Suppelmentary material

Text S1. More details regarding experimental procedures

In our experiments, larvae were reared in 6.6 L tanks that were set up in a module that contained six tanks all connected to the same sump where water was filtered, aerated, and chilled or heated. There were four modules, and each replicate block housed 24 tanks in total (Fig. S1).

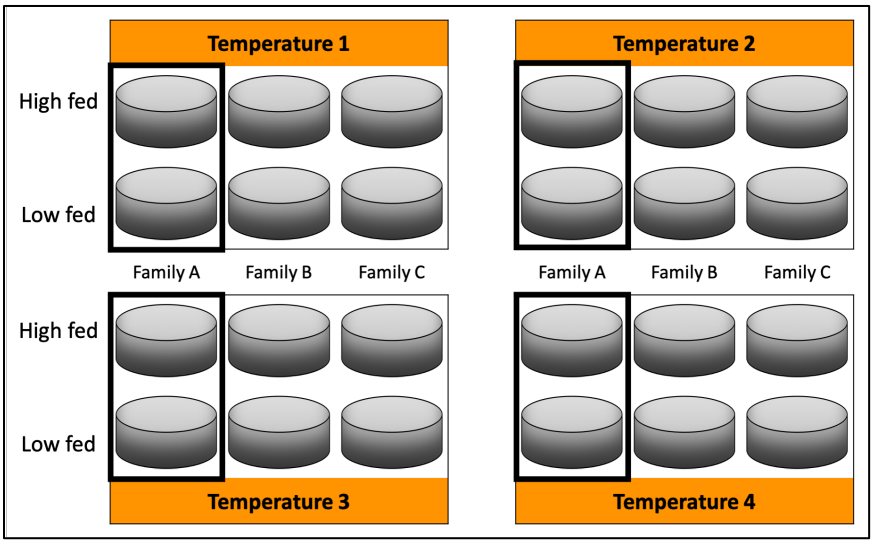

Fig. S1. Experimental setup for temperature and feeding treatments. Each tank began with 100 larvae from a single family. A set of 6 tanks was regulated at one constant temperature at a time and temperatures tested in the experiment ranged from 16-28°C. Families were full-sibling groups and were reared across the various combinations of temperature and feeding treatments. For simplicity, family groups are drawn together, but the spatial arrangement of family and feeding treatment within each temperature block was randomized.

For each replicate block, a group of six tanks was regulated at a constant temperature (Table S1). Throughout the range of temperatures tested, the change in response was linear for all the responses we measured. There was no significant improvement in model fit when a random effect that allowed the slope of the response with temperature to vary with temporal block was included in the analysis.

Table S1. Summary of experimental blocks with each block corresponding to a single two-week trial, and with each temperature corresponding to a set of 6 connected tanks. Experiments performed for a block are listed as growth (G), respiration (R), consumption (C), and excretion (U). Number of unique, full sibling groups (families) are also listed for each block

Block	Start Date	# of Families	Temp 1	Temp 2	Temp 3	Temp 4	Experiment
1	4/18/19	8	16	21	23	28	G, R
2	5/6/19	7	18	25	26	-	G, R
3	5/20/19	4	18	21	25	26	G, R
4	6/4/19	6	16	20	23	28	G, R
5	6/20/19	9	20	23	24	26	G, R
6	7/5/19	7	18	22	23	25	G, R
7	6/26/20	6	22	24	25	28	G, C
8	7/10/20	6	16	19	23	25	G, C
9	7/24/20	6	16	19	23	26	G, C
10	8/21/20	3	16	20	24	27	G, U
11	9/4/20	4	19	21	24	26	G, U

Text S2. Feeding trials: correcting for baseline survival of nauplii

Food consumption rates were assayed in a series of trials where grunion larvae were fed live brine shrimp nauplii and feeding rates were inferred from the loss of nauplii. However, not all nauplii death is due to predation, and before analyzing and interpreting the main feeding experiment, we first examined baseline survival of nauplii in the absence of larval fish. Baseline values for the survival of nauplii in the absence of grunion larvae were calculated as $S_T = N_f / N_i$, where N_f is the final number of nauplii alive, and N_i is the initial number of nauplii alive. We note that the volumetric estimates of N_i and N_f introduced some additional variability to our survival estimates, but the counting procedure was the same for all temperatures.

