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Low Food Availability Narrows the Tolerance of the Copepod *Eurytemora affinis* to Salinity, but Not to Temperature

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Abstract Invasive species perturb food webs, often decreasing resource availability for resident taxa. Low resource availability may interact with abiotic factors to restrict niches, particularly niche axes that influence metabolic demand. The San Francisco Estuary (SFE) provides a case study, as it has low phytoplankton concentrations, likely due to invasive bivalves. We conducted two laboratory experiments to examine how phytoplankton concentrations influence the salinity and temperature tolerance of *Eurytemora affinis*, an important prey taxon for threatened SFE fish. We found that decreased algal concentration narrowed the tolerance of *E. affinis* to salinity, but not to temperature. A third experiment revealed that when food concentration was relatively low (chlorophyll-a of 15 μg L^{-1}) and salinity was elevated (8 vs 4), *E. affinis* did not compensate for osmotic stress by increasing consumption, halving its growth rate. However, when algal concentration was elevated (chlorophyll-a 55 μg L^{-1}), *E. affinis* consumed three times more algae at a salinity of 8 vs 4, allowing copepods to grow equally at both salinities. Our interpretation is that while *E. affinis* can compensate for increased metabolic demand as temperature increases at low food concentrations, it can only compensate for elevated metabolic demand at hypo- or hyperosmotic salinities when food concentrations are high. We therefore propose the hypothesis that low food concentrations narrow the realized salinity, but not the realized thermal, niche of *E. affinis*.

Keywords Osmoregulation · Crustacean · Food · Climate change · Invasive species · Metabolic demand

Introduction

Resource limitation in ecosystems is common, with evidence ranging from heightened primary productivity caused by inputs of nutrients to increases in secondary productivity caused by resource additions (e.g., Elser et al. 2007; Richardson et al. 2010). Due to the prevalence of resource limitation, determining whether it influences niches is a key question in ecology. This is particularly true for niche axes that influence metabolic demand, as resource availability ultimately dictates the metabolic rate an organism can sustain. For euryhaline ectotherms in aquatic ecosystems, two such axes are salinity and temperature, as both deviations from isosmotic salinities and increases in temperature increase metabolic demand (e.g., Brown et al. 2004; Goolish and Burton 1989; Angilletta et al. 2002). Here, we examined whether and how food resources influence the tolerance—which can be considered to encompass an organisms' niche (Helaouët and Beaugrand 2009)—of a euryhaline ectotherm to salinity and temperature.

Among the many causes of resource limitation, including defensive traits of resource species, seasonality, and heterogeneously distributed resources, is exploitative competition. Invasive species can be potent drivers of exploitative competition, particularly when the invader is a bivalve. For example, Eurasian zebra mussels compete with native suspension feeders for food and space, dramatically reducing the abundance of native mussels across a wide geographical range in North America (e.g., Ricciardi et al. 1998). The San Francisco

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Estuary (SFE), which provides the context for the present study, is also heavily invaded by exotic bivalves: *Corbicula fluminea* in freshwater regions and *Corbula amurensis* in brackish regions (e.g., Nichols et al. 1990; Lucas et al. 2002). The arrival and spread of *C. amurensis* correlated with a 3-fold decline in pelagic primary productivity in Suisun Bay, a heavily invaded portion of the SFE (Alpine and Cloern 1992). This decrease in productivity coincided with decreases in zooplankton abundance, including the copepod *Eurytemora affinis* (Feyrer et al. 2003; Glibert 2010; Greene et al. 2011). The decline of *E. affinis* is particularly concerning because the federally threatened delta smelt (*Hypomesus transpacificus*) is thought to be food limited (Feyrer et al. 2003; Bennett 2005; Slater and Baxter 2014), and the relative abundance of *E. affinis* in its gut contents is high (Nobriga 2002; Slater and Baxter 2014). In addition to declining food availability, both salinity and temperature are projected to increase in the SFE with climate change (Cloern et al. 2011). Thus, understanding whether low food limits the thermal and/or salinity niches of *E. affinis* will become increasingly important.

The current salinity range of *E. affinis* in the SFE is surprisingly narrow (Kimmerer et al. 2014), both in comparison to its past salinity range and its range in other regions, possibly because concentrations of its food are low. *E. affinis* occurred at salinities up to 30 in 1980 in the SFE, before the 1986 arrival of *C. amurensis* (Ambler et al. 1985; Alpine and Cloern 1992), while in 1987 the upper salinity range of *E. affinis* was reported to be 10 (Kimmerer et al. 1998). *E. affinis* is found at salinities well over 10 in other regions, including in the Seine Estuary (22.5; Devreker et al. 2008), the Bristol Channel and Severn Estuary (>30; Collins and Williams 1981), and in salt marshes in Europe, Asia, and North America (up to 40; Lee and Petersen 2003). *E. affinis* uses enzymatic pumps to move ions into and out of its body at hyper- and hypotonic salinities, making it an osmoregulator (Roddie et al. 1984; Kimmel and Bradley 2001; Lee et al. 2011; Johnson et al. 2014). Because osmoregulation is energetically costly for euryhaline crustaceans like *E. affinis* (e.g., Goolish and Burton 1989; Allan et al. 2006), one potential cause of the narrow salinity range exhibited by *E. affinis* in the SFE is a reduction in osmoregulatory capability under food-limited conditions. Indeed, Lee et al. (2013) suggest that the same mechanism drives the tendency of *E. affinis* to invade freshwater habitats with abundant food. We hypothesized that low algal concentrations, like those found in the SFE, prevent *E. affinis* from compensating for increased metabolic demand at hyper- or hyposmotic salinities by eating more, narrowing its salinity tolerance and reducing growth. At higher algal concentrations, we hypothesized that *E. affinis* may compensate for osmoregulatory energy expenditure by increasing consumption rates, thereby raising its salinity tolerance. Alternatively, growth could be reduced if copepods are unable to increase feeding rates under salinity stress (e.g., Herbst et al. 2013; Luz et al. 2008).

