifespan (Fig. 2A). The increase in taurine content was described by the equation: taurine content = $0.0054x^2+0.19x-55.8$ (where x is valve length of the larva in μm). The mole-percentage of taurine in the free amino acid pools of different-sized larvae remained at approximately 62 % throughout development (Fig. 2B).

Free amino acid pools of microalgae

Fig. 3A shows a chromatogram of free amino acid pools extracted from cells of the alga *Isochrysis galbana*, a species commonly used as a food source by larvae of *Crassostrea gigas* (Walne, 1974) and used to feed larvae in our study. The arrow indicates the elution time for taurine under the chromatographic conditions used, showing that taurine was not present in the algae (i.e. it was below the detection limit of HPLC). Likewise, free amino acid pools extracted from four other species of microalgae, commonly used as food by larval bivalves, also lacked any detectable amounts of taurine (Fig. 3B–E). Note that the different elution time for taurine in Fig. 3A (cf. Fig. 3B–E) was due to the slightly different HPLC conditions used for separation (i.e. the brand of C-18 column used).

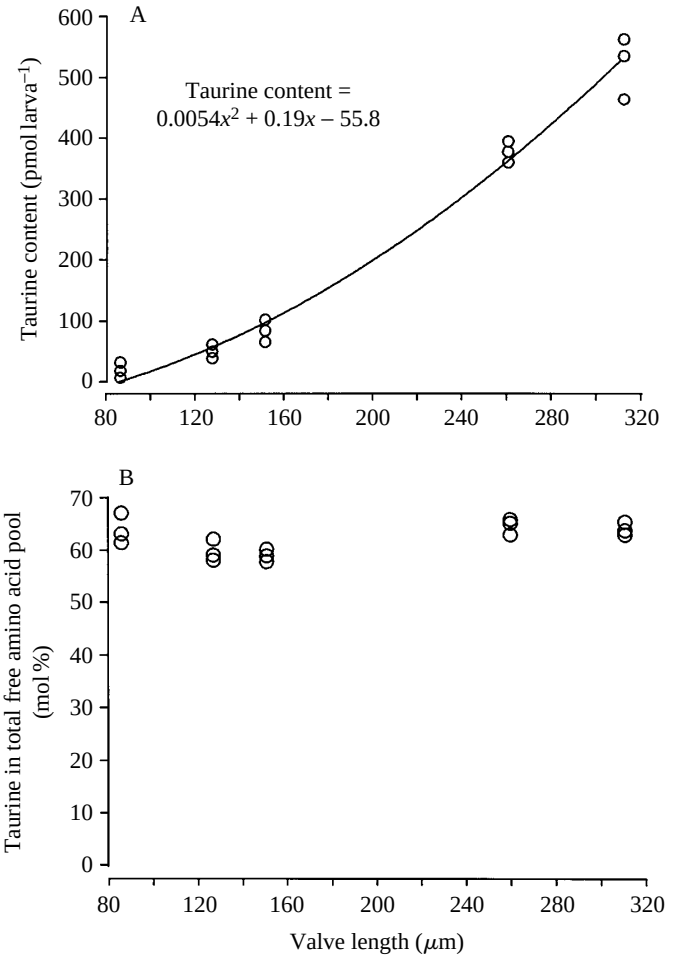

Fig. 2. (A) Taurine content during larval growth (valve length) of the bivalve *Crassostrea gigas*. Each data point represents an independent measurement of pool sizes. The fitted line was calculated with the equation shown, where x is valve length (in μm). (B) The mole-percentage of taurine in free amino acid pools of larvae (*C. gigas*) through development.

Taurine synthesis by bivalve larvae

Unfed larvae in normal-salinity sea water (33 ‰)

When deprived of algal food, larvae of *Crassostrea gigas* did not synthesize measurable amounts of taurine from [^{35}S]cysteine within 3 h (Fig. 4A), although ^{35}S appeared in cysteic acid, cysteine sulfinic acid and hypotaurine. Six other samples taken between 15 min and 3 h also showed no taurine synthesis, but label did appear in sulfur-containing intermediates within the first sampling period (15 min).

