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Effects of ocean acidification and warming on the specific dynamic action of California Grunion (*Leuresthes tenuis*) larvae

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ABSTRACT

Ocean acidification (OA) and Ocean Warming (OW) are ongoing environmental changes that present a suite of physiological challenges to marine organisms. Larval stages may be especially sensitive to the effects of climate change because the larval phase is a time of critical growth and development. Of particular importance to growth is Specific Dynamic Action (SDA) – the energy used in digestion, absorption, and assimilation of food. Relatively little is known about the energetics of SDA for larval fishes and even less is known about how SDA may be affected by climate change. In this study we used feeding experiments and respirometry assays to characterize the functional form of SDA for California Grunion (*Leuresthes tenuis*). In a second set of experiments, we tested the independent and combined effects of ocean acidification and warming on SDA. Our first experiment revealed that an elevated metabolic rate was detectable within an hour of feeding, peaked at 3–6 h post feeding, and lasted about 24 h in total. Experiments testing the effects of acidification and warming revealed that temperature generally increased the maximum rate of postprandial respiration and the total amount of energy expended via SDA. In an experiment where feeding level was the same for fish held at different temperatures, elevated $p\mathrm{CO}_2$ increased the maximum rate of postprandial respiration and shortened the SDA response. However, in an experiment that allowed fish to consume more food at high temperatures, effects of $p\mathrm{CO}_2$ on SDA were minimal. The effects of OA on SDA may depend on a combination of temperature and food availability, and the disruption of SDA with OA may be part of a chain of events where digestion and assimilation efficiency are impaired with potential consequences for growth, survival, and population replenishment.

1. Introduction

Anthropogenic carbon dioxide (CO_2) emissions are causing substantial changes in climate, and it is predicted that the amount of CO_2 in the atmosphere will continue to increase considerably during the coming century (Caldeira, 2005). As a result, average ocean temperatures are expected to rise by 2.89 °C, and average ocean pH is expected to decrease by 0.44 units by the year 2100 under the IPCC's SSP5–8.5 projection (Canadell et al., 2021; Fox-Kemper et al., 2021). Given these ongoing changes in ocean conditions, it is important to understand how marine species will respond to higher temperatures, higher $p\mathrm{CO}_2$ conditions, and the combination of these two stresses (Harvey et al., 2013; Kroeker et al., 2013).

For many marine fishes and invertebrates, the larval stage is particularly susceptible to climate change effects (Cattano et al., 2018; Przeslawski et al., 2015). Negative effects of ocean acidification have been found on survival, growth, behavior, metabolic rate, and tissue health (Baumann et al., 2012; Couturier et al., 2013; Cripps et al., 2011; Frommel et al., 2016; Tasoff and Johnson, 2019). Ocean warming has been shown to negatively affect survival, growth, and feeding behavior (Nowicki et al., 2012; Sswat et al., 2018; Watson et al., 2018) and the responses of organisms to the combination of ocean acidification and warming may be additive, antagonistic, or synergistic (Nowicki et al., 2012; Sswat et al., 2018). Given the importance of the larval stage to population replenishment (Houde, 1987) and the high vulnerability of larvae to climate change effects (Cattano et al., 2018; Przeslawski et al., 2015), more studies of the physiological responses of larvae to climate change are needed. One physiological process that may be important in the context of ocean acidification and warming is Specific Dynamic Action.

Specific Dynamic Action (SDA) is the summed energetic costs of ingestion, digestion, assimilation of nutrients, and synthesis of new molecules and tissue (Andrade et al., 2005; McCue, 2006; Secor, 2009). SDA thus reflects the energetic work involved in food absorption and

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growth and can be evaluated by measuring the increase in metabolic rate post feeding. SDA has been studied for many species (Andrade et al., 2005; McCue, 2006; Secor, 2009) and has been shown to be dependent on a number of external variables, including temperature and meal size (Cui and Wootton, 1988; Di Santo and Lobel, 2016; Frisk et al., 2013; Fu et al., 2005; Guinea and Fernández, 1997; Kleiber, 1961; McCue, 2006), though the responses can be more nuanced than a simple increase or decrease (Di Santo and Lobel, 2016; Frisk et al., 2013; Guinea and Fernández, 1997). Similarly, meal composition can affect the SDA response. For example, low protein, high carbohydrate diets lowered the SDA peak and led to a shorter SDA duration, while low protein high lipid diets led to lower SDA cost in *Silurus meridionalis* (Fu et al., 2005). There have been very few studies of the effects of CO_2 levels on SDA. In a study of juvenile Atlantic Cod (*Gadus morhua*), Tirsgaard et al. (2015a) found that SDA duration increased at elevated levels of $p$$CO_2$. Given the coming changes in the carbonate chemistry of the oceans, it will be important to understand how these changes affect the energetic cost of SDA, especially for the larval phase of marine organisms.

Studies of Specific Dynamic Action in larval fishes are rare, and thus far the ability to resolve the entire SDA profile, and therefore the total energetic costs, has been limited. Studies tend to compare rates of oxygen consumption of fed and non-fed individuals at a single time post feeding (e.g.; Kiørboe et al., 1987; Torres et al., 1996). Such an approach is adequate for testing for a significant SDA response but does not measure the total cost of SDA associated with a feeding event. Other studies have compared the total amount of oxygen consumed by groups of fed and non-fed larvae over much longer periods (e.g. 12 h; Yamashita and Bailey, 1989). This approach implicitly provides a time-integrated measure of SDA costs, but it cannot reveal the profile of the SDA response, and it may underestimate the total cost of SDA per feeding if the duration of the observation period is shorter than the duration of the SDA response. Another approach is to measure respiration at regular intervals post feeding. Lee et al. (2012) used such an approach to test the effects of temperature on the SDA response of larvae of Pacific Cod (*Gadus macrocephalus*). Their results suggest that temperature can alter the profile of SDA, but because the effects of temperature on baseline levels of respiration rate were not reported, a quantitative comparison of SDA cost across temperatures was not made. More research on the SDA responses of larval fishes is needed, and when practical, it may be preferred to evaluate SDA by measuring respiration responses continuously over a time interval that begins immediately after feeding and ends once the SDA response has subsided completely. That way, multiple attributes of the SDA response may be measured (e.g., maximum rate, duration, and total energetic cost).

In this study, we measured the profile of Specific Dynamic Action for larvae of a coastal fish, the California Grunion (*Leuresthes tenuis*). In our first experiment, we characterized the functional form of SDA by comparing respiration of fed and non-fed larvae at various times post feeding. This allowed us to describe general patterns of the rate, timing, and total energetic costs of SDA. In a second experiment, we tested the combined effects of ocean acidification and warming on the SDA response. Our analyses focused on understanding changes in the energetic costs of SDA under these changing environmental conditions.