From the no-fish control treatments (n=26), we estimated survival of nauplii across the range of experimental temperatures. Mean nauplii survival was 92.4%, and there was no significant trend in the proportional survival of nauplii with temperature (linear model: slope = -4.13E-03, P = 0.77). Although the effects were slight and not statistically significant, we still used the relationships between temperature and baseline survival of nauplii to correct the estimates of number of nauplii consumed by grunion larvae in the feeding experiments. In particular, consumption rate of grunion larvae (C, expressed as nauplii $ind^{-1}\ d^{-1}$) was calculated as $C = (C_{est} \times S_T)$, where C_{est} is the number of nauplii lost ($N_i - N_f$), and S_T is the estimated baseline survival of nauplii over a 24-hour period at the same temperature as the feeding trial.

Text S3. Calculating Q_{10} values

Although our main analyses used a linear model to describe how each of the bioenergetic rates we measured changed with temperature, a common way of summarizing the effects of temperature is with a Q_{10} value. Q_{10} describes the factor by which a rate changes with a 10°C increase in temperature. Q_{10} is usually expressed as a two-point comparison in which average rates at a baseline temperature are comparted to averages at another, elevated temperature. However, if one sets the baseline temperature to zero, one can estimate the Q_{10} value across many temperatures at once using a regression approach (Rangel and Johnson 2018, 2019). To calculate Q_{10}, we used the following model to describe variation in energetic rates:

$$R = (aA^b){Q_{10}}^{T/10} \quad \text{(S1)}$$

Where R represents the rate of interest (here, consumption, respiration, or excretion), T is temperature, b is a parameter describing how the rate increases with age (A), and a is a parameter akin to an intercept. a describes the expected rate when age is 1 and temperature is 0. Bioenergetic rates increase with age (and size). We used age to describe this increase because we tested across a range of days but did not have exact sizes for the individual larvae used to measure bioenergetics. Treating the effect of age as a power function offers flexibility. Depending on parameter values, this relationship can be linear (if $b = 1$) or curvilinear (if $b \neq 1$). By definition Q_{10} describes a nonlinear relationship because it describes the multiplicative change with temperature. Taking the logarithm of both sides yields the following expression

$$\log(R) = \log(a) + b\log(A) + \log(Q_{10})\left(\frac{T}{10}\right) \quad \text{(S2)}$$

which is a form that is amenable to parameter estimation using a linear model. The regression of $\log(R)$ on both temperature divided by 10 and the logarithm of age can be used to estimate the parameters in the expression above. The regression coefficient associated with log-transformed age estimates b and the regression coefficient associated with T/10 estimates $\log(Q_{10})$

We used a linear mixed model to estimate the Q_{10} values for consumption and respiration rates. And because it is possible that Q_{10} values change with age, the model included log(age), log(T/10), and their interaction. Family was included as a random effect. For excretion, some of the data included negative numbers. This was because a few of the ammonia measurements with fish had levels that were slightly lower than the average ammonia in the brine-only controls. The negative numbers precluded a log transformation, so the Q_{10} value was estimated by fitting S1 to the data using a nonlinear mixed effects model, with a random effect for variation in Q_{10} among families. Despite being a nonlinear model, the degree of curvature in a Q_{10} model is not necessarily strong, and we note that the predicted values were nearly identical to those derived from a linear model approach.

Text S4. Respiration rates: activity costs and Specific Dynamic Action

Within the respirometry chambers (1.5 ml), larvae could still swim, and our measurements estimated routine rates of oxygen uptake rather than oxygen consumption in an inactive state. Larvae would often move in the chambers, and measurements of oxygen uptake in this study were comparable to rates observed when grunion were measured in much larger containers where grunion larvae had more room to swim and exhibited regular swimming behavior (Fig. S2).