Unfed larvae in hyperosmotic sea water

In larvae of *Crassostrea gigas* incubated for 3 h in 44 ‰ sea water with [^{35}S]cysteine, radioactivity appeared in cysteine sulfinic acid and hypotaurine, but not in taurine (Fig. 4B). Six other samples taken during the experiment also had no measurable label in taurine.

Fed larva

In larvae of *Crassostrea gigas* that were fed microalgae

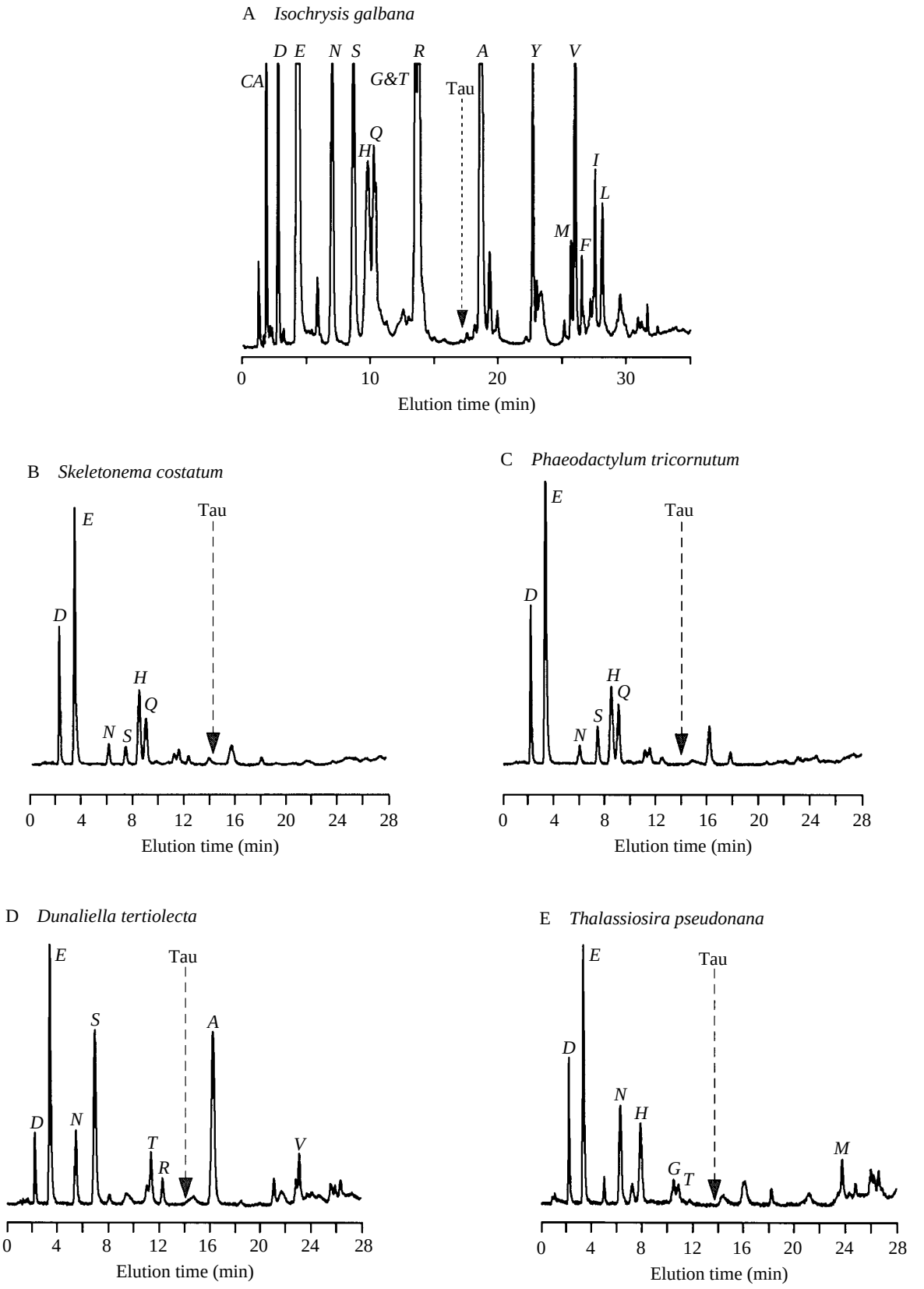

Fig. 3. Chromatograms (A–E five different species) of the free amino acids in algal cells measured with HPLC. Dashed lines and arrows indicate elution times for taurine based on standards. Detection limit for taurine was 17 amol