



World's largest chiton (*Cryptochiton stelleri*) is an inefficient thermoregulator

Lily C. McIntire*, Paul E. Bourdeau

Department of Biological Sciences, Humboldt State University, Arcata, CA 95521, USA and Telonicher Marine Laboratory,
570 Ewing Street, Trinidad, CA 95570, USA

ABSTRACT: Rocky intertidal zones are some of the most thermally stressful environments on earth, where ectotherms deal with tidally driven fluctuations in air and water temperatures that can exceed their maximum thermal tolerance. However, not all intertidal ectotherms face the same exposure risk. In northern regions of the eastern Pacific, summertime low tides occur during midday, exposing ectotherms to potentially stressful temperatures, whereas cooler pre-dawn low tides in southern regions buffer ectotherms from thermal stress. Gumboot chitons *Cryptochiton stelleri* are thermally sensitive intertidal grazers that range from southern California to Alaska, exposing them to a mosaic of thermal stresses. We quantified chiton thermal performance limits in the laboratory by testing the effects of elevated air and water temperatures on grazing. We also compared the thermoregulation efficiency of chitons from thermally benign northern California sites with those from thermally stressful San Juan Island, Washington sites, using 3 components: (1) biomimetic thermal models deployed intertidally, (2) chiton body temperatures in the field, and (3) chiton thermal preference in a laboratory-based thermal gradient. We found that chiton grazing performance was greatly reduced at 18°C in water, and they reached their grazing performance limit after exposure to 20°C in air, confirming previous work documenting thermal limits on chiton respiration. Chitons preferred body temperatures within 3°C of their thermal performance limits, but they rarely achieved body temperatures that would maximize grazing in the field. This suggests that chitons are not thermoregulating efficiently with respect to maximizing grazing performance, but instead are minimizing exposure to temperatures that would be detrimental to their performance.

KEY WORDS: Thermal biology · Risk minimization · Species performance · Intertidal grazer · Temperature stress · Biomimetic models

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1. INTRODUCTION

As the earth warms due to climate change, many species will lose thermally favorable habitat (Easterling et al. 2000, Alley et al. 2007, Pacifici et al. 2015). Because ectotherm body temperatures are dictated by the environment and strongly influence their performance (Huey 1974, Hochachka & Somero 2002), they are at particularly high risk for habitat loss because they must find specific thermal conditions to perform and survive (Kearney et al. 2009). A wide variety of ectotherms use behavioral responses to modify their body temperatures to achieve specific

thermal conditions (Johnson & Kelsch 1998, Martin & Huey 2008), and it is assumed that these responses have evolved to balance the benefits of maximizing overall performance with reducing exposure to thermal extremes (Angilletta et al. 2002, Martin & Huey 2008, Asbury & Angilletta 2010).

As temperatures increase, ectotherms experience an increase in performance (Morelissen & Harley 2007, Miller 2013), which is the rate at which an organism can perform an ecologically relevant activity (Sinclair et al. 2016) and is a function of energy intake and metabolic demand (Nisbet et al. 2012). As temperatures continue to increase, ectotherms achieve

*Corresponding author: Lily.McIntire@humboldt.edu

peak performance. However, once temperatures reach an organism's critical thermal limit at more extreme high temperatures, their performance no longer increases, and the animal becomes stressed, resulting in a sharp decrease in organismal performance (i.e. their thermal performance curves are negatively skewed). The decrease in performance above a certain temperature is called a thermal performance limit (Huey & Stevenson 1979, Sinclair et al. 2016). Ectotherm habitat selection based on thermal quality (i.e. behavioral thermoregulation sensu Weiss & Laties 1961) is thought to reflect tradeoffs between selecting thermal habitat favorable for organismal performance (i.e. preferred temperatures) and minimizing the risk of exceeding thermal limits (Kearney et al. 2009). The balance between preferred temperatures and optimizing performance/minimizing risk has been studied extensively in terrestrial and aquatic ectotherms (reviewed by Martin & Huey 2008 and Crickenberger et al. 2020), and these tradeoffs are not always efficient for the organism. Theory predicts that a combination of increased variation in organismal body temperatures and negatively skewed thermal performance curves should select for an ectotherm's preferred body temperature to be less than its optimal performance temperature and that the greater the variation in body temperatures, the larger the difference between preferred and optimal temperatures (Angilletta et al. 2002, Martin & Huey 2008). The offset between preferred and optimal temperatures will also depend on the organism's mobility and the spatial and temporal variation in habitat temperatures (Woods et al. 2015). For example, in less mobile ectotherms, fitness may be maximized by reducing the risk of being confined to thermally unfavorable habitats rather than maximizing performance.

Intertidal ectotherms experience large spatial and temporal variation in temperature due to the timing of and exposure to tides (Tomanek & Somero 1999, Helmuth 2002, Helmuth et al. 2006). Whereas increased water temperatures during low tides (e.g. in tidepools) can have a detrimental effect on organismal performance (Miller 2013), emersion during low tides can cause organisms to experience temperature increases of up to 20°C (Helmuth et al. 2011). These temperatures can exceed the thermal limits of ectotherms (Harley 2008) and are often coupled with desiccation stress (Helmuth 1998). The combination of aerial exposure and extreme temperatures dehydrates cells within the organism, resulting in decreased respiration rates and performance (Gosling 1992). Intertidal ectotherms may therefore have different thermal tolerances in air versus water (e.g.

