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RESEARCH ARTICLE

Differing thermal sensitivities of physiological processes alter ATP allocation

Francis T. C. Pan, Scott L. Applebaum* and Donal T. Manahan‡

ABSTRACT

Changes in environmental temperature affect rate processes at all levels of biological organization. Yet the thermal sensitivity of specific physiological processes that affect allocation of the ATP pool within a species is less well understood. In this study of developmental stages of the Pacific oyster, *Crassostrea gigas*, thermal sensitivities were measured for growth, survivorship, protein synthesis, respiration and transport of amino acids and ions. At warmer temperatures, larvae grew faster but suffered increased mortality. An analysis of temperature sensitivity (Q_{10} values) revealed that protein synthesis, the major ATP-consuming process in larvae of *C. gigas*, is more sensitive to temperature change (Q_{10} value of 2.9±0.18) than metabolic rate (Q_{10} of 2.0±0.15). Ion transport by Na^+/K^+-ATPase measured *in vivo* has a Q_{10} value of 2.1±0.09. The corresponding value for glycine transport is 2.4±0.23. Differing thermal responses for protein synthesis and respiration result in a disproportional increase in the allocation of available ATP to protein synthesis with rising temperature. A bioenergetic model is presented illustrating how changes in growth and temperature affect allocation of the ATP pool. Over an environmentally relevant temperature range for this species, the proportion of the ATP pool allocated to protein synthesis increases from 35 to 65%. The greater energy demand to support protein synthesis with increasing temperature will compromise energy availability to support other essential physiological processes. Defining the trade-offs of ATP demand will provide insights into understanding the adaptive capacity of organisms to respond to various scenarios of environmental change.

KEY WORDS: *Crassostrea gigas*, Larvae, Metabolic rate, Protein synthesis, Temperature, Phenotypic variation

INTRODUCTION

The environment is a primary driver of biological variation. Among many well-studied environmental factors, temperature is a major controlling factor that governs physiological rates (Fry, 1947; Prosser, 1955; Somero et al., 2017). There is over a 100-year history of studies showing that physiological rates, especially in ectothermic animals, respond to change in environmental temperature (Krogh, 1916; Fry and Hart, 1948; Scholander et al., 1953; Schmidt-Nielsen, 1997; Clarke and Johnston, 1999; Dell et al., 2011; Schulte, 2015; Somero et al., 2017). The increase in metabolism in response to temperature is often used as the foundation for models to predict organismal response and ecological impact under various scenarios of environmental change (Brown et al., 2004; Pörtner and Knust, 2007; Dillon et al., 2010; Pörtner, 2010; Deutsch et al., 2015; Dahlke et al., 2020). Specific models, for instance, predict the impact of thermal stress on oxygen demand and delivery (Pörtner and Knust, 2007; Pörtner, 2010). While the effects of temperature on rates of oxygen consumption are well studied (*op. cit.*), less is known about the relative thermal sensitivity of specific physiological processes within a species that affect the ATP pool at the level of a whole organism. At a cellular level, the causal processes of ATP allocation have been experimentally partitioned, with protein synthesis and ion transport consuming the largest proportion of the ATP pool (e.g. cancer cell lines: Siems et al., 1992). Additional studies on other cell types and tissues reveal similar patterns (Rolfe and Brown, 1997; Hulbert and Else, 2000; Wieser and Krumschnabel, 2001; Cherkasov et al., 2006; Staples and Buck, 2009).

At the level of a whole organism, phenotypic variation from 22 to 74% in the allocation of the ATP pool to protein synthesis is related to genetically determined differences in growth rate of a larval form (Pan et al., 2018). ATP allocation also underlies aspects of organismal resilience to environmental stress (Cherkasov et al., 2006; Deigweiher et al., 2010; Pan et al., 2015a; Podrabsky and Hand, 2015; Frieder et al., 2017, 2018). With regard to ATP allocation in response to environmental temperature, differences in thermal sensitivity between ATP-producing (respiration) and ATP-consuming pathways (e.g. protein synthesis and ion transport) will probably lead to different allocation patterns of the ATP pool which, in turn, may result in variation in thermal tolerance. For instance, with temperature increase there is a corresponding increase in cellular respiration related to protein synthesis activity in marine bivalves (Cherkasov et al., 2006). Temperature sensitivity has also been reported for protein synthesis relative to respiration for different species of isopods (Whiteley et al., 1996; Whiteley and Faulkner, 2005).

The cost of active transport of sodium and potassium ions across the cell membrane by Na^+/K^+-ATPase accounts for ∼20% of total oxygen consumption (Rolfe and Brown, 1997; Hulbert and Else, 2000). The regulation of ion transport is an important metabolic basis of thermal adaptation (Hochachka, 1988). In fish hepatocytes, oxygen consumption related to Na^+/K^+-ATPase enzyme activity increases with temperature (Schwarzbaum et al., 1992; Krumschnabel et al., 1994). The response of isolated gill tissues to warmer temperature results in an elevation of Na^+/K^+-ATPase, with enzymatic thermal sensitivity (Q_{10} value) of ∼3 (Kreiss et al., 2015). In that study, the fraction of tissue oxygen consumption accounted for by Na^+/K^+-ATPase did not increase, suggesting similar thermal sensitivity between respiration and Na^+/K^+-ATPase activity.

In the present study, the effect of temperature on developmental physiology was investigated. Specifically, larval stages of the

Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0371, USA.
*Present address: Environmental Studies Program, University of Southern California, Los Angeles, CA 90089-0156, USA.

‡Author for correspondence (manahan@usc.edu)

F.T.C.P., 0000-0002-1550-4581; D.T.M., 0000-0002-6437-9107

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Pacific oyster (*Crassostrea gigas*) were chosen to study thermal sensitivity (values for Q_{10}) for ATP production as reflected by oxygen consumption, and major ATP-consuming processes such as protein synthesis and ion transport by Na^+/K^+-ATPase. In larvae of this species, Pan et al. (2018) characterized the suite of physiological processes that account for more than 80% of the ATP pool utilization. This characterization allowed for additional studies of ATP allocation strategies in response to environmental change (Frieder et al., 2017, 2018). In addition, as an experimental species for studies of environmental and physiological variation, the energy cost per unit mass of protein synthesis in larvae of *C. gigas* is fixed and independent of genotype, growth phenotype and environmental temperature (Lee et al., 2016). Here, we identify sub-lethal biochemical 'tipping points' (ATP allocation strategies) for growth and survival, based on analyses of the relationship between growth and energy utilization over a range of temperatures known to be environmentally relevant to this species, which has a global distribution (Bayne, 2017).

MATERIALS AND METHODS

Larval cultures

A total of nine different larval cohorts were cultured for the experiments reported in this study. Each larval cohort was started from eggs and sperm obtained from gonads of different gravid males and females of the Pacific oyster, *Crassostrea gigas* Thunberg 1793. Seven of these larval cohorts were used for analysis of temperature sensitivity (determination of Q_{10} values for a range of physiological processes). Experiments were conducted with two additional cohorts of larvae (herein designated as Cohorts 1 and 2) to measure the effect of temperature on growth and survival. Depending upon the fecundity of single males and females, some of these nine cohorts were produced by mixing sperm and eggs from several different individuals. For Cohorts 4, 5, 6 and 7, sufficient gametes were obtained from a single male and female to produce four different sibling larval families, as part of a multi-year breeding program being undertaken for this species (e.g. Pace et al., 2006; Pan et al., 2016, 2018). The availability of sibling larval families is of significance for some of the conclusions presented in the present study (see 'Temperature sensitivities of physiological processes' section in the Discussion).

Fertilization success was confirmed by microscopy. A known number of fertilized eggs was added to a series of 200 liter culture vessels to start each culture at 10 eggs ml^{-1}. Each 200 liter culture vessel contained filtered seawater (0.2 µm pore size) that was pre-heated with flow-through, titanium heat exchangers to the desired rearing temperature. Larval cultures were maintained in a temperature-controlled room and measured continuously using temperature data loggers set to 30 min increments (HOBO U12; Onset Computer Corporation, Bourne, MA, USA). A complete replacement of the seawater in each culture vessel was performed every 2 days. Once larval development had reached the feeding veliger stage (at 2 days old, when cultured at 25°C), larvae in each culture vessel were fed the algae *Isochrysis galbana* at 30 cells $µl^{-1}$. As larvae grew in size, the feeding ration was increased to 50 cells $µl^{-1}$, as per standard culturing protocols for this species (Breese and Malouf, 1975; Helm et al., 2004).

