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ORIGINAL PAPER

Temperature and salinity sensitivity of respiration, grazing, and defecation rates in the estuarine eelgrass sea hare, *Phyllaplysia taylori*

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Abstract

Highly dynamic environments such as estuaries are home to organisms accustomed to wide fluctuations in environmental conditions. However, estuarine temperature and salinity conditions are expected to shift with climate change, potentially altering plant and animal physiology and consequently their ecological interactions. *Phyllaplysia taylori*, a sea hare that lives exclusively in nearshore eelgrass beds in the Eastern Pacific Ocean, is a positive ecological interactor with eelgrass by increasing eelgrass productivity through grazing removal of photosynthesis-blocking epiphytes. The central aim of our study is to understand how increasing temperature and salinity are likely to alter that ecological interaction. First, we determined salinity thresholds for survival of *P. taylori* at 20 °C (typical summer temperature) for 2 weeks, and found that significant mortality occurs at salinity below 25 ppt. Then, we determined respiration rate, grazing rate, and defecation rate of *P. taylori* following a crossed 2-week acclimation at typical summer low- and high temperatures (18 and 22 °C) and salinities (27 and 33 ppt). *P. taylori* respiration and grazing rates were elevated under low salinity and high temperature. To determine how *P. taylori* responds to very warm and extreme summer temperatures, we measured respiration rates at higher temperatures (26 °C—very warm summer and 30 °C—heat shock) and feeding rates following exposure to the 30 °C heat shock. Irrespective of acclimation salinity, *P. taylori* acclimated to 18 °C were more sensitive to heat shock, as they had a larger increase in respiration rate at 30 °C, and had reduced feeding rates following the 30 °C exposure, whereas there was no reduction in feeding rate in 22 °C acclimated specimens. This study provides the first data on the salinity and temperature sensitivity and metabolic physiology of *P. taylori* with relevance to their trophic position in the context of eelgrass ecosystems.

Introduction

Seagrass communities worldwide are threatened by anthropogenic effects that impact climatic conditions and local-scale processes. The effects of shifting environmental conditions on seagrass ecosystem health depend on direct effects (e.g., temperature-dependent physiology) and indirect effects (e.g., shifts in trophic interactions). For example, seagrass growth is sensitive to temperature (Hammer et al. 2018; Mochida et al. 2019), a direct effect, but temperature also influences growth of epiphytic algae (a negative interactor in seagrass beds due to inhibition of eelgrass photosynthesis and O_2 availability) and metabolism of invertebrate grazers (an indirect positive interactor in seagrass beds due to grazing of epiphytic algae), adding complexity to predicting the effect of climate change on seagrass ecosystems (Russell et al. 2013; Hughes et al. 2018). We can improve the prediction accuracy of climate change effects by understanding how changing environmental conditions

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influence key physiological processes underlying trophic interactions (Edwards and Richardson 2004; Walther 2010; Zimmerman et al. 2015). Temperature and salinity, two abiotic drivers of physiology that fluctuate predictably in estuaries, are expected to shift in severity and frequency with climate change (Cayan and Peterson 1993; Cloern et al. 2017; Somero et al. 2017; Cloern 2019). The ecological associations between estuarine eelgrass and the invertebrate grazers living on the eelgrass are key to the overall eelgrass ecosystem function, such as productivity, biomass, and carbon sequestration (Duffy et al. 2003; Jaschinski and Sommer 2008; Lewis and Boyer 2014; Hughes et al. 2018).

The sea hare *Phyllaplysia taylori* (Dall, 1900), a heterobranch mollusk found exclusively living on *Zostera marina* eelgrass in the northeastern Pacific estuaries (Beeman 1963), grazes epiphytes and lays egg masses that hatch into crawl-away juveniles on *Z. marina* blades, thus completing its entire life cycle on eelgrass (Beeman 1966, 1970). The blades of *Z. marina* provide substrate for an epiphytic matrix of algae, diatoms, bacteria, fungi, protozoans, and organic and inorganic debris to settle, inhibiting light and O_2 availability for the plant blades (Neckles et al. 1993; Brodersen et al. 2015; Zimmerman 2017). Invertebrate grazing removes the epiphytic algae that compete with eelgrass for light, and increases seagrass photosynthesis and growth (Duffy et al. 2001; Verhoeven et al. 2012). *P. taylori* have been found to significantly reduce epiphyte growth on *Z. marina* blades, resulting in increased eelgrass biomass (Hughes et al. 2010; Lewis and Boyer 2014), though the specific rate at which *P. taylori* consume epiphyte growth on *Z. marina* leaf blades is unknown. The central aims of our study were to determine the metabolic and grazing rates of *P. taylori* across stressful conditions of increasing temperature and salinity to understand how shifts in the abiotic environment are likely to change the positive indirect effect that *P. taylori* has on *Z. marina*, an important aspect in eelgrass management and restoration practices (Tanner 2018).

The vertical distribution of *P. taylori* mirrors that of intertidal populations of *Z. marina*, from the low intertidal zone to a few meters deep (Beeman 1963). Across seasons and tidal cycles, *P. taylori* individuals experience a wide range of temperatures and salinities (Walters et al. 1985; Kimmerer 2004). During low tide, *P. taylori* regularly are found in shallow pools that can get very warm (Fig. 1), but do not dry out (R. Tanner, pers. obs.). Climate change-associated shifts in temperature and precipitation patterns, as well as watershed management practices are expected to intensify the salinity and temperature variability of the San Francisco Estuary across years, seasons, and tidal cycles (Timmermann et al. 1999; Kimmerer 2002; Cloern et al. 2011). In the future, *P. taylori* are likely to experience warmer and more saline waters during the summer in the San Francisco Estuary (Cloern 2019), as well as exposure to extreme heat during low tide during heat waves, which are increasing in frequency and severity due to climate change (Stillman 2019).