Fusi et al. 2016), which could alter the ability of ectotherms to locate thermally favorable microhabitats. Further, not all intertidal ectotherms are exposed to the same risk of overheating, as the timing of low tides varies geographically, which can either buffer or exacerbate the effects of warming (Helmuth 2002, Helmuth et al. 2006, Mislan et al. 2009). For example, intertidal ectotherms in more northern regions of the Pacific coast of North America (e.g. Washington) are exposed to extreme temperatures more frequently, as low tides occur during the midday in the summertime, when air temperatures are at their warmest. Midday low tides in Washington are more thermally stressful than the pre-dawn low tides that occur in more southern regions (e.g. northern California; Mislan et al. 2009). Consequently, intertidal ectotherms on the northern coast of continental USA may therefore have more difficulty thermoregulating efficiently, and be forced to select cooler temperatures to avoid overheating than those in favorable thermal habitats in more southern regions (Kearney et al. 2009). Very few studies have measured behavioral thermoregulation in intertidal ectotherms or compared preferred temperatures to optimal performance temperatures or thermal limits (Crickenberger et al. 2020), instead focusing on strategies for avoiding thermal extremes by measuring microhabitat associations (Pincebourde et al. 2009, Miller & Denny 2011, Monaco et al. 2016, Ng & Williams 2006). If risk avoidance, due to the high spatiotemporal thermal variability of the habitat and relatively low mobility of the organisms, is the objective of intertidal ectotherms, then behavioral thermoregulation should be relatively inefficient compared to that of fully terrestrial or fully aquatic ectotherms (Crickenberger et al. 2020).

Gumboot chitons *Cryptochiton stelleri*, the world's largest polyplacophoran and the largest intertidal invertebrate herbivore in the northeastern Pacific, provide an excellent system for studying the effects of elevated temperatures on the thermal performance and thermoregulation efficiency (E ; Hertz et al. 1993) of intertidal ectotherms (Petersen & Johansen 1973). They occur from the low intertidal to the shallow subtidal zone (down to 10 m depth), but their abundance in the intertidal zone is variable, and the movement of intertidal gumboot chitons is restricted to a homing range of only a few to ~20 m (Yates 1989, Lord 2011). For example, year-round surveys by Lord (2011) indicated that intertidal gumboot chitons do not show seasonal variation in vertical distribution and do not move up or down in the intertidal with age, suggesting that intertidal individuals maintain their vertical distribution even if temperatures in the intertidal

zone become unfavorable. Gumboot chitons are thought to be thermally sensitive in comparison to many other intertidal species, which often have thermal limits greater than 35°C (Tomanek & Somero 1999, Somero 2002, Pincebourde et al. 2008, Miller et al. 2009). Previous work has shown that gumboot chitons become temporarily immobilized and reduce respiration at 18°C in water and 20°C in air (Petersen & Johansen 1973). Their range extends from southern California to northern Alaska (Yates 1989), which exposes them to a mosaic of temperature stresses caused by tidal timing (Mislán et al. 2009). Further, localized declines in density have been reported in some parts of their range (Jurgens et al. 2015), and in others there is concern about the potential effects of climatic warming (Gotthard & Jansen 2006). Quantifying the *E* of gumboot chitons could therefore provide information useful for predicting how their distribution—both vertically in the intertidal zone and latitudinally across their geographic range—could change under future climatic regimes. At present, however, how elevated temperatures affect their grazing performance and how efficiently they select favorable thermal habitats are unknown.

Here, we explored the air and water temperatures at which gumboot chitons reach their thermal performance limits for grazing. We expected chiton thermal limits for grazing performance to be near their

previously documented respiration thermal limits at 18°C in water and 20°C in air (Petersen & Johansen 1973). Additionally, we compared the *E* of gumboot chitons in the field in a thermally stressful part of their range, San Juan Island, WA (SJI), and a thermally benign part of their range in northern California (CA) with the use of biomimetic models, measurements of live chiton temperatures, and chiton temperature preference in the lab. Quantifying how well gumboot chitons select for thermally favorable habitat in conjunction with their grazing performance limits will be important for projecting the effects of elevated temperatures caused by climate change on these ectothermic species and their interactions with their macroalgal resources.

2. MATERIALS AND METHODS

2.1. Gumboot chiton grazing performance limits

To quantify chiton grazing performance under a range of both air and water temperatures, we did 2 separate laboratory experiments (1 for air and 1 for water) in summers 2018 and 2019. We collected adult chitons at Cape Mendocino and Baker Beach, 2 moderately wave-exposed boulder fields on the northern coast of California (Fig. 1a). Chitons were individu-