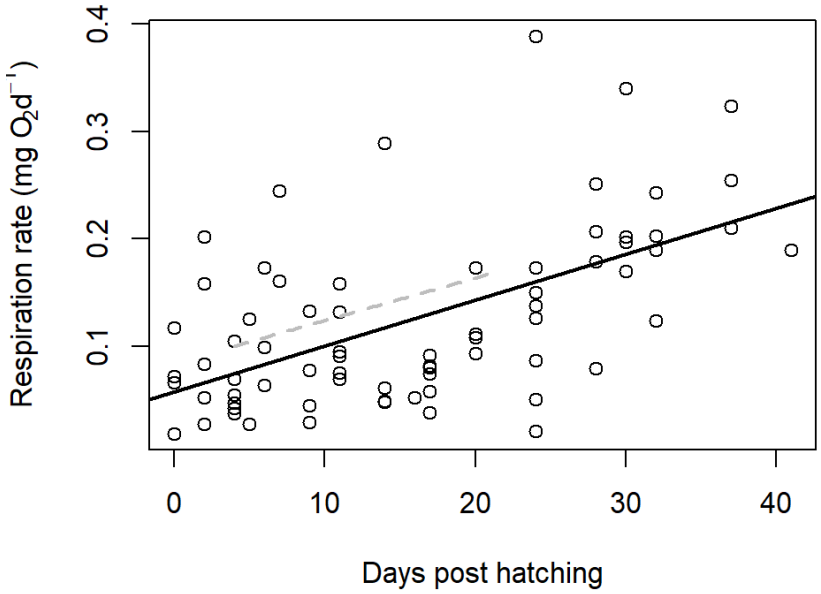

Fig. S2. Respiration rates of California grunion larvae when measured in 80-300 ml beakers over a period of 20 minutes (unpublished data). Solid black line represents the linear model relating respiration to age. Gray dashed line is the relationship for respiration rates measured in the 1.5 ml chambers (this study), and adjusted to 18.8°C – the average temperature in the large beaker trials.

Our measurements of respiration rates likely include some of the effects of Specific Dynamic Action: an increase in metabolism expressed after feeding and thought to represent the energetic costs of biosynthesis. SDA is indicated by an increased rate of oxygen uptake after feeding, and for larval grunion, SDA lasts about 24 hours (D. Johnson, *unpub. data*, also see Geubtner 2003, McCollum et al. 2006, and Chabot et al. 2016 for similar results in other fishes). In our study, the interval between the previous day's feeding and the respirometry measurements was ~ 15 hours, and the cost of SDA and biosynthesis thus make up a component of the energy consumed in these respirometry trials (Kiorbøe et al. 1987).

Text S5. Excretion rates: calculating ammonia production by larval fish

The amount of ammonia excreted by fish larvae (U_{fish}, 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 ($U_{nauplii}$). U_{total} was calculated by multiplying the total volume of seawater by the concentration of ammonia in the 10 mL samples. $U_{nauplii}$ was described by the function $(\alpha_i + \beta T)\, N$, where T is temperature, N is the number of nauplii added, α is the baseline increase in ammonia with number of nauplii added, and β describes how this rate of increase changed with temperature. The subscript i indicates that the baseline slope varied among experimental runs to reflect the differences in ammonia concentration in the stock cultures used to supply nauplii for these experiments. To estimate the α and β parameters, we used a linear mixed-effects model to analyze the amounts of ammonia observed in nauplii-only treatments. In this model α_i was estimated by the fixed effect of nauplii number plus a random

effect deviation describing the differences in slopes among the various experimental runs and stock cultures. The β parameter was estimated as a fixed effect, since a more complex model that allowed the effect of temperature to also vary among runs did not improve model fit significantly. No intercept term was included because the relationship between nauplii added and initial ammonia must go through zero. Because there were five fish per feeding trial, U_{fish} was calculated as ($U_{total} - U_{nauplii}$ [calculated for the appropriate temperature and stock culture of nauplii]) / 5. Data on the amount of ammonia introduced with brine shrimp nauplii are shown in Fig. S3.