2. Methods

2.1. Study species and sample collection

California Grunion (*Leuresthes tenuis*) are a species of new world silversides (Order: Atherinopsidae) that are common in the coastal waters of Southern California and range from Central California to Baja California (Gregory, 2001; Martin et al., 2013; Walker, 1952). Collections were made at Seal Beach, CA (33° 44′ 24.453“, -118° 6′ 54.6516”) during July–August 2021 and March–April 2022. Adult grunion were captured prior to spawning and strip-spawned to collect and fertilize eggs for our experiments. After allowing the embryos to incubate in 450 mL containers of moist beach sand in the lab for approximately 14 d at near constant temperature (20–21 °C), eggs were hatched in seawater using slight agitation of the water to mimic wave action (Griem and Martin, 2000). For each experiment, the larvae of at least 8 pairs of males and females were mixed and subsamples were distributed at random into 6.6 L rearing tanks with a seawater flow of 10 mL/s and a 13:11-h light-dark cycle. Experiments were started on the third day after hatching and ended no later than 12 days.

2.2. Measuring specific dynamic action: General approach

Specific Dynamic Action refers to the energetic costs of digestion and nutrient assimilation and can be measured from the temporary increase in energy consumption after feeding. In general, SDA is expected to result in a profile of energy consumption that rises quickly following a meal then declines more gradually to baseline levels (McCue, 2006). If this process can be measured at frequent intervals post feeding, and if the increase in energy consumption rate can be described as a continuous function, then integrating the rate function can yield a measure of the total SDA cost associated with a meal. In addition, other aspects of the SDA response can be measured (e.g., duration, maximal rate of energy consumption post feeding).

To measure SDA, we compared the oxygen consumption of larvae that were fed with those that were not fed at various intervals post feeding. To do this, we used pairs of rearing tanks and so the larvae were from the same cohort, and thus the same age and average size (ages ranged from 3 to 12 days post hatch). Larvae in one tank were given a full meal (*Artemia* nauplii) and the larvae in the other tank received a sham feeding treatment that mimicked the disturbance of the feeding process but contained no food. At a specific interval after the feeding, oxygen consumption was measured for 5–10 individuals from both groups. The intervals after feeding were 30 min, 1 h, 2 h and so on for intervals up to and including 25 h. To avoid the confounding effects of handling, individual larvae were used only once and for only one time interval. We used the difference in mean oxygen consumption between fed and non-fed larvae as our measure of average SDA at that specific time point. At the end of a feeding trial, both groups were fed to satiation. The next feeding trial did not begin until both groups had been fasted for 24 h. In the following feeding trial, the group that was fed was switched. In each run of the experiment, there were 3–12 pairs on different feeding and measurement schedules, and average SDA was compared across feeding trials and groups to evaluate the overall SDA response at various times post feeding (0.5 to 25 h). See Section S1 in Supplementary Materials for more details about fish housing and feeding.

To measure oxygen consumption, individual larvae were placed within chambers of a closed microplate reader system that uses optical fluorescence to measure oxygen concentration (PreSens, Germany). After a 5-min acclimation period, the change in dissolved oxygen was measured every 30 s over 20 mins. The microplate reader had 2 blocks of 24 wells. In Experiment 1, 20 fish larvae and four blanks were measured per run; in Experiment 2, 40 fish and 8 blanks were done. Blanks were seawater-only treatments and were used to control for background respiration. For all chambers, we ran a linear regression of oxygen concentration on time ($n = 40$ measurements). Respiration rate for each fish (VO_2, expressed in mg O_2 ind^{-1} h^{-1}) was calculated as $VO_2 = V(S - B)$, where S is the slope describing change in O_2 concentration for individual chambers with the fish and B is the average slope for the four chambers with no fish (both in units of mg O_2 L^{-1} h^{-1} and inferred to be per individual since each well held only one larvae), and V is the volume of water in the chamber (1.500×10^{-3} L). Note that the displacement volume of grunion larvae in this study was $<2.5 \times 10^{-6}$ L and thus negligible in these calculations. Fish were assigned to each chamber at random, and larvae were used once in the respiration measurements and then humanely euthanized.

To describe the duration and magnitude of the SDA response, we first calculated $\Delta\overline{VO}_2$, the difference between the mean respiration rate for the pair of fed and non-fed groups:

$$\Delta\overline{VO}_2 = \overline{VO}_{2,Fed} - \overline{VO}_{2,Not\ Fed} \quad (1)$$

The quantity of $\Delta\overline{VO}_2$ (expressed as mg O_2 h^{-1} ind^{-1}l) was compared across times ranging from 0.5 to 25 h post feeding. Note that only one pair was used for one specific time point and the interval length to be tested was randomized such that fish of each age contributed measurements for both short and long intervals. We note that although the comparison of mean respiration rates between samples of fed and non-fed larvae includes appreciable estimation variability for single feeding trials, the focus of the analysis was on the overall trends in the averages of these differences over time (41 trials in Experiment 1, 86 trials in Experiment 2).

2.3. Experiment 1: Measuring the form and duration of the SDA response

The form, magnitude, and duration of SDA were unknown for California Grunion, so the first experiment was designed to evaluate the timing and functional form of SDA. In this experiment, larvae were kept at a temperature of 20.8 °C (SD=1.0). Respiration rate was measured post feeding at approximately every hour for a 24-h period. For each sampling point, respiration rates were measured for 10 larvae from the fed treatment and 10 larvae from the paired, non-fed treatment. Mean VO_2 value of each group was calculated, and the difference between the fed and non-fed individuals ($\Delta\overline{VO}_2$) was used to estimate the rate of postprandial oxygen consumption at that time point. This process was repeated 41 times at intervals ranging from 0.5 to 24 h post feeding.

To describe Specific Dynamic Action, our estimates of $\Delta\overline{VO}_2$ were compared across a range of times post feeding and we conceptualized the change as a continuous process. In our analysis, we fitted four different functional relationships and used Akaike's Information Criterion (AIC) to choose the best model to describe the change in $\Delta\overline{VO}_2$ over time. Our four candidate models included a piecewise regression model with an increasing and decreasing phase; a Ricker model; a 2nd order polynomial; and a 3-parameter Shepherd function (see Table 1). Models were fit using maximum likelihood using the *bbmle* package in R (Bolker and R Core Development Team, 2021), and AIC values were corrected for small sample size. We assumed a normal distribution to describe the error distribution because data were means of 10 fed and 10 non-fed individuals, and the error of means is normally distributed.

We used the AIC-selected model to calculate several metrics of SDA. These included maximum $\Delta\overline{VO}_2$; the time at which $\Delta\overline{VO}_2$ was maximal; the total SDA as measured by the definite integral of the function describing $\Delta\overline{VO}_2$; the time until $\Delta\overline{VO}_2$ returned to 5% of the maximum value; and the time until 95% of the total SDA cost was reached. The latter two metrics are a way of describing the time it takes for postprandial energy consumption to return to a level that is functionally equivalent to pre-feeding levels (e.g., Frisk et al., 2013; Pirozzi and Booth, 2009; Tirsgaard et al., 2015b). In all analyses, derived metrics were calculated from the least-squares estimates of model parameter values, and confidence intervals were calculated based on 1000 resamples of the covariance matrix of the estimated parameters.

