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Larval fish in a warming ocean: a bioenergetic study of temperature-dependent growth and assimilation efficiency

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ABSTRACT: Rising temperatures have important consequences for somatic growth, but observed relationships between temperature and growth can vary in both magnitude and direction. The key to understanding such variation is knowing how temperature affects both the amount of energy available for growth and the efficiency with which surplus energy is assimilated into the body. We tested the hypothesis that patterns of temperature-dependent growth are driven by differential sensitivities of energy intake and expenditure to temperature. Larvae of California grunion *Leuresthes tenuis* were reared across a range of temperatures and 2 levels of food availability. Energy intake was measured from feeding rate, and energy expenditure was evaluated by measuring respiration and excretion rates. When food was abundant, both intake and expenditure increased with temperature, but intake increased more rapidly. These results suggest that high temperatures should lead to faster growth, and these predictions were confirmed by a separate experiment. In contrast, when food was restricted, the increase in energetic demand with temperature outpaced energy intake, suggesting a dwindling surplus of energy at high temperatures. This predicted reversal of the effects of temperature on growth was also confirmed experimentally. Finally, we compared patterns of energetics and growth to test the effects of temperature on food assimilation efficiency. When food was unlimited, assimilation efficiency decreased rapidly with temperature. When food was restricted, assimilation efficiency remained relatively high. Overall, our results emphasize the value of a bioenergetic perspective for illuminating why and how growth rates are likely to change in a warming ocean.

KEY WORDS: Physiology · Energy budgets · Feeding rate · Respiration · Excretion · Development · *Leuresthes tenuis* · Metabolism · Q10

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1. INTRODUCTION

Increasing atmospheric CO_2 levels have caused average global temperatures to increase, and these continuing trends are predicted to have important effects on many species (Hoegh-Guldberg & Bruno 2010, Warren et al. 2011, Gruber et al. 2012, Nolan et al. 2018). Rates of warming in the ocean have increased around the world within the last 2 decades, and the average sea surface temperature is expected to rise by 2 to 4°C globally by the end of the 21st century (Solomon et al. 2007, Alexander et al. 2018). Changes in environmental temperatures will have both direct and indirect effects on the physiology of organisms, and physiological responses to temperature can have effects on higher-order properties such as growth, survival, and population dynamics. The role of temperature in driving variation in growth may be particularly important because it creates variation in body size, and mortality is strongly dependent on size in a wide variety of taxa (Sogard 1997, Maness & Anderson 2013, Iida et al. 2014, Johnson et al. 2014).

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Although there is still much to learn about the effects of warming on organisms, in general, it is expected that as temperatures continue to rise, the energetic costs of homeostasis will also increase (Somero 1969, Doney et al. 2012, Huey et al. 2012, Madeira et al. 2012). In the absence of any compensatory response in energy intake, these costs may result in less energy available for processes such as growth and reproduction (Pörtner & Knust 2007, Schulte 2015). Early life stages of ectothermic organisms (e.g. eggs and larvae) are likely to be especially sensitive to temperature because of their small sizes and low thermal inertia, and any temperature-related change in physiology and energetics may be highly consequential for their subsequent growth and survival. The early larval phase is a critical period for energy acquisition and development, and many studies have demonstrated that even small changes in the physical environment of larvae can ultimately result in considerable differences in rates of growth and mortality (Houde 1989, Rombough 1996, Byrne & Przeslawski 2013, Tasoff & Johnson 2019). Thus, it is important that we understand the effects of temperature on physiology and energetics in greater detail.

In general, energy available for growth (G) is determined by the difference between energy intake (I), which is related to the rate of food consumed and assimilation efficiency, and energy expenditure (E), which is related to respiration, specific dynamic action (SDA, the additional metabolic energy utilized during digestion and biosynthesis), and excretion (Hartman & Brandt 1995). Importantly, each of these processes may be described as a function of temperature (T):

$$G_T = I_T - E_T \quad (1)$$

For example, feeding rate and energy intake may increase with temperature, in part because the increased energetic demand of cells stimulates appetite (Houde 1989, Hartman & Brandt 1995), and in part because digestion occurs more quickly at higher temperatures (reviewed by Blaxter 1963). It is also well known that respiration rate generally increases with temperature, although species vary with respect to their thermal sensitivities (Clarke & Johnston 1999, Rangel & Johnson 2018). Excretion rates may also depend on temperature (Brett & Groves 1979, Checkley 1984, Houde 1989). The net effects of temperature on growth may thus depend on the extent to which temperature affects the relative magnitude of energy intake and expenditure.

Although the exact nature of the intake and expenditure functions are not known for many species, they can be measured experimentally. Depending on the difference in energy intake and expenditure, the relationship between environmental temperature and growth may take on 1 of 4 scenarios that are qualitatively different (Fig. 1). First, it is possible that intake and expenditure may increase at similar rates as temperature increases (Fig. 1A). In this case, growth rate is expected to be constant because the amount of surplus energy, depicted as the vertical

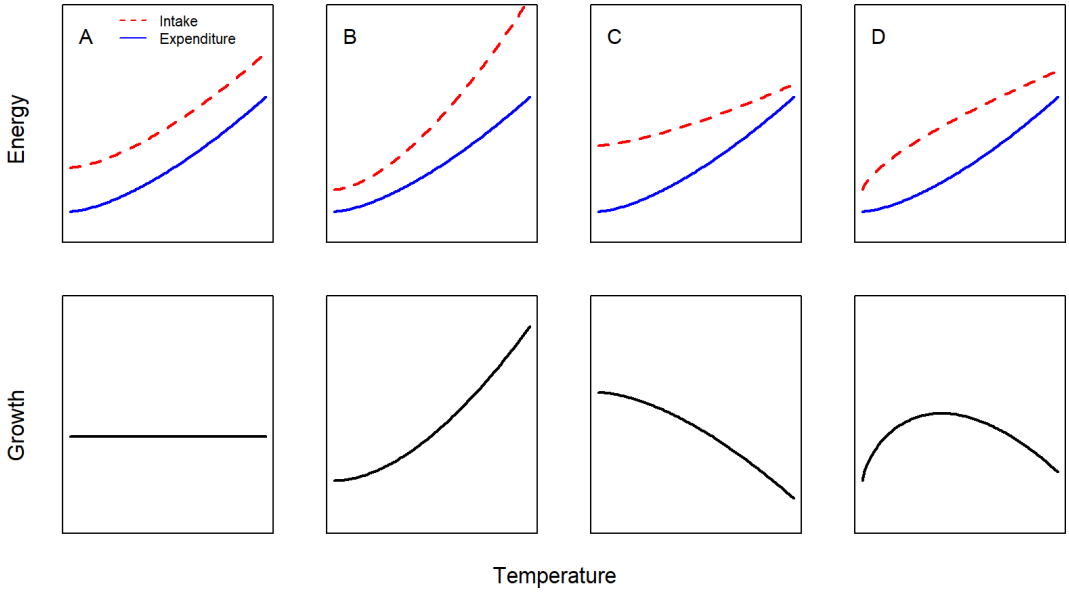

Fig. 1. Hypothesized effects of temperature on bioenergetics, and the resulting patterns of growth. Top row illustrates scenarios of energy intake (red dashed line) and energy expenditure (blue solid line), and energy panels are paired with predicted scenarios of growth rate (bottom row)

distance between the intake and expenditure functions, remains constant across temperatures. Second, consider the case where intake and expenditure of energy increase with temperature, and the rate of intake increases more rapidly than the rate of expenditure (Fig. 1B). In this case, growth rate is expected to increase with temperature because the surplus of energy also increases with temperature. It is also possible that both intake and expenditure are elevated with temperature, but expenditure increases more rapidly (Fig. 1C). In this third case, growth rate is expected to decrease as temperature increases because the surplus of energy declines with temperature. Finally, it is possible that intake increases with temperature at a decelerating rate while expenditure increases at an accelerating rate (Fig. 1D), in which case growth rate will be highest at an intermediate temperature, at which point the surplus of energy is maximized. We note that Fig. 1D encompasses each of the patterns in Fig. 1A–C at parts of the temperature range and thus may be considered as a generalized, expected pattern. We also note that changes in energy acquisition with temperature may be because of changes in feeding efficacy or changes in the efficiency with which ingested food is converted to energy available for growth. Ideally, the relationships among energy acquisition, expenditure, and growth should be considered across a temperature range that is wide enough to define the thermal limits of performance. However, if only part of the temperature range is considered, one must recognize the possibility that relationships among energetics and growth may differ at higher or lower temperatures.

