Supporting Information

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SI Calculations and Estimations

Allocation of Metabolic Energy to Protein Synthesis. The proportion of metabolic energy allocated to protein synthesis was calculated using the measured energy cost per unit of protein synthesized (Fig. 3*C*, grand mean). For example, the rate of protein synthesis for 2-d-old embryos under control treatment was 0.4 ± 0.04 nanograms protein per individual per hour (Table S4, first row). Based upon the measured cost of protein synthesis of 2.4 J/mg protein synthesized, the metabolic cost of synthesizing protein at this rate is 0.86 microJoules per individual per hour. The corresponding metabolic rate for these 2-d-old embryos was 5.2 ± 0.33 picomoles O_2 per individual per hour, equivalent to 2.5 microJoules per individual per hour [484 J/mmol O_2 (1)]. Hence, protein synthesis accounted for 34% of the metabolic rate (Fig. 5*A*, control 2-d-old embryos). Similar sets of calculations from Table S4 form the basis of ATP allocation pie charts in Fig. 5.

Allocation of Metabolic Energy to Ion Transport. The proportion of metabolic energy allocated to in vivo ion transport activity of Na^+,K^+-ATPase was calculated by converting values of K^+ transport to ATP equivalents. The ATP equivalents of sodium–potassium transport activity by Na^+,K^+-ATPase have been established previously for developmental stages of *S. purpuratus* (2). Two moles of K^+ are transported by Na^+,K^+-ATPase for every mole of ATP hydrolyzed, allowing the conversion of the ion transport rate to ATP equivalents. Metabolic rates were converted to ATP equivalents using 5.2 pmol ATP/pmol O_2 (3).

Fractional Rates of Protein Turnover. For developmental stages in which no exogenous feeding on particulate foods occurs, and for which there is no net protein growth (e.g., embryos), measured rates of protein synthesized are equivalent to rates of protein turnover. Fractional rates of protein synthesis, i.e., (protein synthesis rate/whole-body protein content) × 100, represent the percentage of total body protein turned over per unit of time. One- and 2-d-old embryos had an average protein content of 18.5 ng per embryo and a protein synthesis rate of 0.4 ng per embryo per hour (Fig. 2). For these stages, the fractional rate of protein turnover is 2.2%/h.

Accounting for the Increase in Metabolic Rate of Unfed Larvae. The metabolic rates of unfed 6- to 10-d-old larvae increased from an average of 5.7 picomoles O_2 per larva per hour in controls to 7.8 picomoles O_2 per larva per hour under acidification treatment (Fig. 1*B*, *Inset*), an increase of 2.1 picomoles O_2 per larva per hour. To convert oxygen use to energy, an oxyenthalpic equivalent of 484 J/mmol O_2 was used, because developing sea urchins have lipid and protein as the dominant biochemical constituents (4). The increase in metabolic rate corresponds to an increase of 1.0 microJoules per larva per hour. In unfed larvae from the same cultures, rates of protein synthesis increased from 0.19 nanograms per larva per hour under control conditions to 0.37 nanograms per larva per hour under acidification treatment (Fig. 2*B*, *Inset*). Using the measured cost per unit of protein synthesis, the approximately twofold increase in the synthesis rate of unfed larvae is equivalent to 0.43 microJoules per larva per hour [(0.37–0.19) × 2.4 μJ/ng protein] (Fig. 3*C*). Hence, the increased rate of protein synthesis under acidification accounts for 43% of the increase in metabolic rate of 1.0 microJoules per larva per hour. In unfed larvae subjected to acidification treatment, in vivo Na^+,K^+-ATPase activity increased to 14.0 picomoles ATP per larva per hour from 7.0 ATP per larva per hour under control conditions. Converting this increase of 7.0 picomoles ATP per larva per hour to oxygen equivalents [5.2 pmol ATP/pmol O_2 (3)] results in an incremental value for the metabolic cost of ion transport of 1.3 picomoles O_2 per larva per hour. Hence, this increased rate under acidification accounts for 62% of the increase in metabolic rate (1.3 pmol O_2 = 62% of 2.1 pmol O_2). Combined, the energy demand of increased rates of protein synthesis (43%) and ion transport (62%) fully account for the observed increase in metabolic rate (i.e., 105%, within reasonable experimental error) in unfed larvae under acidification.