Low food concentrations may be less likely to limit the thermal niche of *E. affinis*. Like deviations from isosmotic salinities, the metabolic demand of ectothermic animals increases with increasing temperature before declining rapidly (Brown et al. 2004). Unlike deviations from isosmotic salinity, the activity level of ectothermic animals increases with rising temperature until a critical thermal maximum is reached (Angilletta et al. 2002). This allows ectotherms to compensate for increased metabolic demand by increasing feeding rates, at least when the threat of predation is low (Hammock and Johnson 2014). Because calanoid copepods feed by creating a water current towards their mouths with their appendages (Thorp and Covich 2001; Hwang et al. 1993), *E. affinis* may respond to increasing temperature by moving these appendages more rapidly, increasing food consumption (Cloern 1982 and references therein). Therefore, we hypothesized that at low food concentrations, *E. affinis* responds to increased temperature by increasing feeding rate, preventing low food conditions from lowering its tolerance to high temperature. Alternatively, increases in metabolic demand may outpace increases in feeding rate, causing copepods to starve as temperature rises.

Our first aim was to determine whether low algal concentration narrows the tolerance of *E. affinis* to salinity. Our second was to do the same for temperature. In addressing these aims, we found that low food availability narrowed the salinity, but not the temperature, tolerance of *E. affinis*. Our interpretation was that while metabolic demand of copepods increased with both temperature and salinity, under food-limited conditions, *E. affinis* can only compensate by increasing feeding if temperature, and not salinity, is the driver. We examined this interpretation in our final aim by determining whether the extent to which *E. affinis* compensates for osmoregulatory energy expenditure by increasing consumption depends on food concentration.

Methods

To address each of our aims, we conducted three experiments from February 12, 2013 through May 23, 2014 using *E. affinis* raised in our laboratory.

Copepod Cultures

All *E. affinis* used in our experiments were raised in 120-L conical tanks in our laboratory. These cultures have been maintained since their initiation with *E. affinis* collected from the SFE in spring 2006 (Ger et al. 2009). The culture medium was prepared by dissolving 5 g Instant Ocean (Spectrum Brands, Madison, WI, USA) per liter of “moderately hard

synthetic freshwater”(USEPA 2002). The salinity of the culture medium was 4.1. We kept the temperature at 19.5±2.5 °C and fed the copepods a mixture of two types of “Instant Algae” from Reed Mariculture: Nannochloropsis 3600 (Eustigmatophyceae) and Pavlova 1800 (Prymnesiophyceae). Neither alga is alive when shipped from the company (Reed Mariculture *personal communication*). We suspended 0.25 mL of each alga per 100-mL deionized water and delivered 1 mL of this mixture per liter of *E. affinis* culture daily. This feeding rate is equivalent to 1.7×10^5 cells Nannochloropsis and 9.75×10^3 cells Pavlova L^{-1} day^{-1} or 400 μg carbon L^{-1} day^{-1} (Ger et al. 2009). Hereafter, we refer to this feeding rate as “1× food.” Cultures were maintained under low-light conditions (1 lux, photoperiod 16L:8D). Half the water in the tanks was refreshed weekly to prevent accumulation of metabolites. We considered the conditions in our cultures to be the control conditions in our three experiments described below (i.e., water temperature of 19.5±2.5 °C and 5 g L^{-1} Instant Ocean).

Food by Salinity on Mortality

In this experiment, we determined whether the tolerance of *E. affinis* to salinity is influenced by algal concentration. Nine trials were run during which we varied algal concentration and salinity, with each lasting 96 h. To run trials, we transferred water and copepods from our cultures into petri dishes and used 2-mL transfer pipettes to capture juveniles and place them into 600-mL Pyrex beakers filled with 500-mL water (20 juvenile copepods per beaker). We used juveniles because adults can reproduce or senesce, while nauplii (larvae) have higher mortality rates than juveniles (S. Teh *personal observation*). Beakers received either 1× food (see *Copepod Cultures* section) or 3.3 times this feeding rate. That is, we suspended 0.825 mL of each (dead) alga in 100 mL of deionized water and delivered 0.5 mL per beaker per day (hereafter 3.3× food). Salinity was varied in the beakers by manipulating the concentration of Instant Ocean dissolved in “moderately hard synthetic freshwater” (USEPA 2002). During the nine trials, we ran 61 beakers from 0 to 30 g L^{-1} Instant Ocean at 1× food (8 at 0 g L^{-1}, 4 at 2 g L^{-1}, 16 at 5 g L^{-1}, 13 at 10 g L^{-1}, 8 at 15 g L^{-1}, and 4 at 20, 25, and 30 g L^{-1}) and 34 beakers from 0 to 35 g L^{-1} Instant Ocean at 3.3× food (8 beakers at 5 and 10 g L^{-1} and 3 beakers each at 0, 15, 20, 25, 30, and 35 g L^{-1}; Table 1). There was imbalance among treatments in part because we ran replicates at 5 g L^{-1} Instant Ocean as controls during the study to confirm that survival under culture conditions remained high. In addition, we focused our effort on salinities at which mortality was near 50 %.

Once the beakers had 20 juveniles, they were moved into a water bath and gently aerated (~3 bubbles s^{-1}). The temperature of the water bath was maintained between 18 and 21 °C.

Table 1 Treatments and number of beakers (*n*) for the food by salinity (S) experiment on mortality. All beakers were run between 18 and 21 °C and S is in grams per liter Instant Ocean

Food×S on mortality					
Food	S	*n*	Food	S	*n*
1	0	8	3.3	0	3
1	2	4	3.3	5	8
1	5	16	3.3	10	8
1	10	13	3.3	15	3
1	15	8	3.3	20	3
1	20	4	3.3	25	3
1	25	4	3.3	30	3
1	30	4	3.3	35	3

We fed the copepods 1 or 3.3× food every 24 h throughout the course of each 96-h trial, beginning at hour 0. Each beaker had an aluminum foil cover to minimize both inputs of airborne contaminants and evaporation. To prevent stress due to metabolite accumulation, we performed a water change 48 h into each experiment by removing half the water in each beaker using a siphon with a 15-μm screen and replacing it with water at the original salinity. During this step, we also counted and removed mortalities before replacing the beakers in the water bath. Copepods were considered dead if we observed no movement under a dissecting microscope. At 96 h, we quantified live, dead, and missing individuals in each beaker and terminated each trial. By quantifying mortality at both 48 and 96 h, our aim was to limit the number of dead copepods that decomposed before they could be counted. Any missing individuals were assumed to have died and decomposed and were considered dead in our analyses. We recovered either the body or the live animal of 91 % of the juveniles.