Growth and survival

Growth and survival in larvae from all cohorts were monitored. To evaluate effects of temperature on growth and survival, larvae from Cohorts 1 and 2 were reared for up to 16 days post-fertilization at two contrasting temperatures. Within a temperature treatment, larvae were cultured in duplicate 200 liter culture vessels, held at a mean (±s.e.m.) temperature of 21.3±0.09 or 28.1±0.10°C. At each sampling time point of 2- to 4-day intervals, larvae in each culture vessel were enumerated for analysis of: (1) survival; (2) growth (shell length); (3) rates of oxygen consumption; and (4) protein synthesis rates. Survivorship was calculated based on microscopic observation and enumeration of individual viable larvae, corrected for the number of larvae removed for experimental analysis in the previous sampling interval. To control for possible impacts on survivorship of different numbers of larvae across treatments and culture vessel replicates, the number of larvae in all culture vessels was adjusted at each sampling interval to the culture vessel that had the lowest number of surviving larvae. To maintain *ad libitum* feeding conditions across all treatments and replicates, the amount of algal food was frequently monitored throughout the duration of each experiment by counting the number of algal cells in each culture vessel (Z2 Particle Counter; Beckman Coulter, Brea, CA, USA). Additional algal food was added, as appropriate, to maintain a near-constant food source in each culture vessel.

For growth rate determinations, larvae in each culture vessel were sampled at least five times during the growth period studied (up to 16 days post-fertilization). At each sampling interval, the shell lengths of at least 50 randomly selected larvae were measured on calibrated photo-microscopic images using ImageJ (National Institutes of Health, Bethesda, MD, USA). The length of each individual larval shell was quantified as the distance from the anterior to the posterior edge of the shell. Growth rates were calculated for larvae in all treatments based on the slope of a linear regression model for the relationship between shell length and age (days post-fertilization).

Temperature effects on different physiological processes

To determine the temperature sensitivity (Q_{10} values) for a range of physiological processes, larvae from seven different cohorts (Cohorts 3 to 9) were studied. Within each cohort, larvae representing different age and size ranges were also analysed, resulting in a total of 13 independent sets of measurements for Q_{10} values. Larvae used for these analyses were reared at 24.5±0.11°C (mean±s.e.m.), a common temperature used to rear larvae of *C. gigas* (Breese and Malouf, 1975; Helm et al., 2004). For each set of Q_{10} determinations, the effect of temperature was measured for the following *in vivo* physiological processes: (1) oxygen consumption; (2) protein synthesis; (3) amino acid transport (glycine); and (4) ion transport (sodium–potassium). At the start of these *in vivo* physiological assays, larvae from each 200 liter culture vessel were concentrated and enumerated. Four aliquots of a known number of larvae (~50,000 larvae) were transferred to 1 liter Erlenmeyer flasks and pre-exposed to the required experimental temperature treatments, ranging in approximately 5°C increments (exact temperature recorded for each physiological process assayed) over a temperature range of 15 to 30°C. Larvae were exposed at each temperature increment for 2 h before a physiological rate assay was conducted. The temperature range selected for these experimental treatments was based on the known temperature range (11–34°C) for this species in their natural habitats (*C. gigas* has a global distribution: Bayne, 2017). The required temperature was maintained using a circulating water bath with a digital temperature controller (VWR International, Radnor, PA, USA). For the 2 h pre-experimental temperature incubation, larvae in each 1 liter Erlenmeyer flask were aerated and fed algal cells at the same ration as in original rearing conditions in 200 liter culture vessels. Prior to each physiological assay, algal cells were removed by

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passing the seawater through mesh sieves that separated larvae from algae; the larvae were then enumerated. Aliquots of known numbers of filtered seawater-washed larvae were placed in a range of assay containers, and different physiological processes were measured at the respective treatment temperature. As described below, the number of larvae used depended on the specific physiological assay conducted.

To permit comparison of acutely and chronically exposed temperature treatments, larvae from Cohort 2 were grown continuously for up to 16 days at two different temperatures (21 and 28°C, see 'Growth and survival', above). Larvae from this cohort were also analysed for the chronic effect of temperature on respiration and protein synthesis. For both respiration and protein synthesis assays, a range of larval sizes was tested for the effect of chronic exposure to temperature. Specifically, 10 different sizes (range: 80 to 160 µm shell length) were assayed for each physiological process for each chronically exposed temperature treatment (21 and 28°C).

Respiration

Respiration rates of larvae were measured as oxygen consumption (Marsh and Manahan, 1999; Pace et al., 2006; Pan et al., 2016, 2018), with the following modifications. A known number of larvae (range 300–700 individuals, depending on larval size and assay temperature) were placed in a series of 6–10 replicate, ∼600 µl micro-respiration chambers (each a custom-made, sealed glass vial that was independently calibrated for exact volume). The micro-respiration chambers contained air-saturated, filtered seawater equilibrated to the required assay temperature. In this study, respiration measurements were conducted using an oxygen optode system that allowed for time course assays of multiple samples at different temperatures. At each time interval, the tip of a fiber optic cable (Loligo Systems, Viborg, Denmark) was placed over an oxygen sensor spot (2 mm in diameter; PreSens, Regensburg, Germany) that was fixed by Loligo Systems-supplied adhesive to the inside of each micro-respiration chamber. The fiber optic cable was connected to an oxygen meter (Model Witrox 1, Loligo Systems). Optode oxygen readings (arbitrary units) were calibrated and converted to moles of oxygen.

The optode sensor-based measurements in this study were compared with values obtained from end-point assays of micro-respiration chambers, measured with polarographic oxygen sensors (Model 1302, Strathkelvin, North Lanarkshire, UK). The latter method was optimized and cross-calibrated with several independent, micro-respiration methodologies – critical for accurate measurements of respiration in marine invertebrate larvae (Hoegh-Guldberg and Manahan, 1995; Marsh and Manahan, 1999). A further inter-calibration of optode and polarographic oxygen sensors (Fig. 1) demonstrated that these two independent measurement methods resulted in similar values for respiration rates of larvae of different sizes and at different temperatures (Fig. 1: Dunn's pairwise comparison, $P>0.05$ for all pairwise comparisons between optode and polarographic methods).

Respiration rates used to calculate values of Q_{10} were based on a series of three to five optode oxygen measurements taken during a time course assay for each micro-respiration chamber (Fig. 2A). For each temperature tested, six to 10 replicate micro-respiration chambers were used. In this study, a total of 565 respiration rate measurements were conducted on larvae of different sizes, representing different cohorts. Control micro-respiration chambers that contained only filtered seawater (no larvae present) were included with each set of respiration measurements to correct for any background changes in oxygen not attributable to larval respiration (Fig. 2A, dashed lines: regression ANOVA, $P=0.153$; i.e. no significant background consumption of oxygen was observed in these control, micro-respiratory chambers).

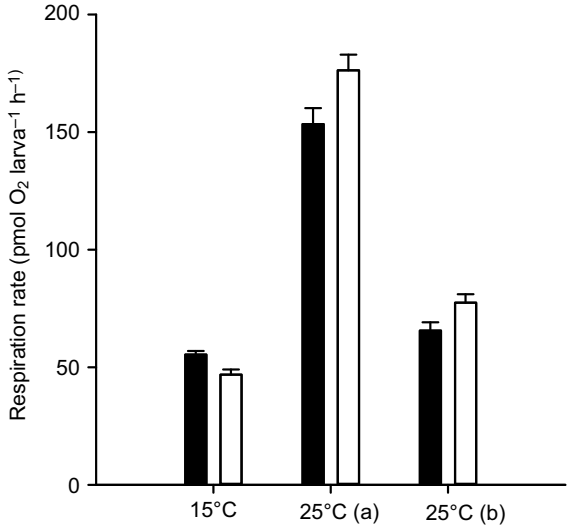

Fig. 1. Comparison of respiration rates in larvae of *Crassostrea gigas* measured using polarographic oxygen sensors and optode oxygen sensors. For each comparison, oxygen in micro-biological oxygen demand (µBOD) respiration chambers was measured at different time points using optode sensors; oxygen consumption by larvae of *C. gigas* is calculated based on the slope of decrease in oxygen over time (optode sensors: open bars). At the end of the incubation for each respiration chamber, oxygen was determined by injecting a sub-sample of seawater taken from each µBOD respiration chamber into a temperature-controlled water bath jacket, which housed the polarographic sensor; oxygen consumption is calculated based on a series of end-point measurements (polarographic sensor: filled bars). Oxygen consumption rates by larvae in the same µBOD respiration chamber were similar, whether measured with either an optode oxygen sensor or a polarographic oxygen sensor ($P>0.05$ for each pair of rates, Dunn's pairwise comparisons after Kruskal–Wallis test). This similarity between methods for measuring oxygen consumption was consistent for larvae of different sizes measured at different temperatures. At 15 and 25°C (a), larval size assayed was 225±2.2 µm. At 25°C (b), a smaller larval size was assayed at 172± 2.7 µm. Each histogram represents the mean (±s.e.m.) of 15 different µBOD respiration chambers.

