Erratum

Pace, D. A. and Manahan, D. T. (2006) Fixed metabolic costs for highly variable rates of protein synthesis in sea urchin embryos and larvae. *J. Exp. Biol.* **209**, 158-170.

Figs 1 and 7 in both the on-line and print versions of this paper were published incorrectly.

Panels A and B in Fig. 1 were not labelled. The correct figure is reprinted below.

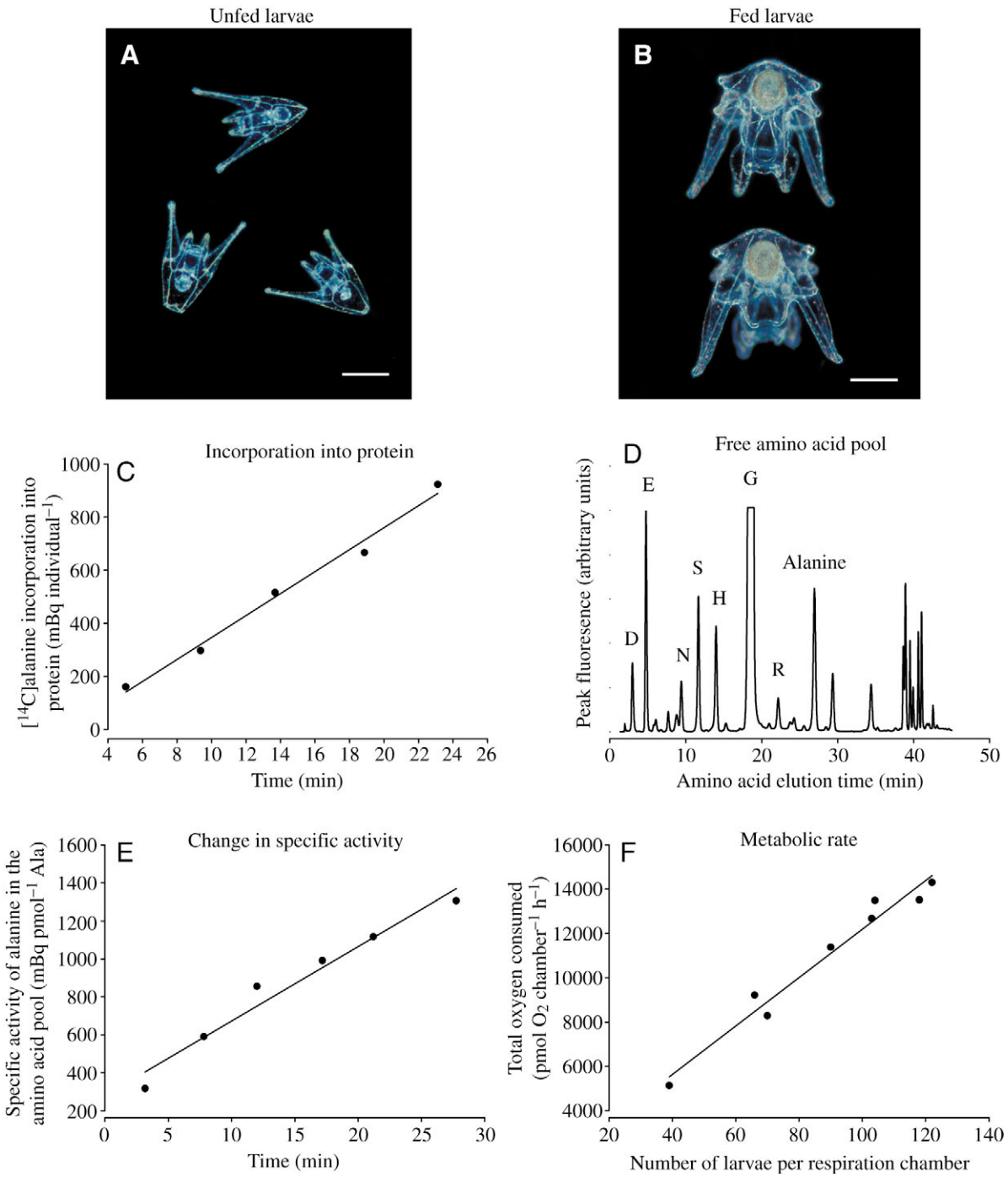

Fig. 1. Photomicrographs and primary data sets for protein synthesis and respiration rate measurements of 13-day-old fed larvae of *Lytechinus pictus*. (A) Photomicrograph of 13-day-old unfed and (B) fed larvae. Scale bars, 200 μm. (C) Linear increase of [^{14}C]alanine incorporation into the protein pool (TCA-insoluble fraction) of 13-day-old fed larvae of *L. pictus*: y=41.4(±2.71)x–68.4 (r^2=0.98; P<0.001). Rates of [^{14}C]alanine

incorporation were converted to absolute rates of protein synthesis (ng protein individual^{-1} h^{-1}) using the change in the specific activity of [^{14}C]alanine in the precursor free amino acid pool (Fig. 1E), the mole-percent of alanine in protein (7.8%; Table 1) and the mole-percent corrected molecular mass for amino acids in protein (129.4 g mole^{-1}; Table 1). (D) Chromatogram of extracted free amino acid pool from 13-day-old larvae of *L. pictus* separated using high performance liquid chromotagraphy (HPLC). D, aspartate; E, glutamate; N, asparagine; S, serine; H, histidine; G, glycine; R, arginine. Alanine was the [^{14}C]amino acid tracer used to determine rates of protein synthesis. (E) Specific activity of [^{14}C]alanine in the free amino acid pool during protein synthesis experiments with 13-day-old larvae of *L. pictus*. Specific activity was measured at the specified sampling intervals by quantifying the moles of total alanine with HPLC (as in Fig. 1D). [^{14}C]alanine was measured by liquid scintillation counting of the radioactivity associated with the alanine peak fraction. Increase of specific activity was described by the linear equation: y=39.2(±3.85)x+279.9 (r^2=0.96; P<0.001 for ANOVA of regression slope). (F) Respiration rate of 13-day-old fed larvae of *L. pictus*. Each data point represents a different respiration chamber. Respiration rate was calculated by determining the slope of total oxygen consumption in each respiration chamber for the known number of larvae in that chamber: y=109.5(±8.4)x+1254 (r^2=0.97; P<0.0001), where the slope of 109.5 has units of pmol O_2 larva^{-1} h^{-1}.

In Fig. 7B, the broken line was labelled incorrectly as 'Correct measured synthesis=183 ng day^{-1}' instead of 'Measured synthesis=183 ng day^{-1}'. The correct figure is reprinted below.