SI Materials and Methods

Animal Culturing and Biological and Technical Replications. A total of 16 million eggs combined from four females were fertilized with pooled sperm from four males. Fertilized eggs were randomly assigned to stock four 200-L culture vessels at 20 eggs/mL. Two of these culture vessels contained control seawater (~400 μatm pCO_2) (Table S1), and two contained CO_2-pretreated seawater (~800 μatm pCO_2). Once the feeding stage was reached on day 4, aliquots of larvae were removed from the control and acidified seawater cultures and were resuspended in a series of eight 20-L cultures at an initial stocking density of 10 larvae/mL. The density was reduced to ~2 larvae/mL as development proceeded and samples were removed from culture vessels for analyses. Four of these 20-L culture vessels at ~400 μatm pCO_2 were treated further by the presence or absence of algal food (fed and unfed treatments). Similar feeding treatments were conducted on larvae at ~800 μatm pCO_2. For all feeding and pCO_2 treatments, measurements of body size, metabolic rate, protein synthesis rate, and in vivo Na^+,K^+-ATPase activity were made on embryos and prefeeding, fed and unfed larvae (ranging in age from 1- to 10-d-old individuals). Approximately 50,000 individuals were analyzed each day of sampling (see Table S4 for details of measurements). Additional batches of 1,000 to 5,000 individuals (depending upon developmental stage) were frozen at −80 °C for later analyses of total Na^+,K^+-ATPase, enzyme, and gene-expression assays. This entire protocol was repeated for 1- to 14-d-old individuals with the exception of the unfed treatment, using pooled gametes obtained from an additional set of three females and two males. Replicate cohorts of developing sea urchins were included in all statistical analyses; cohorts did not differ in body length or metabolic rate (Table S3).

For experiments measuring the cost of protein synthesis, a total of four cohorts of developmental stages obtained from different sets of parents were used. Concurrent measurements of metabolic rate and protein synthesis were made on 1-, 4-, 6-, and 10-d-old embryos and larvae, with and without emetine. To obtain the grand mean for the cost of protein synthesis given in Fig. 3*C*, nine cost values were determined based on nine sets of metabolic rates (with up to 10 replicate respiration measurements each) and nine sets of protein synthesis rates (with up to three five-point time-course assays each, e.g., Fig. 3*A*).

Larval Size. Growth rate was determined by measuring the diameter of 1- and 2-d-old embryos or midline body lengths of 3- to 14-d-old larvae. At least 50 randomly selected individuals from each treatment on each sampling day were measured in size-calibrated digital images from photomicroscopy. Digital images were analyzed using Image J software and converted to sizes in micrometers.

Metabolic Rate. Metabolic rates were determined from O_2 consumption using microrespiration measurements as previously described (5). Known numbers of individuals were incubated for 3–4 h in air-tight microrespiration vials containing filtered seawater that had been pre-equilibrated with either ambient air or elevated pCO_2. At the end of the incubation, the amount of O_2 was measured in samples taken from each respiration vial by injecting subsamples of 200 μL seawater into a temperature-controlled microcell (MC100; Strathkelvin) that was fitted with a polarographic oxygen sensor (Model 1302; Strathkelvin). Changes in the partial pressure of oxygen were monitored on an oxygen meter (Model 782; Strathkelvin) that was calibrated to the concentration of oxygen in moles/L.

Protein Content. Whole-body protein content was determined using the Bradford dye-binding assay, modified for developing marine invertebrates (6).

Protein Synthesis Rate. Absolute rates of protein synthesis were determined following the methods described previously for developing sea urchins (7). In each assay, known numbers of individuals were incubated in 10 mL of filtered seawater containing 74 kBq ^{14}C-alanine (Perkin-Elmer Inc.) and adjusted to a final concentration of 10 μM alanine with cold carrier (nonradioactive alanine; Sigma-Aldrich). A series of 1-mL subsamples of individuals in the incubation seawater was collected approximately every 6 min and gently filtered through a filter membrane (pore size, 8 μm; Nucleopore). Animals were retained on the membrane, gently rinsed with 10 mL of seawater to removed excess radioactivity, and immediately frozen at −80 °C until further analysis. The amount of ^{14}C-alanine incorporated into trichloroacetic acid (TCA)-precipitatable protein at each time interval was measured by scintillation counting and subsequently corrected, using HPLC analysis, for the intracellular specific activity of ^{14}C-alanine in the free amino acids pool in each developmental stage. The amount of alanine incorporated into protein was converted to an absolute rate of protein synthesis. This calculation was based on the amino acid composition of the whole-body protein content of *S. purpuratus* (Table S2). The measured mole-percent–corrected molecular mass of amino acids in protein was 127.4 g/mole, and the mole-percent of alanine was 7.9%. Rates of protein synthesis were calculated from the slope of the linear regression describing the relationship between the amounts of newly synthesized protein and time.