2.4. Experiments 2a & 2b: Effects of ocean acidification and warming on SDA

The purpose of these experiments was to compare the SDA profiles and associated metrics of SDA cost when larvae developed under ocean acidification (OA) and ocean warming (OW) conditions. Two temperature treatments (18.9 ± 1.3 °C and 23.3 ± 1.9 °C, on average; See Table S1 & S2 in Supplementary Materials) were crossed with two ocean acidification treatments (ambient pCO_2 = 366.64 μatm, pH_{NBS} = 8.09; elevated pCO_2 = 913.34, pH_{NBS} = 7.77; Table S1 & S2), for a total of four experimental treatments. The low and high pCO_2 levels represent average conditions for waters of the Southern California Bight (Davidson, 2015; Jones et al., 2016), and an elevation in pCO_2 based on regional projections for the year 2100 (Gruber et al., 2012; Turi et al., 2016). The low and high temperature levels exposed larvae to a range of temperatures grunion larvae are likely to encounter during development (Ehrlich and Muszynski, 1982). For details regarding seawater manipulation and monitoring of carbonate chemistry, see Section 2 in Supplemental Material. As in Experiment 1, a subset of fish of the same age and size were either fed or given a sham feeding. We measured respiration rates at various intervals post feeding to calculate $\Delta\overline{VO}_2$ and make inferences about the SDA profile. Experiments 2a and 2b were each replicated twice.

Experiments 2a and 2b were identical except for differences in how the fish in the various experimental treatments were fed and slight differences in temperature and CO_2 levels (Table S1 & S2). It is known that the feeding capacity of larval fish usually increases with temperature (Dou et al., 2000; Radtke and Dean, 1979) and feeding capacity of larval grunion scales predictably with temperature (Shelley and Johnson, 2022). Inferences about the SDA responses of fish in a warming ocean should therefore account for this increase in feeding. However, independent of the effects of ocean warming on feeding and resulting effects on SDA, temperature may itself affect the form and magnitude of SDA. To parse out these effects, we ran two separate experiments. In experiment 2a, feeding varied by temperature in all treatments according to the following expression:

$$\#of\ nauplii = (14.9a + 5.16T - 97.4)^{*}d \quad (2)$$

where a is the age of the fish expressed as days post hatch, T is temperature, d is the number of the fish in the tank (see Supplementary Materials for more details about feeding procedure). Experiment 2a thus captures any effect of temperature on SDA via natural increases in feeding. In Experiment 2b, fish in all four experimental treatments were given the same amount of food, with levels based on the age of the larvae and the average temperature across all treatments within the experiment. Experiment 2b thus focused on the direct effects of temperature on SDA, whereas Experiment 2a also included the indirect effects of increased feeding with temperature.

For both experiments, we conducted two complementary analyses. The first analysis used a linear mixed effects model to describe the relationship between the oxygen consumption rates of individual fish ($\Delta\overline{VO}_2$) and the fixed effect explanatory variables of age, Ocean Acidification and Warming (OAW) treatment, feeding treatment, and their interaction. Experimental block and seawater module nested within

Table 1
Summary of candidate models used to describe the functional form of Specific Dynamic Action of *Leuresthes tenuis* in Experiment 1. Each model describes the increase in $\Delta\overline{VO}_2$ as a function of time post feeding (t). n = 41 observations of post feeding respiration rates. *Includes parameters of the dynamic equation plus the parameter describing residual variance.

Model	Form	* no. parameters	Log-likelihood	AICc	ΔAICc	Akaike Weights	Evidence Ratio
Ricker	$f(t) = te^{a-bt}$	3	392.3	−778.0	0.0	0.836	0
Piecewise regression	$f(t) = at$ if $t < s$, $= as - b(x - s)$ if $t > s$	4	391.4	−773.8	4.2	0.102	8.166
2nd order polynomial	$f(t) = at + bt^2$	3	389.2	−772.4	5.6	0.051	16.445
Shepherd	$f(t) = \frac{at}{b + t^c}$	4	389.5	−769.3	8.7	0.011	77.478

block were included as random effects. In this analysis, effects of feeding treatment provided a time-averaged measure of the magnitude of SDA, and interactions between feeding and OAW treatments provide a coarse evaluation of whether SDA differed among OAW conditions. However, SDA is a time-dependent process that is best described as a nonlinear function. To examine any differences in the timing and functional form of SDA among the OAW treatments, we used our paired design to conduct a second analysis that compared differences in mean VO_2 among fed and non-fed larvae at various intervals post feeding. Our second analysis of Experiment 2 compared the $\Delta\overline{VO}_2$ profiles for larvae developing under the four experimental treatment combinations (ambient, ocean acidification, ocean warming, and ocean acidification and warming) and estimated the SDA costs under each of these scenarios. Within the microplate reader system, we could measure respiration rates of up to 48 larvae at a time. Given the four treatment combinations and the paired design, and the need for some blanks as procedural controls, each estimate of $\Delta\overline{VO}_2$ was obtained by comparing the means of 5 larvae sampled in each of the fed and non-fed groups. We used the Ricker model as the basis of our analysis of Experiment 2 because results from Experiment 1 indicated that the Ricker model was the best descriptor of post-prandial respiration rate (see *Results* section below).

The Ricker function ($\Delta\overline{VO}_2 = te^{(a-bt)}$) is governed by two parameters and we expanded the model to allow the *a* and *b* parameters to vary with temperature, OA, and their interaction. In the context of our analysis, the *a* parameter describes the initial strength of increase in $\Delta\overline{VO}_2$with a meal, and the *b* parameter describes how quickly $\Delta\overline{VO}_2$ subsides with time (*t*). We modeled the *a* parameter as

$$a = \alpha_0 + \alpha_1 T + \alpha_2 pCO_2 \quad (3)$$

where *T* is temperature, pCO_2 is the partial pressure of CO_2 (in μatm), and the α values estimate the effects that warming and acidification had on the parameter. The α_0 parameter is an intercept term that sets a baseline and expresses *a* when temperature and pCO_2 are equal to 0. The *b* parameter was modeled as

$$b = \beta_0 + \beta_1 T + \beta_2 pCO_2 \quad (4)$$

where the β values estimate the effects that changes in temperature and pCO_2 had on how quickly SDA changed with time post feeding.

The expanded model was fit using nonlinear least squares. Each coefficient was tested against a null hypothesis of zero. We note that within this framework, the effects of temperature and OA on $\Delta\overline{VO}_2$ are inherently interactive because the *a* and *b* parameters are part of an exponential function within the Ricker model. A test for more complex interactions revealed no support for higher-order interactions between temperature and pCO_2 in our submodels of *a* and *b* (see Section 3 in Supplemental Material), so the simpler model (eqs. 3 and 4) was used in the main analysis.