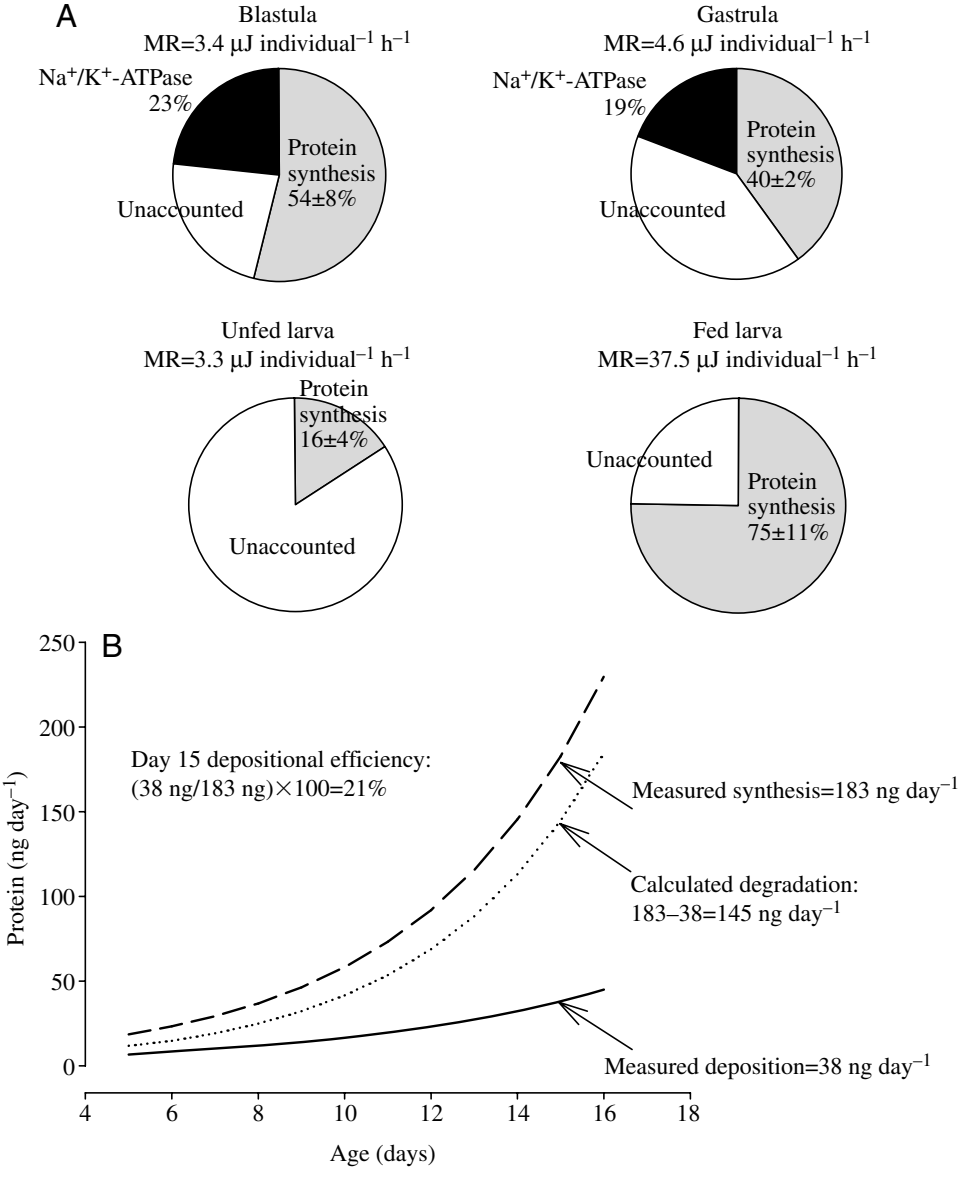

Fig. 7. (A) Setting metabolic rate. Percent of total metabolic rate (MR; given as µJ individual^{-1} h^{-1}) for different developmental stages of *L. pictus* accounted for by protein synthesis and sodium pump activity (data on Na^+/K^+-ATPase taken from Leong and Manahan, 1997). (B) Protein depositional efficiency. Rate of protein deposition (solid line) calculated from data on total protein content (see equation in legend of Fig. 2A). Rate of protein synthesis (broken line) calculated from protein synthesis rate (see equation in legend of Fig. 4). Rate of protein degradation during development (dotted line) calculated as difference between rates of synthesis and deposition. All rates were calculated on a per-larva basis. For 15-day-old larvae, the depositional efficiency was 21%.

In addition, on p. 166, right column, line 8, 'protein dispositional efficiency' should read 'protein depositional efficiency'.

We apologise for these errors and any inconvenience caused.

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Fixed metabolic costs for highly variable rates of protein synthesis in sea urchin embryos and larvae

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Summary

Defining the physiological mechanisms that set metabolic rates and the 'cost of living' is important for understanding the energy costs of development. Embryos and larvae of the sea urchin *Lytechinus pictus* (Verrill) were used to test hypotheses regarding differential costs of protein synthesis in animals differing in size, rates of protein synthesis, and physiological feeding states. For embryos, the rate of protein synthesis was 0.22±0.014 ng protein embryo^{-1} h^{-1} (mean ± s.e.m.) and decreased in unfed larvae to an average rate of 0.05±0.001 ng protein larva^{-1} h^{-1}. Fed larvae had rates of synthesis that were up to 194 times faster than unfed larvae (9.7±0.81 ng protein larva^{-1} h^{-1}). There was no significant difference, however, in the cost of protein synthesis between these larvae with very different physiological states. Furthermore, the cost of synthesis in the larval stages was also similar to costs measured for blastula and gastrula embryos of 8.4±0.99 J mg^{-1} protein synthesized. The cost of protein synthesis was obtained using both direct ('inhibitor') and indirect ('correlative') measurements; both methods gave essentially identical results. Protein synthesis accounted for up to 54±8% of metabolic rate in embryos. Percent of metabolism accounted for by protein synthesis in larvae was dependent on their physiological feeding state, with protein synthesis accounting for 16±4% in unfed larvae and 75±11% in fed larvae. This regulation of metabolic rate was due to differential rates of synthesis for a fixed energy cost per unit mass of protein synthesized. The cost of synthesizing a unit of protein did not change with increasing rates of protein synthesis. We conclude that the cost of protein synthesis is independent of the rate of synthesis, developmental stage, size and physiological feeding state during sea urchin development.

Key words: embryo, larva, sea urchin, *Lytechinus pictus*, protein synthesis, metabolic rate, energetic cost.

Introduction

A longstanding goal of the study of animal physiology has been to understand the processes that regulate metabolism (Schmidt-Nielsen, 1972; Heusner, 1991; Wieser, 1994; Hochachka and Somero, 2002). Early developmental stages have high mass-specific rates of energy utilization relative to adult stages (Zeuthen, 1953), and during larval growth the rates of energy expenditure increase significantly (Widdows, 1991; Hoegh-Guldberg and Manahan, 1995). The biochemical bases that set such changes in mass-specific metabolic rates during development are not fully understood. The sodium pump is one major component that establishes rates of turnover of the ATP pool and, hence, metabolic rate. For instance, in developing sea urchin embryos and larvae, the physiologically active fraction of the sodium pump could alone account for 40% of metabolic rate (Leong and Manahan, 1997).

Protein synthesis and turnover are also processes known to be major determinants of metabolic rate (Waterlow, 1984; Hawkins, 1991). The regulation of protein synthesis is a key component of metabolic depression in response to environmental stress (Guppy et al., 1994; Hofmann and Hand, 1994; Smith et al., 1996; Podrabsky and Hand, 2000). Rapid development and growth correspond with rapid rates of macromolecular synthesis and turnover. In marine invertebrates, protein is a major biochemical constituent of eggs, developing embryos and larval forms (echinoderms: Turner and Lawrence, 1979; McClintock and Pearse, 1986; Shilling and Manahan, 1994; mollusks: Holland and Gabbott, 1971; His and Maurer, 1988; Vavra and Manahan, 1999). While there have been numerous studies of biochemical content of developmental stages of marine invertebrates, far less is known about the metabolic costs of protein synthesis and turnover in these organisms.

Metabolic costs of protein synthesis have been suggested to comprise fixed and variable components (Pannevis and Houlihan, 1992; Smith and Houlihan, 1995). At high rates of protein synthesis the proportion of metabolism accounted for by the fixed costs of synthesis decreases, resulting in up to

tenfold lower costs of protein synthesis per unit mass of protein synthesized by fish cells (Smith and Houlihan, 1995). If costs of synthesis vary as a function of the rate of synthesis, then it would be predicted that developmental stages with highly variable rates of synthesis would have variable costs. In constructing energy budgets for costs of development, such variable costs of synthesis need to be defined. The findings presented in this paper are based upon studies of protein synthesis and metabolic rates measured *in vivo* during development and growth of early life history stages of sea urchins. This work takes advantage of the longstanding experimental tractability of sea urchin embryos for studies of protein synthesis (Davidson, 1968, 1976, 1986, 2001) and adds the physiological perspective of determining the metabolic costs of such synthesis. When combined with other components of the metabolic 'pie-chart' (e.g. the sodium pump; Leong and Manahan, 1997), defining the costs of protein synthesis as a function of developmental stage and size will significantly increase understanding of the fundamental processes that set metabolic rates during animal development and growth.

Materials and methods

Spawning and animal culturing

All seawater used for culturing and for experiments with embryos and larvae was sterile-filtered (0.2 μm pore size) and UV treated. Gravid adults of the sea urchin *Lytechinus pictus* (Verrill 1867), were induced to spawn by intracoelomic injection of 0.5 mol l^{-1} KCl. Fertilization success was checked and only cultures started from eggs having greater than 90% fertilization success were used. Embryos and larvae were reared at a concentration of 6–8 individuals ml^{-1} at 15°C in 20 l culture vessels. Larvae in three replicate 20 l culture vessels received no algal food (the 'unfed' treatment) (Fig. 1A). Larvae in three other 20 l culture vessels were fed algae (the 'fed' treatment) for the duration of the study (to day 16) (Fig. 1B). After each water change every 3 days, the fed larvae received equal amounts of the algae *Rhodomonas* sp. and *Dunaliella tertiolecta* at a total concentration of 30 000 cells ml^{-1}. Larvae in the unfed treatment were observed to remain active and motile during the 14 days without food. These unfed larvae were highly synchronous in size even after 13 days with no food, with a larval length of 438±8.0 μm (N=20; larval length = tip of postoral arm to posterior tip of body). Sea urchin larvae have been reported to recover from such prolonged starvation and, once fed, can continue development to metamorphosis (Moran and Manahan, 2004). Under the rearing conditions used for the fed cultures in this study, the larvae were also synchronous in size at 681±7.9 μm (N=20 for 13-day-old fed larvae).

Protein deposition and amino acid composition

Total protein content was determined independently of protein synthesis measurements on samples of known numbers of individuals using the Bradford assay, modified for use with small samples necessary to study embryos and larvae (Bradford, 1976; Jaeckle and Manahan, 1989). Amino acid composition was determined from total protein extracted from embryos and larvae. Data on the mole-percent composition of amino acids in protein were required for calculations of protein synthesis rates (details below). Protein was precipitated using standard methods (cold, 5% trichloroacetic acid). The precipitates were acid hydrolyzed in 6 mol l^{-1} HCl containing 0.5% phenol at 110°C for 24 h under vacuum. Samples were analyzed on a Beckman System 6300 (Fullerton, CA, USA) amino acid analyzer using cation exchange chromatography and post-column ninhydrin derivatization.