Many empirical studies have investigated the effects of temperature on growth, and although some patterns appear to be more common than others, all 4 of the outcomes in the lower panels of Fig. 1 have been reported numerous times. Even within a single taxonomic group (e.g. fishes) the effects of temperature on growth can be fundamentally different among studies and species. Many studies have observed increases in growth with temperature (e.g. Fig. 1B; Bailey 1982, Boehlert & Yoklavich 1984, Pepin 1991, Shpigel et al. 1992, Claramunt & Wahl 2000, Malzahn et al. 2003, Green & Fisher 2004, Spies & Steele 2016). Others have observed a decrease in growth with temperature (e.g. Fig. 1C; McGurk 1984, Wieser et al. 1988). In some cases, growth is highest at an intermediate temperature (e.g. Fig. 1D; Buckley et al. 2004, Urtizberea et al. 2008), and still others have seen no effect (e.g. Fig. 1A; McGurk 1984, Hurst & Conover 2002). While it is known that the relationships between temperature and growth can vary, reasons for this variability are less clear. In addition, efforts to anticipate the effects of temperature and climate change on growth may not find straightforward guidance by a simple review of case studies, since all possible relationships between temperature and growth are reported frequently, and different species may approach their thermal limits at different temperatures. In contrast, bioenergetic theory may provide a suitable framework for a generalized understanding of why and how temperature is likely to affect growth.

Our study focused on the general hypothesis that the relationships between temperature and growth are ultimately determined by the relative sensitivities of energy intake and energy expenditure to changes in temperature. This was illustrated in Fig. 1 as a set of 4 scenarios, but it is important to note that these patterns are not necessarily static. If the temperature sensitivities of energy intake and expenditure can change with ecological context (e.g. food availability, predation risk, etc.), then the resulting relationship between temperature and growth may also change and may thus shift among 2 or more of the scenarios categorized in Fig. 1. Similarly, variation in the temperature sensitivities of energy intake and expenditure is a mechanism that may help explain why the effects of temperature on growth may differ qualitatively at different periods of time or among different populations, life stages, or species. And from an experimental design perspective, observing such context-dependent shifts would provide a more compelling test of the overarching hypothesis by demonstrating that differential temperature sensitivities of bioenergetic processes can both establish a particular pattern of temperature-dependent growth and change those patterns under different ecological conditions.

In this study, we used bioenergetic theory to evaluate the effects of ocean warming on growth during the larval phase of a marine fish, the California grunion *Leuresthes tenuis*. This was an experimental study with 3 interrelated goals. First, we manipulated temperature and measured the sensitivities of the major bioenergetic processes that comprise the energy budget (food consumption rate, respiration rate, and excretion rate). As a primary test of our hypothesis, we compared whether the observed relationships between temperature and growth matched the pattern predicted by the bioenergetic processes and their relationships with temperature. In addition, we manipulated food levels to test whether temperature sensitivities of bioenergetic processes could change with food availability. This was a secondary

evaluation of our hypothesis in which we tested whether reasonable variation in food availability could force the system from one of the bioenergetic scenarios in Fig. 1 to another, thus altering the relationship between temperature and growth. Finally, we made quantitative comparisons of the observed patterns of growth and the patterns of growth predicted by the combined rates of feeding, respiration, and excretion at various temperatures. This comparison allowed us to quantify assimilation efficiency, which accounts for the conversion of digested food into energy for growth and metabolism, across a range of temperatures and food levels. Assimilation efficiency is a key component of the energy budget, and values may be high for larval fish (Govoni et al. 1986), yet relatively little is known about the degree to which assimilation efficiency is affected by temperature (Radtke & Dean 1979, Checkley 1984). On the other hand, there is evidence to suggest that assimilation efficiency can change with the amount of food consumed (Boehlert & Yoklavich 1984). Understanding effects of temperature on energy budgets, including the component of assimilation efficiency, will be critical for anticipating how animals will respond to climate change and ongoing changes in temperature.

2. MATERIALS AND METHODS

2.1. Study species

The focal species for this study was the larval form of the California grunion *Leuresthes tenuis*, a temperate silverside fish that occupies the coastal waters of the Pacific Ocean from Baja California, Mexico, to central California, USA (Walker 1952). Previous studies on grunion larvae suggested a preferred thermal range of 15–25°C, and some capacity to tolerate temperatures in the range of 13–15°C (Ehrlich & Muszynski 1982) and 25–27°C (Ehrlich & Farris 1972). This species is valuable to human communities because of a recreational fishery and because *L. tenuis* supports a form of ecotourism. Several thousands of people across southern and central California gather on the shore late at night to observe the unusual beach-spawning behavior of these fish (Martin & Swiderski 2001). The spawning events, called grunion runs, occur every 14 d on the nights following the semilunar high tides from February or March to August or September (Walker 1952). Our experiments mirrored the timing of these cycles. During each spawning run, we made collections of embryos, and these embryos were incubated in the lab for 14 d before being used in replicate experimental blocks that lasted ~14 d.

Adult grunion were collected at Seal Beach, California, during a total of 11 spawning runs during the 2019 and 2020 spawning seasons. Grunion were captured with dip nets immediately prior to spawning, and adults were strip-spawned to fertilize and collect eggs. A single female's eggs (a clutch of about 1000–4000 eggs) were extracted by hand into a small plastic container and mixed with milt extracted from a single male. Replicate groups of offspring were thus full sibling families. The fertilized eggs were transferred to containers of moist sand and incubated in the lab at 20°C for 12 to 18 d. Offspring were collected for at least 6 breeding pairs per spawning run, and runs were treated as replicate, temporal blocks within the experimental design (6 blocks in 2019 and 5 blocks in 2020).

2.2. Experimental design

In the main experiment, we manipulated temperature and the abundance of food. Developing grunion larvae were held at 1 of 12 temperatures ranging from 16 to 28°C, and groups of larvae were given either a high or low ration of food to test whether the relationship between temperature and growth differed with food availability. The main experiment examined the effects on growth, but throughout the experiment, samples of larvae were occasionally removed to measure feeding, respiration, and excretion. Given constraints on laboratory access and personnel (especially during the COVID-19 pandemic), we could not measure all of the bioenergetic processes of interest within each block. Instead, some blocks focused on measuring respiration in detail while others focused on feeding rates and/or excretion rates. The temperature range was based on the range of sea surface temperatures that *L. tenuis* currently experience (13–25°C; NOAA station 9410660, https://tidesandcurrents.noaa.gov), and the general increases expected by the end of the 21st century. We used 6 tanks of larvae per temperature level, and each group of 6 tanks was connected to a single sump where seawater was filtered and aerated (Text S1 and Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m691p097_supp.pdf). Water temperatures were regulated by aquatic heaters and chillers, and salinity was maintained at 34 ppt by occasionally adding distilled water. Larvae were reared in the tanks for 14 d (up to 21 d in some cases because of a temporary lab clo-

sure) under a seawater flow regime of 10 ml s^{-1} and a 12:12 h light:dark cycle.

The experiment was replicated in 11 blocks in total, and before each replicate block, tanks were emptied and cleaned, and the seawater was refreshed completely. Each block represents a collection of grunion embryos and larvae that were spawned during spring tides, with multiple families per block. Within a temporal block, 4 temperature treatments were run at one time, and the 4 target temperatures for each block (2 high and 2 low) were selected from the continuous range of temperatures used in the study (Table S1). Each block included a different set of temperature treatments. Replicate families within a block were reared under high- and low-food conditions, and the tank assigned to each feeding level and family was chosen at random. When possible, larvae from the same family (siblings) were reared in all 4 temperature treatments. Each tank began with 100 larvae, and although each family was usually reared at multiple temperatures (mean = 3.18), the number of larvae available per family in these experiments was sometimes limited by the number of eggs produced by females and survivorship during incubation, so replicating families at all 4 temperature treatments was not always possible.