3. Results

3.1. Experiment 1: Describing the functional form of SDA

Examining the difference between average respiration rates of fed and non-fed larvae of the same cohort revealed a substantial amount of energy utilized by Specific Dynamic Action. Shortly after feeding, larvae that were fed had higher $\Delta\overline{VO}_2$ values than non-fed larvae, and the average difference gradually subsided to zero (Fig. 1). Of the candidate models used to describe this pattern, the Ricker function was selected as the best fit (Table 1). Despite being a relatively simple model with just two parameters in the dynamic equation, it had the highest likelihood of producing the observed data, and in this study differences in average $\Delta\overline{VO}_2$ values could be described with a reasonable degree of precision (Fig. 1).

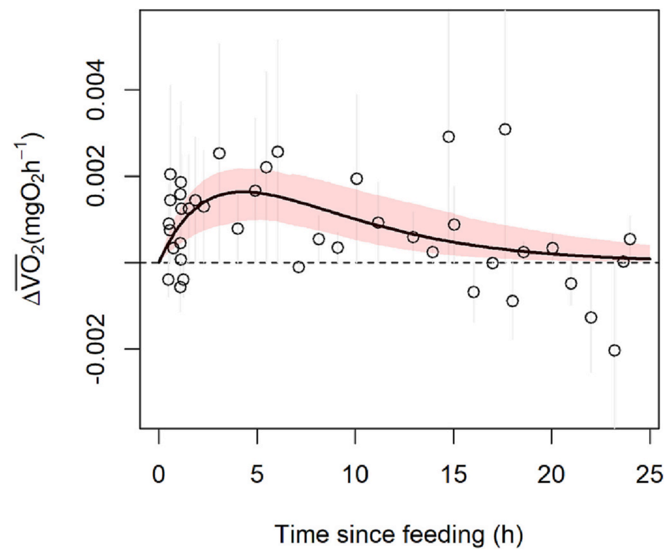

Fig. 1. Experiment 1. Profile of Specific Dynamic Action of *Leuresthes tenuis*. $\Delta\overline{VO}_2$ represents the difference in mean respiration rates between non-fed larvae and larvae fed to satiation (both treatments held at 20.8 °C). If there were no SDA response with feeding, we would expect a mean difference of zero. Each dot represents the difference between the mean of 10 fish in each of the fed and non-fed groups (± 1 SE). Individual fish were measured only once in this experiment. Solid curve represents the fit of a 2-parameter Ricker function. Shaded region depicts the 95% confidence band.

Using the Ricker model to describe the functional form of the SDA profile at 20.8 °C, we found that the increase in respiratory rate ($\Delta\overline{VO}_2$) peaked at a value of 1.63×10^{-3} $mgO_2\ h^{-1}$ (95% CI = 9.87×10^{-4} to 2.22×10^{-3} $mgO_2\ h^{-1}$) at 4.29 h post feeding (95% CI = 3.05 to 7.09). Total SDA cost, estimated from the integral of the Ricker function, was 0.0190 mgO_2 (95% CI = 0.0113 to 0.0345 mgO_2). Assuming an energetic conversion of 13.36 J per mgO_2 respired (Elliott and Davison, 1975), this translates to an average energetic cost of 0.255 J (95% CI = 0.153 to 0.461) for a single feeding to satiation. The time for $\Delta\overline{VO}_2$ to return to 5% of the maximum was 24.6 h (95% CI = 17.6 to 30.0), and the time to reach 95% of total SDA was 20.35 h (95% CI = 14.5 to 30.0).

3.2. Experiment 2: Effects of ocean acidification and warming on SDA

Our experimental manipulation of seawater conditions maintained differences in temperature and carbonate chemistry among the experimental treatments (Table S1 & S2), and larvae developing under these conditions exhibited differences in SDA values. The preliminary analysis of Experiment 2a (feeding varied by temperature) revealed that $\Delta\overline{VO}_2$ increased substantially with larval age, increased with temperature, and was significantly elevated for fed larvae (Fig. 2, Table S3). The time-averaged SDA (difference in elevation of regression lines in Fig. 2) was larger at higher temperatures (Feed x Temperature $P = 0.057$) and slightly smaller under the OA treatments (but not statistically significant; Feed x pCO_2 $P = 0.68$). Our main analysis that examined the timing and profile of SDA (via analyses of $\Delta\overline{VO}_2$) suggested changes in the SDA profile with temperature, but not OA conditions (Table 2). There was no significant, higher-order interactive effect of temperature and pCO_2 on the parameters of the Ricker function ($F_{2,142} = 0.925$, $P = 0.45$), and although some of the individual parameters describing the effects of temperature were associated with marginal *P*-values, the SDA response is reflected in the joint changes in these parameters, and we note that comparisons among the three experiments provided additional evidence that SDA increased with temperature (see below). Similar to the results of Experiment 1, $\Delta\overline{VO}_2$ generally peaked around 3.5 to 5.5 h after feeding and subsided to values near zero by 24 h post feeding (Fig. 3).