Amino acid transport and protein synthesis

Glycine transport and protein synthesis rates were measured as described previously (Manahan, 1989; Lee et al., 2016; Pan et al., 2015b, 2018). At each temperature, duplicate time course assays were conducted (Fig. 2B) in replicate 20 ml glass vials. A total of 130 such assays were conducted for this study on larvae of different sizes and from different cohorts. In each assay, a known number of larvae were incubated in 10 ml of filtered seawater, to which 74 kBq [^{14}C]glycine (PerkinElmer, Boston, MA, USA) was added. The final concentration of glycine was adjusted to 10 µmol l^{-1} with the addition of non-radioactive glycine (Sigma-Aldrich, St Louis, MO, USA). A series of 1 ml samples were collected and vacuum filtered onto an 8 µm (pore size) filter membrane (Nucleopore; GE Healthcare, Pittsburgh, PA, USA). Larvae retained on the filter were gently rinsed with seawater to removed excess radioactivity and immediately frozen at −80°C until further analysis.

Each frozen sample of larvae was sonicated in 500 µl of deionized water. For measurement of glycine transport rates, an aliquot of the larval homogenate was transferred to a 7 ml scintillation vial containing 500 µl of tissue solubilizer (Solvable, PerkinElmer). The

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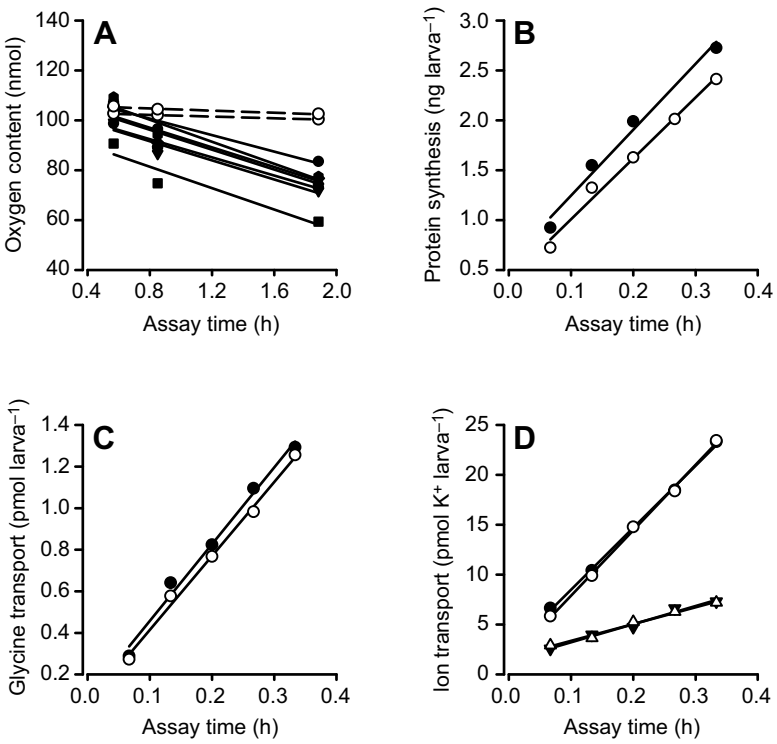

Fig. 2. Time course assays for the determination of different physiological rate processes in larvae of *Crassostrea gigas*. (A) Respiration: filled symbols show oxygen consumption by larvae over time in a series of seven replicate respiration chambers. Respiration rate is calculated from the slope of decrease in oxygen with time in each respiration chamber. Differences in oxygen content at the start of an experiment were caused by minor differences in handling time required to place aliquots of larvae in a series of replicate respiration chambers; such differences do not change the calculation of respiration rate, which is estimated from the slope of oxygen decrease with time. Open circles are oxygen measurements in respiration chambers without larvae (control values). (B) Protein synthesis: filled and open symbols show two independent assays. Protein synthesis rate is calculated as the slope of increase in the amount of newly synthesized protein with time. Minor differences in intercepts do not affect the rate calculation estimated from the slope. (C) Glycine transport: filled and open symbols show two independent assays. Glycine transport rate is calculated as the slope of increase in the amount of glycine transported with time. (D) Ion (sodium–potassium) transport: open and filled triangles represent two replicate assays with ouabain (specific inhibitor for Na^+/K^+-ATPase); open and filled circles show two replicate assays without ouabain. The difference in slope between the two sets of assays (with and without ouabain) is the *in vivo* rate of ion transport by Na^+/K^+-ATPase.

amount of [^{14}C]glycine transported by larvae was measured by liquid scintillation counting, with appropriate quench correction. The amount of radioactivity was corrected for the specific activity of [^{14}C]glycine in seawater to calculate the amount of total glycine transported in moles (Fig. 2C).

For measurement of protein synthesis rates, two aliquots of each larval homogenate were used to determine: (1) the rate of [^{14}C]glycine incorporation into trichloroacetic acid (TCA)-precipitable protein, and (2) the change in the intracellular specific activity of [^{14}C]glycine in free amino acid pool of larvae, analysed by high-performance liquid chromatography (Lee et al., 2016; Pan et al., 2018). For larvae of *C. gigas*, Lee et al. (2016) reported that more than 80% of radioactivity incorporation into the TCA-precipitable fraction was inhibited by emetine, a protein synthesis inhibitor. The amount (moles) of [^{14}C]glycine incorporated into larval protein was calculated by correcting for the change in the intracellular [^{14}C]glycine specific activity. Total moles of glycine incorporated into whole-body protein was converted to mass (grams) of protein synthesized, based on: (1) the measured mole-percent glycine (12±0.2%) present in whole-body protein extracts of larvae of *C. gigas*, and (2) the average molecular mass of amino acids in larval protein (126.6± 0.2 g mol^{-1}: Lee et al., 2016).

Ion transport

The *in vivo* rate of ion transport by Na^+/K^+-ATPase was measured based on methods developed for larvae of *C. gigas* (Pan et al., 2016). In the present study, a total of 116 time course assays were conducted on larvae of different sizes representing different cohorts. At each different temperature tested, a series of four time course assays were conducted (Fig. 2D). For two of these assays, a specific inhibitor of Na^+/K^+-ATPase (ouabain at 2.0 mmol l^{-1}, Sigma-Aldrich) was added to each 20 ml ion transport assay vial that contained 10 ml of seawater and a known number of larvae. A known amount of radioactive tracer was also added [0.9 MBq [$^{86}Rb^+$] (Perkin-Elmer Inc.); Rb^+ is a physiological analog of K^+ (Hilden and Hokin, 1975)]. In parallel, ion transport rate was measured in two separate vials containing the same number of larvae in 10 ml seawater to which no ouabain was added. A series of 1 ml seawater samples containing larvae were collected and vacuum filtered onto an 8 µm (pore size) filter membrane (Nucleopore, GE Healthcare). Larvae retained on the filter were gently rinsed with seawater to remove excess radioactivity and placed in a 7 ml scintillation vial containing 500 µl tissue solubilizer (Solvable, Perkin-Elmer). The amount of radioactivity was quantified using liquid scintillation counting and corrected for the specific activity of K^+ in seawater to calculate the rate of K^+ transport by larvae. The difference in transport rates between assays with and without ouabain represents the rate of ion transport by Na^+/K^+-ATPase in larvae of *C. gigas*.

Modeling bioenergetic changes in ATP allocation strategies

A series of calculations were undertaken to model the impact of temperature on the allocation of the ATP pool during growth for larvae of *C. gigas*. Conversion of rates of protein synthesis and respiration to energy equivalents is based on the cost of protein synthesis in larvae of *C. gigas* [2.1 µJ ng^{-1} protein (Lee et al., 2016)] and an oxyenthalpic value of 484 kJ mol^{-1} O_2 (Gnaiger, 1983). First, for larvae of different sizes (shell length) (Table S1) growing at a constant temperature of 25°C, rates of protein synthesis and respiration were calculated (equations from figs 3 and 5 of Pan et al., 2018). Second, over a range of different temperatures (Table S2), the change in the allocation of ATP to protein synthesis was calculated for a mid-sized larva (200 µm shell length), using the mean thermal sensitivity values for protein synthesis (Q_{10}=3.0) and respiration (Q_{10}=2.0) presented in this study. Third, family-specific differences in ATP allocation (Table 1), based on the different values of Q_{10} for protein synthesis and respiration in larval Cohorts 5 and 7, were calculated for a mid-sized larva (200 µm shell length) at 20 and 30°C.