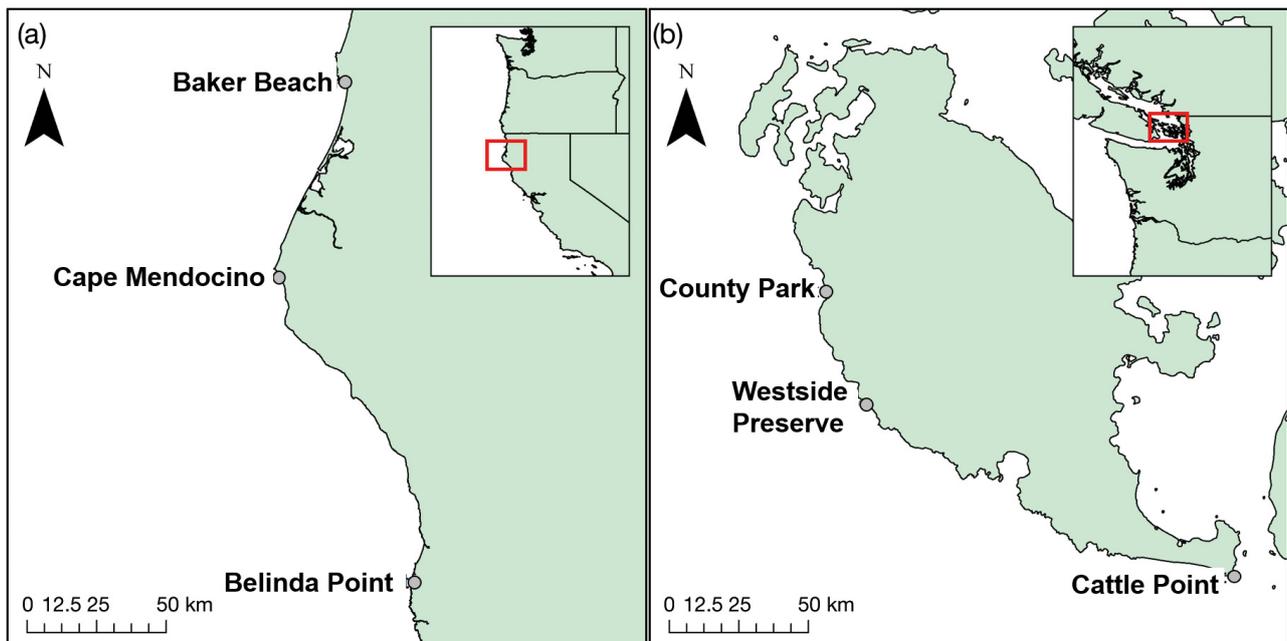


Fig. 1. Survey and collection sites (a) in northern California: Baker Beach (41° 2' 57.37" N, 124° 7' 40.54" W), Cape Mendocino (40° 23' 55.50" N, 124° 22' 53.72" W), and Belinda Point (39° 23' 56.5" N, 123° 49' 10.1" W) and (b) on San Juan Island, WA: County Park (48° 34' 39.49" N, 123° 10' 25.94" W), Westside Preserve (48° 31' 38.90" N, 123° 9' 20.40" W), and Cattle Point (48° 27' 15.07" N, 122° 58' 30.39" W)

ally marked with bee tags glued to a textile tag inserted into the outer mantle of each chiton (Yates 1989). We weighed chitons to the nearest 0.1 g (Mettler Toledo SB16000) after air drying them for 15 min (709.0 ± 220.1 g [mean \pm SD], $n = 111$). Lengths and widths of relaxed chitons were measured with tailor's tape across their dorsal side to the nearest 0.1 cm (length: 24.5 ± 2.7 cm, width: 16.7 ± 1.9 cm, $n = 111$). Prior to experiments, chitons were housed in flow-through seawater tables at the Telonicher Marine Lab (TML) and fed macroalgae (*Mazzaella splendens*) ad libitum before being starved for 4 d to standardize hunger.

During experiments, we housed individual chitons in 40×55 cm Sterilite™ tubs with Vexar™ mesh lids (0.5 cm) in 9.0 cm of flowing seawater. Enclosures were inside 4.0 mm black polyethylene sheeting to insulate experimental enclosures and block out unpredictable lighting in the laboratory. We used light-emitting diode lights timed (12 h light:12 h dark) to match the natural northern California summer photoperiod inside enclosures. Sea tables were lined with 1.25 cm thick interlocking foam mats for insulation. Chitons were acclimated to enclosures 24 h before being exposed to temperature treatments, after which they were allowed to graze for 3 d.

M. splendens was chosen for food as it is the chiton's preferred algae (Yates 1989), and experiences a similar thermal regime to gumboot chitons (Yates 1989). Before each experiment, we identified the reproductive state of *M. splendens* visually or using resorcinol (Shaughnessy & De Wreede 1991). Macroalgae were collected from Cape Mendocino (Fig. 1) 48 h prior to trials, spin dried in a manual centrifuge, and pre-weighed to 25 g of gametophyte tissue (a randomly distributed mixture of reproductive states). Grazer-free algae treatments were used to determine algal weight change due to growth or deterioration. We replicated each temperature treatment in each experiment in 4 temporal blocks, for a total of 11 replicates per treatment.

To quantify the effects of increasing water temperature on chiton grazing, we subjected individual chitons to 5 water temperatures, 11, 13 (ambient), 15, 18, and 20°C (Petersen & Johansen 1973), selected based on local ambient temperatures and previously documented chiton thermal respiration limits (Petersen & Johansen 1973). We generated higher temperatures with a combination of 500 and 300 W aquarium heaters in header tanks and then pumped heated water into treatment enclosures (flow rate: 4–6 ml s⁻¹). Air stones were added to both header and treatment tanks to keep water temperatures from stratifying.

Low-temperature treatments were cooled with a chiller (JBJ Arctica Aquarium Chiller). We monitored temperatures with a thermocouple probe (Omega HH508) in both header and treatment tanks hourly for 12 h d⁻¹ during the experiment. Water temperatures were significantly different across treatments (ANOVA, $F_{4,10} = 172.6$, $p < 0.01$) and also statistically different from one another (Tukey's HSD, all $p < 0.01$).

To prevent grazing before chitons were exposed to treatments, algae were not added until chitons had experienced 6 h of treatment conditions, to be consistent with the low tide treatment. We removed macroalgae daily, spin dried it, and then reweighed it. If chitons had consumed more than 50% of the algae, another 10 g was added so all chitons had algae to consume during the experiment. Feces were removed to prevent re-ingestion. After weighing, algae were re-added so they were exposed to the same temperature as the chitons.

We quantified the effects of elevated air temperatures on chiton grazing by exposing chitons to 5 levels of low tide event air temperatures: 14, 16 (ambient), 18, 20, and 22°C. Temperatures were achieved by draining enclosures and heating the air around chitons with a ceramic heat lamp (Fluiker's 5.5 inch Repta-Clamp Lamp with 75 W bulb) controlled by thermostats (Ink Bird ITC-308). The 14°C treatment was achieved by placing ice packs in the sea table near experimental enclosures. Treatment had a significant effect on air temperature (ANOVA, $F_{4,10} = 280.1$, $p < 0.01$), with each temperature statistically different from one another (Tukey's HSD, all $p < 0.01$). Since chitons experience morning tides in northern California and some chitons show circatidal rhythms (e.g. Ng & Williams 2006), we acclimated chitons by starting low tides daily at 10:00 h by manually draining enclosures. Algae were not added to enclosures until after the first low tide to avoid pre-treatment grazing by chitons. Algae were weighed daily and subjected to the low tide along with the chitons. Temperatures were monitored hourly, and after 6 h, the enclosures were refilled with ambient seawater (~13°C), simulating a realistic low tide for chitons (Pincebourde et al. 2008). Chitons were exposed daily to low tides for 3 consecutive days, but were in flow-through seawater (~12.6°C) between low tides.