cell^{-1}. Tau, taurine; *CA*, cysteic acid; *D*, aspartic acid; *E*, glutamic acid; *N*, asparagine; *S*, serine; *H*, histidine; *Q*, glutamine; *G*, glycine; *T*, threonine; *R*, arginine; *A*, alanine; *Y*, tyrosine; *M*, methionine; *V*, valine; *F*, phenylalanine; *I*, isoleucine; *L*, leucine.

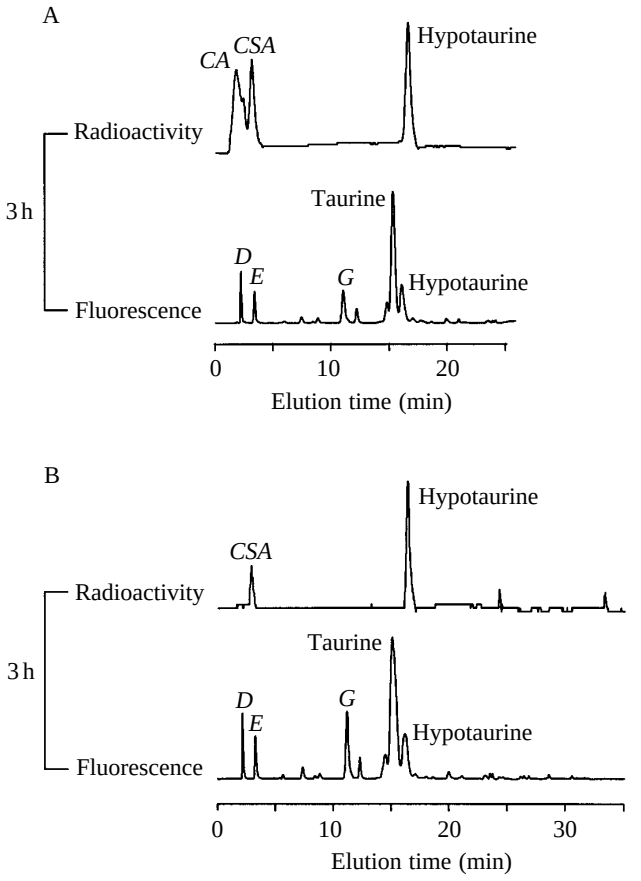

Fig. 4. Chromatograms of free amino acids, measured with HPLC, of unfed bivalve larvae (*Crassostrea gigas*) after (A) a 3 h exposure to [^{35}S]cysteine in normal-salinity sea water (33‰) and (B) 3 h in hyperosmotic sea water (44‰). *CA*, cysteic acid; *CSA*, cysteine sulfinic acid; *D*, aspartic acid; *E*, glutamic acid; *G*, glycine.