We measured salinity, specific conductivity, alkalinity, hardness, and pH at 0, 48, and 96 h during each trial in this and for each subsequent experiment and total ammonia for the trials with elevated food at 96 h. We used a YSI 30 meter to measure salinity and specific conductivity, an Oakton pH 700 meter to measure pH, and Hach test kits to measure hardness, alkalinity, and total ammonia. The mean of the three salinity measurements (at 0, 48, and 96 h) was used to predict copepod mortality in our analyses. Total ammonia ranged from 0 to 0.32 mg L^{-1} (mean 0.15 mg L^{-1}, SD 0.1) at the 3.3 and 4.9× feeding levels. Averaged across all beakers and time points (0, 48, and 96 h, for this and the experiments described below), mean pH was 8.1 (SD 0.18). For all beakers with Instant Ocean concentrations of 5 g L^{-1}, mean salinity over the 96 h was 4.1 (SD 0.1), specific conductivity 7.6 ms (SD 0.41), alkalinity 114 mg L^{-1} (SD 12), and mean hardness 1030 mg L^{-1} (SD 98).

Food by Temperature on Mortality

In our second experiment, we determined whether the high temperature (>18 °C) tolerance of *E. affinis* is influenced by

algal concentration. We only tested for a food-by-temperature interaction at temperatures >18 °C because we did not expect food concentration to influence low temperature tolerance (metabolic demand decreases with decreasing temperature; Brown et al. 2004). We varied temperature in beakers from 18 to 35.1 °C and quantified mortality at three feeding rates (1, 3.3, and 4.9×, 22 beakers at 1×, 17 beakers at 3.3×, and 11 beakers at 4.9×) at 5 g L^{-1} Instant Ocean (Table 2). We also ran 18 beakers between 4.1 and 15 °C at 1× food to explore the tolerance of *E. affinis* juveniles to low temperature (Table 2). There was imbalance because we were most interested in the influence of increased food at stressful temperatures, so we mainly ran the elevated food beakers at higher temperatures, as well as several with elevated food under control conditions to confirm that survival remained high with abundant food. We ran the experiment with three levels of food rather than two to determine whether increasing food from 3.3 to 4.9× decreased mortality. Water temperature was manipulated by placing beakers in plastic water baths (16 qt. Sterilite Storage Boxes) that were maintained at different temperatures. Acclimation was limited to the time it took for beakers to equilibrate to the temperatures in the tubs (e.g., roughly 30 m for beakers to go from 20 to 30 °C). Water temperature was raised with aquarium heaters, lowered with water pumped through the tubs from water chillers (a MGW Lauda T-2 chiller and an Aqua Logic Delta Star Standard Series inline water chiller), or tubs were left at room temperature. Each tub had a submersible water pump to circulate the water and ensure uniform temperature throughout. The temperature of the water in each tub was measured at 0900 and 1700 daily during each 96-h trial. We used the mean of these measurements as a predictor of copepod mortality in our analyses. The average range in temperature for each tub during the 96-h exposures was 0.81 °C (SD 0.63).

Food by Salinity on Consumption and Growth

In our final experiment, we examined the influence of salinity and food concentration on two response variables: copepod consumption and growth. The methodology was identical to the *Food by salinity on mortality* experiment (indeed, mortality was quantified during the final experiment and included in the analysis of the first experiment), except that we also measured loss of chlorophyll-a (Chl-a) from the beakers as a proxy for food consumption and copepod growth. The experiment had five replicate beakers of eight treatments. A 2×3 factorial treatment structure was used in which the three factors were food (1× and 3.3×), salinity (5 and 10 g L^{-1} Instant Ocean dissolved in moderately hard synthetic freshwater; USEPA 2002), and juvenile *E. affinis* (present or absent; Table 2). Temperature was maintained at 18 °C. We used 5 g L^{-1} Instant Ocean as a control (i.e., low osmotic stress), while we chose 10 g L^{-1} Instant Ocean to represent a salinity at which *E. affinis* was likely experiencing osmotic stress at low food (~salinity=8; Fig. 1). We blocked by time during the experiment, running three replicates of each treatment during the first block and two during the second. While Chl-a loss was measured for both blocks, we only measured growth during the second block. To provide the longest possible time for treatment differences to manifest, Chl-a concentration was measured at 96 h in every beaker. We measured Chl-a following the trichromatic method: spectrophotometric procedure (Clesceri et al. 1998). To estimate the amount of Chl-a loss from beakers, Chl-a measurements for beakers with copepods were subtracted from the mean Chl-a measurements for beakers without copepods, specific to the corresponding block and salinity of each beaker. We also calculated the proportion of Chl-a loss per treatment (i.e., the proportion of total Chl-a that was consumed) by dividing mean 96-h Chl-a measurements for treatments with copepods by means without copepods (again, specific to the corresponding treatment and block) and subtracting that proportion from one. In addition to the Chl-a measurements at 96 h, we measured Chl-a at 0 and 48 h for a subset of beakers so that, in conjunction with measurements of beakers at 96 h, we could determine the average level of Chl-a at 1× and 3.3× food.

To measure growth, we subtracted the mean length of 20 juveniles at 0 h from the length of each surviving individual at 96 h from block 2. Length was measured from the tip of the head to the base of the telson for each copepod. Lengths were measured using the image processing program ImageJ 1.45 (Schneider et al. 2012).

Table 2 Treatments and number of beakers (*n*) for the food by temperature (T) experiment on morality (all beakers had copepods) and the food by salinity (S) experiment on consumption and growth. Note: growth was only measured for two out of the five beakers for each treatment. The food by T experiment was run at 5 g L^{-1} Instant Ocean, and the food by salinity experiment was run at 18 °C

Food × T on mortality			Food × salinity on consumption and growth							
Food	T	*n*	Copepods	Food	S	*n*	Copepods	Food	S	*n*
1	18-35.1	22	Yes	1	5	5	No	1	5	5
1	4.1-15	18	Yes	1	10	5	No	1	10	5
3.3	18-35.1	17	Yes	3.3	5	5	No	3.3	5	5
4.9	18-35.1	11	Yes	3.3	10	5	No	3.3	10	5