Cost of Protein Synthesis. The cost of protein synthesis for developmental stages of *S. purpuratus* was determined by measuring the metabolic rate and protein synthesis rates in the absence and presence of the protein synthesis inhibitor (emetine at 100 μM). Emetine is a well-documented specific inhibitor of protein synthesis that does not affect other major ATP-consuming processes such as RNA synthesis in developing sea urchins (8) or whole-cell Na^+,K^+-ATPase activity in cell lines (9). The selected concentration of 100 μM has been shown to inhibit protein synthesis almost completely (Fig. 3*A*) (8) and has been used for several species of developing sea urchins (7, 10). This analysis of the cost of protein synthesis was extended further to measure the synthesis cost in *S. purpuratus* developing in different pCO_2 treatments.

In Vivo Na^+,K^+-ATPase Activity. The physiologically active fraction of total Na^+,K^+-ATPase in the developmental stages of *S. purpuratus* was measured as the ouabain-sensitive portion of potassium transport. The isotope $^{86}Rb^+$ was used for this analysis, as previously described for *S. purpuratus* (2). Briefly, for a given developmental stage, a known number of embryos or larvae was placed in 10 mL of filtered seawater to which was added 0.9 MBq of $^{86}Rb^+$ (PerkinElmer, Inc.). A 1-mL subsample of individuals was removed from the seawater solution every 1–2 min and filtered through a filter membrane (pore size, 8 μm; Nucleopore). Animals on the membrane were rinsed gently with 10 mL of seawater to remove excess isotope. The membrane filter holding the biological sample was placed in a 7-mL liquid scintillation counting vial to which 0.5 mL of tissue solubilizer (Solvable; PerkinElmer, Inc.) was added. After overnight solubilization, liquid scintillation mixture was added, and the radioactivity in each vial was determined. The rate of ion transport was calculated from the slope of the linear regression describing the relationship between $^{86}Rb^+$ transport and incubation time. To convert this value to a rate of K^+ transport (and, hence, in vivo Na^+,K^+-ATPase activity), the rate of $^{86}Rb^+$ transport was corrected for the specific activity of K^+ in seawater, using salinity of 33.5‰ (Table S1).

Total Na^+,K^+-ATPase Enzyme Activity. Total activity was determined using published methods for developmental stages of *S. purpuratus* (2). Frozen embryos and larvae were homogenized in a 10% sucrose buffer. Approximately 30 μg of protein was used for each Na^+,K^+-ATPase enzyme assay. Subsamples of homogenates were placed at a ratio of 1:3 in a reaction buffer (130 mM NaCl, 20 mM KCl, 10 mM $MgCl_2$, 50 mM imidazole, 5 mM ATP, pH 7.7) in the presence or absence of 2 mM ouabain. Reaction mixtures were incubated at 25 °C for 20 min, and the production of inorganic phosphate from ATP was measured at 700 nm in a spectrophotometer.

Na^+,K^+-ATPase Gene Expression. Steady-state levels of *Na^+,K^+-ATPase α* were determined in real-time quantitative PCRs (qPCRs), normalized to that of *EF1α*. Total RNA was extracted using TRIzol reagent (Life Technologies) according to the manufacturer's instructions, treated with RNase-free DNase, and purified with a RNA Clean and Concentrator kit (Zymo Research). Samples were eluted from purification columns in nuclease-free water; RNA concentration in the eluate was determined from absorption at 260 nm, measured with a NanoDrop spectrophotometer (Thermo Scientific). DNA-free RNA was reverse transcribed to cDNA using SuperScript III reverse transcriptase (Life Technologies) and random hexamers. Reverse-transcribed cDNA then was diluted 1:5 with nuclease-free water. Steady-state levels of *S. purpuratus Na^+,K^+-ATPase α* (NM_001123510) were determined in real-time qPCR assays as described (11), using the following primer sequences: forward, GGTGGTCAATTTGACAAGTCTTCA; reverse, TCAGATCGGTTGCAGAGACAA. Expression of *Na^+,K^+-ATPase α* was normalized to that of *EF1α* (NM_001123497), assayed using the primer sequences: forward, CAACGAAATCGTCAGGGAGGTC; reverse, AGATTGGGATGAAGGGCACAG. Relative expression is presented as copies of *Na^+,K^+-ATPase α* per copy of *EF1α*. Copy number values were determined from a standard curve created using plasmids containing clones of *Na^+,K^+-ATPase α* or *EF1α*. All qPCR samples were assayed in duplicate. SYBR Advantage qPCR Premix (Clontech) was mixed with cDNA template, forward and reverse primers (0.2 μM each), and nuclease-free water to a final volume of 25 μL. Samples were cycled on a MX3005P thermocycler (Agilent Technologies, Inc.) as follows: initial denaturation for 30 s at 95 °C followed by 40 cycles of 5 s at 95 °C and 30 s at 60 °C.