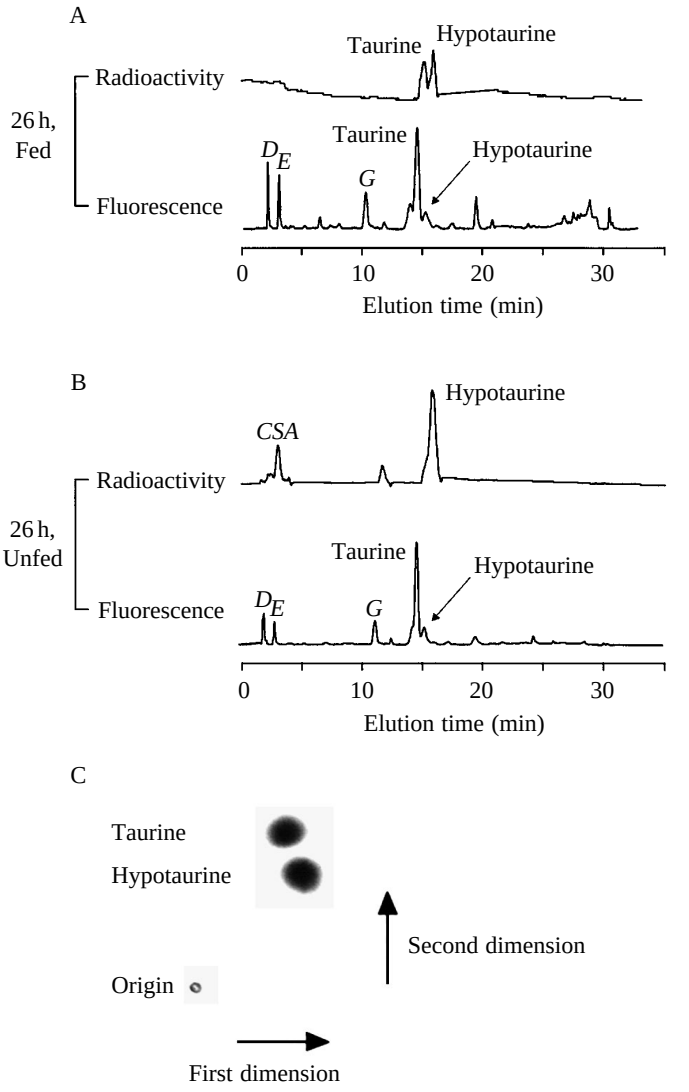

Fig. 5. Chromatograms of free amino acids of bivalve larvae (*Crassostrea gigas*) measured with HPLC. Distribution of ^{35}S after a 26 h exposure of larvae to [^{35}S]cysteine in sea water for (A) fed larvae or (B) unfed larvae. *D*, aspartic acid; *E*, glutamic acid; *G*, glycine; *CSA*, cysteine sulfinic acid. (C) Autoradiogram showing labeling pattern of free amino acids from fed larvae exposed for 26 h to [^{35}S]cysteine (same sample as A above), re-analyzed using normal-phase two-dimensional thin-layer chromatography.

(*Isochrysis galbana*) during incubation with [^{35}S]cysteine, much of the label (approximately 50 %) appeared as taurine after a 26 h exposure (Fig. 5A). In contrast, no label appeared as taurine (Fig. 5B) in a parallel 26 h experiment where larvae from the same culture were not fed algae (control). Normal-phase TLC analysis (Fig. 5C) of extracted pools from fed larvae (26 h sample re-analyzed) confirmed the identity of the radiolabeled products as taurine and hypotaurine, as initially identified using reverse-phase HPLC (Fig. 5A, upper chromatogram).

Effect of feeding state on rates of taurine synthesis

Fed larvae of *Crassostrea gigas* synthesized taurine from [^{35}S]cysteine at 11.7±2.2 fmol larva^{-1} h^{-1} (S.E.M.) (Fig. 6A). In a parallel experiment, unfed larvae had an 11-fold lower rate of taurine synthesis, 1.08±0.33 fmol larva^{-1} h^{-1} (Fig. 6A). Fed larvae also synthesized taurine and hypotaurine from [^{35}S]methionine at rates of 18.4 and 83.1 fmol larva^{-1} h^{-1}, respectively (Fig. 6B).

Amino acid composition of protein in Isochrysis galbana

Fig. 7 shows the amounts of amino acids present in two protein samples extracted from the alga *Isochrysis galbana*. Mean values (N=2) for cysteine and methionine were 0.56 and 4.59 fmol cell^{-1}, respectively.

Discussion

Taurine requirements of larvae during development

Taurine was the major amino acid in the free amino acid pools extracted from larvae of the gastropod *Haliotis rufescens* (Fig. 1C, lower chromatogram) and the bivalve *Crassostrea gigas* (Fig. 4A, lower chromatogram). Lecithotrophic larvae (*H. rufescens*) were able to maintain organic mass (Fig. 1A) and taurine content (Fig. 1B) from the egg (day 0) through