Alanine transport and incorporation rates

[^{14}C]alanine (Perkin Elmer, Wellesley, MA, USA; 6460 kBq μmole^{-1}; 3.7×10^{10} Bq=1 Ci) was used to measure rates of alanine transport from seawater and rates of incorporation of the isotope into the protein fraction of embryos and larvae (for general protocol, see Manahan, 1983). In brief, known numbers of animals (~200–500 individuals, depending on size and rate of transport) were exposed to [^{14}C]alanine and the linear rate of transport was calculated per individual from time-course experiments. An example of the quality of data obtained to measure incorporation rate of [^{14}C]alanine into the protein fraction (TCA-insoluble fraction) is shown in Fig. 1C. For experiments on embryos and early larval stages, the concentration of alanine in seawater was adjusted to 10 μmol l^{-1} by addition of cold carrier (^{12}C-alanine from Sigma Chemical Co., St Louis, MO, USA). For later larval stages (>10 days old), a concentration of 15 μmol l^{-1} alanine was used to minimize possible effects of substrate limitation on transport rate. These substrate concentrations were selected based on previous studies of the kinetics of amino acid transport in developing sea urchins (Manahan et al., 1989; Manahan, 1990). No substrate limitation was observed in any transport assay. For all experiments there was a linear rate of transport during the exposure to [^{14}C]alanine (5–6 points were sampled during each 30–40 min time-course experiment; Fig. 3C). Absolute rates of alanine transport (moles per individual per hour) were determined by correcting the amount of [^{14}C]alanine in animals (determined by liquid scintillation counting after appropriate quench correction) with the specific activity of the isotope in seawater. Alanine transport was also measured to assess if the protein synthesis inhibitor, emetine, decreased alanine transport rate. Emetine was used in the present study for measurements of the cost of protein synthesis.

Protein synthesis

Rates of protein synthesis in embryos and larvae were measured *in vivo* following the well-established protocols for studies of protein synthesis in sea urchins embryos (e.g. Fry and Gross, 1970; Berg and Mertes, 1970). In principle, several different amino acids could be used as radiolabeled precursors to measure rates of protein synthesis. In this study, [^{14}C]alanine

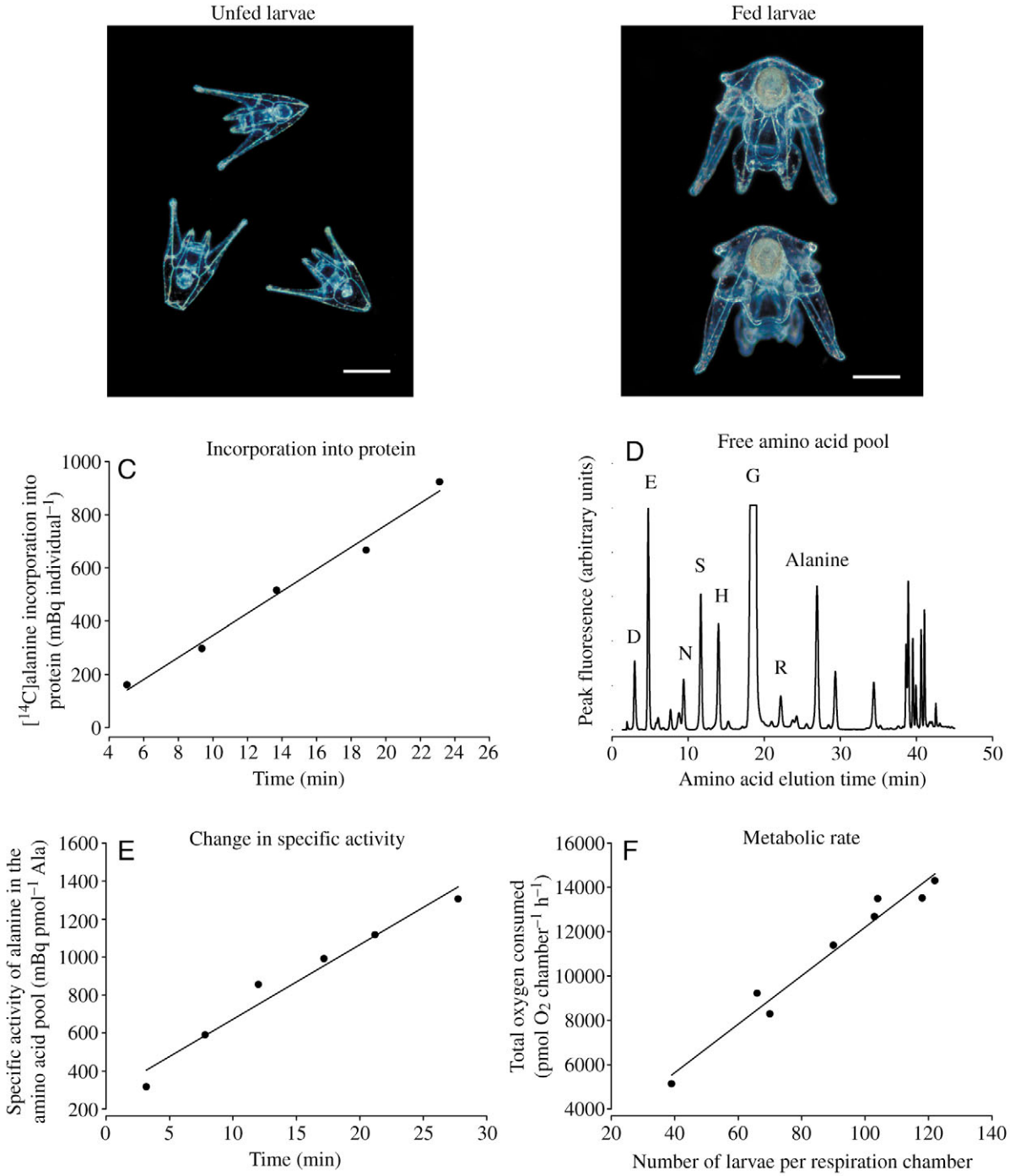

Fig. 1. For legend see p. 161.

was chosen as the tracer to measure rates of protein synthesis. Previously (Marsh et al., 2001) we have shown there to be no differences in protein synthesis rates in developing sea urchins when measured with both 'essential' or 'non-essential' amino acids (leucine and alanine, respectively). This is in accord with other studies of protein synthesis during sea urchin development (summarized in Regier and Kafatos, 1977). Alanine was chosen as the tracer in our study for several reasons. Firstly, it is transported from seawater and rapidly incorporated into protein. Secondly, it is well separated from other constituents of the free amino acid pool of larvae of *L. pictus* (Fig. 1D) and present in sufficient amounts intracellularly to be accurately measured by high performance liquid chromatography (HPLC; for details of methods, see Vavra and Manahan, 1999). The amount of alanine in the free amino acid pool was determined for all measurements of protein synthesis to correct for changes in intracellular specific activity of [^{14}C]alanine (Fig. 1E), necessary for the

Fig. 1. Photomicrographs and primary data sets for protein synthesis and respiration rate measurements of 13-day-old fed larvae of *Lytechinus pictus*. (A) Photomicrograph of 13-day-old unfed and (B) fed larvae. Scale bars, 200 μm. (C) Linear increase of [^{14}C]alanine incorporation into the protein pool (TCA-insoluble fraction) of 13-day-old fed larvae of *L. pictus*: y=41.4(±2.71)x–68.4 (r^2=0.98; P<0.001). Rates of [^{14}C]alanine incorporation were converted to absolute rates of protein synthesis (ng protein individual^{-1} h^{-1}) using the change in the specific activity of [^{14}C]alanine in the precursor free amino acid pool (Fig. 1E), the mole-percent of alanine in protein (7.8%; Table 1) and the mole-percent corrected molecular mass for amino acids in protein (129.4 g mole^{-1}; Table 1). (D) Chromatogram of extracted free amino acid pool from 13-day-old larvae of *L. pictus* separated using high performance liquid chromotagraphy (HPLC). D, aspartate; E, glutamate; N, asparagine; S, serine; H, histidine; G, glycine; R, arginine. Alanine was the [^{14}C]amino acid tracer used to determine rates of protein synthesis. (E) Specific activity of [^{14}C]alanine in the free amino acid pool during protein synthesis experiments with 13-day-old larvae of *L. pictus*. Specific activity was measured at the specified sampling intervals by quantifying the moles of total alanine with HPLC (as in Fig. 1D). [^{14}C]alanine was measured by liquid scintillation counting of the radioactivity associated with the alanine peak fraction. Increase of specific activity was described by the linear equation: y=39.2(±3.85)x+279.9 (r^2=0.96; P<0.001 for ANOVA of regression slope). (F) Respiration rate of 13-day-old fed larvae of *L. pictus*. Each data point represents a different respiration chamber. Respiration rate was calculated by determining the slope of total oxygen consumption in each respiration chamber for the known number of larvae in that chamber: y=109.5(±8.4)x+1254 (r^2=0.97; P<0.0001), where the slope of 109.5 has units of pmol O_2 larva^{-1} h^{-1}.

calculations of protein synthesis rates. HPLC was used to correct the intracellular specific activity of [^{14}C]alanine (Fig. 1E). The assumption of the integrity of the ^{14}C-label as [^{14}C]alanine only, as required for calculations of protein synthesis, was verified in our experiments using HPLC analysis of the free amino acid pools. During the relatively short duration of the protein synthesis experiments (30–40 min), no interconversion of [^{14}C]alanine into other ^{14}C-labeled amino acids was measurable by HPLC and fraction collection. Measurements of the change in specific activity with time of [^{14}C]alanine in the free amino acid pool (Fig. 1E) were used to correct each time point where incorporation rates of [^{14}C]alanine into protein was measured (Fig. 1C). Rates of protein synthesis were calculated by measuring the increase in radioactivity in the protein fraction of embryos and larvae, as follows:

$$PS = d/dt(S_p/S_{faa}) \times MW_p/S_m ,$$

where, PS is the rate of protein synthesis (ng protein individual^{-1} h^{-1}), t is time (h), S_p is the amount of radioactivity in protein ([^{14}C]alanine individual^{-1} h^{-1}), S_{faa} is the specific activity of alanine in the intracellular free amino acid pool (mBq pmol^{-1}), MW_p is the mole-percent-corrected molecular mass of amino acids in protein of *L. pictus* (129.4 g mol^{-1}; Table 1) and S_m is the mole-percent of alanine in protein of embryos and larvae (7.8±0.02%; Table 1).