Of the 6 tanks set to a single temperature, families in 3 of the tanks were given a high ration of food while families in the other 3 tanks were given a low ration of food. Zooplankton prey (*Artemia* sp. brine shrimp nauplii) were introduced into the tanks on Day 3 of the experiment when larval grunion begin to feed (May 1971). To begin with, food rations were 100 nauplii $fish^{-1}$ d^{-1} in the low-food treatment, and 200 nauplii $fish^{-1}$ d^{-1} in the high-food treatment. The concentration of nauplii in the stock solution was estimated by counting nauplii within 4 samples of 200 µl and dispensing the appropriate volume of stock solution to each tank to achieve the target number of nauplii per fish. Previous studies have demonstrated that 160 nauplii $fish^{-1}$ d^{-1} produced regular growth rates of larvae in the lab (May 1971). To account for the fact that older, larger larvae eat more food, feeding levels were increased over time. Low (high) rations were increased to 150 (300) nauplii $fish^{-1}$ d^{-1} at age 5 d, 190 (375) nauplii $fish^{-1}$ d^{-1} at age 10 d, and 200 (400) nauplii $fish^{-1}$ d^{-1} at age 19 d.

2.3. Consumption rate

During the main experiment, we periodically measured the feeding of focal individuals and estimated prey consumption rates at different temperatures to estimate energy intake (I_T). Consumption was measured for larvae at 8 different ages between 3 and 10 d post hatch (dph). In each of these experiments, before food was dispersed among the tanks for the day, larvae from each treatment combination were temporarily isolated in their own container for 1 h before brine shrimp nauplii were added. Containers were 475 ml plastic specimen cups that were filled with approximately 350 ml of filtered seawater drawn directly from the corresponding sump, and containers were immersed in a water bath at the desired temperature. Additional containers of seawater and nauplii without fish larvae were distributed across the temperature treatments as controls. Volumetric estimates of 200–400 nauplii $ind.^{-1}$ were added depending on fish age. After 24 h, fish larvae were removed, and multiple random samples of seawater (5–10 aliquots of 2–4 ml each) were extracted. To determine food consumption at a given temperature (C_T, expressed in nauplii $ind.^{-1}$ d^{-1}), nauplii within these samples were counted to estimate the total number remaining, and thus the total number consumed in each container. When tested on control treatments with nauplii only, beginning counts of nauplii were highly correlated with counts after 24 h ($r = 0.814$; 95% CI = 0.624, 0.913; $n = 26$), suggesting that our volumetric counts had a reasonably high degree of repeatability, while still providing a rapid method of estimating nauplii abundance and therefore prey consumption rate. We also emphasize that the goal of this analysis was to evaluate systematic changes in average consumption rate with temperature, and that imprecision within a single sample would be averaged away when making inferences across many replicate measures of prey consumption.

Survival of nauplii was high in the absence of fish (92.4% on average), and baseline survival was accounted for when estimating consumption rate (see Text S2). To describe how consumption rate changed with larval age and with water temperature, we used a linear mixed-effects (LME) model in which age and temperature were included as explanatory variables and treated as fixed effects. For all experiments, statistical analyses were performed in R version 2021.09.2 (R Core Team 2021), and parameters for LME models were estimated using the 'lme4' package (Bates et al. 2015). To account for the fact that observations of larvae from the same family may not be completely independent, family identity was included as a random effect. In the analysis of consumption rates (and other responses), we could quantify how much of the residual variation in consumption rate was because

of consistent family-to-family differences. In particular, we divided the among-family component of variation by the total variance (estimated by the sum of the among-family component and the residual component). The among-family component of variation provides a measure of the phenotypic covariation within families. In this analysis (and also for respiration and excretion), we began with a full model that included temperature, feeding level, age, and all possible interactions. In this analysis, the term for age also includes effects of body size. Older fish were larger, but because it was impractical to measure size of individual fish (see Section 2.6), we did not include age and size as separate explanatory variables. The term for feeding level tests whether fish that were held at high vs. low levels of food in the main experiment consumed nauplii at a significantly different rate within individual trials where fish were given the same amount of nauplii prey. The full model was then pared back using stepwise, backward elimination using the package 'lmerTest' (Kuznetsova et al. 2017). Reduced models are displayed in tables.

The effects of temperature on rates of consumption (and respiration and excretion) were adequately described by linear models. However, many studies use Q_{10} values, defined as the multiplicative change in a rate over a 10°C increase in temperature, as a summary of the effects of temperature on physiological rates. To facilitate comparisons with other studies, we also estimated Q_{10} values for consumption, respiration, and excretion. To estimate Q_{10} values across the range of data, we used methods outlined by Rangel & Johnson (2018, 2019), and we calculated Q_{10} values for consumption, respiration, and excretion. We note that although the Q_{10} model and the linear model have a different basis (Q_{10} is multiplicative), across the ranges of age and temperature used in this study, the predicted values of all responses were nearly identical for the 2 approaches. See Text S3 for more details on the estimation procedure.

2.4. Respiration rate

Routine respiration rate was estimated for individual larvae at various ages and temperatures to evaluate the main component of energy expenditure (E_T). Respiration was evaluated by measuring rates of oxygen consumption and reflects standard metabolism plus some costs of activity and specific dynamic action (see Text S4 and Fig. S2). Measurements were made on independent samples of larvae at 7 ages between 4 and 14 dph. Respiration rates were measured within chambers of a closed microplate reader system that uses optical fluorescence to measure oxygen concentration (PreSens). A single microplate had 24 respiration chambers, and each chamber was 1.5 ml in volume. The microplate reader system was housed within a hermetically sealed acrylic water bath that was plumbed to recirculate water from the temperature-controlled sumps to hold the testing chambers at the desired temperatures. The experimental system was covered to prevent disturbance of the fish and cleaned between uses. After a 10 min acclimation period for larvae in the chambers, concentration of O_2 in each chamber was measured independently and simultaneously by the plate reader system every 30 s for 10 min, a sufficient amount of time to obtain a robust estimate of oxygen consumption for these larval fish (D. Johnson unpubl. data). For each plate of 24 respiration chambers, 2 or 3 chambers were run as blanks to account for any apparent change in oxygen concentration that was not due to respiration by the grunion larvae. For each set of respiration measurements at a particular temperature, we tested 7 larvae from each family and food treatment (1 larva per chamber and 21 to 22 larvae per microplate). Using 2 plate readers, larvae from all 6 tanks at one temperature were tested at a time, and tests from all of the temperature treatments were completed within a few hours. The order in which the temperature treatments were tested was randomized.

Respiration rate (R, expressed in mg O_2 ind^{-1} d^{-1}) was calculated as $R = V(S - B)$, where S is the slope describing change in O_2 concentration as for individual chambers with fish, and B is the average slope for the chambers with no fish (both in units of mg ml^{-1} d^{-1}), and V is the volume of water in the chamber (1.5 ml). Note that the displacement volume of grunion larvae in this study was <0.0025 ml and thus negligible in these calculations. Respiration data were not corrected for mass at a given age because it was not practical to measure the size of individual larvae. We used a mixed-effects model to analyze how respiration rate changed with temperature and age. Age, temperature, feeding level, and all possible interactions were included as fixed effects. Effects of age and feeding level may both include correlated effects of body size because fish grew throughout the experiment, and at different rates in the high- and low-feeding treatments (see Section 3). Family identity was treated as a random effect. We used backward elimination to find the reduced model that best explained the data.