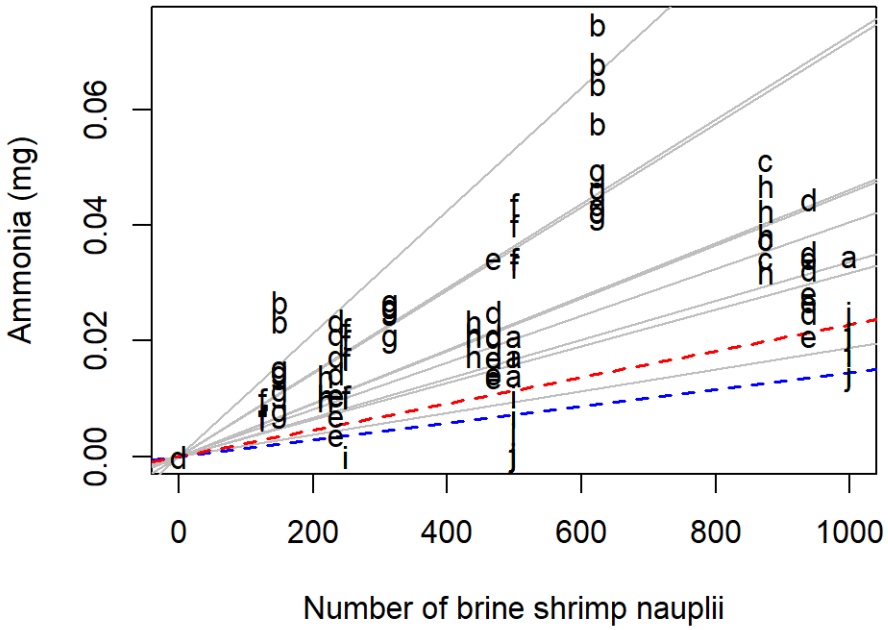

Fig. S3. Relationships between the amount of nauplii added to the no-fish control treatments and the amount of ammonia added and produced by the nauplii. Letters represent the various stock solutions (which varied with respect to initial ammonia concentration). Adding more nauplii meant adding a larger aliquot from the stock solution and a linear increase in amount of ammonia added. The dashed lines illustrate the amount of ammonia introduced at high and low temperatures (red line = 28°C; blue line = 16°C). For simplicity, these are shown for a single stock solution only (j). The effect of temperature was similar across stock solutions.

Table S2. Daily energy equivalents evaluated at average age of larvae (10 dph) and average temperature (22°C) in the experiments. Where applicable, average rates are reported per individual and per unit of dry biomass. Because growth rate and respiration rate differed significantly between food treatments, values for both treatments are reported (high/low).

Process	Daily Average	Energy
Consumption	160.4 nauplii ind^{-1} d^{-1} (0.255 mg nauplii ind^{-1} d^{-1}, (0.334/0.370 mg nauplii mg^{-1} d^{-1})	5.436 J ind^{-1} d^{-1} 7.132/7.888 J mg^{-1} d^{-1}
Respiration	0.134/0.127 mg O_2 ind^{-1} d^{-1} (0.183/0.184 mg O_2 mg^{-1} d^{-1})	1.901/1.722 J ind^{-1} d^{-1} 2.494/2.499 J mg^{-1} d^{-1}
Excretion	0.00752 mg NH_3-N ind^{-1} d^{-1} (0.00986/0.0109mg NH_3-N mg^{-1} d^{-1})	0.187 J ind^{-1} d^{-1} 0.245/0.271 J mg^{-1} d^{-1}
Mass/Energy at 10dph	High fed: 0.762 mg dry tissue Low fed: 0.689 mg dry tissue	High fed:16.743 J Low fed: 15.138 J
Assimilation Efficiency (4-21dph)		High fed: 0.417 Low fed: 0.472

References

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