In Vivo ^{35}S-Met/Cys Labeling of Proteins. Three samples of 1,500 12-d-old larvae were taken from a cohort subjected to either control or acidification treatment. These larvae were radiolabeled in 1.5-mL test tubes for 4 h at 15 °C by the addition of 100 μCi of a protein-labeling mix (^{35}S-Met/Cys; Perkin-Elmer, Inc.), with 500 nM methionine added as cold carrier (Sigma Chemical Co.) to seawater. At the end of the incubation, larvae were gently centrifuged, the seawater and unincorporated isotope were removed, and the pelleted larvae were washed with filtered seawater (three low-speed centrifugation washing cycles) to remove

excess radioactivity. Samples were stored at −80 °C until prepared for analysis by PAGE. Samples were homogenized in radioimmunoprecipitation assay (RIPA) buffer (150 mM NaCl, 0.1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris·HCl, pH 8.0). Protein was precipitated from tissue homogenates by the addition of cold TCA (5% final concentration), incubated for 1 h on ice, and centrifuged for 15 min at 14,000 × *g* (4 °C). Supernatant containing the unincorporated ^{35}S-labeled amino acids was removed. The TCA-precipitated pellet was washed three times with 5% TCA to remove any excess radioactivity, followed by a final wash with methanol to remove residual TCA. Residual methanol was evaporated, and the protein pellet was resolubilized in 10 μL of 0.2 M NaOH. RIPA buffer was added to the resolubilized protein to a final volume of 100 μL. Radioactivity in protein samples was determined by liquid scintillation counting. Volumes containing equal amounts of radioactivity (counts: 75,000 dpm per electrophoretic lane) were combined with Laemmli loading dye (1:1), heated at 80 °C for 5 min, and immediately cooled on ice. Samples were loaded into an SDS polyacrylamide gel (4% stacking; 10% resolving) and separated by electrophoresis at 150 V for ∼2 h. The gel was stained with Coomassie Blue, incubated overnight in 5% glycerol, and then dried onto Whatman filter paper (70 °C for 4 h). The gel was exposed to a phosphoimager plate for 24 h; the plate was scanned with a Molecular Dynamics Storm 860 detection system (GE Healthcare).

Statistical Analyses. When a statistically significant relationship occurred between a biological response and size or age (e.g., regression of increase in metabolic rate with size in growing larvae), ANCOVA was used to test for differences between treatments. In other cases (e.g., absence of growth of unfed larvae), groups were compared by ANOVA. Where necessary, data were log transformed before ANCOVA (indicated in Table S3). Tukey tests were used for post hoc analyses.

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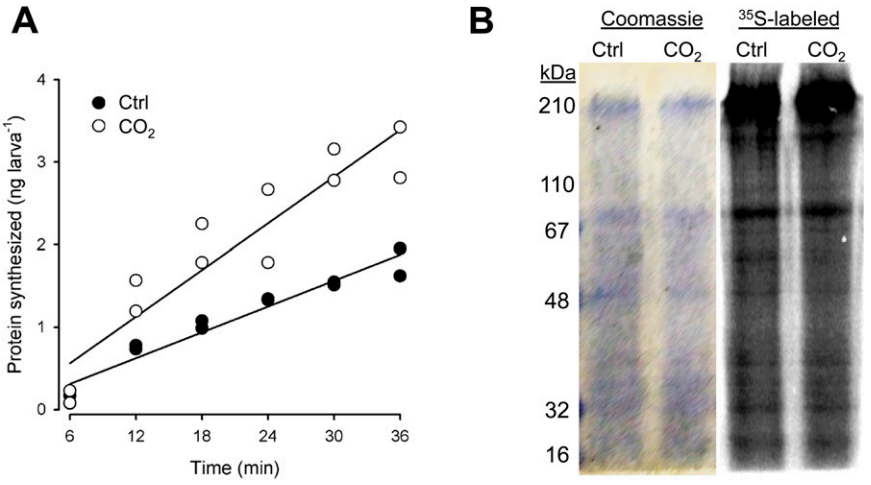

Fig. S1. Rates and patterns of protein synthesis in 12-d-old fed sea urchin larvae under control (Ctrl) and seawater acidification (CO_2) conditions. (*A*) Acidification resulted in an increased rate of protein synthesis compared with control (comparison of slopes, $P = 0.003$). (*B*) Representative images of PAGE separation of total proteins (Coomassie Blue stained) and autoradiogram of ^{35}S-Met/Cys–labeled proteins. Equal amounts of radioactivity (in disintegrations/min) were loaded in each lane.