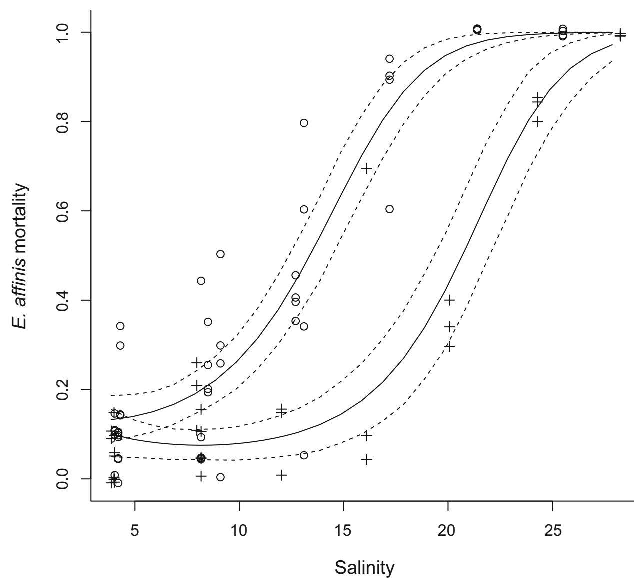

Fig. 1 Proportion mortality of *E. affinis* as a function of measured salinity at 1× (*circles*) and 3.3× (*crosses*) food. The *solid lines* represent the model predictions, and the *dashed lines* are the 95% confidence interval of the model. The experimental trials were run between 18 and 21 °C

Analyses

We used multi-model inference (Burnham and Anderson 2002) to examine our results for evidence of interactions between salinity and food on mortality (experiments 1 and 3), temperature and food on mortality (experiment 2), and salinity and food on both consumption and growth (experiment 3). All analyses were conducted in R. Sets of models were built in which each model corresponded to a hypothesized relationship between conditions in the beakers and mortality, feeding rate, or growth. Each model set included models that ranged in complexity from a null (intercept) model up to models with the interaction of interest (salinity or temperature by food). We expected copepods to exhibit relatively high mortality at both high and low salinities (i.e., exhibit "U-shaped" dose-response curves). However, interactions shift dose-response curves left or right along the *x*-axis, and we considered increasing food more likely to broaden copepod tolerances than to shift entire (U-shaped) dose-response curves. Therefore, we examined the salinity tolerance data for evidence of an interaction at low salinity (0–5 g L^{-1} Instant Ocean) and high salinity (5–35 g L^{-1} Instant Ocean) separately. The model sets at both high and low salinity were: P ~, P~S, P~S+S^2, P~S+S^2+Food and P~S+S^2+Food+S×Food, where P is mortality expressed as a proportion, S is salinity, and Food is the level at which we fed the copepods (1 or 3.3×). We included the quadratic models because linear models often did not fit the data well. To account for over-dispersion, and because the response variable was a proportion, each model had a beta-binomial distribution of error with a logistic link function (Bolker et al. 2009). We used this approach to have both realistic confidence intervals of our parameter estimates and to avoid over-fitting (Bolker et al. 2009). For cases in which the models did not converge, we centered the data by subtracting the mean salinity from each salinity value before fitting the models.

The set of models for the analysis of the second experiment (temperature by food) included all the models used to analyze the salinity by food experiment (experiment one), except that temperature replaced salinity in each model. In addition, we included two models in which we changed the "food" variable from continuous to a dummy variable with only two levels of food. The two levels were low (1×) and high (combined 3.3 and 4.9×). This allowed us to determine whether mortality varied continuously with food or whether 3.3× food represents a threshold above which increasing food caused no additional decrease in mortality.

The set of models fit to the Chl-a data included C ~, C~S, C~S+Food and C~S+Food+S×Food, where C is the loss of Chl-a (in μg L^{-1}), Food is 1 or 3.3×, and S is salinity. The models fit to the growth data were identical, except that the response was growth (in μm). Because the interaction model was strongly supported for both response variables, we also used model comparison to determine whether salinity affected growth and consumption at each level of food. This was accomplished by comparing an intercept model to a model with a linear effect of salinity for both mean growth and Chl-a loss at low and high food. The Chl-a models included a random effect for the two temporal blocks, and the growth models included a random effect for "beaker" because multiple copepods were measured from the same beaker. The growth data were all collected during block 2, so "block" was not included as a random effect in the growth analysis. We used Gaussian distributions of error for the growth and feeding rate models.

The mortality models were fit using maximum likelihood in the bbmle package, and the Chl-a and growth models were fit using the lme4 package (Bates et al. 2014; Bolker 2014). We compared models using sample-size corrected Akaike's Information Criterion (AIC_c; Burnham and Anderson 2002) and used the 95 % confidence intervals of parameters to determine statistical significance (i.e., if the confidence interval did not overlap zero, the associated factor was considered statistically significant).

Our next step was to estimate the salinities at which 50 % mortality occurs under optimal conditions (i.e., LC_{50}s) and the associated confidence intervals. Because low food narrowed the salinity tolerance of *E. affinis* (see "Results"), we excluded trials with low food, thus isolating the influence of salinity on mortality. Then, we fit a quadratic (i.e., U-shaped) beta-binomial model that predicted mortality as a function of salinity to the dataset in which the probability of mortality is:

$$P = \frac{e^{a+bs+cs^2}}{1+e^{a+bs+cs^2}}$$

where s is salinity and a, b, and c are estimated parameters. To calculate the levels of salinity at which 50 % mortality occurred, we first solved for s in terms of P:

$$s = \frac{-b \pm \sqrt{b^2 - 4ca + 4c\ln\left\{ p/_{(1-p)} \right\}}}{2c}$$

Next, we sampled the posterior probabilities of the quadratic model 10,000 times using the R package Rethinking (McElreath 2014) and used each of the models to calculate the upper and lower salinity at which 50 % mortality occurred. That is, we plugged 0.5 in for P, the parameter estimates for a, b, and c for each of the 10,000 models, and thus calculated the two values of s for each model. We then calculated a mean and 95 % CI from the resulting values of s to determine the high and low salinity LC_{50}s and confidence intervals.

We used similar methodology to calculate the high temperature at which 50 % mortality occurred (lethal level 50 %, hereafter LL_{50}). We excluded all trials at 1× food in which temperature was >20 °C because survival was lower at 1× food for *E. affinis* (see "Results"). Then, we fit a quadratic temperature model to the data and used the methodology described above to calculate the high temperature LL_{50}. We only obtained the high temperature LL_{50} because *E. affinis* had >90 % survival at 4.1 °C, the lowest temperature we achieved with our chillers. The R-script used to calculate the LC and LL_{50}s and confidence intervals is available at www.brucehammock.net/r-script.