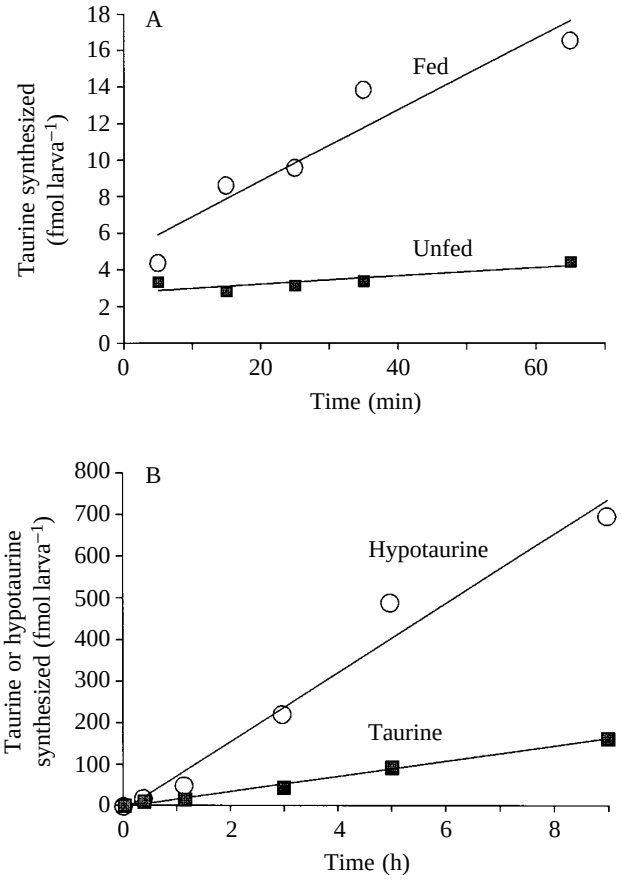

Fig. 6. (A) Taurine synthesis from [^{35}S]cysteine by fed and unfed larvae of the bivalve *Crassostrea gigas*. (B) Taurine and hypotaurine synthesis from [^{35}S]methionine by fed bivalve larvae.

development to metamorphosis. These data on mass stasis are consistent with previously published data showing that the larvae of *H. rufescens* maintain constant mass through development to metamorphosis (Jaeckle and Manahan, 1989*b*). Also, larvae of *H. rufescens* do not have any significant taurine turnover as taurine synthesis was not measurable (Fig. 1C) with the detection methods we used for this part of the study (radioactivity measured with an in-line radiochemical detector). For veliger larvae of *H. rufescens*, taurine efflux into sea water has been reported to be 40 % of the taurine pool per day (Jaeckle and Manahan, 1989*a*), suggesting a high taurine turnover in larvae (taurine content is constant during development, Fig. 1B). At 600 pmol taurine larva^{-1} (Fig. 1B), a 40 % turnover would be 10 pmol taurine larva^{-1} h^{-1}, 50 times greater than the 1000 cts min^{-1} detection limit of the in-line radiochemical detector used (1000 disints min^{-1} = 200 fmol of [^{35}S]taurine synthesized from 74 GBq mmol^{-1} [^{35}S]cysteine). Our current findings suggest that the maternal endowment of taurine in the egg is sufficient for subsequent larval development to metamorphosis. We base this statement on the observations that the taurine pool remains constant during development (Fig. 1B) and that no measurable taurine synthesis occurs (Fig. 1C). High turnover of taurine in larvae of *H. rufescens* is unlikely.

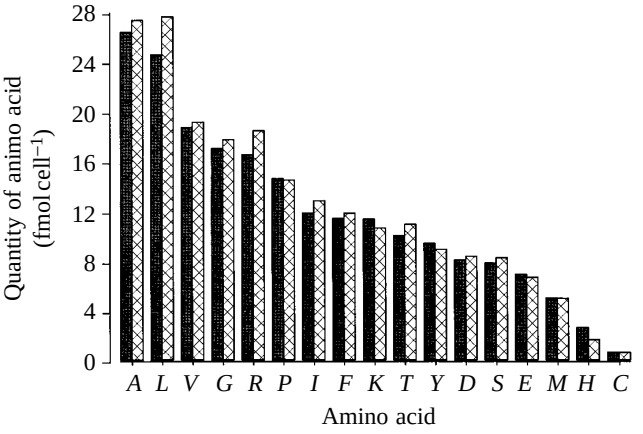

Fig. 7. Amino acid composition of algal proteins (*Isochrysis galbana*) determined using HPLC following acid-vapor hydrolysis. Filled bars and cross-hatched bars represent the results of hydrolysates from two algal protein samples. *A*, alanine; *R*, arginine; *D*, aspartic acid; *C*, cysteine; *E*, glutamic acid; *G*, glycine; *H*, histidine; *I*, isoleucine; *L*, leucine; *K*, lysine; *M*, methionine; *F*, phenylalanine; *P*, proline; *S*, serine; *T*, threonine; *Y*, tyrosine; *V*, valine.