Table 1. *Amino acid composition and mole-percent of amino acids in proteins of embryos and larvae of the sea urchin Lytechinus pictus*

Amino acid	Mean percent composition in protein
Histidine	2.0±0.02
Methionine	2.0±0.06
Tyrosine	2.9±0.08
Phenylalanine	3.8±0.05
Isoleucine	5.0±0.14
Proline	5.0±0.07
Arginine	5.3±0.05
Threonine	5.6±0.04
Valine	6.4±0.01
Serine	6.8±0.07
Lysine	7.3±0.11
Alanine	7.8±0.02
Leucine	7.9±0.19
Glycine	9.7±0.54
Aspartate, Asparagine*	10.9±0.21
Glutamate, Glutamine*	11.8±0.17
†MW_p (g mole^{-1})	129.4±0.40

Values are means ± s.e.m., N=5.

*Asparagine and glutamine are converted to aspartate and glutamate, respectively, during acid-hydrolysis of proteins. Also, cysteine and tryptophan are lost during routine acid-hydrolysis.

†MW_p, mole-percent corrected molecular mass; represents the average molecular mass of amino acids in the protein pool of embryos and larvae of *L. pictus*. MW_p is calculated by multiplying the mole-percent value (given in this table) of each amino acid by its molecular mass, and then adding all mole-percent corrected masses of the amino acids.

Respiration rates

Metabolic rates of embryos and larvae of *L. pictus* were measured as rates of oxygen consumption. For a full description of how oxygen consumption was measured, see Marsh and Manahan (1999). For the measurements made in this study with developing stages of *L. pictus*, embryos and larvae were removed from cultures and resuspended in 0.2 μm of filtered seawater in small (~500 μl) respiration chambers. A range of concentrations of individuals per respiration chamber was used to correct for possible concentration-dependent affects of animal numbers on respiration rates calculated per individual (see x-axis of Fig. 1F as an example). A series of different respiration chambers (e.g. eight, as in Fig. 1F) containing embryos or larvae was used for each set of stage-specific respiration measurements. Incubations were for 3–4 h, depending on rates of respiration of the stages of development under study. At the end of each incubation, a 300 μl subsample of seawater was taken from each chamber with a temperature-equilibrated gas-tight syringe and injected into a temperature controlled oxygen measurement cell (Strathkelvin RC 100, Glasgow, UK). Oxygen tension was measured in each sample with a polarographic oxygen sensor (Model 1302,

Strathkelvin). The number of embryos or larvae in each respiration chamber was then counted and the oxygen consumption per individual calculated as the slope of the regression line of oxygen consumed per hour against number of individuals in each respiration chamber (Fig. 1F). The error of each estimate was calculated as the standard error around the slope of the regression line. An example of the quality of the respiration data obtained with this method for fed larvae of *L. pictus* is given in Fig. 1F. From the regression shown in Fig. 1F, a fed 13-day-old larva had a rate of oxygen consumption of 109.5±8.4 pmol O_2 individual^{-1} h^{-1} (± s.e.m. of slope).

Cost of protein synthesis

Previous studies have reported different costs of protein synthesis depending on the method of measurement used (e.g. Muramatsu and Okumura, 1985; Aoyagi et al., 1988). We tested two widely used methods for calculating costs of protein synthesis to determine if both methods gave similar values for the same species over a range of different developmental stages and physiological states.

Rates of protein synthesis and rates of oxygen consumption were measured in embryos and larvae of *L. pictus* in the presence and absence of the protein synthesis inhibitor emetine. Emetine was used in our study because of its well-documented potency in inhibiting protein synthesis in sea urchin embryos at relatively low concentrations. Concentrations up to 400 μmol l^{-1} emetine in seawater have been used to inhibit protein synthesis in marine invertebrate larvae (Fenteany and Morse, 1993), with concentrations in the 100 μmol l^{-1} range being more commonly used for studies with eggs and embryos of echinoderms (Wagenaar, 1983; Pesando et al., 1995; Yamada, 1998; Sasaki and Chiba, 2001). We used a concentration of 100 μmol l^{-1} for stages of development under 3 days of age and 150 μmol l^{-1} for larger fed larvae (13 days old). In all treatments with emetine, the animals were observed to remain active and swimming before, during, and after the experiments using emetine. In this study, one further step was taken to ensure that emetine was not having any possible negative, non-specific effects on the physiology of the embryos and larvae. We tested for the affect of the inhibitor on rates of amino acid transport throughout the developmental period under study. No decreases in alanine transport rates were observed (detailed below in Results, Fig. 3A–C).

Costs of protein synthesis were also calculated with a method that is not dependent upon the use of inhibitors (‘direct method’). This second approach is based on the quantitative relationship of ‘normal’ physiological changes in metabolic rates and corresponding changes in protein synthesis (Reeds et al., 1985; Lyndon et al., 1989; Marsh et al., 2001). For this ‘indirect method’ (correlative) a series of parallel measurements of metabolic rate and protein synthesis are plotted, with the calculated slope being the estimate of the cost of protein synthesis (in units J mg^{-1} protein synthesized).

Unless indicated otherwise, values are reported as means ± s.e.m.

Results

Protein deposition and amino acid composition

Total protein content remained constant during embryonic development and showed little subsequent change in later, unfed larval stages of *L. pictus* for up to 14 days (Fig. 2A). When larvae were fed on an algal diet, however, their protein content increased rapidly; by day 16 fed larvae had over 10 times more protein than unfed larvae of a similar age (Fig. 2A).

The amount of alanine in the free amino acid pool (Fig. 2B) of embryos and larvae of *L. pictus* increased during larval growth. Relative to fed larvae, embryos and unfed larvae had similar amounts of alanine (2.32±0.05 and 1.94± 0.10 pmol alanine individual^{-1}, respectively). Larvae were fed at day 3 and subsequently the alanine content in the free amino acid pool of fed larvae increased during development from 7.9±0.20 pmol alanine individual^{-1} on day 5 to 54.3±3.9 pmol alanine individual^{-1} on day 16. Compared to unfed larvae at a near-similar age (day 14=1.7± 0.18 pmol alanine individual^{-1}), fed larvae had 32 times more alanine in their free amino acid pool. The mole-percent of alanine in whole-animal protein extracts remained

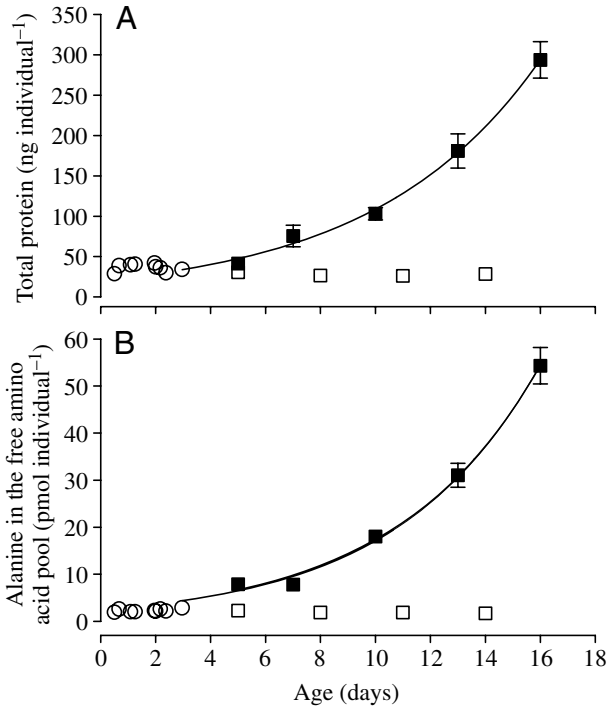

Fig. 2. Changes in (A) total protein and (B) the amount of alanine in the free amino acid pool during development and growth of the sea urchin *Lytechinus pictus*. Embryos, open circles; unfed larvae, open squares; fed larvae, filled squares. Values are means ± s.e.m., and where not shown errors fell within the graphical representation of the data point. (A) Changes in total protein content. Each data point is the mean value of 2–3 determinations. The fitted line shows an increase in protein content for fed larvae: y=20.68e$^{0.17x}$ (r^2=0.99). (B) Changes in the amount of alanine in the free amino acid pool. Each data point is the mean of 6 measurements. The fitted line shows an increase in alanine in fed larvae: y=2.460e$^{0.19x}$ (r^2=0.99).