In this study, we sought to determine the salinity tolerance of *P. taylori*, determine the sensitivity of *P. taylori* respiration rates and grazing rates to shifts in temperature and salinity, and determine the likely consequences of exposure to extreme heat on *P. taylori* metabolism and grazing rates. Since there have been no prior studies on the metabolic physiology of *P. taylori,* we focused our studies on summer-acclimatized specimens likely to experience the highest temperatures and salinities in nature. Respiration and feeding rates were investigated, as they are known to be sensitive to temperature and salinity in mollusks from estuarine (Bedford 1972; Lockwood et al. 1996; Fernández-Reiriz et al. 2005; Paganini et al. 2010; Re et al. 2013) and

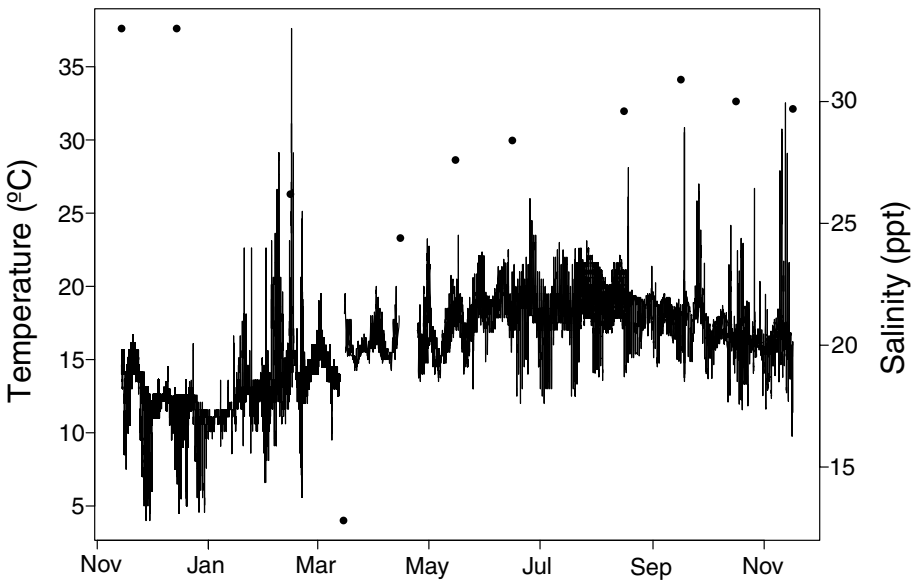

Fig. 1 Temperature and salinity record at Point Molate, California from November 2015 through December 2016. Black circles are salinity and lines are temperatures

marine habitats (Stickle and Sabourin 1979; Baojun et al. 2005), though some estuarine organisms have reduced sensitivity to salinity shifts (Lockwood 1976; Newell 1976). Thermal acclimation is known to influence responses to heat shock in gastropods, with reduction in metabolic rate often observed as a compensatory stress response to minimize energy demand (Sokolova and Pörtner 2003; Padilla-Ramírez et al. 2015). Thus, we sought to determine if heat-stressed *P. taylori* had lower grazing rates, increasing the mismatch between energy availability and demand, e.g., for the cellular stress response (Kültz 2005).

This study exposed summer-acclimatized *P. taylori* to various conditions for 2-week periods to assess salinity tolerance limits and the metabolic and feeding responses to temperature and salinity. To assess salinity tolerance (STE), individuals were exposed to a range of salinity levels, where survival, metabolic, and feeding responses were measured. Metabolic and feeding responses were determined in an orthogonal salinity X temperature (SXT) experiment that crossed typical summer low and high temperatures (18 and 22 °C) with typical summer low and high salinities (27 and 33 ppt). Respiration rates, grazing rates, and defecation rates (to confirm that feeding and ingestion/digestion were coupled) were determined at exposure conditions and at higher temperatures to characterize responses to extreme heat. The 2-week exposure at higher temperature and salinity was expected to reduce the immediate sensitivity to extreme heat.

Materials and methods

Collection site characteristics

Phyllaplysia taylori were collected during June–November 2016 from an eelgrass bed at Point Molate, California in San Francisco Bay (37.9415°N, 122.4101°W). Temperature and salinity sensors were placed within the collection area at tidal heights, where *P. taylori* occurs to measure temperatures and salinities experienced during low tide in small pools on top of the mud flat, where sea hares were routinely found.

Temperatures recorded at the collection site using Maxim Integrated® iButton® thermochron loggers (San Jose, CA, USA) and Onset® UTBI-001 TidbiT® v2 loggers (Bourne, MA, USA), ranged from 11.5 ± 0.01 °C (mean ± SE) during the coldest winter month (December 2015) to 19.0 ± 0.03 °C in the warmest summer month (July 2016), with thermal extremes as high as 38.7 °C during midday low tides (Fig. 1). Salinity at the collection site, which was spot-checked approximately monthly using a hand-held YSI 30 conductivity meter (Yellow Springs, OH, USA), was 27–32 ppt across the months in which specimen collection was performed (Fig. 1).

Specimen collection and common garden

Specimen collections under collecting permit CA DFW SCP-13357 were performed during the monthly lowest (spring) tides at $n = 5$ times at approximately monthly intervals in June, July, August, September and November 2016). At each collection time, $n = 100$ specimens were hand-collected from blades of eelgrass, placed in insulated containers containing a small amount of water from the collection site, and transported within 1 h to the Estuary and Ocean Science Center (EOS Center) in Tiburon, California. At the EOS Center, they were placed into aerated 25L "common garden" aquaria with temperature and salinity conditions set to reflect those measured in the field at the time of collection. The number of common garden aquaria reflected the number of different treatments within the experiment ($n = 2$–4); each common garden aquaria contained specimens exposed to the same treatment. Salinity in common garden aquaria was regulated with twice daily 8 L (32%) water changes, and temperature was maintained by placing the aquaria into a large, temperature-controlled water bath.