Results

The amount we fed the copepods (feeding level) strongly interacted with salinity to influence mortality of *E. affinis* at concentrations of Instant Ocean ≥5 g L^{-1} (the model with a feeding level by salinity interaction received an AIC_c weight proportion of 1; Table 3 and Fig. 1). Feeding level also interacted with salinity at concentrations of Instant Ocean ≤5 g L^{-1}, though with less certainty (the model with a feeding level by salinity interaction received an AIC_c weight proportion of 0.6; Table 3 and Fig. 2). Based on predictions of the high and low salinity interaction models, increasing feeding level from 1 to 3.3× raised the high salinity LC_{50} from 13.4 to 20.7 and lowered the low salinity LC_{50} from 0.8 to 0.3. Thus, the LC_{50}s encompassed a salinity range that was 7.8 wider at the 3.3× than the 1× feeding level. Feeding level interacted with high salinity reliably, and low salinity fairly reliably, as the feeding level by salinity interaction parameter estimate at high salinity was −0.08, 95 % CI: −0.12, −0.04, and at low salinity, it was 0.13, 95 % CI: 0.00, 0.26. The LC_{50}s from the two interaction models fall within the confidence intervals from the quadratic salinity model fit to the high food data (lower LC_{50} 95 % CI: 0, 1.1; higher LC_{50} 95 % CI: 19.7, 22.5; Table 4).

In contrast to salinity, temperature did not interact with feeding level to influence mortality, as the model with additive effects of temperature and food received an AIC_c weight proportion of 0.85 (Table 5, Fig. 3). The top-ranked model differentiated between the 1× and combined 3.3× and 4.9× feeding rates (Table 5). This indicates that while there was mortality caused by food limitation at the 1× level, there was no longer mortality induced by food limitation at the 3.3× level.

Table 3 Model comparison for the salinity × food analysis at high and low salinity. S is salinity and F is food

Model	ΔAIC_c	df	AIC_c wt
	High salinity		
~S + S^2 + F + S × F	0.0	6	1
~S + S^2 + F	15.2	5	<0.001
~S + S^2	44.7	4	<0.001
~S	48.7	3	<0.001
~	142.3	2	<0.001
	Low salinity		
~S + S^2 + F + S × F	0.0	6	0.60
~S + S^2 + F	0.8	5	0.40
~S + S^2	10.3	4	0.00
~S	40.2	3	<0.001
~	76.7	2	<0.001

ΔAIC_c is the change in Akaike information criterion corrected for small sample size, *df* is degrees of freedom, *AIC_c wt* is AIC_c weight expressed as a proportion

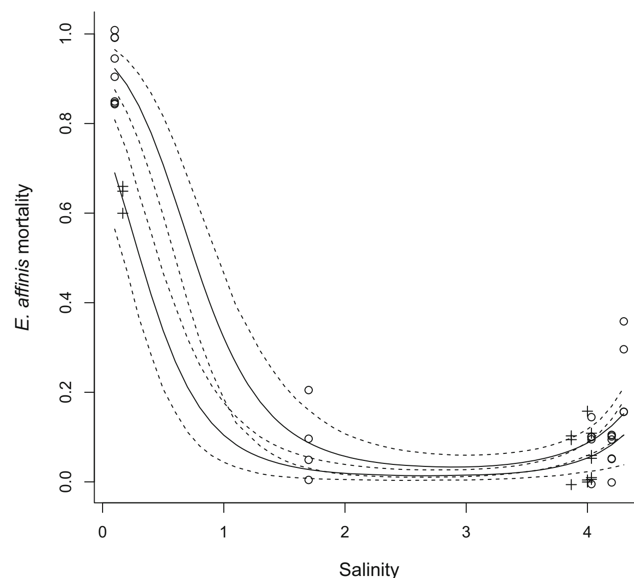

Fig. 2 Proportion mortality of *E. affinis* as a function of measured salinity at 1× (*circles*) and 3.3× (*crosses*) food. The *solid lines* represent the model predictions, and the *dashed lines* are the 95 % confidence interval of the model. The experimental trials were run between 18 and 21 °C

Table 4 Model estimated lethal levels of *E. affinis* to temperature at 5 g L^{-1} Instant Ocean and to salinity at temperatures between 17 and 22 °C at high food (3.3 and 4.9×). The lower LC_{50} estimate for salinity was negative, so we report the estimate at 3.3× food from the salinity by food interaction model for the lower salinity LC_{50}

Stressor	Lower LL_{50}	95 % CI	Upper LL_{50}	95 % CI
Temperature	<4.1	na	29.6	28.6, 30.6
Salinity	0.3	0, 1.1	21.1	19.7, 22.5

Using the top-ranked temperature model, predicted mortality decreased from 12.7 to 5.0 % at 20 °C as feeding level increased from 1 to 3.3×. The high temperature LL_{50} was 29.6 °C, while the low temperature LL_{50} was <4.1 °C (Table 4).

Feeding level also interacted with salinity to affect both growth and food consumption (Table 6; Fig. 4). The models with a salinity by feeding level interaction were the top-ranked models for both response variables, receiving AIC_c weight proportions of 0.95 and 0.96 for growth and Chl-a, respectively (Table 6). The 95 % confidence interval for both interaction parameters was reliably above 0 (salinity by food interaction parameter for growth: 6.1, 95 % CI: 0.6, 11.7; for Chl-a: 1.62, 95 % CI: 0.76, 2.47). At the 1× feeding level, the amount of Chl-a loss was similar at the high and low salinities (the salinity parameter was 0.09, 95 % CI: −0.49, 0.67; Fig. 4). However, growth rates were 2-fold lower at a salinity of 8 than 4 (the salinity parameter was −15.4, 95 % CI: −13.8, −7.0). At the 3.3× feeding level, growth rates were nearly identical between the two salinities (salinity parameter was −1.2, 95 % CI: −11.3, 8.9), but the amount of Chl-a loss was three times higher at a salinity of 8 (the salinity parameter estimate was 3.8, 95 % CI: 2.4, 5.2). The proportions of Chl-a that were lost from beakers at the 1× feeding level with copepods were 0.36 and 0.39 at salinities of 4 and 8, respectively. At the 3.3× feeding level, the proportions of Chl-a loss were 0.15 and 0.35 at salinities of 4 and 8, respectively.