2.2. Effects of air and water temperature on macroalgal palatability

Since algal palatability can be altered by temperature (O'Connor 2009), and to ensure that grazing dif-

ferences in experimental treatments were due to temperature effects on chitons and not algae, we tested the effects of increased temperatures on algal palatability in 2 experiments in which we heat shocked *M. splendens* in high water and air temperatures. We categorized 18 and 20°C as the high water and air temperatures, respectively (Petersen & Johansen 1973). *M. splendens* reproductive state was quantified using the resorcinol method (Shaughnessy & De Wreede 1991), and only gametophytes (which were a mixture of reproductive states) were used to be consistent with the chiton grazing experiment. Additionally, a grazer-free treatment was included in both water and air experiments to account for growth and deterioration of algae.

To test the effect of water temperature on algal palatability, *M. splendens* was divided into control (13°C) and heat-shocked (18°C) groups. Heated water was delivered to the tanks from header tanks that were constructed in the same manner as for the chiton grazing experiments (see Section 2.1). Algae were in the water treatments for 2 d to mirror thermal stress caused by the chiton grazing experiment. For air temperature treatments, we divided algae into 2 groups: control at ambient temperature (16°C) and heat shocked (20°C). We exposed both control and heat-shocked algae to low tides achieved in the same way as during the grazing experiment (see Section 2.1). Algae were exposed to a 6 h low tide once a day for 2 d, so as to match the thermal stress experienced by the algae during the chiton grazing experiment. At the conclusion of 2 d in either air or water treatments, chitons were placed in the middle of the tanks with flow-through ambient (13°C) seawater, and 5 g of both control and heat-shocked algae was placed randomly but equidistant from the chitons in the corners of the tanks. At the conclusion of 2 d, algae were removed from the grazing tanks, spin dried, and reweighed.

2.3. Calculating thermoregulation efficiency (E) and habitat selection of gumboot chitons

To test how efficiently chitons thermoregulate in the field, we quantified the habitat selection of intertidal chitons on SJI, a thermally stressful part of their range, and in CA, a thermally benign region. We analyzed the E of chitons using 3 components of their thermal biology from both regions: (1) the range of possible temperatures for a non-regulating chiton across their vertical distribution in the intertidal zone (T_e), (2) body temperature experienced by live inter-

tidal chitons in the field (T_b), and (3) the set point or preferred temperature chosen by chitons in a laboratory thermal gradient (T_{set} ; Hertz et al. 1993).

We measured the temperatures available to chitons (T_e) at 3 sites on the west side of SJI and 3 sites in CA with biomimetic models (roboboos; Fig. 1), built from synthetic microfiber sponges (20 × 12 cm) with a leather covering (Fig. 2a). Temperatures were

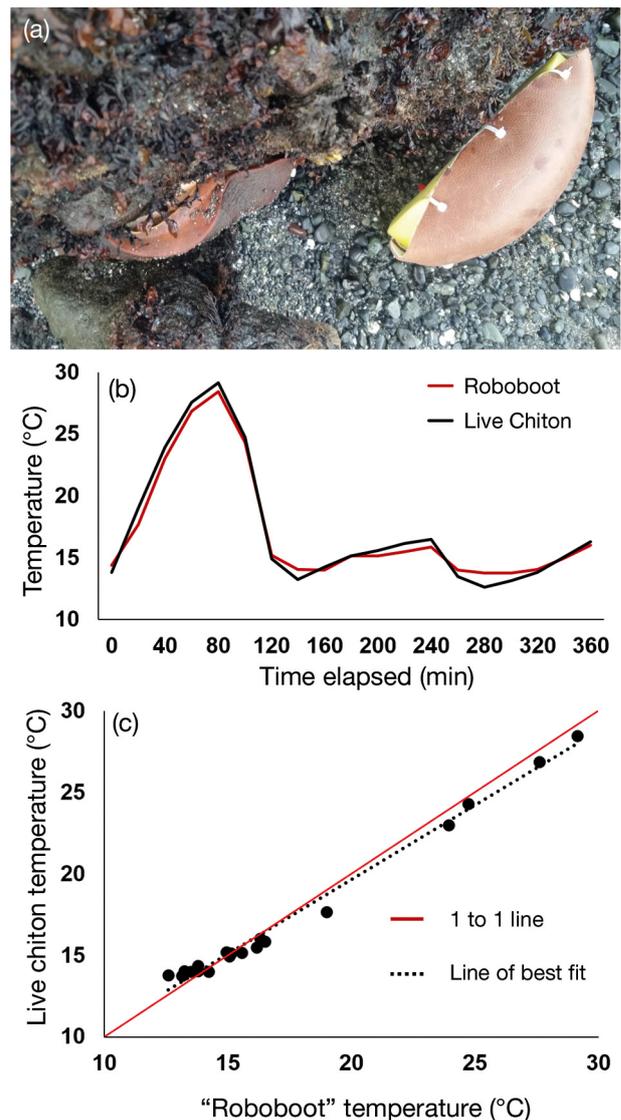


Fig. 2. (a) Live gumboot chiton *Cryptochiton stelleri* (left) next to a roboboot, pre-installation, in the field. (b) Internal temperatures of roboboos (red line, $n = 5$) and live chitons (black line, $n = 5$) taken every 20 min over the course of 6 h. Chitons and roboboos were left in air until they reached 30°C and then placed in flow-through sea tables to quantify how both warmed and cooled. (c) Relationship between live chiton temperatures ($n = 5$) and roboboos ($n = 5$) taken every 20 min for 6 h ($R^2 = 0.99$, $p < 0.01$). Dotted line is the line of best fit (ordinary least squares regression, $y = 0.91x + 1.38$); red line is the 1 to 1 isocline