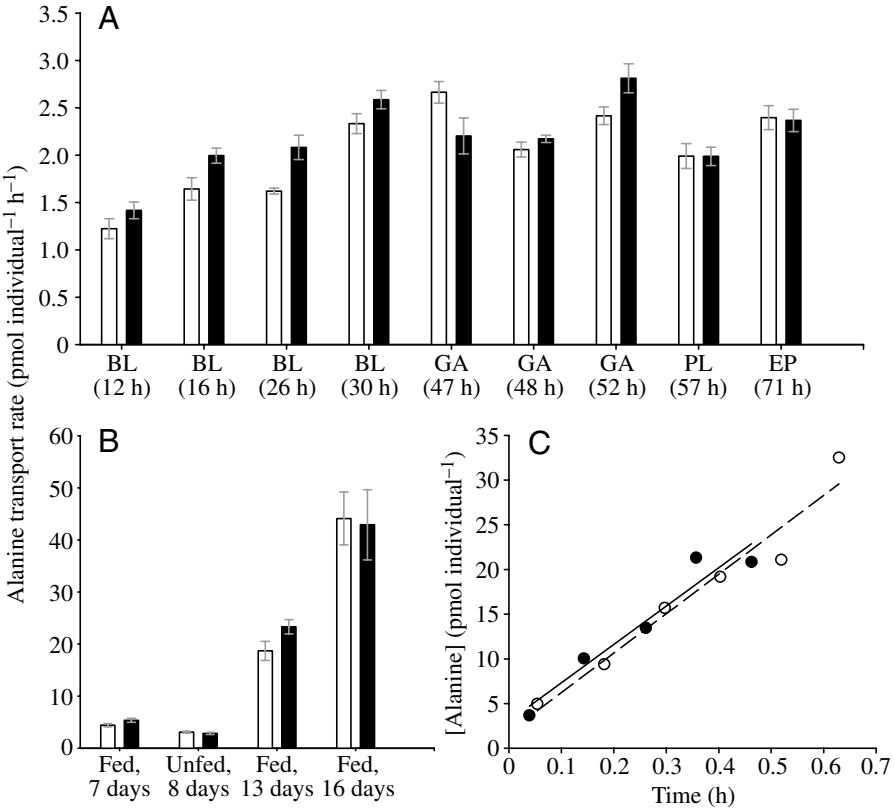

Fig. 3. Comparison of alanine transport rate in embryos and larvae of *L. pictus* in the presence and absence of the protein synthesis inhibitor, emetine (see Materials and methods for details). For all alanine transport measurements, the r^2 values from the linear regressions of the alanine transport rate with time ranged from 0.93 to 0.99. Open bars, without emetine; solid bars, with emetine. (A) Alanine transport rates for stages of development measured before the start of feeding treatment experiments. BL, blastula; GA, gastrula: PL, prism larva; EP, early pluteus. (B) Alanine transport rates in fed and unfed larvae. Values are means ± s.e. slope of the slope of transport rate. (C) Primary data to show comparison of alanine transport rates in the presence and absence of emetine (fed larvae, 16-day-old). Slopes and intercepts were not significantly different (ANOVA). Open circles + broken line, no emetine (r^2=0.95); solid circles + solid line, with emetine (r^2=0.93).

constant at 7.8±0.02% for embryos and larvae of *L. pictus* (Table 1).

Alanine transport rates

Rates of transport of alanine from seawater ranged between 1–3 pmol alanine individual^{-1} h^{-1} in embryos and unfed larvae (Fig. 3A,B). Transport rate increased in fed larvae from 4.4±0.31 pmol alanine individual^{-1} h^{-1} (± s.e.m. of the slope; day 7) to 44.1±5.1 pmol alanine individual^{-1} h^{-1} (Fig. 3B; day 16). Alanine transport rates were also measured in embryos and larvae that were exposed to emetine, the protein synthesis inhibitor used in this study to quantify the cost of protein synthesis. For all the developmental stages studied, alanine transport rates were not significantly reduced in the presence of emetine (Fig. 3A,B; compare open and solid bars). An example of the primary data for the rate of alanine transport by 16-day-old fed larvae in the presence and absence of emetine is given in Fig. 3C. No significant difference in rates was measurable (linear regressions were compared by ANOVA and neither the slopes nor the intercepts were significantly different; P=0.89, slope; P=0.56, intercept).

Rates of protein synthesis

The rates of protein synthesis did not change during embryonic development (Fig. 4), with an average rate of 0.22±0.014 ng protein synthesized individual^{-1} h^{-1}. Larvae were fed algae at day 3, and by day 5 the rate of protein synthesis was 10 times higher than in unfed larvae (5-day-old fed larvae=0.60±0.12 ng protein individual^{-1} h^{-1}; unfed larvae=0.06±0.003 ng protein individual^{-1} h^{-1}). By day 16, rates of protein synthesis in fed larvae had increased to 9.7±0.81 ng protein individual^{-1} h^{-1}. This rate represents an increase of 16 times the rate for the first larval feeding stage measured (day 5), and 194 times more than the average

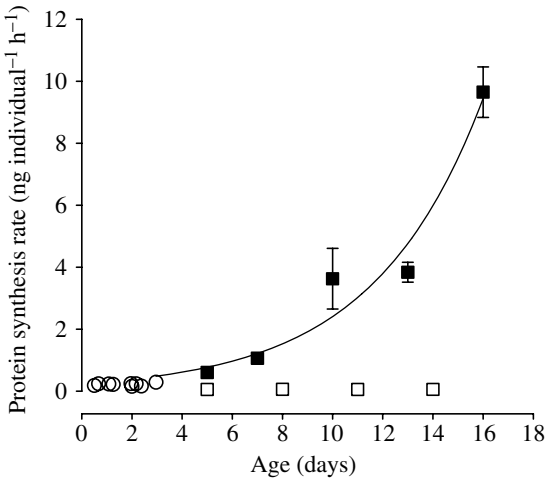

Fig. 4. Rates of protein synthesis in embryos (open circles), unfed larvae (open squares) and fed larvae (filled squares) of the sea urchin *L. pictus*. Each data point is the mean ± 1 s.e.m. of 5–6 determinations; where not shown, errors fell within the graphical representation of the data point. The fitted line shows an increase in rate of protein synthesis for fed larvae: y=0.25e$^{0.23x}$ (r^2=0.96).

Table 2. *Mean fractional rates of protein synthesis in embryos and larvae (unfed and fed) of Lytechinus pictus*

Stage/treatment	*N*	Mean fractional rate	
		% per hour	% per day
Embryo	9	0.60±0.04	14±0.96
Larvae			
Unfed	4	0.20±0.01	5±0.24
Fed	5	2.40±0.45	58±10.8

Values are means ± s.e.m.

Fractional rates of synthesis were determined by dividing each whole-animal rate of synthesis (Fig. 4) by its respective amount of total protein for each developmental time point (Fig. 2A).

synthesis rate in unfed larvae (0.05±0.001 ng protein individual^{-1} h^{-1}).

Fractional rates of protein synthesis represent the percent of the total protein content of an individual that is synthesized per unit time (Table 2). The average fractional rate of protein synthesis in embryos was 0.60±0.04 h^{-1}. Fractional rates of synthesis in unfed larvae, at 0.20±0.01 h^{-1}, were lower than in embryos. In fed larvae, the fractional rates of protein synthesis were higher, at 2.4±0.45 h^{-1}, which is equivalent to 58% per day.

Cost of protein synthesis

Indirect analysis using correlative metabolic cost of protein synthesis

Changes in rates of respiration and protein synthesis were measured simultaneously under normal (no inhibitor present) physiological conditions during development of *L. pictus*. Fig. 5 shows the linear relationship between the rates of metabolism and protein synthesis for all stages of development studied: embryos, unfed and fed larvae. The respiration data for these stages of development were as follows. Blastula stage embryos=7.0±0.6 pmol O_2 individual^{-1} h^{-1} (*N*=4). Gastrula= 9.5±1.3 pmol O_2 individual^{-1} h^{-1} (*N*=4). Unfed larvae= 6.8±1.1 pmol O_2 individual^{-1} h^{-1} (*N*=4). For fed larvae, respiration rates obviously increased with growth and ranged from 21.2 to 164.9 pmol O_2 individual^{-1} h^{-1} (*N*=5). These rates of oxygen consumption were converted to energy equivalents (484 kJ mol^{-1} O_2; Gnaiger, 1983), based on oxyenthalpic values of lipid and protein, the major biochemical constituents of echinoderm larvae (Turner and Lawrence, 1979; McClintock and Pearse, 1986; Shilling and Manahan, 1994). In Fig. 5, metabolic rates are presented as μJ individual^{-1} h^{-1} (e.g. a respiration rate for blastula of 7.0 pmol O_2 is equivalent to 3.4 μJ individual^{-1} h^{-1}).

The relationship of the change in rate of protein synthesis and rate of oxygen consumption for all embryonic and larval stages combined was significant (Fig. 5, broken line; ANOVA, $P<0.0001$; $N=17$). This yielded an energetic cost of protein synthesis of 8.41±0.49 J mg^{-1} protein synthesized. The possibility that this cost estimate is biased by the cluster of low rates of synthesis for embryos and unfed larvae (i.e. the data points in Fig. 5 less than 0.50 ng protein synthesized individual^{-1} h^{-1}), was checked by determining the cost for fed larvae alone (Fig. 5, solid line regression). The value for fed larvae was 7.69±1.36 J mg^{-1} protein synthesized, not statistically different from 8.41±0.49 J mg^{-1} protein synthesized. The slopes and intercepts were not significantly different (ANOVA; $P=0.49$, slope; $P=0.42$, intercept).