Table 5 Model comparison for the temperature×food analysis. T is temperature, F is food, and G is a dummy variable for moderate and high food (i.e., 1× versus combined 3.3 and 4.9× food)

Model	ΔAIC_c	df	AIC_c wt
$\sim T + T^2 + G$	0	5	0.85
$\sim T + T^2 + G + T \times G$	4.7	6	0.08
$\sim T + T^2$	5.6	4	0.052
$\sim T + T^2 + F + T \times F$	7.8	6	0.017
$\sim T + T^2 + F$	49.4	5	<0.001
~T	58.8	3	<0.001
~	93.6	2	<0.001

ΔAIC_c is the change in Akaike information criterion corrected for small sample size, *df* is degrees of freedom, *AIC_c wt* is AIC_c weight expressed as a proportion

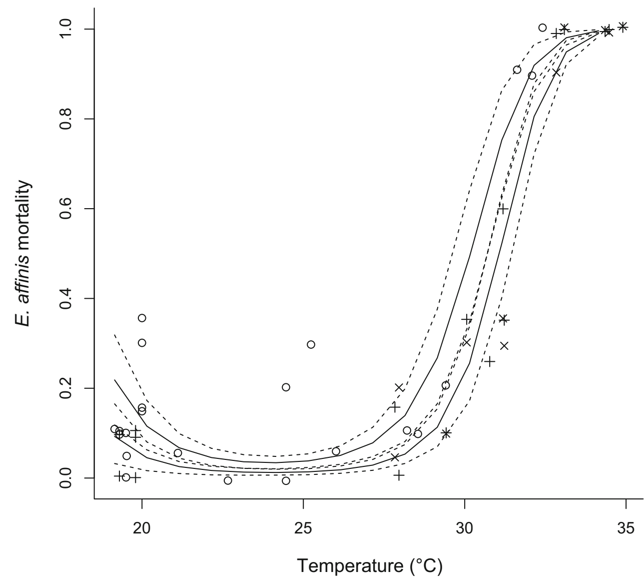

Fig. 3 Proportion mortality of *E. affinis* as a function of temperature at 1× (*circles*), 3.3× (*crosses*), and 4.9× (*multiplication signs*) food. The *solid line* represents the model predictions, and the *dashed lines* show the 95 % confidence interval of the top-ranked model by AIC_c. The experimental trials were run at 5 g L^{-1} Instant Ocean dissolved in moderately hard synthetic freshwater (USEPA 2002, a measured salinity of 4)

Averaged across 0, 48, and 96 h, the concentrations of Chl-a in beakers with copepods were 15.3 μg L^{-1} at the 1× rate and 54.6 μg L^{-1} at 3.3×. The equivalent concentrations of carbon for the same treatments were 314 and 1120 μg C L^{-1} (carbon concentrations based on Ger et al. 2009).

Discussion

For euryhaline ectotherms like *E. affinis*, metabolic demand increases with both temperature and deviations from optimal salinity before declining rapidly as physiological tolerance is exceeded (e.g., Brown et al. 2004; Goolish and Burton 1989; Angilletta et al. 2002). Therefore, the food requirements of *E. affinis* would be expected to increase from control

Table 6 Model comparison for the growth and food consumption experiments. S is salinity and F is food (1× and 3.3× food).

Model	df	Growth		Chl-a	
		ΔAIC_c	AIC_c wt	ΔAIC_c	AIC_c wt
~S+F+S×F	6	0.0	0.95	0.0	0.96
~S+F	5	5.9	0.05	6.4	0.04
~	4	23.3	<0.001	17.1	<0.001
~S	3	28.3	<0.001	13.9	<0.001

ΔAIC_c is the change in Akaike information criterion corrected for small sample size, *df* is degrees of freedom, *AIC_c wt* is AIC_c weight expressed as a proportion

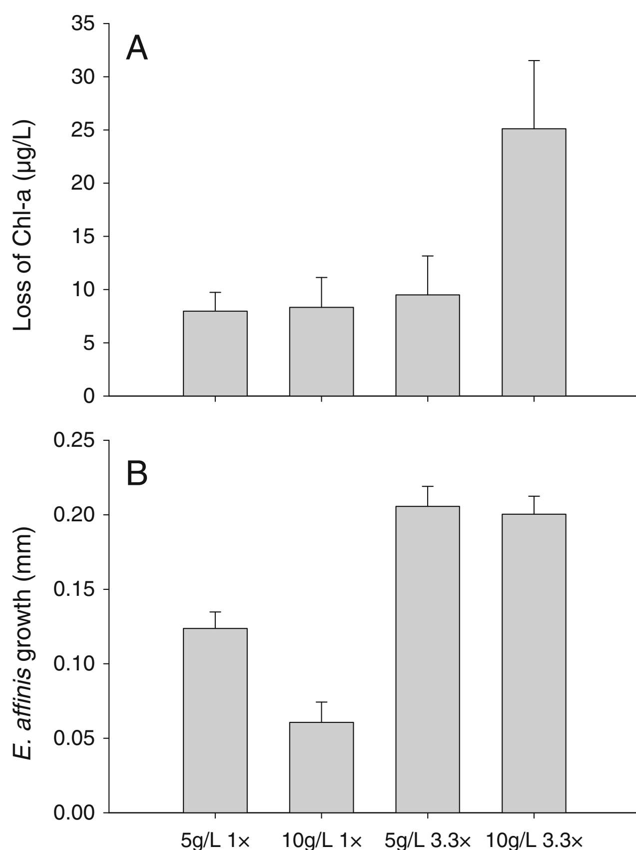

Fig. 4 Loss of Chl-a (as a proxy for food consumption by *E. affinis*; **a**) and growth in length of *E. affinis* juveniles (**b**) by treatment. Treatments are concentrations of Instant Ocean dissolved in moderately hard synthetic freshwater (USEPA 2002; measured salinities of 4 and 8) crossed with feeding rate (1 and 3.3×). Mean Chl-a concentrations at the 1 and 3.3× feeding levels are 15 and 55 μg L^{-1}. The experiment was run at 18 °C

conditions in both tolerance experiments. However, the level at which we fed the copepods had an additive effect on mortality in the food by temperature tolerance experiment, but an interactive one in the food by salinity tolerance experiment, with low food only narrowing the salinity tolerance of *E. affinis*. In the food by temperature tolerance experiment, predicted mortality declined 2.6-fold as food increased from 1 to 3.3× at 20 °C, indicating that *E. affinis* was food limited at 1× food (Fig. 3). Because the difference in mortality rate between 1 and 3.3× food held fairly constant as temperature increased, individuals appeared to compensate for increased metabolic demand at higher temperatures by increasing consumption (Fig. 3). In contrast, mortality rates diverged rapidly with increasing salinity between 1 and 3.3× food (Fig. 1). For example, as salinity increased from 8 to 15, predicted mortality increased from 19 to 65 % at 1× food, but only 8 to 15 % at 3.3× food. Thus, low food narrowed the tolerance of *E. affinis* to salinity, but not to temperature. These results agree well with the work of Lee et al. (2013), who found that the tolerance of *E. affinis* to fresh water increases with increased food.