Individuals collected were of the average size in the population at time of collection, and only individuals that had likely reached sexual maturity (≥ 3 cm in length) were collected. Wet masses were 1.3 ± 0.6 g, 1.0 ± 0.3 g, and 1.1 ± 0.4 g (mean ± SD) for specimens used in the salinity tolerance experiments of June, July and August 2016, respectively. Wet masses were 1.3 ± 0.4 g for the specimens used in the two salinity X temperature (SXT) experiments of September and November 2016. There were no statistically significant differences in body size across collections (ANOVA). All rate processes were mass-corrected (see below).

Phyllaplysia taylori individuals were fed throughout experimentation using epiphyte-covered screens (Fig. 2a). Framed by plastic drinking straws, fiberglass window screen and plastic ribbons were used replicate an eelgrass-like habitat (Hovel et al. 2016). To accumulate epiphyte growth, screens were incubated for 1 week in outdoor aquaria exposed to sunlight and a constant flow of water from San Francisco Bay. Epiphyte-covered screens were added to the 25 L common garden aquaria with a ratio of approximately one screen to every two *P. taylori* individuals. Epiphyte screens were changed every 3 days to minimize sea hare handling stress and maximize food availability.

After 5 days in common garden aquaria, final exposure conditions were approached gradually: salinity shifted during water changes at a rate of 0.5 ppt every 12 h, and temperature was shifted by no more than 1 °C every 12 h. Once exposure conditions were reached, and individuals were transferred to acrylic cylinders placed into 500 mL glass beakers filled with water of the correct salinity and temperature (Fig. 2b). Acrylic cylinders had mesh bottoms, which

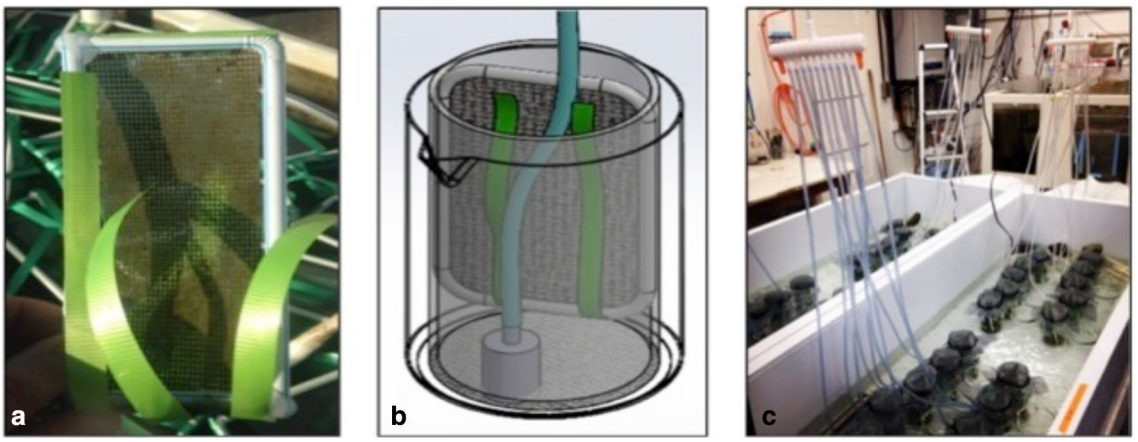

Fig. 2 Experimental apparati used in the study of *Phyllaplysia taylori* physiological response to temperature and salinity. **a** Epiphyte screen, approximately 15 cm by 6 cm, comprised of fiberglass window screen, two plastic drinking straws, and plastic ribbon to mimic seagrass. **b** Mesh-bottomed acrylic cylinder nested inside a 500 mL beaker, with air stone and epiphyte screen inside acrylic cylinder. **c** Two-week exposures were conducted using water baths for temperature control and individual cylinder/beaker combinations with mesh covering to prevent specimens from escaping

allowed for easy water changes, which were performed every 12 h by picking up the cylinder and moving it to an adjacent beaker filled with water at the correct salinity and temperature. Water temperature was maintained in a recirculating water bath within 0.5 °C of treatment temperature (Fig. 2c). Specimens were checked twice daily for mortality; dead specimens were removed, with no replacement specimens. All experiments lasted 2 weeks from the time that specimens were transferred to acrylic cylinders. Two weeks is adequate time for acclimation to steady state in the majority of physiological phenotypes (Somero 2015; Khlebovich 2017).

Salinity tolerance experiments (STE)

Salinity tolerance limits were determined during $n = 3$ salinity tolerance experiments (STE) at monthly intervals in summer 2016. In June 2016, specimens ($n = 24$ per treatment) were held at 20 ± 1 °C and salinities of 24 ± 0.5 ppt and 32 ± 0.5 ppt. In July 2016, specimens ($n = 12$ per treatment) were held at 20 ± 1 °C and salinities of 24 ± 0.5 ppt, 27 ± 0.5 ppt, 30 ± 0.5 ppt, and 33 ± 0.5 ppt. In August 2016, specimens ($n = 24$ per treatment) were held at 20 ± 1 °C and salinities of 24 ± 0.1 ppt, 25 ± 0.1 ppt, 26 ± 0.1 ppt, and 27 ± 0.1 ppt. Survival was estimated for each STE, whereas feeding and respiration rates were determined in the July and August STEs.

Salinity X temperature experiments (SXT)

The combined effects of salinity and temperature on respiration, feeding, and defecation rates in *P. taylori* were investigated in $n = 2$ replicated crossed salinity X temperature experiments (SXT) in September and November 2016. Temperature and salinity levels reflected average summertime high and low values at the collection site. Specimens ($n = 24$ per treatment per experiment) were held at $18 \pm 1°$ or 22 ± 1 °C crossed with salinities of 27 ± 0.5 ppt and 33 ± 0.5 ppt and used to assess respiration rates, grazing rates and defecation rates.

Respirometry

Respiration rates were determined at the 2-week exposure salinity for each specimen. Individuals were held without food during the last 3 days of each 2-week exposure to reduce specific dynamic action in measuring metabolic rate (Jobling and Davies 1980). Individuals were placed in 70 mL respirometry vials equipped with PreSens Oxygen Sensor Spots (PreSens, Germany; as described in Paganini et al. 2014). Vials were calibrated within 1 week prior to each measurement at 0% oxygen saturation using 2% sodium sulfite (Arcos Organics) and 100% oxygen saturation using water of the appropriate salinity and temperature that had been vigorously bubbled with air for approximately 20 min.