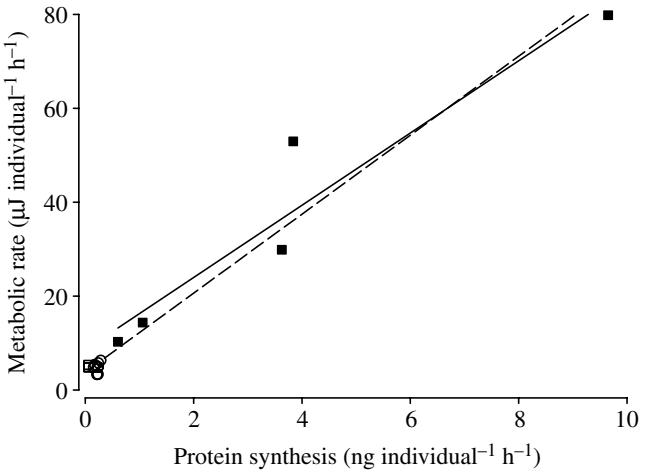

Fig. 5. Correlative cost of protein synthesis in embryos (open circles), unfed larvae (open squares) and fed larvae (filled squares) of *L. pictus*. Cost of protein synthesis was calculated from the slope of the relationship between protein synthesis and metabolic rate. The broken line is the regression of metabolic rate and protein synthesis for all stages of development studied (embryos, unfed and fed larvae). Slope of regression=8.41±0.49 J mg^{-1} protein; $r^2=0.95$; $N=17$). Solid line is regression for fed larvae alone=7.69±1.36 J mg^{-1} protein; $r^2=0.91$; $N=5$).

Direct analysis using an inhibitor of protein synthesis

The value given above of 8.41±0.49 J mg^{-1} protein synthesized calculated from the correlative analysis of the metabolic cost of protein synthesis during development of *L. pictus* is essentially identical to the cost measured by direct inhibitor analysis of 8.40±0.99 J mg^{-1} protein synthesized obtained with the protein synthesis inhibitor, emetine. Fig. 6 shows metabolic and protein synthesis rates and, by difference, the respective energy costs of protein synthesis for several different stages of development (blastula to fed larval stages) in the presence and absence of emetine. Normal rates of metabolism and protein synthesis (i.e. those measured with no emetine present) are represented by the maximum height of each histogram bar shown for each developmental stage. The solid bars represent the rates when measured in the presence of emetine. The costs of protein synthesis were calculated from differences in respiration and protein synthesis rates measured in the presence and absence of emetine. These costs ranged from 8–10 J mg^{-1} protein synthesized by embryos and larvae of *L. pictus* (exact values for each developmental stage are given in the legend to Fig. 6). The values for blastulae, gastrulae, and larvae were not significantly different (ANOVA; $P=0.60$, $N=8$). When all

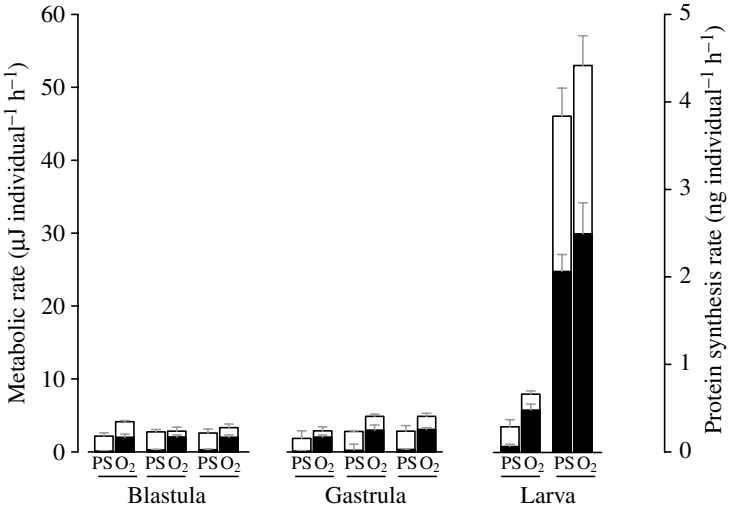

Fig. 6. Inhibitor analysis to determine the cost of protein synthesis in embryos and larvae of *L. pictus*. Protein synthesis (PS) and metabolic rates (O_2) in the presence and absence of emetine (see Materials and methods for details) are shown for each replicate measurement for each developmental stage studied (blastula, gastrula and larva). Total height of each bar represents metabolic rate or protein synthesis rate when no inhibitor was present. The height of the solid component represents the rate in the presence of emetine. Error bars are calculated as s.e.m. of the slope for each linear regression analysis (error of the estimate of protein synthesis calculated as in Fig. 1C; error for metabolic rate as in Fig. 1F). Costs of protein synthesis (all means ± s.e.m.) were: blastula, 7.76±2.55 J mg^{-1} protein (N=3); gastrula, 7.75± 0.90 J mg^{-1} protein (N=3); larva, 10.34±0.57 J mg^{-1} protein (N=2). The costs were not significantly different for all stages of development analyzed (ANOVA; P=0.60, N=8). The average cost of protein synthesis=8.40±0.99 J mg^{-1} protein.

cost estimates for all stages of development were pooled, the average cost of protein synthesis in *L. pictus* was 8.40±0.99 J mg^{-1} protein synthesized.

Discussion

Understanding the processes that regulate metabolism still remains a major goal in physiology, even after 50 years of intensive study (Zeuthen, 1953; Kleiber, 1961; Schmidt-Nielsen, 1972; Hochachka and Somero, 1984, 2002). During animal development, embryos and larval stages are known to have high mass-specific metabolic rates (Zeuthen, 1953) with large, mass-independent changes in metabolism at specific stages of development (e.g. gastrulation; Fujiwara and Yasumasu, 1997; Leong and Manahan, 1997). The biochemical bases of such changes, however, are not fully understood. In the present study we have defined up to 75% of the causes of metabolic expenditure during early animal development, as detailed below (Fig. 7A, fed larva).

For fed larvae, both rates of protein synthesis and metabolism increased during growth and development (Fig. 5). To calculate the proportion of metabolic energy used to maintain the measured rates of protein synthesis in fed larvae, the rate of protein synthesis (Fig. 4) for each developmental stage measured was converted to energy equivalents (8.4 J mg^{-1} protein synthesized; Figs 5, 6). The percentage of total metabolic rate accounted for by the cost of protein synthesis was then calculated. Five such sets of calculations for different-sized fed larvae were performed to yield an average value of 75±11%, based upon an average metabolic rate of 37.5 μJ h^{-1} (Fig. 7A, fed larva).

Previous research has described the ontogenetic changes in the sodium pump (Na^+/K^+-ATPase) during embryonic development in *L. pictus* (Leong and Manahan, 1997). Thus for embryonic stages, the percentage of total metabolism used for protein synthesis and the sodium pump can be determined in *L. pictus*. For the blastula and gastrula stages of development, Leong and Manahan (1997) reported that 23% and 19% of metabolism, respectively, could be accounted for by the *in vivo* activity of the sodium pump. The fraction of metabolism accounted for by protein synthesis in these same stages of development (Fig. 7A) was 54±8% and 40±2% for blastula and gastrula stages, respectively. Combined, the percentage of metabolism accounted for by the sodium pump and by protein synthesis is 77% (23% + 54%) for a blastula and 59% (19% + 40%) for a gastrula (Fig. 7A).

These data for metabolic partitioning of protein synthesis during embryonic development of 40–50% of metabolic rate are similar to values reported for other animals. Some specific examples include the octopus *Octopus vulgaris*, where protein synthesis is 35–51% of metabolism (Houlihan et al., 1990a); the crab *Carcinus maenas* at 19–37% (Houlihan et al., 1990b); embryos of the killifish *Austrofundulus limnaeus* at 36% (Podrabsky and Hand, 2000); and the mussel *Mytilus edulis* at 19–26% (Hawkins et al., 1986). For larval stages of *L. pictus,* the pattern of metabolic partitioning is strikingly different to that of earlier embryonic stages. The percentage of metabolism accounted for by rates of protein synthesis varied as a function of feeding state. Unfed larvae had a metabolic rate of 3.3 μJ larva^{-1} h^{-1}. Calculating the costs of protein synthesis from the low, constant synthesis rate shown in Fig. 4 and the corresponding age-specific metabolic rates (Fig. 5), a value of 16±4% was obtained for the percent of metabolic rate accounted for by protein synthesis (Fig. 7A, unfed larva). In contrast, protein synthesis accounted for 75±11% of metabolic rate in rapidly growing fed larvae. These results highlight the importance of environmental factors, such as food availability, on the internal partitioning of energy use *via* up- and downregulation of protein synthesis. Unfed larvae of *L. pictus* have a low protein turnover of 5% per day (Table 2). This strategy of reducing protein synthesis is also seen in other animals experiencing environmental stress (Hand and Hardewig, 1996; Smith et al., 1996). For fed larvae, fractional rates increased tenfold (58%, Table 2). Such high fractional rates of protein synthesis have been reported for developmental stages of fish (Conceicao et al., 1997). This large increase in