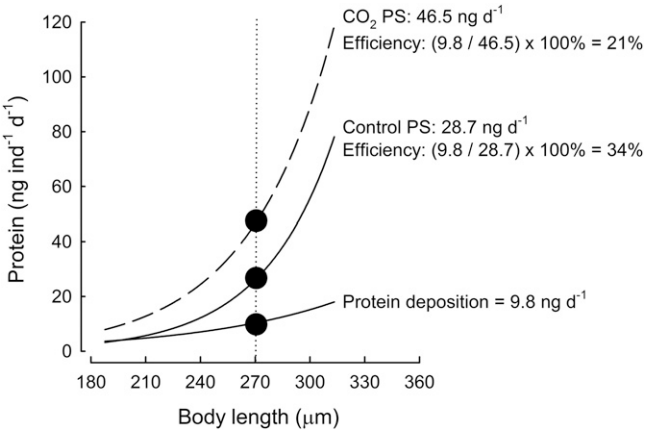

Fig. S2. Protein depositional efficiencies under control and seawater acidification treatments. Protein depositional efficiency (i.e., the ratio of protein accreted to protein synthesized) was calculated for a 270-μm larva, shown as a vertical dashed line near the midpoint of the regressions (calculated values are shown as solid circles). For developing sea urchins of this size, protein depositional efficiency was reduced to 21% under acidification, relative to 34% for those grown under control conditions. Calculations were based on equations for rates of protein deposition (9.8 ng/d) and protein synthesis (PS) under control (Control PS: 28.7 ng/d) and seawater acidification (CO_2 PS: 46.5 ng/d) conditions. Primary data are given in Fig. 2 *A* and *B*. The relationship between age and protein content of developing sea urchins in the current study is described by the regression equation: Protein content = $10.8 \times e^{(0.2 \times Age)}$ ($r^2 = 0.93$). The first derivative of this equation gives the equation for the daily rate of protein growth: rate of protein deposition = $10.8 \times 0.2 \times e^{(0.2 \times Age)}$. Body length was substituted for age using the equation for the regression shown in Fig. 1*A* (body length = $117.5 \times Age^{0.4}$). Daily rates of protein synthesis were calculated from the regression fit to the data in Fig. 2*B* [shown here on linear scale; Control: Log PS = 0.01 × (body length) − 1.4; Acidification: Log PS = 0.01 × (body length) − 0.8].

Table S1. Environmental conditions in experimental cultures

Seawater parameter	Control	CO_2 treatment
Temperature, °C	15.3 ± 0.10	15.3 ± 0.07
pH_{NBS}	8.1 ± 0.01	7.9 ± 0.00
Salinity	33.5	33.5
DIC, μmol/kg	1,921.8 ± 53.33	2,180.1 ± 17.71
pCO_2, μatm	431.9 ± 16.78	800.6 ± 9.07
Ω calcite	3.1 ± 0.09	2.2 ± 0.02
Ω aragonite	2.0 ± 0.06	1.4 ± 0.02
TA, μmol/kg	2,097.1 ± 56.84	2,283.3 ± 18.18
CO_3^{2-}, μmol/kg	126.8 ± 4.93	90.7 ± 0.99

Temperature, pH (calibrated with National Bureau of Standards buffers, pH_{NBS}), and total dissolved inorganic carbon (DIC) are measured values. Temperature was monitored continuously throughout the duration of culture experiments with automatic temperature loggers that were placed in culture vessels (Model HOBO U12; Onset Computer Corp.). Measurements of pH and water samples for DIC were collected on each day of biological experiments. A handheld pH meter (Orion Star Handheld pH meter; Thermo Scientific), calibrated with National Institute of Standards and Technology standard was used for pH measurements. DIC was measured using a carbon coulometer (CM 5015; UIC, Inc.) calibrated with certified reference materials (batch 120) supplied by Andrew Dickson (Scripps Institution of Oceanography, San Diego). Salinity is from reported values for seawater near Santa Catalina Island where the seawater used for cultures was obtained (1). Total alkalinity (TA), partial pressure of CO_2 (pCO_2), calcite and aragonite saturation states, and carbonate ion (CO_3^{2-}) concentrations were calculated from measured variables using CO2SYS (2) with selected dissociation (3, 4) and HSO_4^- constants (5). The average pCO_2 value (431.9 μatm) used to culture larvae under control conditions is within the range of values reported for coastal California waters (6). Values in the table are mean ± SEM. Ten culture vessels were assayed for carbonate chemistry throughout the developmental period studied, five for control and five for CO_2-treated seawater. The errors were calculated for pCO_2 for all statistical variation across culture vessels and between water sampling days and represent a maximum of 3.9% (SEM/mean) for control (431.9 μatm) and 1.1% for CO_2-treated (800.6 μatm) seawater. As expected, pCO_2 and pH differed significantly between treatments (Mann–Whitney *u* tests, pCO_2: $U = 0.0$, $P < 0.001$; pH: $U = 0.0$, $P < 0.001$). The values for TA calculated from CO2SYS are different by 8% ($U = 52.0$, $P < 0.001$) between treatments. By CO2SYS analysis, this 8% difference in TA for a given pCO_2 would change the reported pH values by no more than 0.036 units, thus not altering the major conclusion of this table showing a large difference in pH and pCO_2 between treatments.