2.5. Excretion rate

Excretion of nitrogenous waste requires energy, and although previous studies suggest that excretion is a relatively small component of the total metabolic losses in teleost fishes (e.g. 4–15%, Wootton 1990, Peck et al. 2003), rates of excretion may be affected by temperature. In this study, we measured ammonia as the primary excretory product, as typically observed in similar carnivorous larval fish (Wood 1993, Zimmer et al. 2017). The contributions of non-ammonia sources of waste (e.g. urea, feces) to the total energy budget were not measured but are expected to be very low. Data for both fecal egestion and urea excretion from larval fishes are rare (Buckley & Dillmann 1982), but in adults of a related species (common minnow *Phoxinus phoxinus*), non-ammonia excretion is a minor component of energy from waste products (e.g. 10–15%, Cui & Wootton 1988). Thus, energy lost via non-ammonia waste is a small fraction (~12.5%) of the total energy lost to excretion, and excretion is generally a small fraction (~10%) of the total energy budget.

We measured relative excretion rates at various ages and across our experimental temperatures. This allowed us to estimate excretion as an additional component of energy expenditure (E_T). Over a 24 h period, we measured excretion rates for samples of larvae at 7 ages between 3 and 18 dph. Groups of 5 larvae were placed in a 475 ml container of artificial seawater (with a verified starting concentration of 0 mg l^{-1} ammonia; 8 control samples had concentrations lower than the 0.005 mg detection threshold), and approximately 200–400 nauplii $ind.^{-1}$ (depending on age of the fish) were added to each container. In this experiment, brine shrimp nauplii were added by dispensing a small volume of the stock culture of nauplii to the experimental treatments. The exact volume depended on the concentration in the stock culture and the desired number of nauplii in the excretion trials. This process introduced some ammonia to the seawater, but in a predictable way that could be described by analyzing control treatments with nauplii only. Each day the excretion experiment was run, a set of controls containing only nauplii at 3 different concentrations (ranging from 150 to 1000 nauplii per container) was established to account for any ammonia introduced by the brine solution.

At the end of the 24 h period, 10 ml water samples were drawn through a 150 µm nylon mesh. Concentration of total ammonia nitrogen (NH_3-N, in mg l^{-1}) was measured via the Bower & Holm-Hansen (1980) method with a pocket calorimeter (DR300, Hach). The amount of ammonia excreted by fish larvae (U_T, in mg NH_3-N $ind.^{-1}$ d^{-1}) was calculated as the total ammonia in the container (U_{total}) minus the expected amount introduced with and produced by the brine shrimp nauplii ($U_{nauplii}$). For additional details see Text S5 and Fig. S3. To analyze how excretion rate (U_T) varied with temperature and age of the fish, we used an LME model. Age, temperature, feeding level, and all interactions were included as fixed effects, and family ID was included as a random effect. Backward elimination was used to find the reduced model that best explained the data.

2.6. Growth rate

To estimate growth rate (G_T, in mg d^{-1}), we measured larvae and compared the change in average mass over time at various ages and temperatures. Mass measurements were taken for independent samples of larvae on the day of hatching (0 dph) to determine initial size, and then for larvae at 11 ages ranging from 4 to 21 dph. It was necessary to limit the number of days we weighed fish because weighing required destructive sampling. For each treatment combination and replicate family, samples of 20 individuals were euthanized in a solution of tricaine methanesulfonate (MS-222) in seawater. Groups of larvae were dried for 24 h at 40°C and weighed on a microbalance to the nearest 0.1 mg. It was necessary to weigh 20 individuals at a time because the average individual mass was <1 mg, and we did not have access to a scale with high enough precision to detect differences is mass between individuals.

To analyze variation in growth, we used mixed-effects models of the change in average mass with age. These models focused on how the change in size with age varied across temperatures and in response to feeding treatments. Specifically, we included fixed effects for age, the temperature by age interaction, the feeding treatment by age interaction, and the 3-way interaction among age, temperature, and feeding treatment. No main effects of temperature or feeding treatment were included because these variables do not affect size directly. Rather, their effects, if present, are to modify growth rate (expressed here as the change in size with age). As random effects, we allowed the effect of age to vary among families. This allowed us to account for natural variation in growth rates among the different families while focusing on the main effects of temperature and feeding.

2.7. Bioenergetic analyses

The energy available for growth is proportional to the difference between energy intake (I_T) and energy expenditure (E_T) at each temperature. Both of these components include multiple physiological processes and can be expanded and described as $G_T = AE_T\ C_T - R_T - U_T$, where G_T is energy accumulated in the body in the form of biomass. The major processes that govern intake are assimilation efficiency (AE_T) and food consumption (C_T), whereas the major processes that govern expenditure are respiration (R_T) and excretion (U_T). Each of these component processes is expected to vary with temperature (T), but may do so to different extents (Winberg 1960, Fry 1971, Wootton 1990, Koch et al. 1992). In the analysis, we compared the temperature-dependent processes of consumption, respiration, and excretion to the temperature-dependent patterns of growth. To determine energy budgets, each of the measured rates was converted to a common currency of total energy acquired or lost (J ind.$^{-1}$) by summing the expected, daily rate over an 18 d period (the duration of our growth study) for the range of temperatures sampled (16–28°C). To convert consumption rate (nauplii ind.$^{-1}$ d^{-1}) to energy, we multiplied the number of nauplii consumed by the average energy content of 1 brine nauplius (3.39×10^{-2} J, Vanhaecke et al. 1983) To convert number of nauplii consumed to biomass consumed, we multiplied by the average mass of 1.59×10^{-3} mg nauplius^{-1} (Vanhaecke et al. 1983). To convert respiration rate (mg O_2 ind.$^{-1}$ d^{-1}) to energy (J), we multiplied by the oxycalorific constant of 13.6 J mg^{-1} O_2 respired (Elliott & Davison 1975). To convert excretion rate (mg NH_3-N ind.$^{-1}$ d^{-1}), we multiplied by 24.8 J mg^{-1} NH_3-N excreted (Elliott & Davison 1975). To convert growth in mass (mg d^{-1}) to the amount of energy accumulated per fish per day, we multiplied by the energy density of 21.97 J mg^{-1} dry *L. tenuis* tissue (D. Johnson unpubl. data).

2.8. Inferring assimilation efficiency

After we experimentally determined the rates and energy equivalents of food consumption, respiration, excretion, and growth across temperatures, we compared patterns of growth to patterns of the underlying energetic processes that were measured in this study. To account for additional energy used for converting digested food into energy for the body, AE_T was calculated by taking the sum of the energetic equivalent of growth and the amount of energy lost from respiration and excretion, divided by the amount of food energy consumed.

3. RESULTS

3.1. Consumption rate

We measured prey consumption rates for a sample of 93 grunion larvae from 15 families. Consumption rate was adjusted for natural mortality of nauplii and increased significantly with both age and temperature (Table 1). The interaction between these 2 variables was not significant, indicating that the increase in consumption rate with temperature did not change appreciably with age (Fig. 2). The feeding levels in the main experiment did not have a significant effect on consumption rates in 1 d trials, indicating no compensatory response by larvae growing under low-food conditions, nor were any of the higher-order interactions significant. For a fish at the average age (10 dph) and temperature (22°C), average consumption rate was 160.4 ± 13.1 (SE) nauplii ind.$^{-1}$ d^{-1} (see Table S2 in the Supplement for conversions to biomass of nauplii and energy consumed per unit biomass of fish). For every degree increase in temperature, food consumption increased by 5.2 ± 1.47 nauplii ind.$^{-1}$ d^{-1} ($p = 6.16 \times 10^{-4}$). For every day increase in age, food consumption increased by 14.9 ± 2.59 nauplii ind.$^{-1}$ d^{-1} ($p = 6.47 \times 10^{-8}$). The Q_{10} value for the effect of temperature on daily consumption rate was 1.778 (95% CI: 1.312, 2.382).