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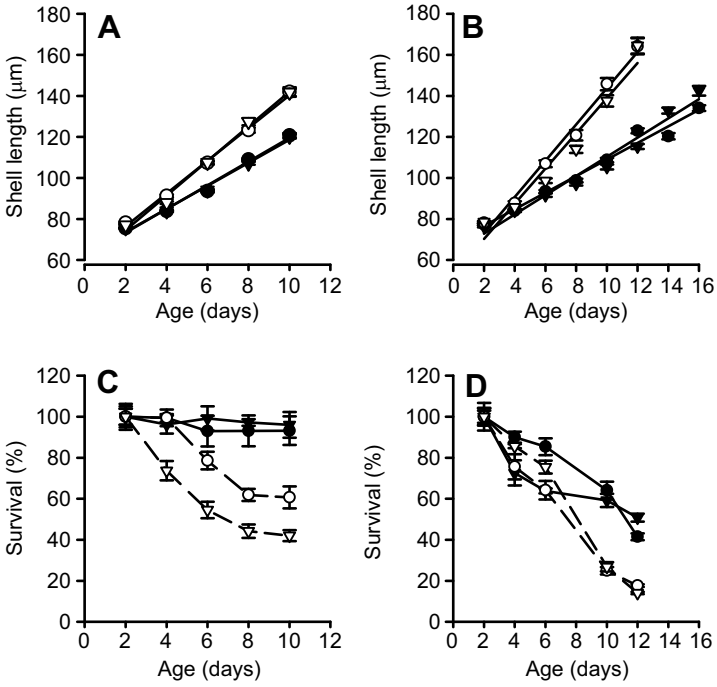

Fig. 3. Growth and survivorship for larvae of *Crassostrea gigas* reared at two different temperatures (21 and 28°C). Growth rates (A and B) and percentage survival (C and D) were determined using two larval cohorts (Cohort 1: A and C; Cohort 2: B and D). Circles and triangles represent replicate 200 liter culture vessels held at either 21°C (filled symbols) or 28°C (open symbols). (A) Growth rates of larvae from Cohort 1 (N≥50 independent shell length measurements for each data point). Filled circles represent a replicate 200 liter culture vessel where larval growth rate at 21°C was 5.8±0.11 µm day^{-1} (slope±s.e. of regression between shell length and age). Filled triangles represent a second replicate 200 liter culture vessel where larval growth rate at 21°C was 5.6±0.11 µm day^{-1}. Open circles and open triangles represent growth rate at 28°C of 8.4±0.20 and 8.0±0.20 µm day^{-1}, respectively, in replicate 200 liter culture vessels. (B) Growth rates of larvae from Cohort 2 (N≥50 independent shell length measurements for each data point). Filled circles represent a replicate culture vessel at 21°C: growth was 4.1±0.09 µm day^{-1}. Filled triangles represent a second replicate culture vessel at 21°C: growth was 4.7±0.11 µm day^{-1}. Open circles and open triangles represent growth rate at 28°C of 8.9±0.29 and 8.7±0.28 µm day^{-1}, respectively, in replicate 200 liter culture vessels. (C) Survival of larvae from Cohort 1. Each data point represents the percentage survival, calculated from the visual enumeration of the number of live larvae on a given day, relative to day 2 (newly formed veliger larvae). Error bars represent calculated coefficients of variation for survivorship on each sampling day. (D) Survival of larvae from Cohort 2. Analysis for each data point was calculated with the same method used for Cohort 1.

RESULTS

Chronic exposure temperature treatments

Growth and survival

The survivorship of larvae of *C. gigas* was measured for all nine cohorts in all 200 liter culture vessels used in these experiments. For larvae reared at 25°C, a standard rearing temperature for *C. gigas* (Breese and Malouf, 1975; Helm et al., 2004), survivorship averaged 70% by day 11, averaged across the 15 different 200 liter culture vessels that were used to rear larvae at this temperature. There was, as expected in rearing larvae, a difference in survivorship across different cohorts. For instance, for larval Cohort 8, five replicate vessels were assayed, yielding an average survival of 43±3% (coefficient of variation). Cohort 9 had three replicate culture vessels with a higher average survival at 68±5%. The highest surviving cohort was Cohort 3 at 83%; Cohorts 5 and 7 were 80 and 77%, respectively.

Thermal dependence of growth and survivorship in larvae of *C. gigas* was tested in two different cohorts (Cohorts 1 and 2) that were reared from fertilized eggs at either 21 or 28°C. Larvae from both cohorts showed consistent growth rates between duplicate culture vessels at each rearing temperature tested. The growth rates in replicate culture vessels for larvae from Cohort 1 reared at 21°C were 5.8±0.11 µm day^{-1} in one 200 liter culture vessel (Fig. 3A: slope±s.e.m., R^2=0.92, P<0.001) and 5.6±0.11 µm day^{-1} in the replicate 200 liter culture vessel (R^2=0.92, P<0.001). When reared at 28°C, growth rates were 8.0±0.20 µm day^{-1} (Fig. 3A: R^2=0.88, P<0.001) and 8.4±0.20 µm day^{-1} (R^2=0.86, P<0.001). For larvae of Cohort 2 reared at 21°C, growth rate in replicate culture vessels was 4.1±0.09 µm day^{-1} (Fig. 3B: R^2=0.81, P<0.001) and 4.7±0.11 µm day^{-1} (R^2=0.80, P<0.001), and at 28°C rates were 8.9±0.29 µm day^{-1} (R^2=0.73, P<0.001) and 8.7±0.28 µm day^{-1} (R^2=0.73, P<0.001) (Fig. 3B). Between the two temperature treatments for both cohorts, growth rate increased at the higher temperature (Fig. 3A, Cohort 1: slope comparison of growth at 21 and 28°C: P<0.001, N=1004 shell length measurements; Fig. 3B, Cohort 2: slope comparison: P<0.001, N=1698 shell length measurements).

Table 1. Altered ATP allocation in larval families of *Crassostrea gigas* with differing thermal sensitivities (Q_{10}) for protein synthesis and respiration

Cohort	Temperature (°C)	Protein synthesis (ng larva^{-1} day^{-1})	Respiration (pmol O_2 larva^{-1} day^{-1})	ATP allocation (%)
5	20	200	1708	51
	30	561	4442	55
7	20	182	2178	36
	30	618	3485	77

Physiological rates at 25°C were modeled based on Fig. 5A,B. The respective physiological rates at 25°C and the range of Q_{10} values for Cohorts 5 and 7 analysed in the present study (see below for additional statistics and Q_{10} calculation) are used to calculate temperature-corrected rates at 20 and 30°C. Cohort 5: respiration Q_{10} of 2.6 (mean of 3.0 and 2.3 for 114 and 146 µm larvae, respectively, from Table 3); protein synthesis Q_{10} of 2.8 (mean of 2.5 and 3.0 for 114 and 146 µm larvae, respectively). The respective regression analyses for each of these Q_{10} values are as follows. Respiration for a 114 µm larva, slope=0.47±0.06 (P<0.001, N=38), equivalent to Q_{10} of 3.0. Each Q_{10} value is calculated from the slope (±s.e. of slope) of the semi-logarithmic regression relationship between physiological rate and different temperatures (Schmidt-Nielsen, 1997). A semi-logarithmic slope of 0.47 yields a Q_{10} of 3.0 for a 114 µm larva. Respiration for a 146 µm larva, slope=0.37±0.05 (P<0.001, N=27), equivalent to Q_{10} of 2.3. Protein synthesis, 114 µm larva, slope=0.40±0.03 (P<0.001, N=6), equivalent to Q_{10} of 2.5. Protein synthesis for a 146 µm larva, slope=0.48±0.12 (P<0.01, N=8), equivalent to Q_{10} of 3.0. Cohort 7: respiration Q_{10} of 1.6 (mean of 1.4 and 1.8 for 124 and 145 µm larvae, respectively; Table 3); protein synthesis Q_{10} of 3.4 (mean of 2.6 and 4.2 for 124 and 145 µm larvae, respectively). The respective regression analyses for each of these Q_{10} values are: respiration for a 124 µm larva, slope=0.15±0.04 (P<0.01, N=32), equivalent to Q_{10} of 1.4. Respiration for a 145 µm larva, slope=0.26±0.06 (P<0.001, N=28), equivalent to Q_{10} of 1.8. Protein synthesis for a 124 µm larva, slope=0.42±0.09 (P<0.01, N=8), equivalent to Q_{10} of 2.6. Protein synthesis for a 145 µm larva, slope=0.62±0.07 (P<0.001, N=8), equivalent to Q_{10} of 4.2.