logged with waterproofed iButton temperature loggers (Thermochron DS1921G) embedded in the sponge. We verified how closely robo boots mimicked live chitons by comparing robo boot temperatures ($n = 5$) to live chiton temperatures ($n = 5$) every 20 min for 6 h. Live chiton temperatures were taken by inserting a thermocouple probe into their pallial groove. Robo boot temperatures matched the body temperatures of live chitons in air and water (Fig. 2b) within 1°C (Fig. 2c, $R^2 = 0.99$, $p < 0.01$). Prior to installation, we calculated the mean tidal heights (relative to mean lower low water) of live chitons at all sites using published NOAA tide charts and a laser level (Spectra LL100N). We attached robo boots to intertidal rocks using two 2 cm wide strips of Vexar™ mesh that were crossed over the robo boot and fastened to the rock with stainless steel lag bolts and masonry anchors. Temperature loggers were programmed to record temperatures every 20 min to account for chiton thermal inertia—the amount of time it takes for their bodies to come to equilibrium with their environment (Pincebourde et al. 2009; Fig. 2b). Robo boots were installed at the average tidal height of live chitons (SJI: -0.17 ± 0.21 m [mean \pm 2 SD], CA: -0.20 ± 0.15 m [mean \pm 2 SD]) on horizontal and vertical surfaces both in and out of shade to quantify temperatures in all habitats that chitons had access to in the field. We installed 4 to 12 robo boots at each of our 3 sites on the west side of SJI ($n = 17$; Fig. 1b) during the month of July 2019 and 6 to 10 robo boots at each of our 3 sites in northern California from May to August 2019 ($n = 22$; Fig. 1a). Since ectotherms equilibrate with water temperatures when immersed (Gilman et al. 2006), we installed 1 waterproofed iButton in tidepools that never drained during low tide at each site, to quantify temperatures of submerged chitons. To ensure we were quantifying low tide air temperatures, we used NOAA tide data and robo boot tidal heights to extract temperatures that occurred during emersion.

We quantified habitat selection and air, water, and substrate temperatures; wind speed; and humidity in each habitat while taking chiton body temperature (T_b) in conjunction with habitat type and tidal height at low tide at each site in CA (Fig. 1a) and on SJI (Fig. 1b). Since lunar cycle can impact habitat selection (Hayford et al. 2015), surveys were done during all low tides lower than 0.0 m (26 total days in CA and 17 on SJI), which encompassed multiple lunar cycles. We searched for chitons in a 400 to 600 m^2 area in CA and a 200 to 940 m^2 area on SJI. We categorized habitat type as sheltered (crevices and under boulders), tidepool, or exposed (on cobbles, on sand,

or on the sides and tops of boulders). In CA, where chiton abundances are high ($n > 40$ per site; L. C. McIntire pers. obs.), we took T_b 's between predicted low tide and 2 h after low tide. To remove bias toward any particular tidal height, chitons were marked with numbered flags, and a random number generator was used to determine the order in which they were analyzed. On SJI, chiton abundance was low ($n < 6$ per site; L. C. McIntire pers. obs.), allowing us to measure T_b 's at all 3 sites during each daily low tide. Sites were done in random order, starting 1 h before predicted low tide and ending 2 h after low tide, to avoid bias for any one site.

To quantify the preferred body temperatures of chitons in the lab (T_{set}), we constructed a laboratory-based thermal gradient that was heated to 25°C at one end and chilled to 11°C at the other. The gradient was a plexiglass tank built on an aluminum block ($1.29 \times 0.30 \times 0.04$ m [length, width, depth, respectively]). Insulation foam was placed along the edges of the block to prevent heat loss through the bottom and sides of the gradient. This created a linear temperature gradient that varied in temperature by $\sim 3^\circ\text{C}$ every 22 cm. We covered the aluminum with an adhesive PVC sheet and 0.5 cm of sea water so chitons could move along the gradient unimpeded. Chitons were deterred from leaving the block by a coat of paraffin/soy wax on the acrylic siding and a polystyrene crate (1.5 cm mesh size to avoid shading) attached to the sides of the gradient above the block.

We placed chitons (length: 25.9 ± 0.5 cm, width: 16.2 ± 0.42 cm, weight: 644.19 ± 38.9 g) individually in the center of the gradient with orientation randomly assigned to avoid directional bias. We randomly switched the warm and cool ends of the gradient between chitons to avoid gradient orientation effects on chiton movement. Since chitons explored their thermal habitat, we took photographs of chitons every 20 min, and once it was determined that they had not moved for 20 min—the amount of time it takes their body to equilibrate with the block (Fig. 2)—we took their T_b . Chitons were given up to 1.5 h to select their T_{set} . We recorded T_b rather than block temperatures, since chitons could span across several centimeters and thus a range of block temperatures. We quantified the T_{set} of 22 chitons (11 from Cape Mendocino and 11 from Baker Beach) from CA and 11 from SJI. T_{set} was not measured for chitons from our CA site Belinda Point because there were only 7 chitons found in the entire sampling area. All T_{set} values were quantified within 6 d (the average time between summer low tides low enough

to expose chitons in the field) of collection from the field to minimize acclimation time.

Using the parameters measured above, we calculated how frequently chitons experience temperatures outside of their preferred temperature range (d_b) and the thermal quality of their environment (d_e), which were used to calculate the efficiency of temperature regulation (E ; Hertz et al. 1993) for chitons at each site in CA and on SJI, where

$$E = 1 - \frac{\bar{d}_b}{d_e}$$

and values for E closer to zero indicate inefficient thermoregulation, while those close to 1 indicate efficient thermoregulation (Hertz et al. 1993).