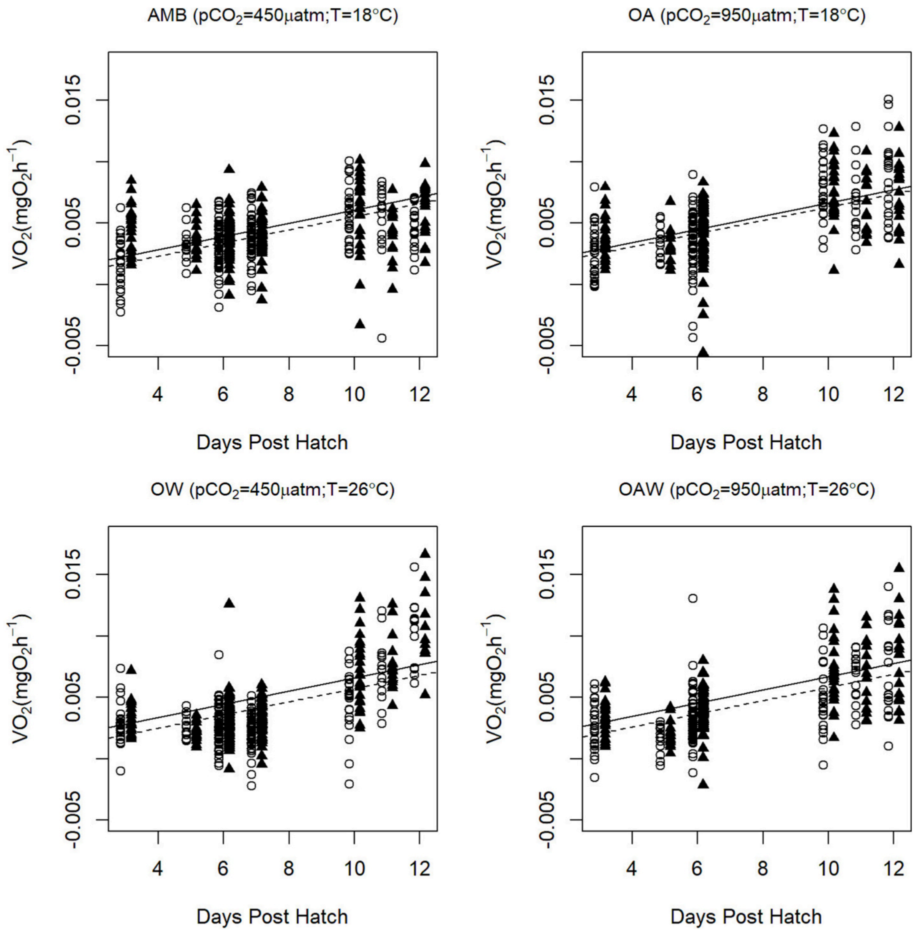

Fig. 2. Respiration rates of *Leuresthes tenuis* larvae at various ages and under the four Ocean Acidification and Warming treatments in Experiment 2a (feeding increased with temperature). Filled triangles and solid lines represent fed larvae and open circles and dashed lines represent non-fed larvae. Regression lines were generated from a linear mixed effects model and are evaluated pCO_2 and temperature values representative of our OA and OW treatments (see main text).

Table 2
Effects of temperature and ocean acidification conditions on the functional form of SDA in Experiment 2a (feeding increased with temperature). Parameters were estimated using nonlinear regression and describe the effects of experimental treatment conditions on the initial steepness of a Ricker model (parameter *a*, now modeled as a combination of the α parameters), and the eventual decline with time (parameter *b*, now modeled as a combination of the β parameters).

Effect	Parameter	Estimate	SE	t	P
Ambient	α_0	−9.874	1.790	−5.516	1.65×10^{-7}
Temperature	α_1	0.125	0.069	1.823	0.071
pCO_2	α_2	-1.579×10^{-4}	8.370×10^{-4}	−0.189	0.851
Ambient	β_0	0.055	0.240	0.230	0.818
Temperature	β_1	−0.013	0.009	−1.389	0.167
pCO_2	β_2	9.257×10^{-7}	1.319×10^{-4}	0.001	0.994

The profiles of $\Delta\overline{VO_2}$ were higher in the high-temperature treatments (Fig. 3), and this was reflected in higher peaks of $\Delta\overline{VO_2}$ values (1.59×10^{-3} mgO_2 h^{-1} at 450 μatm; 1.47×10^{-3} mgO_2 h^{-1} at 950 μatm) and lower peaks for treatments at ambient temperatures (9.24×10^{-4} mgO_2 h^{-1} at 450 μatm; 8.50×10^{-4} mgO_2 h^{-1} at 950 μatm). Values of total SDA over a 24-h period were elevated at high temperatures, but similar for fish held under low versus high pCO_2 conditions (18 °C and 450 μatm = 0.0130, 18 °C and 950 μatm =0.0120, 26 °C and 450 μatm = 0.0150, 26 °C and 950 μatm = 0.0139; all values in mgO_2).

Preliminary analysis of Experiment 2b revealed that $\Delta\overline{VO_2}$ increased significantly with age and feeding (Table S4 and Fig. 4). In Experiment 2b, which had the same feeding levels across temperature treatments. SDA profiles changed significantly with OA conditions, but not temperature (Table 3). There was no significant higher-order interaction between the effects of temperature and OA on the parameters of the Ricker function ($F_{2,132} = 1.373$, $P = 0.257$), but we emphasize that the Ricker model is nonlinear, and our data suggested that the effect of pCO_2 on the SDA response was magnified at higher temperatures. SDA profiles peaked sooner and at slightly higher values for fish under OA conditions (at 17 °C, peak $\Delta\overline{VO_2}$ was 8.80×10^{-4} mgO_2 h^{-1} at 450 μatm and 1.83×10^{-3} mgO_2 h^{-1} at 950 μatm; at 23 °C, peak $\Delta\overline{VO_2}$ was 1.56×10^{-3} mgO_2 h^{-1} at 450 μatm and 4.03×10^{-3} mgO_2 h^{-1} at 950 μatm). The total energy expended via SDA over a 24-h period was elevated for fish held under OA conditions at 23 °C (0.0159 mgO_2 at 950 μatm vs 0.0138 mgO_2 at 450 μatm) and slightly smaller for fish held under OA conditions at 17 °C (0.0110 mgO_2 at 950 μatm vs 0.0083 mgO_2 at 450 μatm). Effects of pCO_2 on SDA may thus scale multiplicatively with temperature and may also be dependent on food availability.

4. Discussion

For larval grunion, Specific Dynamic Action was detectable within an hour of feeding and lasted about 24 h in total. The peak of the SDA profile occurred around 3–6 h post feeding, and most of the total energy exerted occurred within the first 12 h. The energy used during SDA accounts for a modest but significant portion of the total energy budget. In Experiment 1, which we designed to investigate the form of SDA