Specimens were given a 3-h recovery period following placement in respirometry vials to ameliorate handling stress. During the recovery period, water was changed every 40 min to prevent hypoxia. While oxygen tension was not measured during the recovery period, based on a calculation of the fastest respiration rate we measured (~ 8 µmol O_2 g^{-1} h^{-1}) for a sea hare much larger than we used (5 g) at the warmest, saltiest conditions used (30 °C and 33 ppt), oxygen tension would not have been reduced to less than 93% saturation during the recovery period, which is a level unlikely to induce a behavioral or physiological to hypoxia. Following the recovery period, water in the vials

was replaced with fully aerated water at the measurement temperature and the vials were sealed underwater to prevent inclusion of air bubbles. Sealed respirometry vials were incubated in a water bath at the measurement temperature for 30 min and then dissolved oxygen measurements were made every 20 min for a total of seven measurements per specimen. Vials without a sea hare (blanks) were included to account for any change in water oxygen content due to background processes. Respiration rates were determined at four temperatures (18, 22, 26, and 30 °C), in increasing order, with the two lowest temperatures being the acclimation temperatures and the two highest temperatures reflecting warm and heat shock temperatures. Between respirometry measurement temperatures vials were opened and flushed with aerated water, and temperatures were increased to the next measurement temperature over a 30 min period, at which time water in the vials was replaced with air-saturated water at the new measurement temperature and vials were sealed to start the next set of measurements. Oxygen tensions did not fall below 70% saturation during respirometry trials. Respiration rates were calculated and expressed in units of μmol O_2 g^{-1} h^{-1} as previously described (Miller et al. 2014; Paganini et al. 2014).

The temperature sensitivity of respiration rate (Q_{10}) for each individual was calculated between the highest and lowest measurement temperatures ($T_1 = 18$ °C, $T_2 = 30$ °C), where R is respiration rate at each measurement temperature:

$$Q_{10} = \frac{R_2}{R_1}^{10/(T_2 - T_1)}. \quad (1)$$

Feeding and defecation rates

Feeding rates were determined immediately following respirometry. In the SXT experiments, an additional feeding trial was performed 3 days prior to respirometry, because the post-respirometry feeding rates followed exposure to 30 °C and thus potentially reflected a response to heat stress.

To measure feeding rate, *P. taylori* individuals from each exposure group were blotted dry, weighed, and placed individually in 500 mL beakers. Epiphyte-covered screens were patted dry, weighed, and added to the beakers (one screen per beaker). Specimens were allowed 10 h to graze at temperature and salinity levels from their 2-week exposure treatment. A control screen (no sea hare) was included in each treatment group. At the end of 10 h, individuals and screens were reweighed. The average change in weight of the control screens was subtracted from each individual's change in screen weight to account for any epiphyte removal from screens due to handling or aeration. To measure defecation rate, detritus was filtered from the water in each beaker through a paper towel and dried for 24 h at 60 °C. Dried detritus was then removed and weighed. Defecation rate was calculated as the mass of detritus produced by each individual minus the average mass of detritus from control beakers without a sea hare. Feeding and defecation rates were normalized to wet weight of each specimen. Any measurements below zero were adjusted back to zero, as this would have only happened due to sampling error.

Statistical analyses

All statistical analyses were performed in R (R Team Core 2017). Differences in specimen weight among collection times were examined by one-way ANOVA. A one-way ANOVA was used to analyze survival in the STEs. Differences in respiration, feeding, and defecation rates across salinity in the STEs were analyzed using one-way ANOVA. Respiration rates in the SXTs were analyzed using a linear mixed effects model with sea hare individual as a random factor to account for repeated measures across respiration rate measurement temperatures. Linear regressions were used to identify the main and interactive effects of exposure temperature and salinity in the SXTs on respiration rate temperature sensitivity (Q_{10}) and feeding and defecation rates separately. ANOVA was used to determine the differences in feeding and defecation rates before and after 30 °C exposure in the SXTs. In all cases, pairwise differences were estimated using a Tukey's HSD.

Results

Survival

We observed a large decrease in survival following 2 weeks of exposure at 24 ppt salinity. On average, 25% of *Phyllaplysia taylori* survived after exposure to 24 ppt for 2 weeks, significantly lower than ≥ 75% survival of *P. taylori* exposed to salinities within the 25–33 ppt range (Fig. 3). Though survival at 24 ppt differed among experimental replicates [survival was 25% in June, 0% in July, and 50% in August (Fig. S1)], it was always lower compared to individuals held at ≥ 25 ppt salinity.

Respiration rates

Respiration rate across the STEs ranged from 1.05 to 4.01 μmol O_2 h^{-1} g^{-1} and had a mean of 2.32 ± 0.74 μmol O_2 h^{-1} g^{-1} (mean ± SE, see Fig. S2). While respiration rate did not significantly differ among salinities because of high variability within treatment, the highest respiration rates were in the 26 ppt and 30 ppt acclimation conditions (~ 50% higher than 24 ppt) and the lowest were in the 24 ppt and 25 ppt acclimation conditions.