While growth increased with temperature, larvae reared at higher temperatures had greater mortality. For Cohort 1, survivorship was 94% after 10 days of continuous rearing at 21°C (Fig. 3C). In contrast, after the same rearing time interval, only ∼51% (mean of two culture vessels) of larvae from Cohort 1 survived at 28°C (Fig. 3C). This difference in survivorship between rearing temperatures for Cohort 1 was also apparent for Cohort 2 (Fig. 3D). Comparing the survivorship within each cohort across the two temperature treatments revealed a 43% difference in survival by day 10 for Cohort 1, and a 30% difference for the corresponding end-point analysis for Cohort 2. Clearly, higher temperature resulted in lower survivorship.

Respiration and protein synthesis

Increases in growth rate require elevated rates of protein synthesis, which results in a higher energy investment in biosynthesis (ATP allocation strategy: Pan et al., 2018). In the current study, respiration and protein synthesis were measured for Cohort 2 larvae that were reared for up to 16 days ('chronic' exposure) at either 21 or 28°C. To compare the relative impact on ATP production (from respiration) and ATP demand (for protein synthesis) at different temperatures, the analysis is based on comparisons of regression models for these two physiological processes to account for differences in size-at-age between temperature treatments (Fig. 3A,B): i.e. larvae at 21°C had a lower growth rate than larvae of the same cohort reared at 28°C. This analysis revealed that protein synthesis has a higher temperature sensitivity than respiration (Table 2). For each physiological process, the slopes of regressions for physiological rates and larval sizes are similar between temperatures (respiration: $F_{1,154}$=3.75, P>0.05; protein synthesis: $F_{1,33}$=0.79, P>0.05), but the intercepts are significantly different (respiration: $F_{1,155}$=54.50, P<0.001; protein synthesis: $F_{1,34}$=5.38, P=0.03). This finding of similar slopes with different intercepts illustrates that for both physiological processes, rate differences (intercepts) between 21 and 28°C were consistent (slopes) throughout the rearing and growth period studied. The temperature sensitivity (Q_{10} value) for respiration is 1.4±0.05. This calculation is based upon solving the respiration rate equations given in Table 2 for a mid-sized larva of 140 µm in size: at 21°C, respiration was 36.6 and 45.9 pmol O_2 larva^{-1} h^{-1} at 28°C (i.e. Q_{10} is 1.4). The standard error of this Q_{10} value is 0.05, propagated from the sum of squared errors of both regressions. The temperature sensitivity (Q_{10} value) for protein synthesis is 2.3±0.02. This calculation is based on solving the protein synthesis rate equations given in Table 2 for a larva of 140 µm in size: at 21°C, protein synthesis was 116.3 and 210.1 ng protein larva^{-1} day^{-1} at 28°C (i.e. Q_{10} is 2.3). Based on 95% confidence intervals for each error value, these Q_{10} values for respiration and protein synthesis are significantly different: confidence intervals for respiration Q_{10} are 1.30 (lower limit) and 1.50 (upper limit); corresponding lower and upper confidence intervals for protein synthesis Q_{10} are 2.26 and 2.34, respectively. The upper confidence interval for the Q_{10} value for respiration at 1.50 is lower than the lower confidence interval for the Q_{10} value for protein synthesis at 2.26. This analysis shows that protein synthesis is more sensitive to temperature increase than respiration during the chronic exposure temperature treatments tested over a period of 10–16 days (depending upon cohorts).

Acute exposure temperature treatments

Q_{10} values for physiological processes

The analysis of temperature response was expanded to include the fundamental physiological processes of amino acid and ion transport, in addition to respiration and protein synthesis. Each Q_{10} value is calculated from the slope (±s.e. of slope) of the semi-logarithmic regression relationship between physiological rate and different temperatures (Schmidt-Nielsen, 1997). Table 3 presents all Q_{10} values for larvae from each of seven cohorts. Fig. 4 illustrates a representative analysis of how Q_{10} values are calculated for specific physiological processes. In each case, rates were measured on larvae of similar age and size within a specific cohort (Table 3: Cohort 4, 111±1.3 µm, mean±s.e.m.). Fig. 4 shows the effect of temperature on respiration (Fig. 4A), protein synthesis (Fig. 4B), glycine transport (Fig. 4C) and ion transport by Na^+/K^+-ATPase (Fig. 4D). Within the temperature range analysed, the $\log_{10}$-transformed rates scaled linearly with assay temperature, which permitted calculation of a single Q_{10} value and error estimate from the slope of the regression for each of the four physiological processes measured. Notably, the Q_{10} value for protein synthesis was the highest at 3.6, with lower values for respiration (2.4), glycine transport (2.0) and ion transport (2.0) (Fig. 4).

Temperature dependence of physiological rates during growth

For the seven cohorts studied, the effect of temperature was analysed over a wide range of larval sizes, spanning from 99 to 256 µm in shell length. Two findings emerge from this analysis. First, the 45 Q_{10} values, combined for each of the four physiological processes measured, were analysed by a series of four ANOVA linear regression analyses of larval size and the corresponding physiological process (respiration, protein synthesis, glycine transport or ion transport). This revealed that for each physiological process, larval size did not co-vary with Q_{10} values across 13 different larval sizes and seven cohorts. For the values given in Table 3, the ANOVA of linear regressions of different larval sizes and corresponding Q_{10} values showed no significant

Table 2. Rates and thermal sensitivity (Q_{10}) of respiration and protein synthesis for larvae of *Crassostrea gigas* reared at different temperatures

	Respiration rate (pmol O_2 larva^{-1} h^{-1})				Protein synthesis rate (ng larva^{-1} day^{-1})			
Rearing temperature (°C)	Equation	R^2	N	P	Equation	R^2	N	P
21	log Resp=0.010×size+0.051	0.75	80	<0.001	log PS=0.017×size−0.209	0.75	18	<0.001
28	log Resp=0.012×size+0.045	0.92	78	<0.001	log PS=0.015×size+0.214	0.88	19	<0.001
	Q_{10}=1.4±0.05				Q_{10}=2.3±0.02			

Chronic exposure temperature treatments for Cohort 2 larvae. Ten different sizes of larvae were assayed during the ∼16 day growth period studied (see Materials and Methods for details). Regression equations describe the relationships between $\log_{10}$-transformed physiological rates with increasing size (shell length) in larvae. P-values indicate significance of regressions. For respiration (Resp) measurements at 21 and 28°C, the slopes are not statistically different, while y-intercepts are elevated at 28°C (slope comparison, $F_{1,154}$=3.75, P>0.05; intercept comparison, $F_{1,155}$=54.50, P<0.001). Similarly, for protein synthesis (PS), the slopes are not statistically different, while y-intercepts are elevated at 28°C (slope comparison, $F_{1,33}$=0.79, P>0.05; intercept comparison, $F_{1,34}$=5.38, P=0.03). The Q_{10} values of 1.4 and 2.3 for respiration and protein synthesis, respectively, are calculated for similar-sized larvae of 140 µm shell length (see statistical analyses in Results).