2.4. Statistical analyses

We adjusted grazing rates for individual chiton biomass (g) and time submerged (h), since emersed chitons do not graze, for both water and air experiments. Data for the water experiment were both non-normal and heteroscedastic, so we used Welch's ANOVA on rank-transformed data followed by a Games-Howell test (Shingala & Rajyaguru 2015). Since data in the air experiment were non-normal, even after transformation, but homoscedastic, they were analyzed with a Kruskal Wallis test followed by multiple Mann-Whitney U -tests for pairwise comparisons (Tomarken & Serlin 1986). Statistical significance of all tests was <0.01 , so we did not use Bonferroni corrections, as it was unlikely that results were due to random chance (Moran 2003). We tested the effects of trial on chiton grazing rates with Welch's ANOVA for water and a Kruskal Wallis test for air experiments and found no significant effects of trial for either the water (Welch's ANOVA, $t = 1.01$, $df = 3$, $p = 0.41$) or the air (Kruskal Wallis, $\chi^2 = 5.10$, $df = 3$, $p = 0.16$) experiment, so data were analyzed without including trial as a factor.

Palatability grazing rates were non-normal, even after transformation, but homoscedastic. Since it was a paired design and the data were non-normal, we used a Wilcoxon signed rank test to compare the grazing rates on heat-shocked and control algae (Blair & Higgins 1985). For graphical visualization, we subtracted the mass of heat-shocked algae consumed from the mass of ambient algae consumed and added 95% CIs to determine if there was a difference in consumption between heat shocked and ambient algae within each experiment.

We compared the proportion of chitons in each habitat between SJI and CA with a χ^2 test of inde-

pendence (Sokal & Rohlf 1995), and compared the relationships between body temperatures and abiotic variables (humidity; wind; and substrate, air, and water temperatures) in microhabitats with simple linear regressions (Sokal & Rohlf 1995). We also used simple linear regression to assess the relationship between air and roboboot temperatures (Sokal & Rohlf 1995). Even though T_b values were non-normal, they were homoscedastic, so we compared them between habitat types in the 2 different regions using an ANOVA, followed by Tukey's HSD test since they are robust to deviations from normality (Sokal & Rohlf 1995). We tested for differences in tidal height between the 2 regions with a mixed-model ANOVA with site as a random factor. To test for differences between the T_{set} values of chitons between the 2 regions, we used a t -test (Sokal & Rohlf 1995). For T_{set} , chitons selected a wide range of temperatures, so we used the middle 50% for analysis (Hertz et al. 1993). T_e , d_b , and d_e values were not normally distributed and the residuals were heteroscedastic, so we compared these metrics between the 2 regions using a generalized linear model (GLM) with site included as a random factor nested within region (Zuur et al. 2009). Our sample sizes were somewhat low, and power analysis revealed low power (52%) to detect regional differences in d_e , d_b , and E values. Adjusting the alpha from 0.05 to 0.10 allowed increased power (71%), so the significance of p -values was assessed at $\alpha=0.1$ for these tests (Fisher 1950). To assess whether E was different from zero (absence of thermoregulation) for both SJI and CA chitons, we used bootstrapping (10 000 iterations, with replacement) to compute distributions of \bar{d}_e and \bar{d}_b for each site and used those values to calculate mean and 90% CIs for E for chitons from each region (Hertz et al. 1993, Efron & Tibshirani 1994). If E is significantly different from zero at $\alpha = 0.10$, then 90% CIs will not contain zero. All statistics were done in R (v 3.4.4).

3. RESULTS

The highest water temperatures negatively affected chiton grazing (Welch's ANOVA, $t = 8.3$, $df = 4$, $p < 0.001$; Fig. 3a, Table A1 in the Appendix). Grazing rates were similar at 11, 13, and 15°C, with a moderate increase between 13 and 15°C. Chitons grazed significantly less at 20°C compared to all treatments below 15°C (Fig. 3a, Table A1). Aerial exposure during experimental low tides caused chitons to graze less overall compared to those in water,

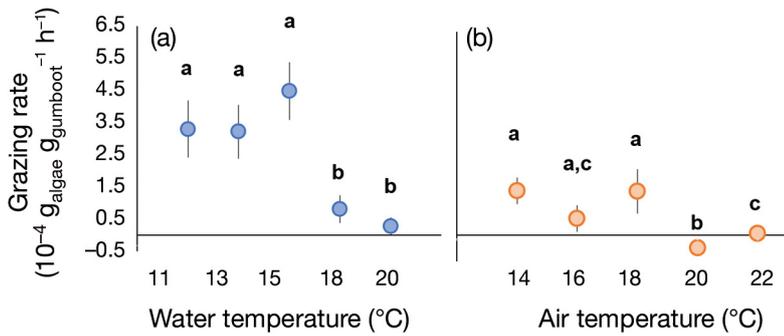


Fig. 3. Effects of elevated (a) water and (b) air temperatures on gumbboot chiton *Cryptochiton stelleri* grazing rates adjusted for chiton biomass and hours spent grazing. Negative values could indicate algal growth or water retention in treatments that were negligibly grazed. Letters represent treatments that are statistically similar. Error bars are ± 1 SE

even at low air temperatures (Kruskal-Wallis $\chi^2 = 28.69$, $df = 4$, $p < 0.001$). Chitons grazed at a similar rate at 14, 16, and 18°C but dropped off steeply at 20 and 22°C (Table A2). The lowest grazing rates were at 20°C, which was 166% lower than all other treatments (Table A2). Heat shocking algae had no effect on chiton grazing rates in either air (Wilcoxon signed rank, $V = 10$, $df = 4$, $p = 0.08$) or water ($V = 27$, $df = 4$, $p = 1.0$), indicating that the among-treatment variation in chiton grazing in the water and air experiments was due to the direct effect of temperature on grazers and not indirectly via temperature effects on algal palatability.