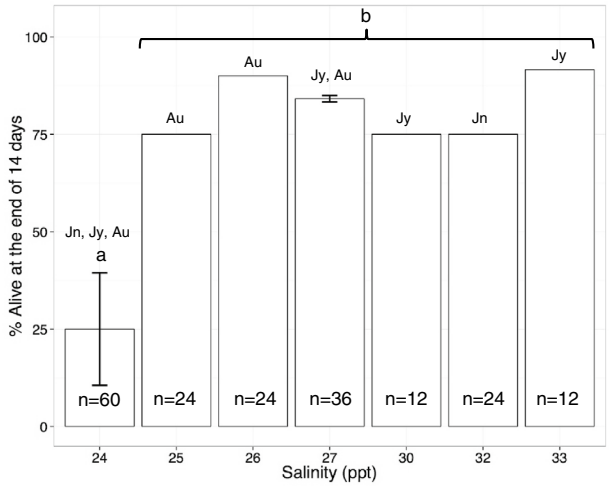

Fig. 3 Percentage of *Phyllaplysia taylori* surviving for 14 days after exposure to one of the seven salinity treatments at 20 °C during the salinity tolerance experiments. The month in 2016 during which the salinity was tested is indicated by letters on top of bars (Ju = June, Jy = July, Au = August), where test salinity was replicated across multiple months, values represent means ± 1 SE

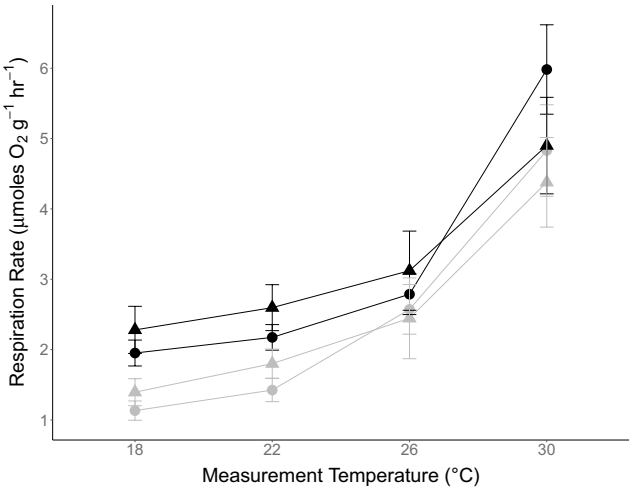

Fig. 4 Average respiration rates of *Phyllaplysia taylori* after 2-week exposure to one of the four treatments, measured at acclimation temperatures (18 °C, 22 °C) and "heat wave" temperatures (26 °C, and 30 °C). Colors are acclimation salinity (black = 27 ppt, grey = 33 ppt) and shapes are acclimation temperatures (circles = 18 °C, triangles = 22 °C). Points are mean ± SE

Respiration rate across the SXTs ranged from 0 to 7.70 µmol O_2 h^{-1} g^{-1} and had a mean of 2.82 ± 1.79 µmol O_2 h^{-1} g^{-1}. Looking at overall trends across acclimation conditions, respiration rate significantly increased with measurement temperature by ~ 110% at 26 °C and by ~ 350% at 30 °C when compared with 18 °C [LME; $t_{(84)} = 4.73$, $p < 0.0001$ and LME; $t_{(84)} = 14.73$, $p < 0.0001$]. Individuals exposed for 2 weeks to 33 ppt salinity had significantly lower respiration rates across measurement temperatures [LME; $t_{(26)} = -2.18$, $p < 0.05$]. Two-week exposure to 22 °C resulted in slightly elevated respiration rates compared to 18 °C exposed specimens at the 18 °C and 22 °C measurement temperatures (Fig. 4), but the differences were not statistically significant (Table 1). There was no interactive effect between 2-week acclimation salinity and temperature on respiration rates. Though temperature sensitivity of respiration rates between 26 °C and 30 °C appears higher in 18 °C exposed specimens (especially at 27 ppt salinity), due to high within-group variance, there was no statistically significant difference in the respiration rates at the 30 °C measurement temperature among acclimation groups.

Q_{10} did not significantly vary among treatment groups in the SXTs and averaged 2.68 ± 0.99 across all treatments. Exposure to high salinity trended towards increasing Q_{10} [ANOVA; $F_{(2,25)} = 4.055$, $p < 0.054$], whereas exposure to high temperature did not have any significant effect. One individual was excluded as an outlier in Q_{10} calculation due to no detected respiration rate at 18 °C. Q_{10} evaluated between the 26 °C and 30 °C measurement temperatures did not show significant differences between treatments, supporting the earlier statement that temperature sensitivity of 18 °C acclimation groups is not statistically different from other acclimation groups [ANOVA; $F_{(3,24)} = 0.614$, $p > 0.05$].

Table 1 Full contrasts for SXTs LME model

	Estimate ± SE	*t* value	*p* value
Temperature	0.329 ± 0.647	0.508	0.616
Salinity	−0.818 ± 0.562	−1.456	0.158
Meas. temp. 22	0.222 ± 0.472	0.471	0.639
Meas. temp. 26	0.836 ± 0.472	1.769	0.081
Meas. temp. 30	4.031 ± 0.472	8.532	< 0.0001***
Temperature: salinity	−0.067 ± 0.901	−0.074	0.941
Temperature: MT 22	0.094 ± 0.695	0.135	0.893
Temperature: MT 26	0.006 ± 0.695	0.009	0.993
Temperature: MT 30	−1.410 ± 0.695	−2.028	0.0461*
Salinity: MT 22	0.069 ± 0.604	0.115	0.909
Salinity: MT 26	0.603 ± 0.604	0.998	0.322
Salinity: MT 30	−0.333 ± 0.604	−0.552	0.583
Temperature: salinity: MT 22	0.020 ± 0.969	0.021	0.983
Temperature: salinity: MT 26	−0.394 ± 0.969	−0.407	0.685
Temperature: salinity: MT 30	0.697 ± 0.969	0.720	0.474

***Significance $p < 0.001$

*Significance $p < 0.05$

Feeding rates

Feeding rates in the STE, or milligrams of epiphytes (mgE) removed from feeding screens per gram *P. taylori* wet weight (pre-feeding trial, gPt) per hour, ranged from 5.40 to 91.33 mg(E) $g(Pt)^{-1}$ h^{-1} and averaged 45.46 ± 4.14 mg(E) $g(Pt)^{-1}$ h^{-1} (mean ± SE). Feeding rate was negatively correlated with 2-week salinity exposure, with *P. taylori* held at 30 ppt and 33 ppt feeding at about half as fast as individuals held at 24 ppt [ANOVA; $F_{(5,28)} = 4.90$, $p < 0.001$; Fig. S3].