In addition to the general increases with temperature and age, the families (which were full-sibling

Table 1. Summary of the linear mixed-effects models of the effects of age and temperature on the feeding rates of larval grunion *Leuresthes tenuis* (n = 93 larvae)

Source	Coefficient	SE	Estimated df_{Resid}	t	p
Fixed effects					
Intercept	−97.431	38.534	76.857	−2.528	1.40×10^{-2}
Age	14.939	2.594	51.206	5.760	4.81×10^{-7}
Temperature	5.166	1.471	89.999	3.512	6.97×10^{-4}
Random effect variances					
Family	632.800				
Residual	2131.100				

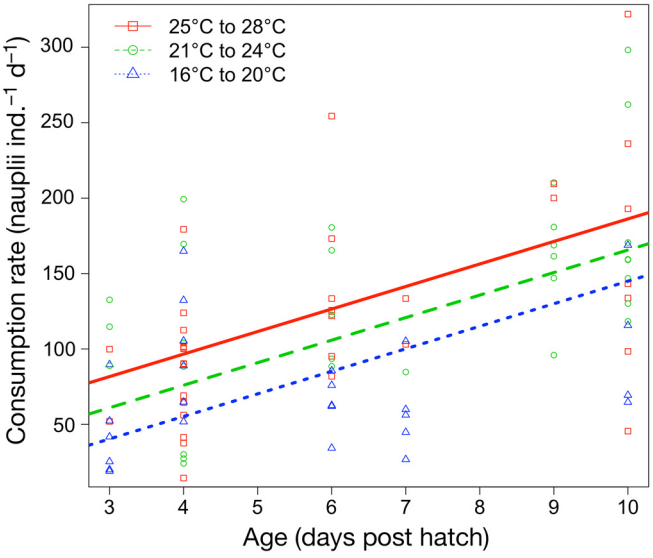

Fig. 2. Food consumption rate measured from 24 h feeding experiments with single *Leuresthes tenuis* larvae of various ages and housed at a range of temperatures (16–28°C). Temperature was included as a continuous variable in all analyses, but for display, data are grouped by temperature and lines evaluated at the midpoint temperature for each group are included to illustrate how average consumption changed with both age and temperature (blue, dotted = 18°C; green, dashed = 22°C; red, solid = 26°C). n = 93 larvae from 15 families

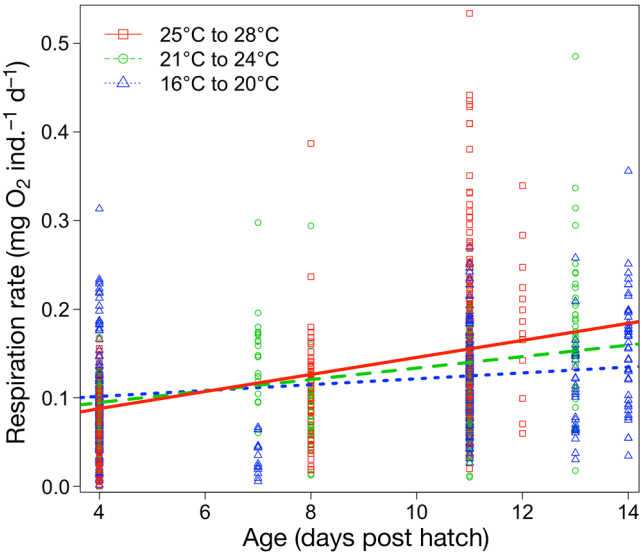

Fig. 3. Respiration rates of larval grunion *Leuresthes tenuis* measured across various ages and temperatures (16–28°C). Lines summarize the change in average respiration rate with age and are evaluated at the midpoint of each temperature group for display (blue, dotted = 18°C; green, dashed = 22°C; red, solid = 26°C). n = 1274 larvae from 40 families

groups) exhibited a moderate degree of consistency in consumption rates. Expressed as percentages of the total variation, and calculated from the random effect variances, the among-family component of variation was 20.9%, suggesting that prey consumption rates exhibit a moderate degree of repeatability among larvae of a single family. Some families tended to feed at higher-than-average rates (for a given age and temperature) and other families tended to feed at lower-than-average rates, regardless of the particular larva being tested. For context, temperature explained 8.81%, of the overall variation in feeding rates.

3.2. Respiration rate

Rates of respiration were measured for a total of 1274 larval fish from 40 families. Respiration rate increased significantly with age and temperature, was lower overall for fish from the low-food treatments, and the interaction between age and temperature was significant (Table 2). Overall, temperature sensitivity increased with age, and respiration was highest for older fish at high temperatures (Fig. 3). The increase in respiration rates with age reflects the growth and increase in size of larvae, and the decrease in respiration rates with feeding level is consistent with the smaller average size of fish that were fed lower rations throughout the experiment. In addition, the mixed-effects analysis revealed consistent variation in respiration rates among families. Expressed as percentages of the total variation, the among-family component of variation was 46.0%.

Table 2. Summary of the linear mixed-effects model of the effects of age and temperature on the respiration rates of larval grunion *Leuresthes tenuis* (n = 1274 larvae)

Source	Coefficient	SE	Estimated df_{Resid}	t	p
Fixed effects					
Intercept	0.183	2.35×10^{-2}	1043	7.806	1.43×10^{-14}
Age	-1.10×10^{-2}	2.57×10^{-3}	1247	−4.272	2.09×10^{-5}
Temperature	-4.93×10^{-3}	9.83×10^{-4}	1233	−5.105	6.09×10^{-7}
Feeding (Low)	-1.31×10^{-2}	4.80×10^{-3}	1143	−2.730	6.43×10^{-3}
Age:Temperature	7.93×10^{-4}	1.16×10^{-4}	1245	6.846	1.19×10^{-11}
Random effect variances					
Family	2.08×10^{-3}				
Residual	2.44×10^{-3}				

These results suggest that larvae from the same family were highly similar with respect to respiration rate (for a given age and temperature). Temperature explained 9.15% of the overall variation. For a fish at the average age (10 dph) and temperature (22°C), average respiration rate was 0.134 (7.56 × 10^{-3} SE) mg O_2 ind.$^{-1}$ d^{-1} for the high-food treatment and 0.127 (7.20 × 10^{-3} SE) mg O_2 ind.$^{-1}$ d^{-1} for the low-food treatment. Rates of respiration per unit biomass of fish were nearly identical for high- and low-food fish, further suggesting that the lower respiration rates of low-food fish was because of their smaller size at a given age (Table S2). The Q_{10} for a fish at 10 dph was estimated to be 1.230 (95% CI: 1.073, 1.381), although it should be noted that because of the interactive effects of age and temperature, Q_{10} values became slightly larger with age.

Table 3. Summary of the linear mixed-effects model of the effects of age and temperature on excretion rates of larval grunion *Leuresthes tenuis* (n = 88 containers of larvae)

Source	Coefficient	SE	Estimated df_{Resid}	*t*	p
Fixed effects					
Intercept	-1.11×10^{-2}	4.12×10^{-3}	80.78	−2.684	8.82×10^{-3}
Age	1.56×10^{-3}	4.67×10^{-4}	78.13	3.348	1.25×10^{-3}
Temperature	5.71×10^{-4}	1.84×10^{-4}	77.88	3.111	2.60×10^{-3}
Age:Temperature	-4.37×10^{-5}	2.11×10^{-5}	78.20	−2.076	4.11×10^{-2}
Random effect variances					
Family	2.30×10^{-6}				
Residual	7.86×10^{-6}				

3.3. Excretion rate

Rates of ammonia excretion by larval fish were measured during 88 trials, and on larvae from 8 families. Excretion rate increased significantly with both age and temperature (Table 3), but with a significant interaction indicating stronger effects of temperature on excretion at younger ages (Fig. 4). Larvae from the same family tended to have similar rates of excretion (at a given age and temperature), and the among-family component of variation accounted for 22.6% of the total. Temperature explained 4.90% of the overall variation. For a fish at the average age (10 dph) and temperature (22°C), average excretion rate was 7.52 × 10^{-3} (6.40 × 10^{-4} SE) mg NH_3-N d^{-1}. The Q_{10} value for the effect of temperature on excretion rate was 1.231 (95% CI: 0.838, 1.623; also see Table S2 for amount of ammonia produced and energetic equivalent per unit of fish biomass).