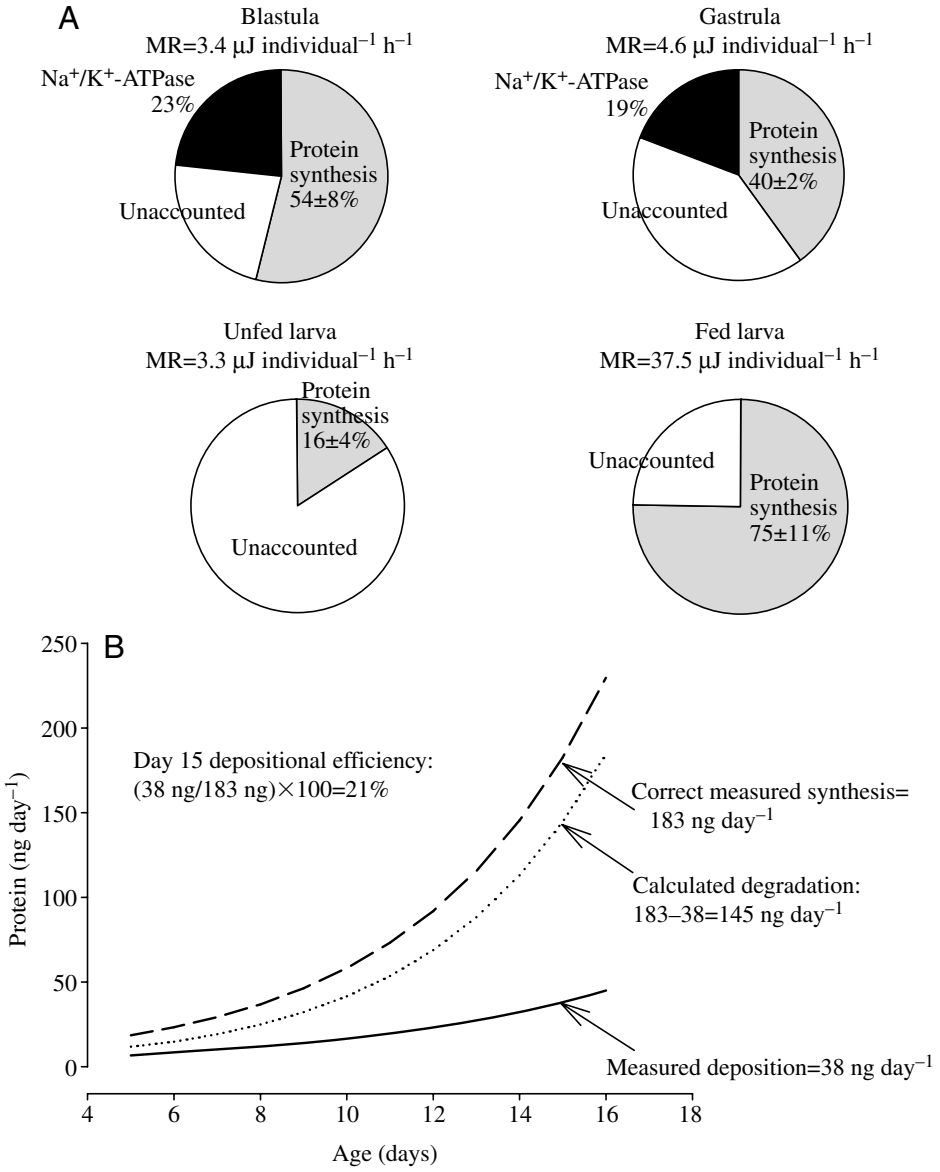

Fig. 7. (A) Setting metabolic rate. Percent of total metabolic rate (MR; given as μJ individual^{-1} h^{-1}) for different developmental stages of *L. pictus* accounted for by protein synthesis and sodium pump activity (data on Na$^+$/K$^+$-ATPase taken from Leong and Manahan, 1997). (B) Protein depositional efficiency. Rate of protein deposition (solid line) calculated from data on total protein content (see equation in legend of Fig. 2A). Rate of protein synthesis (broken line) calculated from protein synthesis rate (see equation in legend of Fig. 4). Rate of protein degradation during development (dotted line) calculated as difference between rates of synthesis and deposition. All rates were calculated on a per-larva basis. For 15-day-old larvae, the depositional efficiency was 21%.

fed larvae of *L. pictus* still falls within the metabolic 'pie-chart', accounting for 75% of metabolism (Fig. 7, fed larva). While the proportion of metabolism accounted for by the sodium pump has not been measured for larvae of *L. pictus,* that percentage is as high as 40% for unfed pluteus-stage larvae of another species of temperate sea urchin, *Strongylocentrotus purpuratus* (Leong and Manahan, 1997). It is likely that for unfed larvae of *L. pictus,* with relatively lower metabolic rates (3.3 μJ individual^{-1} h^{-1}), a large proportion of the unaccounted 84% of metabolism could be accounted for by the sodium pump. For fed larvae, with an order-of-magnitude higher metabolic rate (37.5 μJ individual^{-1} h^{-1}), the percentage accounted for by the sodium pump must be accommodated within the 25% of metabolism not accounted for by protein synthesis (Fig. 7A, fed larva).

From the data presented on rates of protein deposition and rates of protein synthesis for fed larvae (Figs 2A, 4), the depositional efficiency of protein synthesis can be calculated as a function of different growth rates (Fig. 7B). From the equations for the rates of protein synthesis and growth (see legends in Figs 2A, 4), the measured rate of synthesis and the resultant rate of protein deposition can be calculated. As shown in Fig. 7B, the rate of protein synthesis increased dramatically and had higher rates during faster growth than did the corresponding rate of protein deposition (i.e. protein dispositional efficiency decreased during growth). For instance, in Fig. 7B a 15-day-old fed larva had a rate of protein synthesis of 183 ng day^{-1} and a protein deposition rate of 38 ng day^{-1}. Hence of the 183 ng of protein synthesized, only 38 ng resulted in growth. Thus 145 ng of synthesized protein were degraded (183–38=145). The ratio of the rate of deposition to rate of synthesis is the depositional efficiency and, for a 15-day-old larva, the value is 21% (Fig. 7B). In earlier stages of development, the curvilinear nature of depositional efficiency results in higher efficiency with lower rates of protein synthesis and growth. For 6-day-old larvae, for

example, the depositional efficiency is 37%. This range of values for depositional efficiency is consistent with studies of other animals (Houlihan, 1991; Bayne and Hawkins, 1997). The analysis in Fig. 7B shows that developmental stages with the highest rates of protein synthesis and protein deposition, have the lowest depositional efficiency. It is noteworthy that this high rate of protein accumulation with low depositional efficiency is very 'expensive' metabolically, consuming 75% of total metabolic rate of fed larvae (Fig. 7A). This value decreases considerably in unfed larvae, which have low rates of protein synthesis, to only 16% of metabolism (Fig. 7A).

We have shown that the cost of protein synthesis during development of the sea urchin studied is independent of developmental stage, size, rate of protein synthesis, and physiological feeding state. A consequence of this fixed cost of synthesis is that the regulation of metabolic rate is achieved by altering rates and hence allocation of energy (i.e. the metabolic partitioning as in Fig. 7A), not by changing costs of synthesis as a function of the rate of synthesis. The energetic cost is fixed per unit of protein synthesized in developing sea urchin embryos and larvae. Given the importance of this fixed cost for the arguments presented above, we next consider the methodological bases for our conclusion that metabolic costs of protein synthesis are fixed, even for highly variable rates of synthesis.

Sea urchins have long been an ideal experimental organism for studies of developmental biology. The comprehensive reviews provided by Davidson (1968, 1976, 1986, 2001) highlighted the seminal research on sea urchins, ranging from analysis of expression of individual genes, to measurements of biochemical and physiological processes in embryos and larval stages. In fact, some of the earliest rate measurements of DNA replication, RNA synthesis and protein synthesis were conducted on sea urchin embryos (reviewed in Davidson, 1968, 1976, 1986, 2001). Research on cellular activation and regulation of protein synthesis following fertilization (Epel, 1967; Grainger and Winkler, 1987; Rees et al., 1995) has allowed for the formulation of highly reliable experimental protocols, with minimal perturbation to the experimental organism, and the attendance to critical assumptions regarding rate measurements in sea urchin embryos and larvae.

Our intent in this study was to build upon well-established methods for studies of protein synthesis in developing sea urchins to define the costs and energetic implications of such synthesis for energy metabolism. The protocol used in our study for measuring rates of protein synthesis was based largely on previous studies of rates of protein synthesis in sea urchin embryos (e.g. Fry and Gross, 1970; Marsh et al., 2001). An important addition to this protocol was the use of high-performance liquid chromatography (Vavra and Manahan, 1999) to measure the specific activity of the free amino acid pool (Fig. 1E). This allowed for continuous measurement of the size (moles) of the free amino acid pool and the specific activity of [^{14}C]alanine throughout the entire labeling experiment. This analysis also ensured that no interconversion of the [^{14}C]alanine tracer occurred during experiments (i.e. in Fig. 1E, all radioactivity was found under the alanine peak). Changes in the intracellular specific activity of [^{14}C]alanine in the free amino acid pool (Fig. 1D,E) and the rate of incorporation of [^{14}C]alanine into protein (Fig. 1C) were measured simultaneously. The rate of incorporation of [^{14}C]alanine into the protein fraction was then corrected for the changing specific activity of [^{14}C]alanine in precursor pool, allowing for a calculation of an absolute rate of protein synthesis.