Planktotrophic larvae of the bivalve *Crassostrea gigas* increased their taurine content by 43-fold as they grew (Fig. 2A). As planktotrophic larvae feed and grow, they will increase in mass (volume) and need to increase their individual taurine content to maintain osmotic balance. In support of this, the mole-percentage of taurine remained constant (at 62 %) in the free amino acid pool during growth (Fig. 2B). Given the high growth rates of larvae compared with that of adult bivalves, the larva's requirement for taurine (as a percentage increase per day) is much more pronounced.

Adult molluscs are thought to obtain taurine *per se* from their diet (Simpson *et al.* 1959; Bishop *et al.* 1983). However, taurine as a dietary source is unlikely for larval forms. Very few species of microalgae have been reported to contain taurine (Flynn and Flynn, 1992; Jackson *et al.* 1992) and larvae of *Crassostrea gigas* can be grown (Walne, 1974; also larvae used in this study) on an algal diet that is taurine-free (*Isochrysis galbana*, Fig. 3A). In general, the species of microalgae used as food by bivalve larvae do not contain measurable amounts of taurine (Fig. 3B–E). Uptake of taurine from sea water by larvae of *C. gigas* does occur (Manahan, 1989), but this input cannot be quantitatively important to requirements during growth because taurine is not measurable in natural sea water (Mopper and Lindroth, 1982; J. R. Welborn and D. T. Manahan, unpublished observations). In larvae, it is more likely that taurine is synthesized *de novo* from sulfur-containing precursors obtained from microalgae.

De novo *synthesis of taurine by larvae and factors affecting synthesis*

Taurine synthesis from cysteine by unfed bivalve larvae (*Crassostrea gigas*) was not measurable (by in-line radiochemical detection) even when using incubations of up to 26 h (Fig. 5B). Appreciable taurine synthesis from radiolabeled

cysteine appeared in larvae only if they were fed microalgae (Fig. 5A, HPLC analysis; Fig. 5C, TLC analysis). As cysteic acid, cysteine sulfinic acid, hypotaurine and taurine were labeled from [^{35}S]cysteine, taurine synthetic pathways in bivalve larvae are similar to those found in mammals (Jacobsen and Smith, 1968; Huxtable, 1986) and other marine invertebrates (Bishop *et al.* 1983). Feeding the larvae up-regulated taurine synthesis from [^{35}S]cysteine by 11-fold, from 1.08 to 11.7 fmol taurine larva^{-1} h^{-1} (Fig. 6A). This low rate of taurine synthesis in unfed larvae was only measurable by collecting HPLC fractions for liquid scintillation counting (cf. in-line radiochemical detection). Taurine synthesis was also demonstrated in fed larvae of *C. gigas* given methionine as a precursor (Fig. 6B). The taurine synthesis rate from methionine was in the fmol range (18.4 fmol larva^{-1} h^{-1}), similar to the rate of taurine synthesis from cysteine.

The specific activities of [^{35}S]cysteine and [^{35}S]methionine used for calculating taurine synthesis rates were based upon the known additions of labeled and unlabeled amino acids to sea water (e.g. Fig. 6A,B: 37 kBq ^{35}S ml^{-1} with 500 nmol substrate l^{-1}). The rates of taurine synthesis we present for larvae of *Crassostrea gigas* are conservative as they are based on specific activities of precursors in the sea water (74 GBq mmol^{-1}) and do not take into account the unknown dilution of the label with intracellular substrate following transport of ^{35}S into the larvae. We attempted to measure with HPLC the specific activity of ^{35}S in larvae but, owing to the very low amounts of intracellular substrate (e.g. methionine was present at 0.53 pmol larva^{-1}, 178 μm valve length), the measurements were unreliable. We stress here not the absolute rates of taurine synthesis, but rather that feeding up-regulates taurine synthesis in unfed bivalve larvae.