3.4. Growth rate

Growth rate was measured as the change in dry mass with age for 293 samples of 20 larvae from 68 families. Dry mass of larvae ranged from 0.30 to 1.90 mg ind.$^{-1}$ and for a fish at the average temperature (22°C), the average growth rate for a fish from the high-food group was 2.28 × 10^{-2} (5.29 × 10^{-3} SE) mg d^{-1}, and the average growth rate for a fish from the low-food group was 1.55 × 10^{-2} (5.28 × 10^{-3} SE) mg d^{-1}. Patterns of growth were complex, and this was reflected in the analysis by a significant, 3-way interaction describing how the change in mass with age (our measurement of average growth) also depended on level of feeding and temperature (Table 4). In other words, feeding level significantly altered the relationship between temperature and growth rate. For the high-food group, growth rate increased by 1.14 × 10^{-3} (4.89 × 10^{-4} SE; p = 1.98 × 10^{-2}) mg d^{-1} for every degree increase in temperature (see increase in slopes of solid lines in Fig. 5). In contrast, for the low-food group, growth rate decreased by 4.15 × 10^{-4}

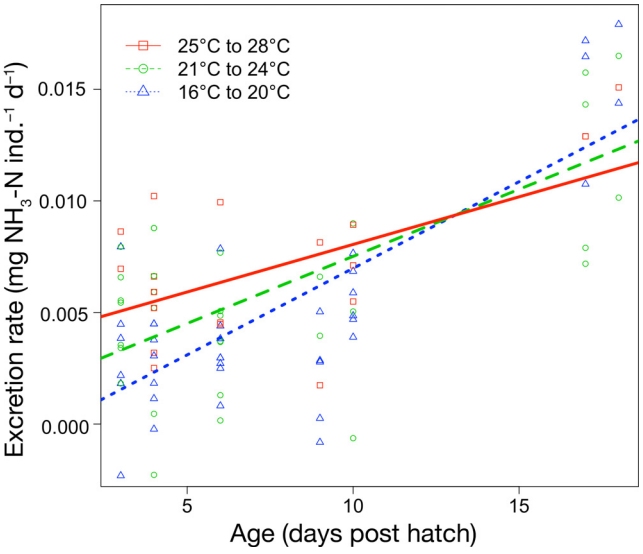

Fig. 4. Ammonia excretion rate measured from 24 h feeding experiments. There were 5 *Leuresthes tenuis* larvae per container, and excretion was evaluated across various ages and temperatures (16–28°C). Lines summarize change in average excretion rate with age and are evaluated at the midpoint of each temperature group for display (blue, dotted = 18°C; green, dashed = 22°C; red, solid = 26°C). n = 88 containers of larvae from 8 families

Table 4. Summary of the linear mixed-effects model of growth in mass of larval grunion *Leuresthes tenuis* (n = 293 samples of 20 larvae each). In this analysis, growth was measured from the change in average mass with age, and the coefficient associated with the effect of age represents average growth rate. Coefficients for the interaction terms describe how growth rates were modified by temperature, feeding, or a combination of the two factors. Residual df = 284

Source	Coefficient	SE	df	*t*	p
Fixed effects					
Intercept	0.534	6.98×10^{-2}	1	7.651	2.57×10^{-10}
Age	-2.42×10^{-3}	1.19×10^{-2}	1	−0.204	0.839
Age:Temperature	1.14×10^{-3}	4.89×10^{-4}	1	2.344	1.98×10^{-2}
Age:Feeding	2.70×10^{-2}	1.53×10^{-2}	1	1.765	7.69×10^{-2}
Age:Temperature: Feeding	-1.56×10^{-3}	6.89×10^{-4}	1	−2.267	2.35×10^{-2}
Random effect variances					
Family	0.191				
Age|Family	5.74×10^{-4}				
Residual	3.16×10^{-2}				

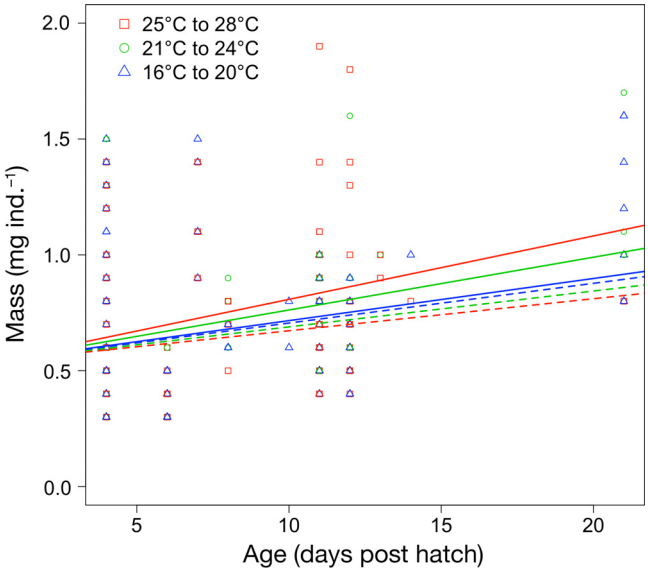

Fig. 5. Growth as evaluated by changes in dry mass with age. Points represent average mass per *Leuresthes tenuis* larva and were derived from groups of 20 larvae weighed together. Lines represent change in average mass with age and are grouped by feeding level (solid = high food; dashed = low food) and evaluated at the midpoints of temperature groups for display (blue = 18°C; green = 22°C; red = 26°C). n = 293 samples of larvae from 68 families

(4.90×10^{-4} SE; $p = 1.98 \times 10^{-2}$) mg d^{-1} for every degree increase in temperature (see decline in slopes of dashed lines in Fig. 5). When evaluating variation in mass (at a given age and temperature), the among-family component of variation accounted for 85.8% of the total. It should be noted that mass measurements were made on groups of larvae. The variance summarized by the mixed-effects model does not include among-individual variation in mass that is undoubtedly prevalent in nature but was not practical to measure within this study because the mass of a single larva was near the limit of precision of the electronic balance.

3.5. Energy budgets and assimilation efficiency

Rates of food consumption, respiration, and excretion all increased with temperature. However, when converted to units of energy, and integrated over the 18 d period for which growth was measured, the overall magnitude of energy and degree of change with temperature was large for food consumption (Fig. 6A), moderate for respiration (Fig. 6B), and minor for excretion (Fig. 6C). A comparison of these components of the energy budget suggested that higher temperatures resulted in a greater surplus of energy (Fig. 6D), largely because increases in the energy acquired by increased food consumption at higher temperatures outpaced the increases in metabolic costs. Note that of the general, bioenergetic patterns summarized in Fig. 1, the results most closely matched scenario B. Based on the observed difference between energy intake and expenditure when food was unrestricted (solid lines in Fig. 6D), energy surplus increased strongly with temperature. When food was more limiting (dashed lines in Fig. 6D), the results shifted to a pattern more similar to scenario D, with a peak in energy surplus followed by a decline at higher temperatures.

When food was not limiting, biomass growth increased with temperature (Fig. 5), but not as strongly as one would expect based on the increase in surplus energy revealed by the comparison of food energy consumed vs. energy used in respiration and excretion (Fig. 6D). These results indicated that assimilation efficiency decreased steadily with temperature when food was abundant (Fig. 7). When food was limiting, the pattern was more complex. The comparison of energy budgets suggested a slight peak in surplus of energy within the range of temperatures tested, and thus a slight, hump-shaped relationship between temperature and growth. However, our measurements of mass suggested an incremental and consistent decline in growth across the temperature range (see Fig. 5; also note that analyses revealed no significant nonlinearities in growth with