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Table 3. Temperature sensitivity (Q_{10}) of physiological rate processes in different larval cohorts of *Crassostrea gigas*

Larval cohort	Shell length (µm)	Respiration (pmol larva^{-1} h^{-1})		Protein synthesis (ng larva^{-1} day^{-1})		Glycine transport (pmol larva^{-1} h^{-1})		Ion transport by Na^+/K^+-ATPase (pmol K^+ larva^{-1} h^{-1})	
		N	Q_{10}	*N*	Q_{10}	*N*	Q_{10}	*N*	Q_{10}
3	99±0.5	38	1.4	8	2.2	8	1.7	8	2.1
	117±1.2	38	2.9	7	3.7				
4	111±1.3	27	2.4	8	3.6	8	2.0	8	2.0
	163±3.4	28	2.0	6	3.2	6	2.2		
5	114±1.2	38	3.0	6	2.5	8	2.0	6	1.8
	146±1.8	27	2.3	8	3.0	8	2.2	4	2.2
6	117±1.2	30	1.7	8	3.2	8	2.8	8	1.8
	133±3.2	28	1.9	8	2.4	6	3.2		
7	124±1.7	32	1.4	8	2.6	8	2.1	8	2.1
	145±1.7	28	1.8	8	4.2	8	2.4		
8	184±2.0	39	1.4	8	2.0			8	2.6
	256±2.9	15	1.7	4	2.3	4	1.9		
9	203±3.1	39	1.6	8	2.4	8	4.3	8	2.1
Mean Q_{10}±s.e.m.			2.0±0.15[a]		2.9±0.18[b]		2.4±0.23[a,b]		2.1±0.09[a]

Acute exposure temperature treatments. Larval sizes (shell length) are means±s.e.m. (N≥50). Sample size (N) for each physiological process represents the total number of assays across different temperatures (spanning 15 to 30°C, see Materials and Methods for details). Values of Q_{10} were calculated from the slopes of regressions describing the relationship between $\log_{10}$-transformed physiological rates and temperature (see Fig. 4 as an example). All slopes used to calculate Q_{10} values are statistically significant by regression ANOVA (P<0.05). Mean Q_{10} values differ among the four physiological processes assayed (one-way ANOVA, $F_{3,41}$=5.64, P=0.002). Superscript letters (a,b) show Tukey's test results of the ranking of mean values for Q_{10}. Values sharing the same superscript letters are not statistically different.

slopes for the physiological processes tested (respiration: P>0.05, N=13; protein synthesis: P>0.05, N=13; glycine transport: P>0.05, N=11; ion transport: P>0.05, N=8). Second, all 45 Q_{10} values in Table 3 were used in a one-way ANOVA, which revealed significant differences ($F_{3,44}$=5.64, P=0.002) across mean Q_{10} values for each of the four physiological processes. There is a higher temperature sensitivity for protein synthesis (Q_{10}=2.9) than measured for respiration (Q_{10}=2.0). Post hoc analysis by Tukey's test revealed that the Q_{10} value for protein synthesis of 2.9±0.18 (mean±s.e.m.) is higher than that of respiration at 2.0±0.15 (q=5.45, P=0.002) and ion transport (Na^+/K^+-ATPase) at 2.1±0.09 (q=4.11, P=0.03). The Q_{10} value for glycine transport of 2.4±0.23 is not different from protein synthesis (q=2.48, P>0.05), respiration (q=2.74, P>0.05) and ion transport (q=1.78, P>0.05).

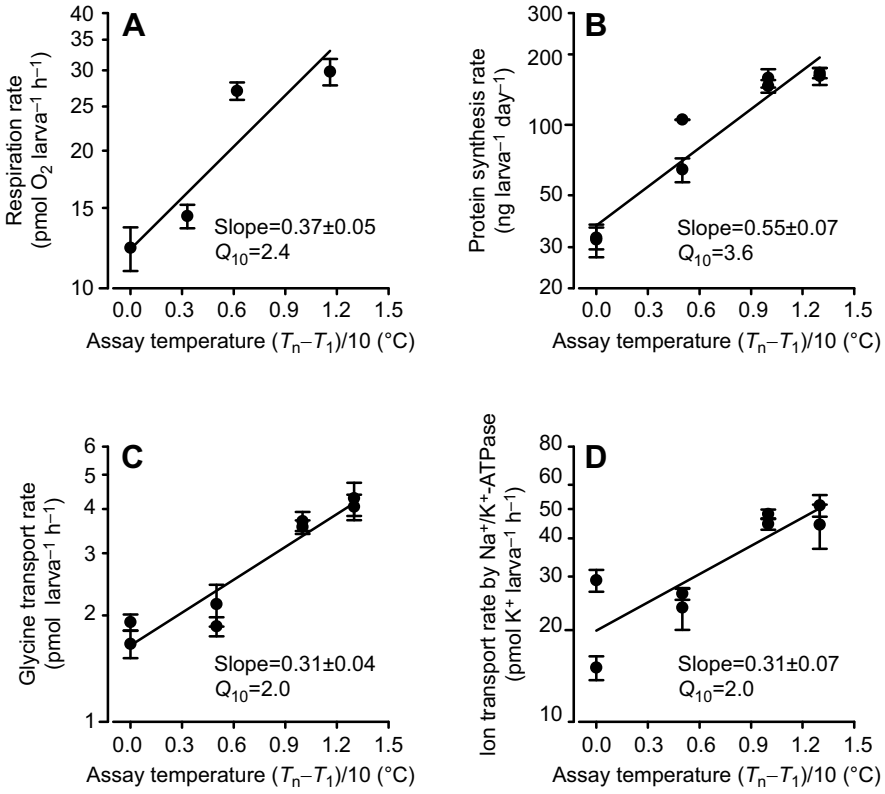

Fig. 4. Calculation of temperature sensitivity (Q_{10} values) for four physiological processes in larvae of *Crassostrea gigas*. Rates measured at four different temperatures using larvae of 111 µm (mean shell length) from Cohort 4 (Table 3) are illustrated as an example. Each Q_{10} value is calculated from the slope of the regression between logarithm-transformed rates and assay temperatures [converted to temperature increments: (T_n – T_1)/10; Schmidt-Nielsen, 1997]. (A) Respiration rate: each data point is the mean (±s.e.m.) of six to seven replicate respiration chambers, as illustrated in Fig. 2A, repeated for each of the four different temperatures tested. (B) Protein synthesis rate: each data point is a slope (±s.e. of slope) of a time course assay as illustrated in Fig. 2B; two assays were conducted at each temperature. (C) Glycine transport rate: each data point is a slope (±s.e.) for a time course assay as illustrated in Fig. 2C; two assays were conducted at each temperature. (D) Ion transport by Na^+/K^+-ATPase: values are differences in slopes between assays conducted with and without ouabain as illustrated in Fig. 2D; four assays were conducted at each temperature (two with the inhibitor ouabain and two without). Error bars are s.e. of slopes of time course assays.

In summary, protein synthesis is the major ATP-consuming process for developmental stages of *C. gigas*, requiring the allocation of ∼50% of the ATP pool (Lee et al., 2016; Frieder et al., 2018; Pan et al., 2018). The higher temperature sensitivity for protein synthesis reported in this study (Tables 2 and 3) will result in a higher percentage of the total ATP pool being allocated to biosynthesis as environmental temperature increases.

DISCUSSION

A major goal of physiological ecology is to connect whole-organism processes to the underlying mechanisms of environmental effects on cellular processes. The present study provides evidence of the different sensitivities to temperature of energy-producing and energy-consuming processes. This results in trade-off limits for allocation of the ATP pool to meet the physiological demands of rising temperature which, in turn, set thermal limits at the level of the whole organism. Metabolic trade-offs are often invoked as explanations for organismal adaptive potential under various scenarios of environmental change (Cherkasov et al., 2006; Deigweiher et al., 2010; Kreiss et al., 2015; Podrabsky and Hand, 2015). The differential thermal sensitivity reported here (Tables 2 and 3) between the production and consumption of ATP in larvae of *C. gigas* provides insight into the metabolic limits of thermal tolerance. Specifically, the higher thermal sensitivity of protein synthesis, relative to respiration, coupled with measured increases in growth and decreased survival at increased temperatures (Fig. 3), supports a hypothesis that reallocation of the ATP pool could define a biochemical 'tipping point' for fitness and survival with rising temperature. As modeled in Fig. 5, the 'tipping point' is defined here as when the value for ATP allocation exceeds 50% to support a single process.

Metabolic cost of thermal stress and modeling of ATP allocation

In this study, the relative Q_{10} values for respiration and protein synthesis were compared in both acute and chronic temperature treatments (Tables 2 and 3). For larvae exposed continuously over a period of up to 16 days to chronic temperature treatments, the Q_{10} values differ 1.6-fold between respiration and protein synthesis (Table 2). Similarly, in the acute temperature treatments, there was a 1.5-fold difference in the mean Q_{10} values between respiration and protein synthesis (Table 3). This comparison of acute and chronic temperature treatments showed similar thermal responses in larvae. Notably, the Q_{10} values of 1.4 and 2.3 for respiration and protein synthesis, respectively, measured for larvae in the chronic temperature treatments (Table 2) fall within the range of Q_{10} values observed in the acute temperature treatments (Table 3).