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Table S2. Amino acid composition of proteins in developmental stages of the sea urchin *S. purpuratus* under control and seawater acidification treatments

Protein	Control treatment, nmoles				Acidification treatment, nmoles			Grand mean, nanomoles	Composition of protein, mole-percent
	Day 1	Day 4	Day 9	Day 14	Day 1	Day 4	Day 14		
Tryptophan	0.9	0.7				0.8		0.8 ± 0.07	0.8
Cysteine	1.0	0.9		1.1	1.1	1.0		1.0 ± 0.04	1.0
Methionine	1.4	1.2		1.5	1.4	1.3	1.6	1.4 ± 0.06	1.4
Histidine	1.7 ± 0.01	1.6 ± 0.01	1.9 ± 0.05	1.9 ± 0.03	1.7 ± 0.02	1.6 ± 0.04	1.9 ± 0.02	1.8 ± 0.03	1.7
Tyrosine	3.0 ± 0.04	3.0 ± 0.04	3.1 ± 0.04	3.2 ± 0.06	3.0 ± 0.01	3.1 ± 0.05	3.2 ± 0.02	3.1 ± 0.02	3.0
Phenylalanine	4.3 ± 0.06	4.3 ± 0.06	4.1 ± 0.05	4.2 ± 0.07	4.4 ± 0.02	4.4 ± 0.05	4.3 ± 0.03	4.3 ± 0.03	4.2
Arginine	4.7 ± 0.03	4.3 ± 0.01	5.2 ± 0.05	5.0 ± 0.08	4.8 ± 0.01	4.6 ± 0.12	5.1 ± 0.07	4.8 ± 0.07	4.7
Proline	5.0 ± 0.02	5.7 ± 0.11	5.1 ± 0.03	5.1 ± 0.09	5.0 ± 0.00	5.4 ± 0.04	5.0 ± 0.04	5.2 ± 0.06	5.1
Isoleucine	5.3 ± 0.07	5.1 ± 0.05	4.9 ± 0.04	5.1 ± 0.07	5.4 ± 0.04	5.2 ± 0.05	5.1 ± 0.02	5.2 ± 0.04	5.1
Threonine	6.5 ± 0.06	7.1 ± 0.10	5.9 ± 0.03	6.0 ± 0.05	6.6 ± 0.01	7.0 ± 0.03	5.9 ± 0.03	6.4 ± 0.11	6.3
Serine	6.8 ± 0.04	6.9 ± 0.04	6.5 ± 0.05	6.2 ± 0.06	6.9 ± 0.01	6.7 ± 0.07	6.3 ± 0.09	6.6 ± 0.06	6.5
Lysine	7.0 ± 0.07	5.9 ± 0.03	7.3 ± 0.12	7.0 ± 0.11	6.7 ± 0.42	6.0 ± 0.08	7.2 ± 0.07	6.7 ± 0.13	6.6
Valine	7.4 ± 0.10	7.1 ± 0.09	6.5 ± 0.01	6.6 ± 0.09	7.3 ± 0.05	7.1 ± 0.02	6.6 ± 0.02	6.9 ± 0.08	6.8
Leucine	8.3 ± 0.11	7.3 ± 0.07	7.6 ± 0.07	7.9 ± 0.13	8.5 ± 0.03	7.5 ± 0.11	8.1 ± 0.08	7.9 ± 0.09	7.8
Alanine	7.6 ± 0.06	8.2 ± 0.04	8.3 ± 0.04	8.1 ± 0.05	7.6 ± 0.04	8.1 ± 0.04	8.2 ± 0.04	8.0 ± 0.06	7.9
Glycine	8.0 ± 0.05	9.7 ± 0.06	11.0 ± 0.03	10.2 ± 0.13	7.9 ± 0.04	9.5 ± 0.06	10.1 ± 0.13	9.5 ± 0.24	9.4
Aspartate/asparagine[†]	10.7 ± 0.09	10.3 ± 0.12	10.8 ± 0.18	10.7 ± 0.05	10.5 ± 0.12	10.4 ± 0.02	10.1 ± 0.68	10.5 ± 0.10	10.4
Glutamate/glutamine[†]	11.8 ± 0.09	11.9 ± 0.06	10.9 ± 0.09	10.9 ± 0.07	11.8 ± 0.05	11.6 ± 0.01	10.9 ± 0.04	11.4 ± 0.10	11.2
MW_p, g/mole									127.4