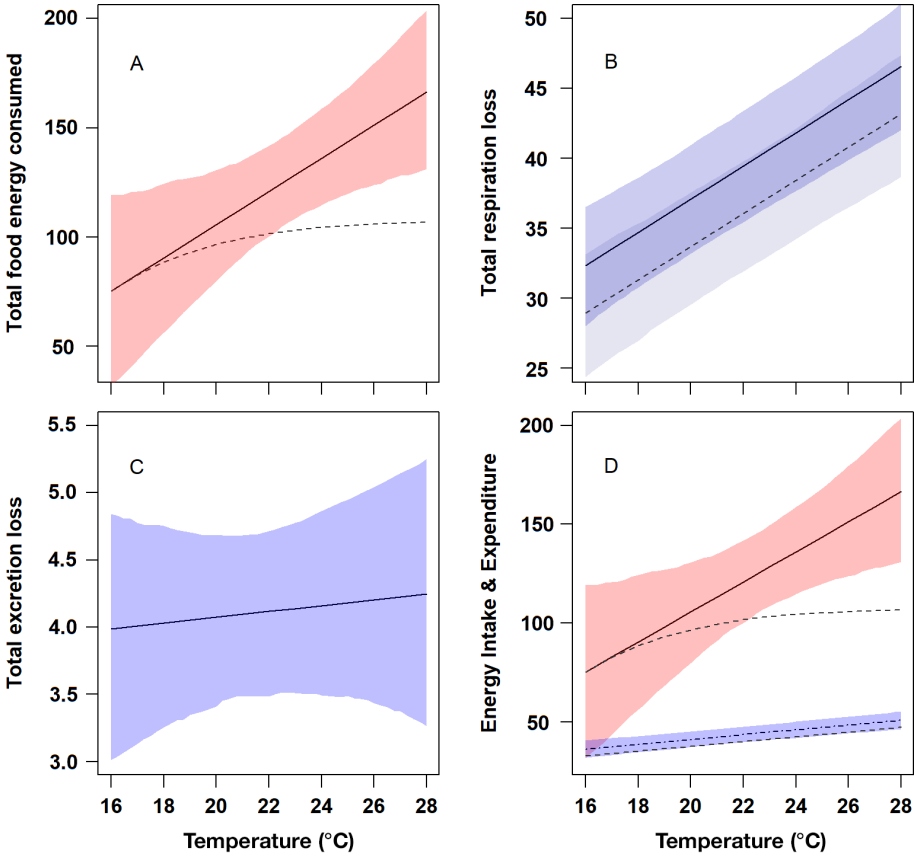

Fig. 6. Summary of the energy budget of *Leuresthes tenuis* larvae over ages 3–21 d post hatching. *Y*-axes are in units of J fish^{-1}. (A) Energy consumed from food. (B) Respiration loss. (C) Excretion loss across temperature. (D) Difference between energy intake (solid line) and energy expenditure (respiration plus excretion; dotted-and-dashed line) represents surplus energy available for growth. Shaded regions represent 95% confidence bands. Dashed lines in panels A, B, and D illustrate bioenergetic rates in the low-food treatment in which food consumption was limited to a maximum of ~150 nauplii fish^{-1} d^{-1} (see Section 2). Rates of excretion loss did not differ significantly between high- and low-food treatments and are not separated for display

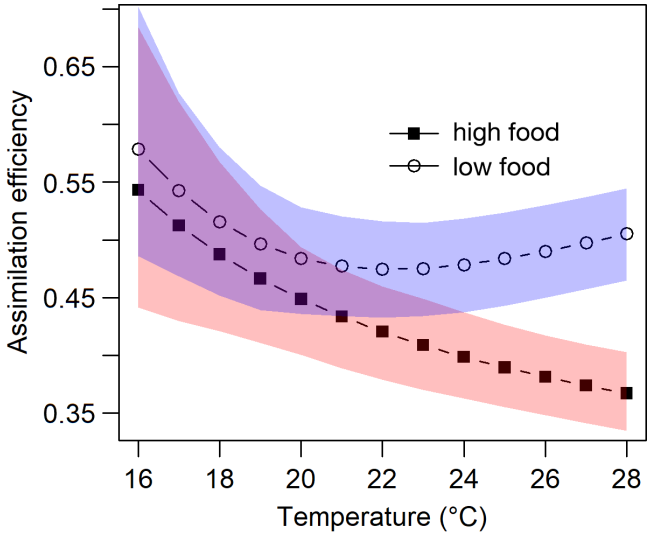

Fig. 7. Temperature-dependent patterns of assimilation efficiency of *Leuresthes tenuis* larvae evaluated under food saturation (high food) and food limitation (low food). Shaded regions represent 95% confidence bands

temperature). These results suggest that in the low-food treatments, assimilation efficiency remained fairly high across temperatures, with a slight dip in the middle of the temperature range (Fig. 7).

4. DISCUSSION

The patterns of growth observed in this study were generally consistent with predictions derived from independent measures of the effects of temperature on food consumption rates, respiration rates, and excretion rates. Our results suggest that these processes are the main mechanisms that determine energy available for growth, and that the relationships between temperature and growth are ultimately determined by the relative sensitivities of energy intake and energy expenditure to changes in temperature. In this study, as temperature increased, energy intake and energy expenditure both increased, but the rate of intake increased more rapidly than the rate of expenditure, suggesting that as long as food was not limited, there was a greater surplus of energy for growth at the higher range of temperatures. The observed patterns of growth aligned well with this prediction, and we observed faster growth of larvae at higher temperatures when food was not limiting.

However, by manipulating food availability, we forced a shift in the temperature sensitivities of bioenergetic processes. As predicted by our hypothesis, this was accompanied by a change in the relationship

between temperature and growth (see Fig. 1B,D). Restricting food affected the relationship between temperature and energy intake but did not alter the relationship between temperature and energy expenditure. At both levels of feeding in this study, enough food was offered to promote growth across the range of experimental temperatures. However, over the duration of the experiment, the feeding levels in the low-food treatment restricted intake in a manner that depended on temperature. In the low-food treatment, there was more food available than what an average fish could consume at low temperatures; however, at high temperatures, fish had the capacity to consume more than what was offered, and increases in feeding rates were hindered by food availability. Despite an initial increase in intake with temperature, limited food availability caused the intake function for the low-food treatment to slow and ultimately plateau with temperature (Fig. 6A). As a result, expenditure increased more rapidly than intake did at high temperatures. Consistent with our general hypothesis, growth in mass increased with temperature when food was unlimited and feeding capacity could increase rapidly with temperature (as predicted in Fig. 1B and observed in the high-food treatment). In contrast, when food was limited such that the relationship between temperature and intake was decelerating over much of the temperature range (as predicted in Fig. 1D), growth decreased with temperature. These results support the hypothesis that temperature sensitivities of bioenergetic components can influence growth, but they also emphasize that the effects can depend on ecological context (e.g. food availability). Importantly, the feeding levels used in our experiments were by no means extreme. Similar limitations in food availability may be common in nature, especially for planktivorous fishes (Werner & Blaxter 1980, Anderson & Sabado 1995, Donelson et al. 2010), and in general, the relationship between temperature and growth may hinge on the overall availability of food.

It is difficult to project the long-term effects of ocean warming on growth, but at above-average ocean temperatures, marine productivity is expected to decrease overall (Doney et al. 2012, Gregg & Rousseaux 2019). If the decrease in production is strong enough, our results suggest that larval growth may ultimately decline with ocean warming because even though larvae have the capacity to consume more food when temperatures are high, restrictions on food availability combined with increased energetic costs of maintenance under higher temperatures may result in less energy available for growth. Understanding how ocean productivity will change as ocean temperatures rise is a major challenge, but our study emphasizes the importance of knowing how the abundance of zooplankton is expected to respond to climate change, since the availability of zooplankton prey may have large effects on the growth and subsequent survival of larval fish (Houde 1989).