There are no reported cases of taurine synthesis by bacteria (see reviews by Jacobsen and Smith, 1968; Huxtable, 1986, 1992 and references therein), so we attribute the patterns of taurine synthesis reported here to metabolism by the animals under study. Appreciable taurine synthesis in bivalve larvae could only be demonstrated under conditions when larvae were fed (Figs 5A and 6A), yet conversion of [^{35}S]cysteine to cysteic acid, cysteine sulfinic acid and hypotaurine was rapid regardless of feeding state. This suggests that in bivalve larvae taurine synthesis may be regulated at either hypotaurine (oxidase) aminotransferase or cysteic acid decarboxylase. Regulation at these steps is unusual, as in mammalian systems the rate-limiting step in taurine synthesis is the conversion of cysteine to cysteine sulfinic acid *via* cysteine dioxygenase (Huxtable, 1986) (assuming that the same enzyme responsible for this step in mammals is also present in bivalve larvae).

Rate of supply of taurine precursors in the diet of a bivalve larva

The information on taurine synthesis in adult marine invertebrates suggests that taurine synthesis rates are too low to supply requirements (Bishop *et al.* 1983). We have demonstrated that larvae of *Crassostrea gigas* have the capacity for *de novo* synthesis of taurine from both cysteine and methionine (Fig. 6A,B). Can larvae supply their taurine requirements *via de novo* synthesis from precursors found in algal proteins? Larvae of 110 μm valve length (*C. gigas*) feed on *Isochrysis galbana* at a rate of 214±55 cells larva^{-1} h^{-1} (S.D.), when cultured using algae present at 50 000 cells ml^{-1} (Douillet, 1991). Although larvae are unlikely to see such concentrations of phytoplankton in the field, such high algal concentrations are necessary for optimal larval growth during culturing and are similar to algal concentrations used in our experiments (45 000 cells ml^{-1}). The equation in Fig. 2A for the rate of taurine content increase was calculated from measurements obtained for larvae of *C. gigas* grown with 45 000 cells ml^{-1}. At 5 fmol of cysteine and methionine (combined) per algal cell (Fig. 7), a feeding rate of 214 cells larva^{-1} h^{-1} would supply a larva with a total of 25.7 pmol of taurine precursor per day. Under our culturing conditions, larvae of *C. gigas* increased in size at a rate of 5–10 μm day^{-1}. Taking an average size increase of 7.5 μm day^{-1}, a 110 μm larva would increase to 117.5 μm with a corresponding increase of 10.6 pmol of taurine (taurine content for each size calculated from the equation given in Fig. 2A). A growing larva of *C. gigas* could supply its total requirement for taurine (10.6 pmol) if 41 % of the available precursors from the larva's food (25.7 pmol) were converted to taurine. A 41 % conversion value is a reasonable estimate to supply the larva's taurine requirement from *de novo* synthesis, given that we measured approximately 50 % conversion of [^{35}S]cysteine to taurine in fed larvae of *C. gigas* (Fig. 5A).

Our findings show that, for molluscan larvae with lecithotrophic development (the gastropod *Haliotis rufescens*), taurine initially present in the egg is sufficient for larval development to metamorphosis. For planktotrophic larval forms (*Crassostrea gigas*), the requirement for taurine during development can be supplied by *de novo* synthesis from dietary precursors. In molluscan physiology, the relative contribution of *de novo* taurine synthesis compared with that obtained in the diet has been subject to discussion for many years (Simpson *et al.* 1959; Allen and Garrett, 1971; Bishop *et al.* 1983). The lack of significant *de novo* taurine synthesis reported for adult bivalve molluscs has led to the conclusion that taurine is a dietary requirement for adults. Our findings for larval forms differ from this, in that there is significant *de novo* synthesis of taurine during development and that feeding can up-regulate rates of taurine synthesis by 11-fold in unfed larval forms.

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