The total number of protein samples analyzed for amino acid composition was 21 for the range of developmental stages, ages, and pCO_2 treatments given above (see Table S1 for chemistry conditions of control and seawater acidification treatments). The average molecular weight of amino acids in proteins (MW_p) of *S. purpuratus* was calculated to be 127.4 g/mole. This value was calculated from the grand mean of the 20 protein amino acids analyzed. The grand mean column represents average moles of amino acid present in developmental stages. Within replicate samples, the mass of protein sample that was acid-hydrolyzed before each amino acid analysis differed slightly. After analysis, the resultant moles of amino acids were normalized across different samples to a total of 100 nmol per sample. The ^{14}C-labeled tracer used for determination of absolute rates of protein synthesis was alanine, which is present at 7.9 mole-percent in protein. Seawater acidification had no significant effect on amino acid composition of proteins in developmental stages, compared with control samples ($P > 0.05$). Data in each column labeled by Day are mean values ± 1 SEM, $n = 3$. For the column labeled Grand mean, values are mean ± 1 SEM for the pooled samples of all days and treatments ($n = 21$ for all amino acids other than tryptophan, $n = 3$; cysteine, $n = 5$; methionine, $n = 6$). Separate preparatory steps for amino acid analyses were conducted for individual measurements of tryptophan (mercaptoethanesulfonic acid hydrolysis), and cysteine and methionine (performic acid oxidation) as per standard protocols for amino acid analysis conducted at the University of California, Davis, Proteomics Core Facility where this analysis was conducted.

[†]Asparagine and glutamine form aspartate and glutamate, respectively, during the acid hydrolysis analysis step.

Table S3. ANCOVA and ANOVA results for measured variables in developmental stages of the sea urchin *S. purpuratus* under control and seawater acidification treatments

	Source	df	F	*P*
ANCOVA variable				
Body length*, Fig. 1*A*	Model	3, 2,080	7,009.30	<0.001
	Age	1	13,940.10	<0.001
	pH	1	2.78	0.095
	Cohort	1	2.91	0.088
Metabolic rate*, Fig. 1*B*	Model	3, 254	731.70	<0.001
	Body length	1	1,205.79	<0.001
	pH	1	0.97	0.326
	Cohort	1	1.75	0.187
Total protein content*, Fig. 2*A*	Model	2, 48	189.10	<0.001
	Body length	1	378.17	<0.001
	pH	1	0.04	0.847
Protein synthesis rate*, Fig. 2*B*	Model	2, 19	56.79	<0.001
	Body length	1	104.85	<0.001
	pH	1	8.73	0.009
Ion transport rate, Fig. 4*B*	Model	2, 19	34.07	<0.001
	Body length	1	61.02	<0.001
	pH	1	7.12	0.016
ANOVA variable				
Body length, Fig. 1*A*	Model	7, 480	11.35	<0.001
	Age	3	25.65	<0.001
	pH	1	0.38	0.537
	Age × pH	3	0.71	0.544
Metabolic rate, Fig. 1*B* and *Inset*	Model	3, 96	17.25	<0.001
	Stage†	1	26.53	<0.001
	pH	1	18.81	<0.001
	Stage × pH	1	2.06	0.155
Total protein content, Fig. 2*A* and *Inset*	Model	3, 48	5.14	<0.001
	Stage†	1	0.11	0.743
	pH	1	3.60	0.063
	Stage × pH	1	9.00	0.004
Protein synthesis rate, Fig. 2*B* and *Inset*	Model	3, 19	14.11	<0.001
	Stage†	1	21.48	0.003
	pH	1	8.07	0.012
	Stage × pH	1	8.62	0.010
Ion transport rate, Fig. 4*B* and *Inset*	Model	3, 15	11.27	0.008
	Stage†	1	15.78	0.002
	pH	1	8.20	0.014
	Stage x pH	1	9.83	0.009
Na^+,K^+-ATPase gene expression, Fig. 4*C*	Model	11, 47	5.35	<0.001
	Age	5	6.74	0.002
	pH	1	4.16	0.049
	Age × pH	5	4.25	0.004
Total Na^+,K^+-ATPase activity, Fig. 4*C*	Model	11, 37	0.28	0.984
	Age	5	0.37	0.863
	pH	1	0.02	0.877
	Age × pH	5	0.25	0.936

As stated in *Materials and Methods*, ANCOVA was used to test for differences between treatments when a statistically significant relationship occurred between a biological response and size or age (e.g., Fig. 1*B*, regression of increase in metabolic rate with size in growing larvae). In other cases (e.g., Fig. 1*A*, absence of growth of unfed larvae), groups were compared by ANOVA. If significant interactions are evident, post hoc Tukey analyses were performed. The term "cohort" refers to groups of developmental stages obtained from different parents and used in replicate sets of experimental treatments and measurements.