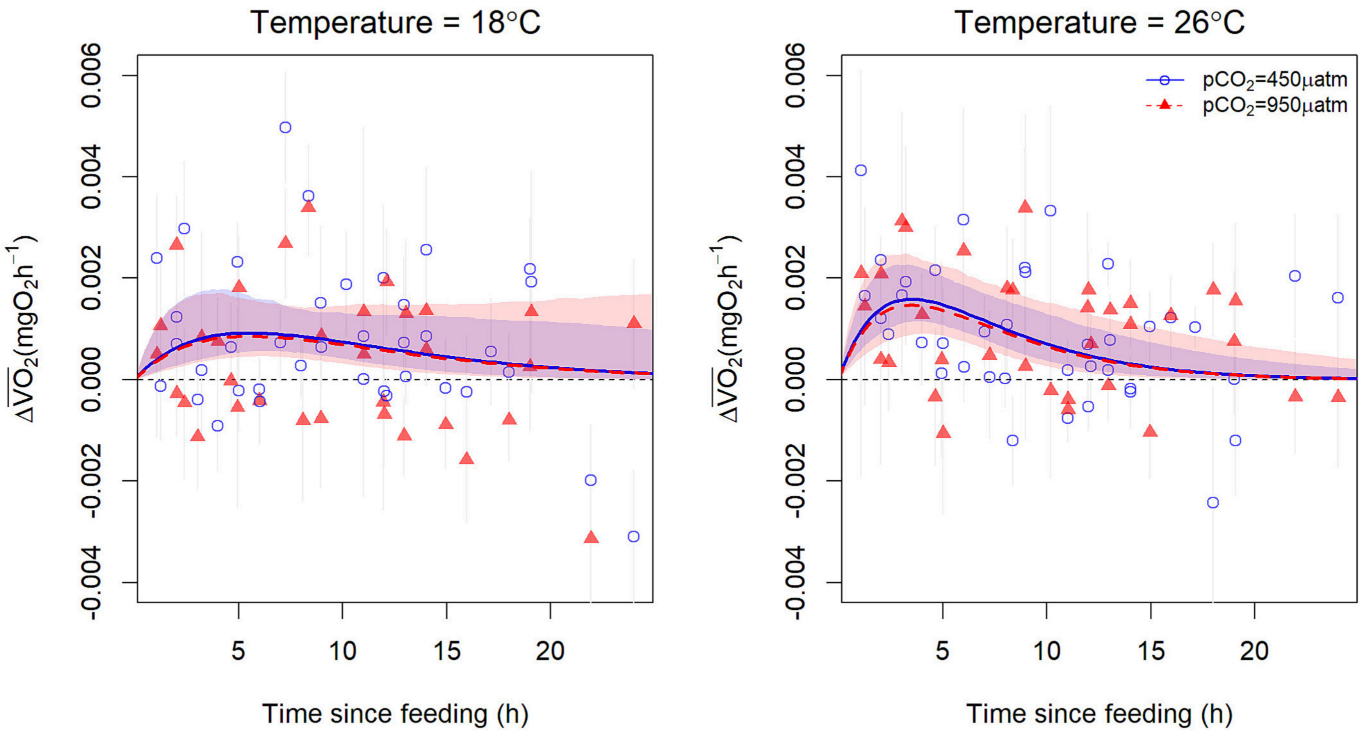

Fig. 3. Effects of ocean acidification and ocean warming conditions on Specific Dynamic Action of *Leuresthes tenuis* when feeding level varied with temperature (Experiment 2a). Each dot represents the difference between the mean VO_2 of 5 fish in each of the fed and non-fed groups ($\pm$ 1 SE). Curves represent the fit of a Ricker model evaluated at pCO_2 and temperature combinations representative of our experimental treatments. Shaded regions represent 95% Confidence Bands.

metabolism, SDA accounted for the consumption of 0.255 J of energy per day. To place that value in context, prior research suggests that at temperatures equal to those used in Experiment 1 (20.8 °C), a 10-day old grunion larva on a similar diet of *Artemia* will consume 1.7 J of metabolic energy per day via routine activities (Shelley and Johnson, 2022). Based on these values, SDA constitutes approximately 15% of the daily metabolic energy costs of a grunion larva. Estimates of total SDA costs are rare for larval fishes, but the relative value for California Grunion is similar to values for at least two other species. The amount of the energy budget used by SDA in larval Walleye Pollock (*Theragra chalcogramma*) was estimated to be 13.3% of the daily metabolic expenditure (Yamashita and Bailey, 1989). For larval whitefish (*Coregonus* sp.), SDA was estimated to account for 22% of the total metabolic costs (Karjalainen et al., 2003).

In addition to the total energetic costs of SDA, we may also consider the magnitude of the increase in respiration rate due to SDA (i.e., $\Delta\overline{VO}_2$), since there are more studies that have summarized this aspect of the SDA response. The rate of SDA for California Grunion was comparable to other larval fishes. When the peak value of $\Delta\overline{VO}_2$ was standardized to dry weight (inferred from previous data on the effects of temperature on size at age when reared in the same apparatus; Shelley and Johnson, 2022), our observed value of 2178 $mgO_2\ kg^{-1}\ h^{-1}$ was within the range of values reported for larvae of herring, mackerel, and red drum (631, 3275, and 3311 $mgO_2\ kg^{-1}\ h^{-1}$ repectively; Giguère et al., 1988; Kiørboe et al., 1987; Torres et al., 1996). In general, standardized values of SDA rates may be higher for larvae than they are for juveniles. When standardized to wet weight, the $\Delta\overline{VO}_2$ value for larval grunion was 582 $mgO_2\ kg^{-1}\ h^{-}1$. This value is higher than values reported for juvenile Cod (32.6 $mgO_2\ kg^{-1}\ h^{-1}$; Tirsgaard et al., 2015b), juvenile mulloway (45.6 $mgO_2\ kg^{-1}\ h^{-1}$; Pirozzi and Booth, 2009; average taken from all size groups at 20 °C), and juvenile cobia (166 $mgO_2\ kg^{-1}\ h^{-1}$ for fish 20 g of mass; Feeley et al., 2007). In general, the higher relative SDA costs in larval fish appear to be consistent with high specific growth rates during this stage (Dutta, 1994) and the idea that SDA values reflect the energetic costs of growth (Jobling, 1985; Kiørboe et al., 1987).

4.1. Effects of ocean acidification and warming on SDA

Warming and acidification conditions both affected the SDA response, but in differing ways and to different degrees. Higher temperatures generally resulted in an SDA response that had a higher peak and larger total area. These patterns were observed in Experiment 2a and Experiment 2b, and the SDA response in Experiment 1 (20.8 °C) was larger than those in the low-temperature treatments for Experiments 2a and 2b (18 and 17 °C). These results are consistent with general expectations of an increase in SDA at higher temperatures (McCue, 2006) and with other studies of SDA rate in fishes (Guinea and Fernández, 1997; Luo and Xie, 2008; Peck et al., 2003).

We found evidence that pCO_2 affected the SDA response, but the results differed between Experiments 2a and 2b and may be dependent on the context of food availability and/or season. When food was constant across temperatures (Experiment 2b), the peak of the SDA curve was both higher and sooner for fish held under high pCO_2 conditions, and this change was amplified at higher temperatures. In addition, fish experiencing high pCO_2 conditions exhibited little to no elevation in $\Delta\overline{VO}_2$ beyond about 8 h post feeding, whereas fish housed under ambient CO_2 conditions exhibited a more prolonged response with VO_2 remaining elevated for 15–20 h post feeding. Although the goal of this study was to examine whole-organism performance rather than physiological processes, these changes in the SDA profile under OA conditions might at least suggest which processes are likely to be affected by pCO_2. SDA processes that occur early include an increase in gut motility and the excretion of gastric acid (Diefenbach, 1975a, 1975b), whereas processes that occur later include nutrient absorption and protein synthesis (Robertson et al., 2001). It is possible that increased pCO_2 increases energetic costs associated with early-acting processes such as peristalsis and acid secretion, and that these costs may be exacerbated at high temperatures (Burnstock, 1958). In addition, the depressed $\Delta\overline{VO}_2$ values