The differing mortality responses to salinity and temperature across levels of food likely reflect dissimilarity in the feeding response of *E. affinis* to increasing temperature and salinity. As ectotherms, copepods can move their feeding appendages more quickly at higher temperatures (Hwang et al. 1993; Angilletta et al. 2002; Thorp and Covich 2001), allowing them to roughly double food consumption for a 10 °C temperature increase (Cloern 1982 and references therein). While deviations from isosmotic salinity also increase energetic demand (e.g., Goolish and Burton 1989), there is not a corresponding increase in the upward limit of activity as with increased temperature. Therefore, our interpretation is that, unlike for increasing temperature, individuals cannot compensate for increased metabolic demand at high salinity by increasing consumption when algal concentration is low. This interpretation is supported by the growth and Chl-a results. At high (3.3×) food, consumption tripled as salinity increased from 4 to 8 while growth was unaffected. The increase in food intake likely occurred because copepods were compensating for increased energetic demand at the higher salinity as in Goolish and Burton (1989). However, while metabolic demand would be expected to increase similarly in the low food treatments with increased salinity (it was an identical 4 to 8 increase in salinity), we did not observe increased loss of Chl-a. Therefore, we conclude that the filtering rate of *E. affinis* was maximized at 1× food, leaving no room for compensatory feeding at a suboptimal salinity.

Feeding rates may be similarly maximized in the San Francisco Estuary (SFE), although applying our results directly to the estuary is difficult. On one hand, there is extensive evidence that food for zooplankton is limited in the SFE. The mean concentration of Chl-a in Suisun Bay, SFE habitat for delta smelt and *E. affinis*, declined from 11±2 μg L^{-1} from 1975 to 1986 (before the invasion of the bivalve *C. amurensis*) to 2.2±0.2 μg L^{-1} from 1987 to 2010 (after the invasion; Cloern and Jassby 2012). There is evidence from several taxa that levels of Chl-a below 10 μg L^{-1} limit zooplankton in the SFE (Müller-Solger et al. 2002; Kimmerer et al. 2005; Cloern and Jassby 2012). For example, Kimmerer et al. (2014) found that egg production and growth of *E. affinis* in the SFE are well below those determined in the laboratory at food-saturated rates. Similarly, the growth rates of *E. affinis* were depressed at 15.3 μg L^{-1} Chl-a in our third experiment, particularly at high salinity, indicating that *E. affinis* could not consume enough food to grow optimally at 1× food (Fig. 4). Thus, we found that copepods were relatively intolerant to salinity and exhibited relatively low growth rates at a level of Chl-a roughly seven times higher than the mean Chl-a measurements in Suisun Bay from 1987 to 2010. On the other hand, detritus and microzooplankton—two components of seston—are not included in Chl-a measurements and were absent from our experiments. While detritus may not be an important component of the diet of *E. affinis* (Müller-Solger

et al. 2002; Heinle et al. 1977, although see Heinle and Flemer 1975), microzooplankton are important (Merrell and Stoecker 1998; York et al. 2014; Heinle et al. 1977). Thus, relating the levels of food in our beakers to the SFE is difficult without further experimentation.

Nevertheless, there is evidence that *E. affinis* exhibits a relatively narrow salinity range in the SFE, potentially due to low food concentrations. In 1980, before the 1986 invasion of *C. amurensis*, the upper salinity range of *E. affinis* in one study was ~30 (Ambler et al. 1985). By 1987, the upper range had declined to 10 (Kimmerer et al. 1998), markedly lower than what both its tolerance (salinity LC_{50}~20 in the present study) and ranges in other regions (upper salinity limits >20; Devreker et al. 2008; Collins and Williams 1981; Lee and Petersen 2003) would indicate (Kimmerer et al. 2014). The apparent decline in the upper salinity bound of *E. affinis* in the SFE is consistent with the hypothesis that low food concentrations, linked to the invasion of *C. amurensis*, have narrowed the salinity range of *E. affinis* by limiting its ability to osmoregulate. Lee et al. (2013) present experimental evidence that the same mechanism may operate during invasions of freshwater by *E. affinis*. Invasions are more likely to occur when food is abundant, likely because increased osmoregulatory costs prevent invasions into freshwater if food concentrations are low (Lee et al. 2011, 2012, 2013). While an argument could be made that selection or genetic drift caused the salinity range of *E. affinis* to decline in the SFE, it seems unlikely given the short time span over which it appears to have occurred (7 or fewer years; Ambler et al. 1985; Kimmerer et al. 1998). Given that low food availability narrows the salinity tolerance of *E. affinis* (Figs. 1 and 2, Lee et al. 2013), that ecological niches can be considered subsets of tolerances (Helaouët and Beaugrand 2009) and the extensive evidence for food limitation in the SFE, we hypothesize instead that decreased food availability, presumably caused by the invasion of *C. amurensis*, has narrowed the realized salinity niche of *E. affinis*.

Our results are applicable to efforts to optimize salinity for euryhaline ectotherms. That is, determining the salinity at which food is most efficiently converted into growth rather than osmoregulation. At the lowest level of feeding, we found that growth rates were two times higher at a salinity of 4 than 8, while rates of food consumption were similar. At the higher level of feeding, we found that growth rates were similar between the two salinities, but the rate of food consumption was three times higher at a salinity of 8. Thus, when food was limited, growth was sacrificed at the higher salinity, likely in favor of osmoregulation. When food was abundant, compensatory feeding allowed copepods to grow equally quickly at both salinities. To optimize salinity for an animal, typically growth (or another fitness correlate) is measured across salinities at ad libitum feeding, with the optimal salinity occurring where growth peaks (e.g., Staples and Heales 1991). However, our work suggests that if food is abundant, for euryhaline ectotherms that osmoregulate growth is insensitive to changes in salinity because of compensatory feeding. Compensatory feeding could explain, for example, why Cotton et al. (2003) found no difference in growth between black sea bass fed to satiation between treatments of 20 and 30 % salinity. We suggest that a better method would be to determine the salinity at which food conversion efficiency peaks (i.e., the growth to food consumption ratio; e.g., De Silva and Perera 1976). This would obviate the need to determine the level of food at which compensatory feeding does not dampen the influence of salinity on fitness correlates (e.g., our 1× food treatment for *E. affinis* juveniles) and should make salinity optimization experiments more sensitive.