SJI reached stressful operative air temperatures ($>18^\circ\text{C}$; Petersen & Johansen 1973) more often than CA during July (GLM, $F_{1,4} = 11.34$, $p = 0.03$). On SJI, roboobots recorded temperatures $>18^\circ\text{C}$ 8% of the time, whereas CA exceeded stressful operative temperatures $<1\%$ of the time. Chitons from both regions selected the same T_{set} (median: SJI = 17.5°C , CA = 17.3°C ; t -test: $t = 0.24$, $df = 29$, $p = 0.81$; Fig. 4), but thermal habitat quality (d_e) was more favorable on SJI (4.08) than in CA (5.65), as roboobots deviated less on average from T_{set} on SJI (Fig. 4a) than in CA (Fig. 4b; GLM, $F_{1,4} = 4.81$, $p = 0.09$) since environmental temperatures are generally cooler. In both regions, chiton body temperatures deviated from T_{set} similarly ($d_b = 4.1$ [SJI], 4.4 [CA]; GLM, $F_{1,4} = 0.02$, $p = 0.90$).

Chitons on SJI used sheltered habitats more than those in CA (Fig. 5; chi-squared test of independence; $\chi^2 =$

52.7; $df = 219$, $p < 0.01$), occurring in sheltered habitats 62% of the time compared to 19% of the time in CA. CA chitons were found in pools (29%) or in exposed habitats (52%) more frequently than SJI chitons, which occurred in those habitats 8 and 14% of the time, respectively. SJI chitons also occurred lower on the shore (-0.18 ± 0.02 m) than CA chitons (-0.02 ± 0.02 m; ANOVA, $F_{1,2} = 4.09$, $p = 0.08$).

Chiton T_b values varied among habitat (Table A3; exposed = $13.0 \pm 0.1^\circ\text{C}$, sheltered = $13.0 \pm 0.1^\circ\text{C}$, tidepool = $12.2 \pm 0.2^\circ\text{C}$; ANOVA: $F_{1,2} = 5.40$, $p < 0.01$) and region (SJI = $13.6 \pm 0.2^\circ\text{C}$, CA = $12.8 \pm 0.1^\circ\text{C}$; ANOVA, $F_{1,2} = 20.88$, $p < 0.01$), but these factors did not interact (ANOVA, $F_{1,2} = 0.90$, $p = 0.41$). Exposed SJI chiton T_b values were also higher than both CA sheltered (Tukey's HSD, $p < 0.01$) and tidepool (Tukey's HSD, $p < 0.01$) chitons. Tidepool chiton T_b values in CA were also lower than tidepool chitons on SJI (Tukey's HSD, $p < 0.01$), but chiton T_b values did not differ between sheltered and exposed habitats on SJI (Tukey's HSD, $p = 0.29$) or in CA (Tukey's HSD, $p = 0.96$). T_b values were the same in all other habitats.

SJI was slightly less humid than CA (SJI: $67.0 \pm 1.55\%$ relative humidity [RH], CA: $71.5 \pm 0.56\%$ RH); however, humidity did not differ between exposed and sheltered habitats in either region (ANOVA, $F_{1,2} = 2.03$, $p = 0.15$ [SJI]; ANOVA, $F_{1,2} = 0.52$,

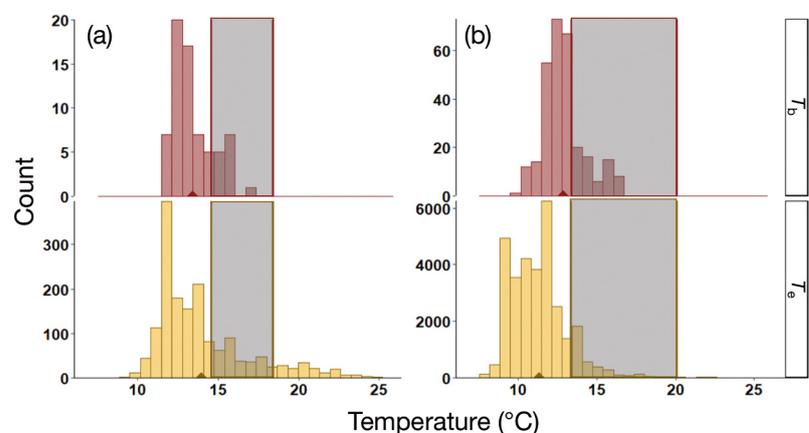


Fig. 4. Frequency distributions of gumbboot chiton *Cryptochiton stelleri* body temperature (T_b) and roboobot temperature (T_e) (a) on San Juan Island, WA (SJI), and (b) in northern California (CA). Shaded region represents the middle 50% of laboratory-selected temperatures for both populations (T_{set} : 14.6–18.4 [SJI], 13.6–20.1 [CA]). Triangles represent the mean T_b or T_e . The y-axes are different lengths since more measurements were taken in CA (T_b : $n = 282$; T_e : $n = 30875$) than on SJI (T_b : $n = 70$; T_e : $n = 1035$)

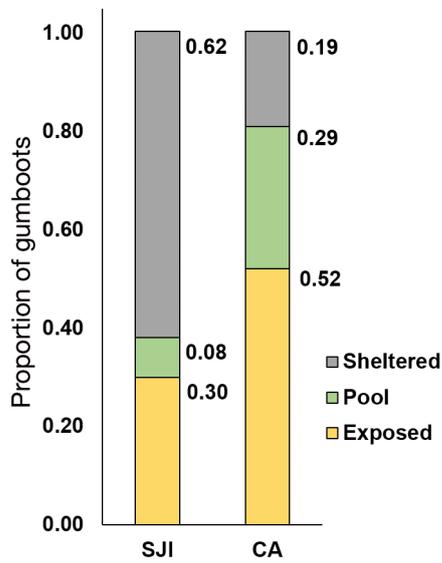


Fig. 5. Intertidal habitat used by gumboot chitons *Cryptochiton stelleri* on San Juan Island, WA (SJI) and in northern California (CA)