Fig. 5 illustrates a bioenergetic model of how changes in growth and temperature affect the allocation of the ATP pool in larvae of *C. gigas*. This model is based on the following calculations: (1) Q_{10} values for temperature sensitivity of protein synthesis and respiration (Table 3); (2) equations for size-specific rates of respiration and protein synthesis (Pan et al., 2018); (3) an oxyenthalpic value of 484 kJ mol O_2^{-1} (Gnaiger, 1983) to convert respiration to energy equivalents, based on predominance of protein and lipid in the biochemical composition of larvae (Moran and Manahan, 2004); and (4) the energy cost to synthesize a unit mass of protein, known to be independent of genotype, growth phenotype and environmental temperature for larvae of this species [Lee et al., 2016: 2.1±0.2 J (mg protein synthesized)$^{-1}$]. The justification for the use of 484 kJ mol^{-1} O_2 (the average value of 441 and 527 kJ mol^{-1}, oxyenthalpic values for lipid and protein, respectively) is that these oxyenthalpic values are recommended for conversion of oxygen consumption to enthalpies of combustion of catabolized biomass in the context of physiological energetics (Gnaiger, 1983). Furthermore, under normoxic conditions, the calorimetric-respirometric ratio for larvae of *C. gigas* (478 kJ mol^{-1} in Hand, 1999) is within 1% of the oxyenthalpic value used in this study (484 kJ mol^{-1} O_2). Fig. 5 presents an analysis over a range of conditions for larvae of: (1) different sizes at constant temperature (Fig. 5A); (2) same size at different temperatures (Fig. 5B); and (3) different sizes at different temperatures (Fig. 5C).

Fig. 5A illustrates the size-dependent change in energy production from respiration and energy demand for protein synthesis (see Materials and Methods: 'Modeling bioenergetic changes in ATP allocation strategies' section). At a constant temperature of 25°C (the routine rearing temperature for this species), the allocation of the ATP pool to protein synthesis increases from 36% in a 100 µm larva to 66% in a 300 µm larva. This gradual increase in the percentage allocation of available ATP to support protein synthesis highlights that, as larvae grow and approach the size at which metamorphosis occurs, a smaller proportion of the ATP pool is available to support other energy-demanding cellular functions that are likely to be essential for morphological and associated physiological changes at that later stage of development. Fig. 5B illustrates how the allocation of the ATP pool changes with increasing temperature (see Materials and Methods: 'Modeling bioenergetic changes in ATP allocation strategies' section). The higher Q_{10} value for protein synthesis (Table 3) results in a disproportional increase in the cost of supporting biosynthesis relative to respiration as environmental temperature increases. For instance, at 15°C, only 35% of the ATP pool in a 200 µm larva is required to support protein synthesis, but at 30°C that allocation increases to 65% (Fig. 5B) (30°C is within the temperature range of *C. gigas*: Breese and Malouf, 1975; Helm et al., 2004; Rico-Villa et al., 2009; Bayne, 2017).

A metabolic 'landscape' for the energy demand of protein synthesis at different larval sizes and environmental temperatures is modeled in Fig. 5C. Values used to model the metabolic responses for larvae of different sizes in different temperature environments are from Tables S1 and S2, respectively. Values for protein synthesis and respiration for larvae of different sizes were each calculated for a different temperature (as represented in Fig. 5C), using Q_{10} values for protein synthesis and respiration averaged across seven larval cohorts (Table 3). The area of 'optimal allocation' (blue graphical area in Fig. 5C) illustrates the ∼50% allocation value of the ATP pool across a range of sizes and temperatures; 'stressful allocation' is shown as the green-to-red area to illustrate the increasing demands with regard to ATP utilization. For instance, at 30°C, larvae near metamorphic size (∼300 µm) would need to allocate over 80% of their ATP pool to support the cost of protein synthesis. Allocation of such a large proportion of the ATP pool to just a single process would severely limit trade-offs to support other essential physiological functions (Fig. 5D). Protein synthesis is the single biochemical process with the highest metabolic cost during development and growth of marine invertebrates (echinoderms: Pace and Manahan, 2006; Pan et al., 2015a; molluscs: Lee et al., 2016; Frieder et al., 2018; Pan et al., 2018). As illustrated in Fig. 5D, protein synthesis and degradation account for 48% of the total ATP pool in a larva of *C. gigas*. This allocation diagram illustrates that sufficient ATP is available to support other essential processes, such as ion regulation (20% of ATP pool), calcification (14%) and nucleic acid biosynthesis. When the ATP allocation to protein metabolism exceeds 50% (e.g. ∼80%

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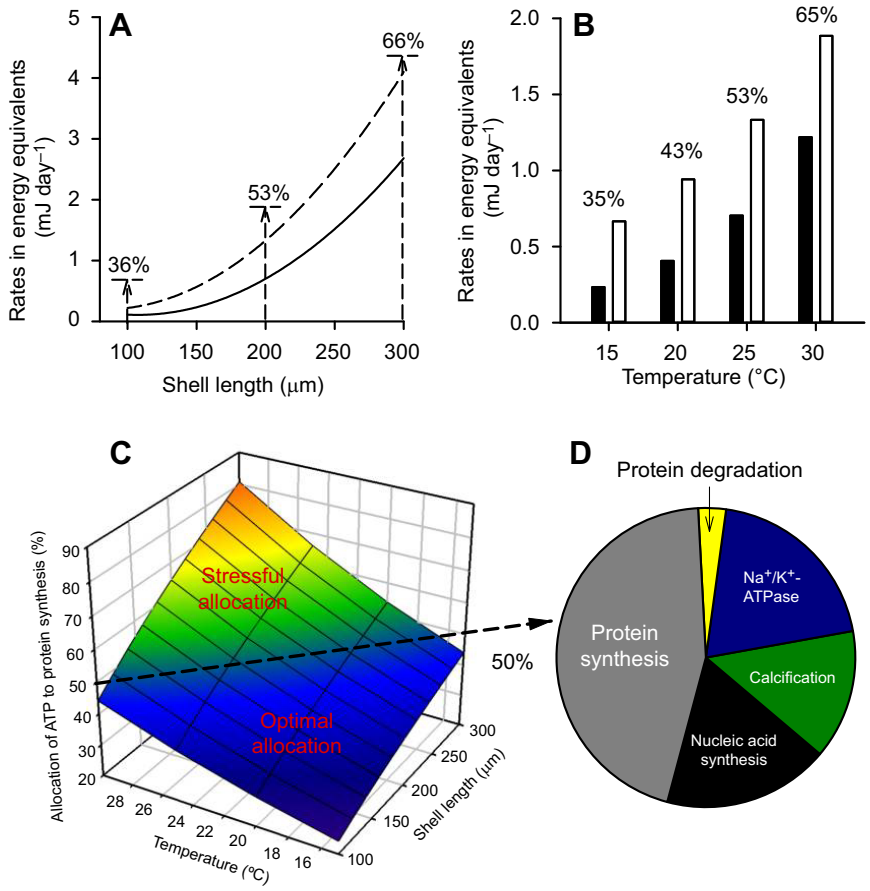

Fig. 5. A three-dimensional allocation landscape model of bioenergetic changes in allocation of the ATP pool in larvae of *Crassostrea gigas* growing at different environmental temperatures. (A) Rates of respiration (dashed line) and protein synthesis (continuous line) expressed as energy equivalents for larvae of different sizes. Physiological rates are calculated based on known relationships between respiration, protein synthesis and larval size at 25°C (Table S1). Percentage values indicated with an arrow above the dashed line show proportion of ATP allocated to protein synthesis for larvae of different sizes (shell lengths of 100, 200 and 300 µm). (B) Effect of temperature on respiration (open bars) and protein synthesis (filled bars), converted to energy equivalents. Physiological rates are calculated for a mid-size 200 µm larva (Table S2). Temperature effects are modeled based on Q_{10} values for respiration and protein synthesis (Table 3). Percentage values show allocation of ATP to protein synthesis at different temperatures from 15 to 30°C, with corresponding ATP allocation range from 35 to 65%. (C) The interaction of larval size and temperature on ATP allocation to protein synthesis. The area of the graph in blue ('Optimal allocation') illustrates the range of larval sizes and temperatures for which <50% of the ATP pool is allocated to protein synthesis. Areas in green-to-red ('Stressful allocation') depict increasing demand for energy to over 80% of the ATP pool to support protein synthesis at higher temperatures. The dashed line across the allocation landscape marks the 50% value for allocation of the ATP pool to protein synthesis. (D) Allocation of the ATP pool in a larva of *C. gigas* (redrawn from Pan et al., 2018). The combined metabolic cost of protein synthesis (gray) and protein degradation (yellow) accounts for 48% of ATP demand. At that value [~50% ATP 'optimal allocation' in (C)], there is sufficient ATP to support other essential processes: ion regulation by Na^+/K^+-ATPase (blue, at 20% of ATP pool); calcification (green, at 14%); nucleic acid biosynthesis (black, see fig. 8 of Pan et al., 2018 for details of allocation estimate). At higher temperatures, the metabolic allocation to protein metabolism increases up to 80% of the ATP pool ('stressful allocation' in C), resulting in insufficient ATP to sustain development and growth.

in a larger larva) with increasing temperature (Fig. 5C), clearly there would be insufficient ATP to sustain development and growth. Such imbalance of ATP allocation, combined with cohort-to-cohort variation, could explain the observations presented in Fig. 3 of increasing mortality when larvae of *C. gigas* experience temperatures above 25°C. Beyond temperature, under conditions of compounding environmental stressors resulting from anthropogenic climate change (such as ocean acidification; Doney et al., 2009), the metabolic demands to respond to multiple stressors become unsustainable within a fixed ATP pool (Fig. 5D). Ocean acidification alone is frequently invoked to be the cause of large-scale, catastrophic die-off of larval stages of *C. gigas* (Service, 2012; Barton et al., 2015). Under ocean acidification stress, larvae are known to alter the allocation of their ATP pool (Pan et al., 2015a; Frieder et al., 2017, 2018). These studies highlight the importance of understanding the cellular bases of sub-lethal stress that, once a critical threshold is reached, result in mortality. Modeling biochemical strategies of response, such as presented in Fig. 5C, is necessary for prediction of organismal performance over a range of environmental conditions.