Whether euryhaline animals exhibit compensatory feeding in response to salinity stress likely depends on where along the dose response curve salinity treatments lie. Studies like that of Herbst et al. (2013) on the damselfly *Enallagma clausum* in which both consumption and growth declined as salinity increased probably were conducted at salinities where the negative influences of salinity on predatory capabilities overcome any increase in metabolic demand (71 % of its salinity LC_{50}, Herbst et al. 2013). At a less extreme salinity, De Silva and Perera (1976) found that young grey mullet raised at a salinity of 30 consumed roughly ten times more food than mullet raised at a salinity of 10 (estimated from the first month of feeding on Fig. 2; De Silva and Perera 1976), results similar in direction to our study. However, the authors also found that mullet raised at a salinity of 30 grew ~14 times slower than at a salinity of 10 (De Silva and Perera 1976). Thus, unlike *E. affinis*, it appears that grey mullet could not completely compensate for increased metabolic demand at the higher salinity with increased food intake, drastically reducing growth. This difference in growth response may have occurred because grey mullet were closer to the limit of their tolerance at a salinity of 30 (60 % of its salinity LC_{50}; Hotos and Vlahos 1998) than *E. affinis* was at a salinity of 8 (38 % of its "high food" salinity LC_{50}; Table 4). At hyposmotic salinity, Normant and Lamprecht (2006) found that consumption by the euryhaline crustacean *Gammarus oceanicus* increased roughly 2 % per unit decrease in salinity. Thus, while the direction of effects on growth and consumption are generally consistent with our study, the effect sizes appear to depend on the relationship between salinity treatments and tolerances of experimental taxa.

Our results, in combination with climatic projections, suggest that temperature maxima will limit *E. affinis*, though independently of food concentration. Juvenile *E. affinis* were quite tolerant of high temperature, with an LL_{50} of 30 °C (Table 4). Temperature acclimation was limited to the time it took for the water in the 600-mL beakers to equilibrate to the temperature in water baths. While longer periods of acclimation may have increased the tolerance of *E. affinis* (Bradley

1978), our findings agree well with the upper temperature tolerance of 30 °C reported by Bradley (1975) in which temperature was increased by 1 °C every 5 min. Given that half the juveniles died at 30 °C, temperature in shallow (<1 m) SFE habitats currently has the potential to limit *E. affinis* populations, as temperatures exceed 30 °C during the summer at Liberty Island (Chris Foe, *personal communication*). In addition, the model of Cloern et al. (2011) projects that 62 days in the next hundred years will exceed 30 °C at a subset of 8 delta locations under the "fast warming" scenario by Cloern et al. (2011; Larry Brown, *personal communication*). Thus, water temperature peaks are likely to increasingly limit populations in shallow SFE habitats, but independently of food concentration.

In contrast to temperature, low food concentrations have the potential to increasingly limit the upper salinity range of *E. affinis* with climate change. For the northern San Francisco Bay, Cloern et al. (2011) project up to a 4.5 increase in salinity over the latter third of this century. The 75th percentile for salinity from 1969 to 2010 in Suisun Bay was 10.7 (median=5.8; Cloern and Jassby 2012). Thus, the 75th salinity percentile already exceeds the upper salinity bound of *E. affinis* in Suisun Bay reported by Kimmerer et al. (1998) of ~10 and is projected to increase substantially (Cloern et al. 2011). We therefore suggest that *E. affinis* is likely to become increasingly rare in Suisun Bay if food concentrations remain low and salinity increases as projected. However, this will remain speculative until it is determined whether the concentration of food in Suisun Bay is generally high enough to allow compensatory feeding of *E. affinis* at hyperosmotic salinities.

Several attributes of our experiments make the salinity LC_{50} values only narrowly applicable. First, we did not acclimate the copepods to salinity in our experiments, though acclimation is known to improve the tolerance of both *E. affinis* and other crustaceans (e.g., Sprague 1963; Roddie et al. 1984). It is therefore likely that their tolerances would have increased if we had acclimated the copepods. The lack of acclimation could explain why *E. affinis* was more sensitive to low salinity in the laboratory (the salinity LC_{50} was 0.3 at high food, Table 4) than its lower range in both the SFE (a lower salinity limit of 0.1, Kimmerer et al. 1998) and higher ranges in other estuaries (e.g., salinity >30 in the Bristol Channel and Severn Estuary, Collins and Williams 1981) would suggest. Second, the tolerance of *E. affinis* to salinity likely increases with life stage (Devreker et al. 2008), so nauplii are potentially less tolerant and adults more tolerant than the values we report. Finally, we used animals from our laboratory cultures to determine the LC_{50}s, which have been isolated from the SFE since 2006. During this time, the animals may have evolved lower tolerances to salinity, especially given the evidence for rapid evolution by *E. affinis* (Lee et al. 2011). Because of these issues, we consider the salinity LC_{50} values to be only narrowly applicable and consider the shifts that the LC_{50} values exhibited with changing algal concentration to be of far greater significance.

In conclusion, we found that temperature and algal concentration influenced mortality of *E. affinis* independently, while salinity and algal concentration interacted to influence mortality, with low food narrowing the salinity tolerance of *E. affinis*. In the SFE, invasive bivalves have likely lowered the algal concentration, and *E. affinis* exhibits a surprisingly narrow realized salinity niche (Kimmerer et al. 2014). A potential cause of the narrow salinity niche of *E. affinis* is a degraded ability to osmoregulate due to food limitation. If this hypothesis is correct, *E. affinis* will have increasing difficulty osmoregulating in the more saline portions of its range in the SFE under climate change. Temperature is also likely to limit *E. affinis* populations under climate change, as a climatic model projects an increasing number of days and locations at which the temperature LL_{50} will be exceeded. Any management actions to increase the concentration of food in the SFE are unlikely to reduce mortality caused by high temperatures, but may broaden the salinity range of an important prey item for delta smelt. However, determining whether *E. affinis* exhibits compensatory feeding at hyper- or hyposmotic salinities at the levels of food found in the SFE remains a key question. Finally, the metabolic niches of other euryhaline ectotherms may be similarly affected because the mechanisms we describe are unlikely to be unique to *E. affinis*.

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