Of the 3 bioenergetic rates measured, consumption was the most sensitive to temperature (Q_{10} of 1.778; greater slope in Fig. 6). Sensitivity was less for respiration (Q_{10} = 1.230) and excretion (Q_{10} = 1.231). Excretion was by far the smallest component of the energy budget (Fig. 6), and although we did not measure non-ammonia wastes, studies on other fish species suggest that these wastes account for ~15% of the energy devoted to excretion (Cui & Wootton 1988). Even if the production of non-ammonia wastes responded to temperature differently than ammonia production did, it is unlikely that this would have much of an effect on the overall relationship between temperature and surplus energy. The Q_{10} values for respiration of larval grunion may be slightly below average, but are within the range that is commonly reported for fishes. For example, a recent review of larval fishes found that respiration Q_{10} values ranged from 1.23 to 4.77 in a sample of 14 studies (Peck & Moyano 2016). Published Q_{10} values for feeding rate are rare, but from data published in 3 studies of larval fishes (mummichog *Fundulus heteroclitus*, Radtke & Dean 1979; olive flounder *Paralichthys olivaceus*, Dou et al. 2000; yellowtail clownfish *Amphiprion clarkii*, Ye et al. 2011), we calculated Q_{10} values of 13.99, 2.06, and 5.54, respectively. Reported Q_{10} values for excretion rates are similarly rare, but from published data we were able to estimate Q_{10} values of 3.85, 1.60, and 1.29 for larvae of turbot *Scophthalmus maximus* (Finn & Rønnestad 2003), Pacific cod *Gadus macrocephalus* (Lee et al. 2012), and *P. olivaceus* (Lee 2015), respectively. Although comparative data are relatively rare, it seems that bioenergetic rates of larval grunion exhibit a relatively low thermal sensitivity. It is possible that the low sensitivity of grunion energetic rates to temperature is an adaptation to living in a temperate environment where wide fluctuations in temperature are the norm (e.g. Gilbert & Miles 2019). During the spawning season (March–July), water temperatures near our collection site can range from 12 to 25°C, especially if one includes nearby estuaries inhabited by grunion (Allen & Horn 1975). It is easy to see how a low sensitivity of energy expenditure to temperature would be an advantage under such conditions.

In general, it may be hypothesized that the capacity to acquire surplus energy diminishes as organisms near their thermal limits (e.g. as depicted in

Fig. 1D). Although we evaluated energetics and growth across the purported thermal range of this species (Ehrlich & Muszynski 1982), we did not see a decline in the surplus energy available for growth when food was not limited experimentally. Mortality rates increased exponentially with temperature, and it was difficult to keep grunion larvae alive at temperatures above 28°C. It is possible that other aspects of physiology fail and lead to mortality before a decline in surplus energy can be detected. If survival of grunion larvae could have been sustained at higher temperatures, perhaps we would have seen a decline in surplus energy. Another possibility is that there was selective loss of individuals at high temperatures. If the shapes of the bioenergetic curves varied among individuals such that some individuals did experience a loss in surplus energy at high temperatures, these individuals would be more likely to die under higher temperature conditions. The degree and importance of such individual variation is not yet known. Our study identified substantial variation in the overall rates of consumption, respiration, and excretion among families (see below), but the study was not designed to evaluate variation in the shape of their responses with temperature. Future studies of how the functional form of energy budgets varies among individuals or families will be valuable for understanding the selective effects of ocean warming on populations.

The design of our experiment allowed us to evaluate assimilation efficiency across temperatures, and we found that grunion developing in a high-food environment exhibited a consistent drop in assimilation efficiency with temperature. In contrast, grunion in the low-food treatments exhibited higher assimilation efficiencies, and a slight, U-shaped pattern of change with temperature. Effects of temperature on assimilation efficiency can be direct or indirect (Bobka et al. 1981, Cui & Wootton 1988). One indirect effect that may be particularly important is a decline in assimilation efficiency that occurs simply because feeding rate is increased (Sprung 1984). With more food moving through the gastrointestinal tract, absorption of nutrients may be less efficient (reviewed by Blaxter 1963), perhaps because increased rates of consumption stimulate peristalsis and quicker movement of food through the intestine, and perhaps because larger volumes of food traveling through the intestine as a bolus have a smaller surface area to volume ratio, and proportionately less contact with the absorptive surfaces of the intestine. Whatever the mechanism, reductions in assimilation efficiency with feeding rate appear to be common. For instance, Boehlert & Yoklavich (1984) observed a decrease in carbon assimilation efficiency for larval herring as food density increased, suggesting that the associated gross growth efficiency also decreased. In another study of larval herring, Kiorbøe et al. (1987) observed a plateau in growth at high feeding rates, most likely due to decreased assimilation efficiency. Others have observed a similar decrease in gross growth efficiency at high levels of feeding (Houde & Schekter 1983, Checkley 1984, Rendleman et al. 2018).

A direct effect of temperature on assimilation may be because of effects of temperature on digestive enzymes (Somero 1969, Dong & Somero 2009) or the action of involuntary muscles of the gastrointestinal tract (Jobling & Davies 1979). In general, enzymatic reactions speed up with temperature, perhaps contributing to higher assimilation efficiency. In addition, there may be some degree of plasticity in digestive enzyme expression. However, these effects may be more noticeable when feeding levels are low. Plasticity in digestive enzyme expression is likely to be much smaller than plasticity in feeding rate, and when food is consumed more quickly, the increases in enzymatic efficiency may be overwhelmed by declines in efficiency associated with faster feeding rates. When less food is available, less food moves through the gastrointestinal tract, and slower but more efficient digestion along with boosted enzymatic activity at higher temperatures may explain the slight increase in assimilation efficiency observed in the low-food treatments.

In response to warming, assimilation efficiency is an important process to consider. Our results suggest that assimilation efficiency can decrease sharply with temperature, and a decline in assimilation efficiency as temperature increases may be a general expectation for several reasons. Feeding rates often increase with temperature (Radtke & Dean 1979, Fonds et al. 1992), and direct measurements of assimilation efficiency have found that efficiency can decline with feeding rate (e.g. Houde & Schekter 1983, Boehlert & Yoklavich 1984, Theilacker 1987). In a review of the effects of temperature on various species of marine fish larvae, Houde (1989) used an energy budget model to reason that assimilation efficiency should decline an average of 17% over a temperature range of 10 to 30°C. This analysis highlighted an important effect, but it should be noted that species found in warm water may be adapted to assimilate their food more efficiently under warm conditions, and such effects may diminish the magnitude of within-species responses. In our study, we observed a much greater decline in assimilation efficiency with temperature

(approximately 30% from 16 to 28°C when food was not limited). These results suggest that changes in assimilation efficiency can be an important effect of changes in seawater temperature. Even though assimilation efficiency is a difficult process to measure, it can be highly sensitive to temperature, and any forecasts of the effects of climate change on growth of fishes should account for this process carefully.

In addition to the overall effects of temperature on energetics and growth, we found that grunion larvae from the same family were similar with respect to rates of food consumption. In our analyses, the among-family component of variance provides a measure of within-family repeatability, and values were 20.9% for consumption, 46.0% for respiration, 22.6% for excretion, and 85.8% for growth, though mass was measured at the group level and this last percentage is high because total variation did not include among-individual variation. Physiological traits of larvae may covary, and some of the family to family variation in respiration and excretion may reflect similarity in other traits such as body size. Moreover, the consistency among family members suggests that some of these traits may be appreciably heritable. Heritability summarizes the degree to which the phenotypic variation in a trait reflects genetic variation and thus provides a measurement of evolutionary potential (e.g. Johnson et al. 2010, Satterfield & Johnson 2020). Future studies examining heritability of physiological and behavioral responses to temperature will be important for measuring the potential for populations to evolve in response to climate change (Munday et al. 2013, Reusch 2014).

5. CONCLUSIONS

Our study was motivated by the observation that the responses of growth rate to temperature can vary across studies, species, and locations. Studying the bioenergetics of growth may help explain much of this variability. The temperature dependence of growth varies because the processes that determine energy for growth are also influenced by temperature, but at varying rates. The temperature sensitivity of bioenergetic processes can also depend on ecological context. Here we tested the effects of food availability, but it is possible that other factors such as predation risk, salinity, or environmental oxygen levels affect bioenergetic processes and thus modify the relationships between temperature and energy available for growth (Lima & Dill 1990, Angilletta et al. 2002, Torres & Giménez 2020). In general, a more detailed understanding of the bioenergetics underlying growth will be useful for projecting how growth rates are likely to change in a warming world. Growth of fish larvae can have a major influence on their probability of survival (Johnson et al. 2014) and is thus critical for successful recruitment and population replenishment (Sissenwine 1984, Houde 1987, Anderson 1988). Despite the inherent complexities involved, further studies of bioenergetics and growth will be important for anticipating the long-term health of populations as the climate continues to change (Angilletta et al. 2002, Humphries et al. 2004, Hoegh-Guldberg & Bruno 2010, Madeira et al. 2012).

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