Temperature sensitivities of physiological processes

Biochemical reactions are constrained by fundamental thermodynamic principles. The average Q_{10} values for the range of physiological processes reported in the present study (Table 3) agree well with the reported range for Q_{10} values (Hochachka, 1988; Clarke and Johnston, 1999; Somero et al., 2017). Within this range, however, processes with a higher Q_{10} of ~3 – such as protein synthesis (Table 3) – will have a larger increase in rate with increasing temperature, relative to processes with a Q_{10} of ~2 (respiration and ion transport). There is extensive literature on the study of temperature effects on biological processes. For instance, specific enzymes in aerobic metabolic pathways respond differently to temperature relative to other enzymes in the same aerobic pathway, by having

differences in the slope of the thermal performance curve (Schulte, 2015). Logan et al. (2012) reported latitudinal differences in thermal physiology among adult mussels from different natural populations. Dell et al. (2011) summarized information for 112 traits in 309 species. In general, thermal sensitivities for individual traits are variable across species. What is lacking, however, as stated by Dell et al. (2011), is an understanding of how temperature influences different traits within single species. Such information will improve an integrative understanding of how temperature has an impact on different levels of biological organization. What is noteworthy in this context in the present study is that the process of energy supply (respiration) is less thermally sensitive than the major process of energy demand (protein synthesis). This uncoupling of ATP balance has important physiological consequences.

With respect to thermal sensitivity of protein synthesis, recent work has highlighted the importance of RNA thermosensing mechanisms that facilitate rapid increase of protein synthesis from previously silenced mRNA templates (Kortmann and Narberhaus, 2012; Mortimer et al., 2014; Somero, 2018). For the relationship between heat shock response and metabolic cost, genetically modified fruit flies that have extra copies of the *Hsp70* gene show an increase in respiration when exposed to higher temperatures (Hoekstra and Montooth, 2013). Analysis of the genome of the Pacific oyster, *C. gigas*, reveals that the *Hsp70* gene family is expanded to contain 88 genes (Zhang et al., 2012). In response to heat stress, a ∼2000-fold increase in gene expression of five highly inducible *Hsp70* genes occurred in adult *C. gigas*, in addition to lesser increases in all heat-shock genes assayed (Zhang et al., 2012). Our finding of increased sensitivity of protein synthesis to temperature in larvae of *C. gigas* (Tables 2 and 3) suggests that expression of heat-shock genes could have important physiological consequences requiring higher metabolic costs to cope with thermal stress. A high fraction of the proteome is dedicated to maintaining protein folding and structure (Müller et al., 2020). In addition, preferential synthesis of different types of proteins occurs in response to thermal shock due in part to differential effects of temperature on degradation and translation of different classes of pre-existing mRNA, as well as on gene transcription per se (Su et al., 2018; Somero, 2020). In our previous study of protein synthetic response to the stress of seawater acidification, we found no chromatographic evidence for preferential synthesis of different types of proteins (Pan et al., 2015a). Nonetheless, the findings of protein responses to thermal perturbation warrant further investigation into changes of specific protein classes synthesized and the link between these changes and the metabolic bases of trade-offs between growth and stress response.

Defining standing genetic variation is important for predicting the potential to adapt to a changing environment. This theme can be illustrated from data obtained with the different larval cohorts of *C. gigas* presented in Table 3 (four of these seven cohorts were produced from different crosses of a single male and female, i.e. each of these four cohorts was a sibling larval family; see Materials and Methods). Comparisons between larval cohorts highlight potential variation in thermal sensitivity for different physiological processes. Specifically, underlying the mean Q_{10} values of 2.0 and 2.9 for respiration and protein synthesis, respectively, respiration Q_{10} values ranged from 1.4 to 3.0 and protein synthesis Q_{10} values ranged from 2.0 to 4.2. Based upon analyses of the upper and lower 95% confidence intervals (calculated from the standard error of the slope for each Q_{10} value; Fig. 4 as an example), these respiration Q_{10} values of 1.4±0.27 and 3.0±0.88 are significantly different based on the non-overlap of the upper and lower 95% intervals. Similarly for protein synthesis, the 95% confidence intervals for Q_{10} values of 2.0±0.65 and 4.2±1.32 do not overlap and are significantly different. The biological significance of these differences in temperature sensitivity is illustrated by an analysis of ATP allocation strategies across specific larval families. For instance, for a natural environmental temperature range between 20 and 30°C experienced by this species (Bayne, 2017), a larva in the Cohort 5 family maintains a narrow range of 51% at 20°C to a similar value of 55% at 30°C of the available ATP pool to support protein synthesis (Table 1). Notably, because of the closeness of the Q_{10} values for respiration and protein synthesis for Cohort 5 (Table 3), the range of ATP allocation remains very similar across all temperatures. In contrast, for the Cohort 7 larval family that has differing Q_{10} values for respiration and protein synthesis, a much wider range of ATP allocation strategies occurs across temperatures, from 36% at 20°C, increasing to 77% at 30°C. A conclusion from this analysis is that larvae from Cohort 5 probably have genetically based physiological capacities that are more resilient (i.e. maintain physiological homeostasis) to environmental change.

Variance for a wide range of physiological processes is known to be genetically determined in larvae of *C. gigas* (Pace et al., 2006; Lee et al., 2016; Pan et al., 2015b, 2016, 2018; Frieder et al., 2017). The analysis in the present study reveals family-dependent variation in thermal sensitivity of the major physiological traits regulating energy supply and demand (respiration and protein synthesis). Further studies are warranted, using long-term breeding programs to measure standing genetic variation in existing populations (e.g. Applebaum et al., 2014), to test for a possible genetic basis for temperature adaptation. Such genetically based variations in sensitivities of metabolic processes to temperature change and other factors will be primary determinants of fitness and selection under future scenarios of rapid environmental change.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: F.T.P., S.L.A., D.T.M.; Methodology: F.T.P., S.L.A., D.T.M.; Validation: F.T.P., S.L.A., D.T.M.; Formal analysis: F.T.P., S.L.A., D.T.M.; Investigation: F.T.P., S.L.A., D.T.M.; Resources: D.T.M.; Data curation: F.T.P., S.L.A., D.T.M.; Writing - original draft: F.T.P., D.T.M.; Writing - review & editing: F.T.P., S.L.A., D.T.M.; Visualization: F.T.P., S.L.A., D.T.M.; Supervision: D.T.M.; Project administration: D.T.M.; Funding acquisition: D.T.M.

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SUPPLEMENTARY TABLES

Table S1. Allocation of ATP to protein synthesis in larvae of *Crassostrea gigas* of different sizes

Shell length (µm)	Protein synthesis (ng larva^{-1} d^{-1})	Respiration (pmol O_2 larva^{-1} d^{-1})	ATP allocation (%)
100	33	398	36%
120	61	662	40%
140	102	1018	43%
160	159	1478	47%
180	236	2053	50%
200	335	2755	53%
220	461	3594	56%
240	616	4581	58%
260	805	5728	61%
280	1032	7043	64%
300	1299	8538	66%

Table S2. Allocation of ATP to protein synthesis in larvae of *Crassostrea gigas* at different temperatures

Temperature (°C)	Protein synthesis (ng larva^{-1} d^{-1})	Respiration (pmol O_2 larva^{-1} d^{-1})	ATP allocation (%)
15	112	1378	35%
20	194	1948	43%
25	335	2755	53%
30	581	3896	65%