In the SXTs, feeding rates measured 3 days prior to respirometry ("pre-heat-shock") ranged from 6.30 to 46.45 mg(E) $g(Pt)^{-1}$ h^{-1} and averaged 21.53 ± 1.44 mg(E) $g(Pt)^{-1}$ h^{-1} (Fig. 5a, filled symbols). Feeding rates after respirometry and exposure to 30 °C ("post-heat-shock") ranged from 0 to 34.80 g(E) $g(Pt)^{-1}$ h^{-1} and averaged 10.63 ± 2.56 g(E) $g(Pt)^{-1}$ h^{-1} (Fig. 5a, open symbols). Pre-heat-shock, higher acclimation salinity significantly reduced feeding rate and higher acclimation temperature increased feeding rate (Table 2). The 18 °C/33 ppt treatment group had 7% significantly lower feeding rates from the rest of the treatment groups pre-heat shock ($p < 0.05$), but no other pairwise differences were found among treatment groups. Post-heat shock, specimens acclimated at 18 °C had significantly decreased feeding rate compared to those acclimated to 22 °C [ANOVA; $F_{(2,19)} = 50.92$, $p < 0.0001$], but no significant effect of salinity was found.

Defecation rates

Feeding and defecation rates were positively correlated, showing a distinct relationship between the two processes (example of STE data in Fig. S4). Defecation rates, or milligrams of feces (mgF) per gram *P. taylori* per hour, in the STEs ranged from 1.27 to 12.84 mg(F) $g(Pt)^{-1}$ h^{-1} and averaged 4.71 ± 0.51 mg(F) $g(Pt)^{-1}$ h^{-1}. All salinity level defecation rates were significantly different from each other, with the lowest defecation rate at 33 ppt (~ 600% lower than at 24 ppt, $p < 0.0001$; see Fig. S3). Individuals at 24 ppt had the highest defecation rate by at least a measure of 300% [ANOVA, $F_{(5,28)} = 4.11$, $p < 0.01$].

In the SXTs, pre-heat-shock defecation rates ranged from 0.61 to 4.13 mg(F) $g(Pt)^{-1}$ h^{-1} and averaged 1.66 ± 0.08 mg(F) $g(Pt)^{-1}$ h^{-1} (Fig. 5b). Defecation rates post-heat shock ranged from 0 to 3.33 mg(F) $g(Pt)^{-1}$ h^{-1} and averaged 0.98 ± 0.20 mg(F) $g(Pt)^{-1}$ h^{-1}. Temperature had a positive effect on increasing defecation rate, while salinity showed the opposite trend; defecation rates decreased overall after heat shock (Table 2). Mirroring the observed differences in feeding rates post-heat shock, there was a strong reduction in defecation rates in 18 °C, but not

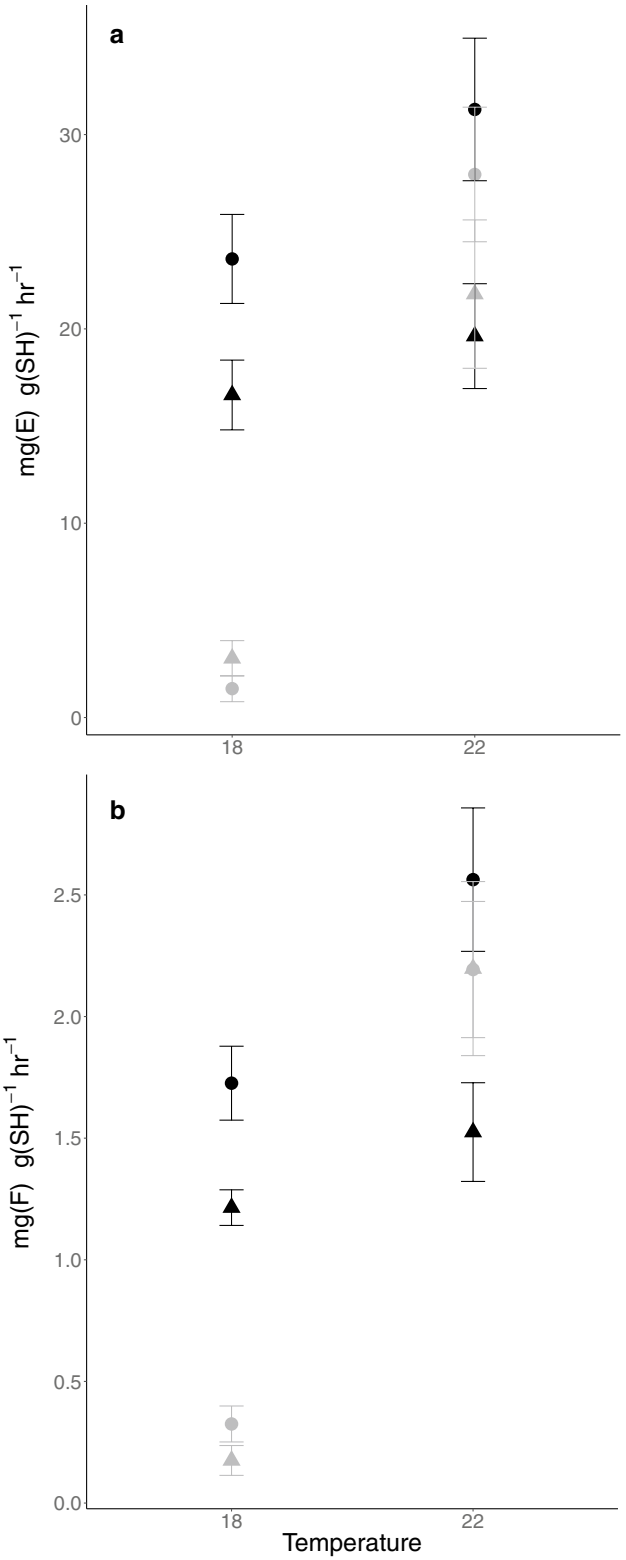

Fig. 5 **a** Feeding rate expressed in average milligrams of epiphytes removed from screen (mgE) per gram *Phyllaplysia taylori* initial weight (gPt) per hour. **b** Defecation rate expressed in average milligrams of fecal production (mgF) per gram *P. taylori* initial weight (gPt) per hour. Symbol shape represents 2-week exposure salinity at 33 ppt (triangles) and 27 ppt (circles), black symbols represent before heat shock, and grey symbols represent after heat shock measurements. Points are mean ± SE