One of the most important aspects in determining accurate rates of protein synthesis is the measurement of the intracellular, precursor specific activity pool. The most immediate precursor pool to peptides being manufactured is the aminoacyl-tRNA pool. In this study, as in most current studies of protein synthesis, we measured the specific activity of the free amino acid pool, the next immediate precursor to aminoacyl-tRNA. We recognize that it is possible that subcellular compartmentalization may result in the specific activity of the free amino acid pool not reflecting the specific activity of the aminoacyl-tRNA pool, as observed in some higher animals (Airhart et al., 1974; Ilan and Singer, 1975). For sea urchin embryos, however, Regier and Kafatos (1977) reported insignificant differences between the specific activity of the free amino acid pool and the aminoacyl-tRNA pool when using tracer amounts of amino acid.

The protocols in common use for studies of protein synthesis in sea urchin embryos do differ from protocols that use a 'flooding dose' of labeled amino acid, rather than a 'tracer dose'. The rational for the flooding dose is to minimize any potential compartmentalization in the free amino acid pool, so that the specific activity of the free amino acid pool is functionally the same as the specific activity of aminoacyl-tRNA. Marine invertebrates in general (Yancey et al., 1982), and sea urchin embryos specifically, have very high amounts of intracellular free amino acids (used as organic osmoltyes). It is well established in studies of protein synthesis in sea urchin embryos (e.g. Berg, 1965; appendix III in Davidson, 1986) that an effective 'flooding dose' of label is not practical due to the very large size of the free amino acid pools in these animals. Synthesis and turnover rate measurements for sea urchins are more commonly obtained from measurements of the kinetics of incorporation of tracer amounts of precursor into a specific, newly synthesized macromolecule (e.g. this study: Fig. 1C,E). Also, using tracer amounts permits experiments based on the amino acid transport capacities of the embryos under study and minimizes possible perturbation to cellular metabolism. Finally, an important validation of the method employed in the present study to measure protein synthesis in sea urchins is that our results are in good agreement with those from earlier studies of protein synthesis in sea urchin embryos. For instance, Berg and Mertes (1970) reported a fractional rate of protein synthesis in embryos of *L. anamesus* (= *L. pictus*) of 0.96% h^{-1} at 19°C. Allowing for temperature differences (i.e. a Q_{10} between 2 and 3), that value is similar to the rate calculated in our study for embryos of the same species measured at 15°C (0.60±0.04% h^{-1}; Table 2).

We are confident that our measurements of the absolute rates of protein synthesis are accurate (Fig. 4). The values presented in Fig. 5 for the respiration rates of developing stages of *L. pictus* measured for the present study are within the ranges reported in the literature for this species (Manahan, 1990; Shilling and Manahan, 1990; Jaeckle and Manahan, 1992; Leong and Manahan, 1997). For instance, Jaeckle and Manahan (1992) determined the oxygen consumption rate of gastrula-stage *L. pictus* to be 10.0 pmol O_2 individual^{-1} h^{-1}. This value agrees well with the respiration rate reported in the present study for gastrulae of 9.5±1.3 pmol O_2 individual^{-1} h^{-1} (see Results, 'Cost of protein synthesis' section). While measurements of synthesis rates and metabolic rates are robust and well established in the literature, the approach is less obvious as to how to combine these measurements for determinations of the metabolic cost of protein synthesis (Muramatsu and Okumura, 1985; Muramatsu et al., 1987; Aoyagi et al., 1988). Muramatsu et al. (1987) showed that the correlative cost of protein synthesis can be dependent upon the physiological feeding state of the organism, implying that measurements on starving animals would be more reliable due to less interference from non-protein synthetic related energy consumption. Our results with developing sea urchins show no difference in the correlative cost of protein synthesis between embryos and larvae with very different physiological feeding states (Fig. 5).

A common criticism of using any inhibitor for studies of metabolism is that the inhibitor itself might alter other cellular processes than the one under study. This would confound interpretations of metabolic energy consumption, such as the cost of protein synthesis (Aoyagi et al., 1988; Wiesner and Zak, 1991; Wieser and Krumschnabel, 2001). Protein synthesis inhibitors, such as cycloheximide, can at certain concentrations reduce transport rates of glucose (Evans, 1971) and potassium (Reilly et al., 1970). Another commonly used protein synthesis inhibitor, puromycin, has been shown to reduce by up to 20% the rates of amino acid transport by sea urchin embryos (Berg, 1965). For our studies of developing sea urchins, alanine was used to measure rates of protein synthesis. At the concentration used in the present study, emetine did not significantly decrease the rate of alanine transport (Fig. 3). Our results are consistent with the general findings that have shown emetine to be a specific inhibitor of protein synthesis in eukaryotes. Emetine is known to bind to specific sites on eukaryotic ribosomes (Pestka, 1971) and has been found to be very useful in studies of protein synthesis in a wide range of developmental stages of marine invertebrates (sea urchin: Wagenaar, 1983; Pesando et al., 1995; Yamada, 1998; sea star: Sasaki and Chiba, 2001; gastropod: Fenteany and Morse, 1993). The concentration of emetine used in the current study was within the range known to inhibit protein synthesis in marine invertebrates. Specifically, emetine at a concentration of 100 μmol l^{-1} inhibited over 90% of protein synthesis in embryonic stages (Fig. 6, blastula and gastrula). For an early larval stage (3-day-old), emetine inhibited 80% of protein synthesis. For a more advanced larval stage (13-day-old), 46% of protein synthesis was inhibited by emetine even at a higher concentration of 150 μmol l^{-1}. The different levels to which the inhibitor decreased rates of protein synthesis at different stages of development are likely due to the ease with which the inhibitor, dissolved in seawater, can enter the smaller number of cells in embryos, *vs* access to cells in larval stages with more complex morphology and resultant tissue layers. These differences in relative potency of emetine did not, however, alter estimates of protein synthesis costs because the same concentration of emetine was used in experiments to measure both respiration and protein synthesis rates with and without inhibitor (Fig. 6).

In light of the discussions in the literature regarding the selection of the 'best' method to measure costs of protein synthesis, we compared both of the methods in common use. The correlative method is an indirect measurement, in that the estimate of cost is calculated from the relationship between rates of synthesis and respiration, with the slope of this relationship representing the cost of protein synthesis (Fig. 5). The inhibitor method is a more direct analysis, in which the cost of protein synthesis is measured as the decrease in rates of synthesis and respiration in the presence of an inhibitor (Fig. 6). An advantage of the inhibitor method is that it can measure the cost of protein synthesis at single developmental stages and does not require a broad range of protein synthesis and metabolic rate measurements to estimate a cost of protein synthesis, as is required for correlative cost estimates. In our studies, both methods gave essentially identical results of 8.4 J mg^{-1} protein synthesized. The embryos and larvae studied varied significantly, by our experimental design, in developmental stage, physiological feeding state, size (Fig. 1A,B), and rates of protein synthesis (Table 2, Fig. 4). Yet the amount of energy required to synthesize protein remained fixed at 8.4 J mg^{-1} protein synthesized. This fixed cost of protein synthesis for developing sea urchins is similar to costs reported for a wide range of adult animals, e.g. *Mytilus edulis*, 11.4 J mg^{-1} protein synthesized (Hawkins et al., 1989), cod fish, 8.7 J mg^{-1} (Lyndon et al., 1989), chickens, 5.4 and 13.0 J mg^{-1} (respectively, Aoyagi et al., 1988; Muramatsu and Okumura, 1985) and mammals, 11.5 J mg^{-1} (Reeds et al., 1985). Estimates of the cost of protein synthesis measured *in vivo* usually give higher and more variable values than those based upon the minimal energy required for peptide bond formation. Possible reasons for these higher than theoretical values have been discussed extensively and are usually explained by the fact that other metabolic processes are included in estimates of protein synthesis costs conducted on cells and whole organisms, ranging from mammals to marine invertebrates (Reeds, 1985; Hawkins, 1991; Houlihan, 1991).

In this study, we have addressed a central question in physiology – what are the mechanisms underlying the 'cost of living'. The focus of the current work has been on stages of development that undergo rapid cell division (embryos) and growth (increase in size and mass). We conclude that the energetic cost of protein synthesis in developing sea urchins is fixed at 8.4 J mg^{-1} protein synthesized and is independent of

large variations in protein synthesis rates during development and growth. This finding for sea urchin development is in contrast to studies with fish, where tenfold variable costs of synthesis within a species have been reported (Smith and Houlihan, 1995). While we report here that the cost of protein synthesis for a temperate species of sea urchin is 8.4 J mg^{-1} protein synthesized, we previously reported that Antarctic sea urchin embryos and larvae have a very low cost of protein synthesis at 0.45 J mg^{-1} protein synthesized (Marsh et al., 2001). These ranges of cost estimates for protein synthesis highlight the need to understand the mechanisms that could result in markedly different quantities of ATP being required for protein synthesis in eukaryotes. Additionally, understanding such differences in the context of developmental biology and metabolic energy expenditure under different thermal environments are of importance for studies of the evolutionary physiology of animals.

Analysis of the amino acid composition of sea urchin protein was conducted at the Molecular Structure Facility, University of California, Davis. We thank Allison Green for assistance with culturing and for providing data on respiration of embryos and larvae. This work was supported by the National Science Foundation under Grant No. 0130398.

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