*Log-transformed data.

†Stage includes embryos and unfed larvae.

Table S4. The allocation of ATP to protein synthesis and ion regulation in developmental stages of the sea urchin *S. purpuratus* under control and seawater acidification treatments and under different feeding states

CO_2 treatment	Feeding state	Age, d	Body length, μm	Metabolic rate, picomoles O_2 per individual per hour	Protein synthesis rate, nanograms protein per individual per hour	ATP allocation to protein synthesis, %	In vivo Na^+,K^+-ATPase activity, picomoles P_i per individual per hour	ATP allocation to Na^+,K^+-ATPase, %	Unaccounted fraction, %
Control	Prefeeding	2	145.6 ± 1.93	5.2 ± 0.33	0.4 ± 0.04	34	4.1	15	51
		3	194.0 ± 2.36	7.7 ± 0.71	0.3 ± 0.03	18	4.6	12	70
		4	222.3 ± 1.62	6.7 ± 0.40	0.3 ± 0.02	19	5.3	15	66
	Fed	6	253.6 ± 1.87	14.1 ± 1.14	0.9 ± 0.08	31	18.6	25	44
		8	282.1 ± 2.71	23.2 ± 1.28	1.1 ± 0.39	24	27.4	23	53
		10	318.5 ± 2.47	51.0 ± 2.59	5.3 ± 0.52	51	28.0	11	38
	Unfed	6	253.1 ± 2.01	5.0 ± 0.2	0.2 ± 0.03	21	7.8	30	49
		8	260.0 ± 3.01	6.1 ± 0.3	0.2 ± 0.03	17	6.2	20	63
Acidification	Prefeeding	2	137.3 ± 1.46	7.2 ± 0.72	0.4 ± 0.06	25	8.0	22	53
		3	203.8 ± 2.74	10.8 ± 1.40	0.7 ± 0.06	30	13.9	25	45
		4	218.8 ± 1.50	7.3 ± 0.85	0.6 ± 0.16	41	10.6	28	31
	Fed	6	259.1 ± 1.90	11.5 ± 0.74	1.1 ± 0.14	46	21.1	35	19
		8	288.1 ± 3.66	20.1 ± 1.12	2.4 ± 0.44	59	27.2	26	15
		10	300.8 ± 3.66	42.6 ± 2.30	5.6 ± 0.50	66	45.3	20	14
	Unfed	6	252.7 ± 2.55	7.7 ± 0.16	0.4 ± 0.04	24	18.4	46	30
		8	255.2 ± 2.69	6.0 ± 0.26	0.3 ± 0.03	26	14.1	45	29

Physiological rates used for the calculation of ATP allocation pie charts shown in Fig. 5. At each age, measurements were made on different aliquots of larvae from the same cohort of individuals cultured under control or acidification conditions. Data are shown as mean ± 1 SEM; $n = 50$ individuals for body midline length measurement; $n = 8$–10 respiration vials (up to 800 individuals in each vial) for metabolic rate. Each protein synthesis rate is the slope (± SE) of duplicate six-point time-course assays. A total of 192 time points (with known number of individuals at each point, ~1,000) were sampled for synthesis rates presented in this table. At each time point, the amount of newly synthesized protein is corrected for intracellular specific activity of ^{14}C-alanine with HPLC. Each in vivo Na^+,K^+-ATPase activity is calculated as the difference between duplicate six-point time-course $^{86}Rb^+$ transport assays with ouabain and two assays without ouabain. A total of 384 time-point samples (with known number of individuals at each point, ~1,000) were collected for the calculation of physiological rates of ion transport by Na^+,K^+-ATPase. Because of the subtractive nature of the determination of in vivo Na^+,K^+-ATPase activity (difference in rates with and without ouabain), error analyses are not listed for the column in the table; however, each of these $^{86}Rb^+$ transport assays had an error of 20% or less (i.e., on average regression r^2 values = 0.80 for 64 time-course assays; see Fig. 4*A* for an example). Calculations of ATP allocation for rates of protein synthesis and ion transport are described in in *SI Calculations and Estimations*.