Table 2 Full ANOVA results for SXTs feeding and defecation rates, showing the effects of exposure temperature, exposure salinity, and heat shock

	Estimate ± SE	*t* value	*p* value
Feeding rates			
Temperature	2.770 ± 0.525	5.273	2.32e−06***
Salinity	−1.104 ± 0.343	−3.222	0.00214**
Heat shock	−10.584 ± 2.099	−5.041	5.35e−06***
Defecation rates			
Temperature	0.280 ± 0.038	7.301	5.51e−10***
Salinity	−0.083 ± 0.025	−3.329	0.00145**
Heat shock	−0.674 ± 0.150	−4.483	3.12e−05***

***Significance $p < 0.001$

**Significance $p < 0.01$

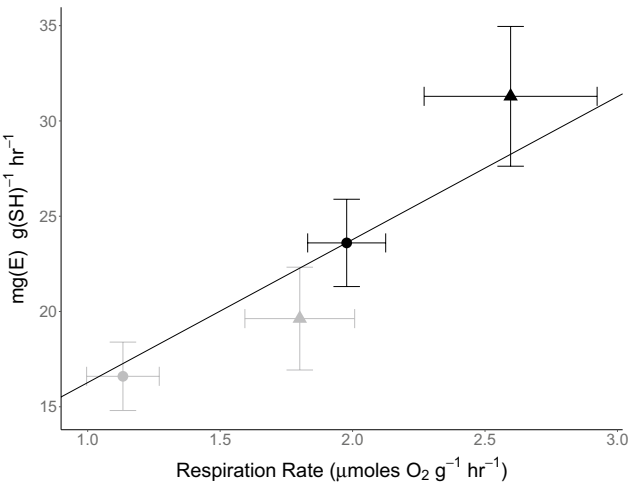

Fig. 6 Milligrams of epiphytes removed from screen per gram *Phyllaplysia taylori* initial weight per hour (pre-heat shock) vs. respiration rate measured at the 2-week salinity and temperature exposure condition. Colors are acclimation salinity (black = 27 ppt, grey = 33 ppt) and shapes are acclimation temperatures (circles = 18 °C, triangles = 22 °C). Points are mean ± SE. Regression coefficients are $y = 8.763 + 7.505x$, adjusted $R^2 = 0.348$, $p < 0.001$

22 °C-acclimated individuals [ANOVA; $F_{(3,64)} = 29.65$, $p < 0.0001$, see Table 2].

Relationship between feeding rate and respiration rate

There was no significant linear relationship between feeding rate and respiration rate observed in the STEs (Fig. S5). However, in the SXTs, there was a significant positive linear relationship between pre-heat-shock feeding rate and respiration rate measured at acclimation salinity and temperature ($y = 8.763 + 7.505x$, adjusted $R^2 = 0.348$, $p < 0.001$, Fig. 6).

Discussion

This study assessed salinity tolerance and the respiration, feeding, and defecation rates of summer–autumn collected *Phyllaplysia taylori* following 2-week exposures at a range of salinity and temperature levels reflective of summer extremes in San Francisco Bay. We hypothesized that increased temperatures and reduced salinities would increase respiration and grazing rates. Exposure to low salinity resulted in low survival as well as increased respiration, feeding, and defecation rates when compared to high salinity conditions. In addition, we examined whether environmental temperature and salinity exposure changed the metabolic response to extreme heat, reflecting temperatures when midday low tides occur during heat waves. We hypothesized that *P. taylori* exposed to warmer and saltier conditions would have a muted response to heat shock due to plasticity in stress response traits (i.e., heat hardening). We found the 2-week exposure to high summertime temperature ameliorated the effect of heat stress on grazing. We present the first characterization of *P. taylori* metabolic physiology. These metrics of *P. taylori* performance in response to a changing environment are important to characterize to facilitate better predictions of the ecological interactions between *P. taylori* and *Zostera marina* eelgrass in a climate change context.

Low salinity is stressful for *P. taylori*

We found that elevated salinity increased survival and decreased feeding rate in *P. taylori* during the summer. The relationship between elevated salinity and *P. taylori* success in the wild is indicated by a substantial increase in *P. taylori* throughout the San Francisco Bay (SFB) during the 2006–2016 decade (K. Boyer, pers. comm.), a period of intense drought that strongly influenced the SFB ecosystem (Chang et al. 2018). Stable populations of *P. taylori* are commonly found in oceanic and hypersaline habitats, such as in Tomales Bay, CA (Hearn and Largier 1997) and in subtidal eelgrass beds of the Channel Islands, CA (L. Sadler, pers. comm.). This suggests that *P. taylori* are euryhaline organisms that prefer higher salinity water, despite their native habitat of estuarine and nearshore coastal eelgrass beds that may experience bouts of low salinity waters.

Respiration rates in this study ranged from ~ 1 to 6 µmol O_2 g^{-1} h^{-1}, well within the range of rates reported in other marine gastropods. For example, *Aplysia californica* respiration rates range from 0.5 to 4 µmol O_2 g^{-1} h^{-1} and *Littorina saxatilis* respiration rates range from 0 to 10 µmol O_2 g^{-1} h^{-1} (Sokolova and Pörtner 2001; Idrisi

et al. 2006). Acclimation to lower salinity resulted in an increase in respiration rate, indicating increased stress. This trend is also observed in other marine mollusks, including the mussel *Mytilus edulis*, which demonstrated increased oxygen consumption with decreased salinity (Stickle and Sabourin 1979), and in the clam *Meretrix meretrix* (Baojun et al. 2005). In addition, prolonged exposure to chronic high or low salinity compared with a fluctuating salinity regime resulted in increased respiration rates in the estuarine clam *Potamocorbula amurensis* (Paganini et al. 2010).