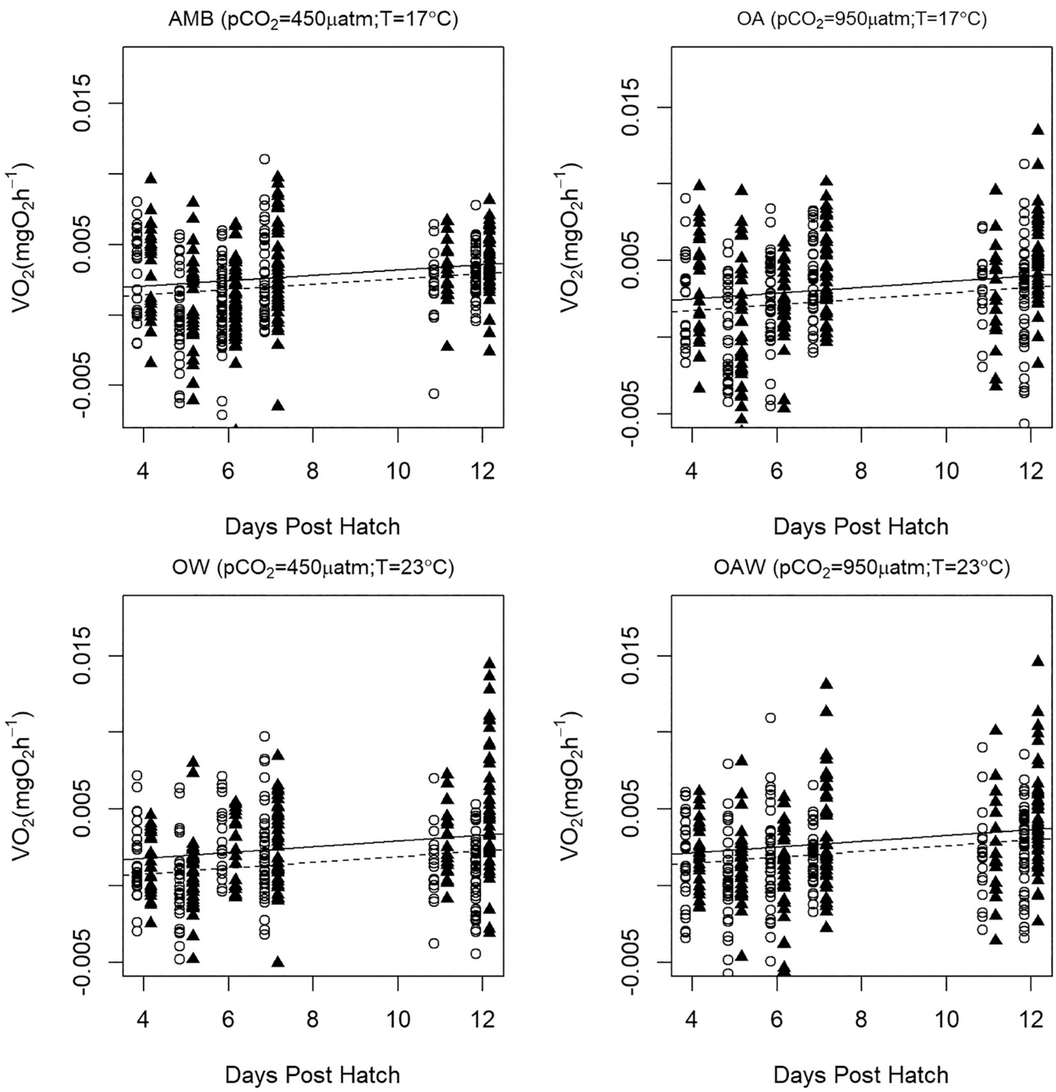

Fig. 4. Respiration rates of *Leuresthes tenuis* larvae at various ages and under the four Ocean Acidification and Warming treatments in Experiment 2b (feeding level constant across experimental treatments). Filled triangles and solid lines represent fed larvae and open circles and dashed lines represent non-fed larvae. Regression lines were generated from a linear mixed effects model and are evaluated pCO_2 and temperature values representative of our OA and OW treatments (see main text).

Table 3
Effects of temperature and ocean acidification conditions on the functional form of SDA in Experiment 2b (feeding was the same across temperatures). Parameters were estimated using nonlinear regression and describe the effects of experimental treatment conditions on the initial steepness of a Ricker model (parameter *a*, now modeled as a combination of α parameters), and the eventual decline with time (parameter *b*, now modeled as a combination of β parameters).

Effect	Parameter	Estimate	SE	t	P
Ambient	α_0	−11.81	2.267	−5.209	6.98×10^{-7}
Temperature	α_1	0.115	0.086	1.813	0.072
pCO_2	α_2	3.525×10^{-3}	1.299×10^{-3}	2.712	0.008
Ambient	β_0	0.398	0.429	0.927	0.356
Temperature	β_1	−0.016	0.018	−0.858	0.393
pCO_2	β_2	-7.692×10^{-4}	3.130×10^{-4}	−2.457	0.015

during what is likely to be the post-absorptive phase (i.e., > 8 h post feeding) are consistent with our observations of reduced biomass growth when grunion larvae develop under high pCO_2 and food limited conditions (E. Siegfried, *unpublished data*).

Despite the evidence for a shift in SDA response to pCO_2 in Experiment 2b, there was little effect of OA on the SDA curve when food varied across temperature (Experiment 2a). One explanation for this result may be that when larvae can obtain more food at high temperatures, the response of SDA to pCO_2 is somehow masked. There may also be seasonal differences in responses. Experiment 2a was conducted near the end of the reproductive season (August), whereas Experiment 2b was conducted near the beginning (April). Although the experiments were conducted in the same apparatus, some evidence suggests that the sensitivity of growth and survival to elevated pCO_2 can vary throughout the season (e.g., Murray et al., 2014; Johnson, 2022), and it is possible that fish born early versus late in the reproductive season also have different responses of SDA to pCO_2. Another explanation concerns statistical power. Although our approach allowed us to examine SDA in an innovative way by comparing mean VO_2 rates between groups of fed and non-fed fish, our results revealed substantial variation among individual VO_2 values (see Figs. 2 and 4 and Tables S3 and S4). We were able to detect overall shifts in SDA in this study, but based on our findings we would recommend sampling at least 15 larvae from each of the fed and non-fed groups for such an approach in future studies. We also recommend sampling many times early within the SDA response (between 0.5 and 6 h post feeding for grunion larvae) so that the rise and fall of postprandial energy consumption can be described with a high degree of precision.

There were some constraints to this study that affect our scope of inference. First, we note that SDA is often measured by monitoring respiration rates of individuals before and after a feeding event. Such an approach requires that individual animals can be kept isolated for monitoring and often involves a respiration measurement system that can be intermittently switched between a closed-circuit system (to measure respiration) and an open system (to ensure adequate oxygen levels throughout the digestive period and the entire assay). Our system for measuring respiration used closed chambers, and it was not practical to perform repeated measurements on individual larvae, which were < 9 mm in length and susceptible to damage if handled many times. Our cohort-based approach includes a little more sampling variation in each data summary since individuals naturally vary with respect to their overall respiration rates. However, larvae can be reared in large numbers, and respiration rates of many larvae can be measured at one time. A cohort-based approach thus allows a reliable means of measuring *average* SDA, even if there is necessarily more spread in the primary data. We note that average rates of VO_2 at a given age and temperature were similar to those in earlier studies by our research group (Shelley and Johnson, 2022), and we also note that the estimated SDA component is relatively small (maximum $\Delta\overline{VO_2}$ was always <1/4 of the Routine Metabolic Rate).

In addition to limitations in the ability to measure the metabolic rate before and after feeding on one individual, the number of larvae that can be measured at one time was limited. The microplate system used in this experiment has a total of 48 wells, of which eight were used as controls. In Experiment 2 with four OAW treatments, this meant that mean $\Delta\overline{VO_2}$ was calculated from 5 fed and 5 non-fed fish. Because each individual data point used in the main analyses was a measure of the mean difference between fed and non-fed individuals, there was less precision in the individual estimates of measures of SDA under OAW than in the first experiment where there were 10 fed and 10 non-fed individuals used (e.g., there is less scatter in Fig. 1 than Figs. 3 and 5). Although this is the case, this experiment was designed to make inferences about the mean differences between treatments and to describe the functional form of SDA. In this case, having more replicate feeding events (41 in Experiment 1 and 86 in Experiment 2) and resulting measurements of SDA under OAW resulted in comparable precision of the estimate of the mean SDA between the two experiments.

Finally, *Artemia* are not the natural prey of larval grunion, and it is conceivable that the SDA response observed in this study may be different than the SDA response in natural conditions. However, such effects, if present, are likely to affect the baseline SDA response and not the relative differences in SDA among OA treatments and temperatures. Even so, *Artemia* have similar energy densities to plankton of similar sizes found in the Pacific (21.3 J/mg for *Artemia*; Vanhaecke et al., 1983 versus 20.5 J/mg for *Tigriopus* copepodites; Theilacker and Kimball, 1984), suggesting that *Artemia* were appropriate for use in our study. Similarly, it is possible that fed and non-fed fish responded differently to the stress of the feeding and/or handling. Our paired approach was designed to remove the main effects of handling, but if the responses to stress were appreciably different between fish in our fed and non-fed treatments, then it is possible that our estimate of SDA includes a component that is due to the differential stress responses. Although we cannot rule out this scenario, we view it as unlikely because visual assessment of the fish did not suggest that they reacted differently to handling, and because fish were not chronically starved in this study as the terms fed and non-fed might imply. Rather, feeding treatments were transitory and alternating, and over the course of the experiment, larvae in the fed and non-fed groups received the same total amount of food.

It is likely that the changes in SDA observed under ocean acidification conditions reflect changes in the ability of larval fish to assimilate food and synthesize new biomass. This has overall implications for growth and development of larvae as the carbonate chemistry of seawater changes (Bignami et al., 2017; Cominassi et al., 2020). Growth will depend on several factors (e.g., food availability, temperature), but OA conditions can result in lower growth rates for grunion larvae (E. Siegfried, *unpublished data*) and other larval fishes (Baumann et al., 2012; Chambers et al., 2014; Frommel et al., 2014). In addition, smaller body size is associated with higher rates of mortality in general (Houde, 1989; Pepin, 1991), and size-dependent mortality may be intensified under OA conditions (Johnson, 2022). Deleterious effects of OA on SDA

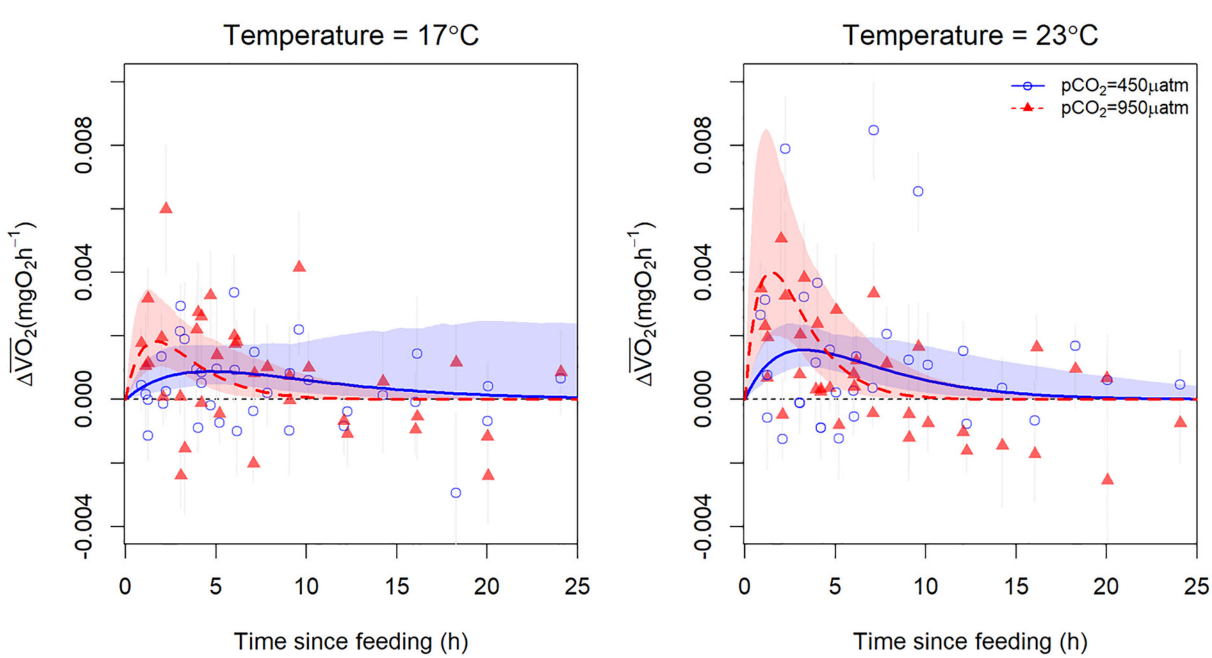

Fig. 5. Effects of ocean acidification and ocean warming conditions on Specific Dynamic Action of *Leuresthes tenuis* when feeding level was held constant across temperatures (Experiment 2b). Each dot represents the difference between the mean VO_2 of 5 fish in each of the fed and non-fed groups (± 1 SE). Curves represent the fit of a Ricker model evaluated at pCO$_2$ and temperature combinations representative of our experimental treatments. Shaded regions represent 95% Confidence Bands.

may therefore be part of a chain of events in which assimilation efficiency, molecular synthesis, and growth are impaired with potential downstream consequences for survival and population replenishment. Even small changes in larval mortality rates can have large, cumulative effects on recruitment (Houde, 1987; Houde, 2009; Johnson et al., 2014), so further study of the mechanistic links between physiology, growth, and survival of larvae will be critical for anticipating the effects of climate change on fish populations (Cattano et al., 2018; Kroeker et al., 2013).

Data statement

Data from this study are archived with the Biological and Chemical Oceanography Data Management Office: https://www.bco-dmo.org/project/852257

Author statement

Both authors contributed equally to all aspects of the study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jembe.2023.151893.

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