While many marine invertebrates do not show a direct relationship between respiration and feeding rate as a function of temperature (Newell and Branch 1980), we observed a positive linear correlation between these two traits (Fig. 6). The relationship we observed is not a direct temperature relationship, rather a summation of low-to-high stress conditions in our study, influenced strongly by salinity acclimation. Low salinity treatments resulted in higher feeding and metabolic rates, suggesting that increased energy may be needed by *P. taylori* to maintain homeostasis at lower salinity, possibly for osmotic and ionic physiology or for a stress response. Little is known about the osmotic physiology of sea hares in general (Deaton 2008; Martillotti and Tsai 2018), and there have been no prior published studies on the osmotic physiology of *P. taylori*. In species of the best studied sea hare genus, *Aplysia*, which is considered to be an osmoconformer, neuronal and epithelial control are likely involved in restoring osmotic homeostasis in response to hypoosmotic shock (Scemes and Cassola 1995; Souza and Scemes 2000; Keeton et al. 2004; Kadakkuzha et al. 2013). Whether similar mechanisms are present in *P. taylori* is unknown.

Although summertime populations of *P. taylori* appear to be highly sensitive to low salinity, this species is bi-voltine and persisted as a winter generation throughout the winter months during the 2006–2016 decades when salinities in San Francisco Bay dipped well below the 24 ppt threshold (Fig. 1; see the San Francisco Bay National Estuarine Research Reserve data repository, China Camp). The winter generation may have a distinct physiology from the summer generation, but that remains to be investigated. The heavy precipitation of winter–spring 2016 caused extensive freshening of the SFB and populations of *P. taylori* were gone during the subsequent year (R. Tanner, pers. obs.). Whether the sea hares disappearance was due to freshening beyond the salinity tolerance limits of the winter population, or due to other environmental factors remains to be determined.

Heat hardening of *P. taylori*

Organismal response to long-term exposure to high temperature is well known to involve elevated expression of heat shock proteins (hsp) as well as suppressed energetic demand (Low et al. 2018; Harada and Burton 2019). Heat shock proteins can provide protection to extreme heat, and reduced respiration rates in stressful conditions can result in lower energy demands, and, therefore, elevate tolerance to acute stress (Sokolova 2013). *Phyllaplysia taylori* acclimated to warm and salty conditions were functionally insensitive to heat stress, resulting in a diminished response of respiration rate during heat stress (Fig. 4) and normal grazing rates following heat stress (Fig. 5). Acclimation to elevated temperatures has been shown to increase future feeding competence in warmer waters across a range of invertebrates (Beiras et al. 1995; Peck et al. 2008). In *P. taylori*, respiration rates did not indicate a strong metabolic suppression in warm acclimated specimens, as there is no evidence for temperature compensation in respiration rate (Fig. 4). The extent to which warm acclimation caused heat hardening through induction of hsps or other aspects of the cellular stress response is unknown, and would be worth further study (Kültz 2005).

The elevated sensitivity to heat shock seen in respiration rates of 27 ppt salinity acclimated specimens might have been due to osmotic shock influencing immediate response to heat shock. It has been demonstrated that osmotic shock can regulate the generalized cellular stress response, e.g., through MAP kinase signaling pathways that are also involved with mechanisms of thermal adaptation and response to heat stress, which would also influence heat hardening of intertidal organisms (Burg et al. 1996; Kültz 2001; Picard and Schulte 2004; Lockwood et al. 2015; Clark et al. 2018; Li et al. 2018; Harada and Burton 2019).

Putting *P. taylori* physiology in an ecological context

Because eelgrass beds are a critical habitat for estuarine ecosystems, providing nursery habitat, erosion attenuation, and carbon capture, understanding the effects of environmental changes on the trophic interactions of eelgrass and epiphytic grazers such as *P. taylori* is an important aspect of improving the accuracy of climate change impact predictions (Duffy et al. 2003; Boyer and Wyllie-Echeverria 2010; Zimmerman 2017). The results of this study suggest that shifts in environmental temperature and salinity are likely to shift the epiphyte consumption of *P. taylori,* and thus the magnitude of the indirect effect that *P. taylori* has on the productivity and growth of *Zostera marina* eelgrass (Lewis and Boyer 2014). The data presented here will allow for modeling the bulk grazing rates of *P. taylori,* as a function of salinity, temperature and sea hare biomass, data which may be important in choosing eelgrass restoration locations and estimating eelgrass bed growth rates (Tanner 2018). Our findings indicate that increased salinities in San Francisco Bay will not hinder *P. taylori* population persistence, while the impact of heat

waves will depend on thermal acclimatization; if waters are consistently warm, heat stress responses may be buffered. These findings allow stronger predictive inferences for an important ecological interaction in a climate change context.

Conclusion

Understanding the range of salinities and temperatures in which *P. taylori* has maximal ecosystem impacts through grazing is an important consideration for eelgrass management and restoration. The findings of this study suggest that areas of San Francisco Bay with relatively high annual salinities are able to host the most successful *P. taylori* populations, with climate change related salinity increases adding suitable habitat over the next century. While temperature extremes will negatively impact *P. taylori* at a greater frequency in the hottest months, their ability to acclimatize to increased temperature and salinity suggest continued epiphyte grazing of *P. taylori* populations, exerting a positive effect on *Z. marina* eelgrass ecosystems.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests. All animal care and sampling protocols outlined in CA SCP-13357 were followed; no formal national or international guidelines are explicitly stated for the taxon in this study. This work was supported by the San Francisco State University Romberg Tiburon Center Bay Discovery Grant (2015 and 2016 cycles) and the CSU Council on Ocean Affairs, Science and Technology travel Grant (2016 and 2017 cycles) to LEF.

Data accessibility Data have been uploaded to the PANGAEA Biosphere Database (https://doi.org/10.1594/PANGAEA.903705).

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