$p = 0.48$ [CA]), and there was a weak but significant relationship between T_b and humidity ($R^2 = 0.11$, $p < 0.01$). Wind speeds were higher in CA than on SJI (SJI: 0.04 ± 0.02 m s⁻¹, CA: 0.74 ± 0.12 m s⁻¹), but there was no relationship between T_b and wind speed ($R^2 = 0.00$, $p = 0.28$). Air temperature taken within microhabitats and right next to live chitons had a stronger relationship with chiton body temperature ($R^2 = 0.40$, $p < 0.01$) than air temperatures outside of microhabitats and those recorded by the closest robochitons (linear regression, $R^2 = 0.10$, $p < 0.01$). In emersed chitons, substrate temperature explained variation in T_b better than other abiotic variables ($R^2 = 0.70$, $p < 0.01$), and in tidepools, T_b 's were strongly related to water temperature ($R^2 = 0.66$, $p < 0.01$).

Overall, chiton E did not differ between regions (Fig. 6). CA chitons were thermoregulating, though not efficiently (Fig. 6; $E = 0.23 \pm 0.085$), and the E of SJI chitons was not different from zero (Fig. 6; $E = 0.11 \pm 0.178$).

4. DISCUSSION

Our results strongly indicate that gumboot chitons both on SJI and in CA are poor thermoregulators with respect to achieving their preferred temperatures in the field (Fig. 6), as E for chitons in both regions was less than 0.3 and in other species, values

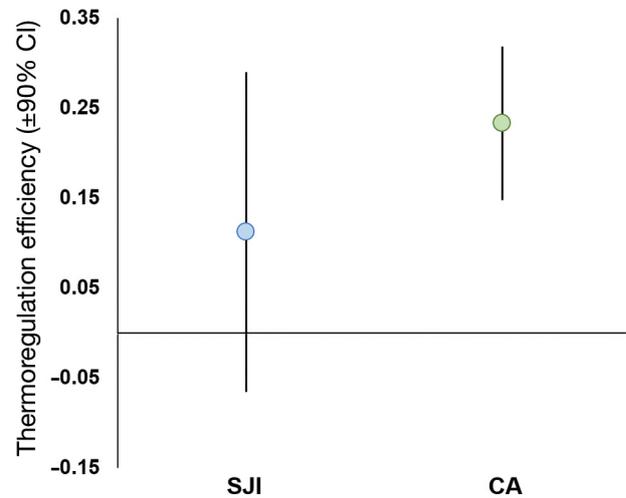


Fig. 6. Mean thermoregulation efficiency (E) of gumboot chitons *Cryptochiton stelleri* from San Juan Island, WA (SJI), and northern California (CA). Error bars are 90% CIs calculated from 10 000 bootstrap samples with replacement

less than 0.4 are considered poor (Hertz et al. 1993). On SJI, chitons do not appear to be thermoregulating to reach their preferred temperatures at all, and in CA they are thermoregulating poorly. Our comparisons were only made between 2 regions; however, our conclusions are supported by a range of biomimetic model measurements, laboratory experiments, and a well-documented pattern of regional variation in thermal stress (Helmuth 2002, Helmuth et al. 2006, Mislán et al. 2009). Conceptual models of ectotherm performance predict that ectotherms, like reptiles and insects, should select temperatures within $\sim 1.8^\circ\text{C}$ of their preferred temperature to maximize performance in the field (Martin & Huey 2008). However, reptiles and insects are highly mobile and able to retreat from extreme temperatures into burrows or other thermal refugia relatively quickly (Cooper 2000). Slower-moving ectotherms may be unable to access thermal refugia if there are sudden, rapid fluctuations in temperature, like in the intertidal zone. Consequently, ectothermic organisms in the intertidal will often select body temperatures 3 to 5°C cooler than their preferred temperatures to avoid overheating (Asbury & Angilletta 2010, Tepler et al. 2011). Our results are consistent with these findings, and gumboot chitons, unable to quickly move to cooler habitats when temperatures rapidly increase during low tide, are likely thermoregulating to avoid overheating.

Increased water temperature had a detrimental effect on chiton grazing, but aerial exposure had an even greater negative effect on chiton grazing, even

at ambient temperatures (14–16°C). Grazing reduction was due to direct temperature effects on chitons and not indirectly through effects on algae, as evidenced by our palatability study, which showed that even if there was a temperature-driven change in the physical or chemical defenses of *Mazzaella splendens*, it did not affect gumboot chiton grazing rates. Aerial exposure likely had a more detrimental effect on grazing than warming water due to alterations in chiton cellular processes. In intertidal molluscs, aerial exposure is often coupled with desiccation, which results in the loss of cellular water and has negative effects on heat shock protein synthesis and function, resulting in a decrease in respiration and other cellular processes (Gosling 1992). Thus, our findings support previous work in pointing to the importance of considering temperatures reached during both submersion and emersion separately (Yamane & Gilman 2009). Future studies should determine whether warming air and water act additively or interactively on chiton grazing performance (King & Sebens 2018). Nevertheless, since both elevated air and water temperatures reduce chiton grazing, intertidal chitons are likely to face a reduction in grazing performance owing to climate change-associated warming. However, because chitons could be changing their grazing behavior to compensate for metabolic stress, metabolic rates would also need to be measured in conjunction with grazing rates to determine the effects of elevated temperatures on overall chiton performance.

Interestingly, gumboot chitons from both CA and SJI selected T_{set} values in the laboratory that were only ~3°C below their thermal performance limit in air (20°C; Fig. 3). Since gumboot chitons experience a sharp decrease in grazing and respiration (Petersen & Johansen 1973) at 20°C and beyond, they may be playing it safe when selecting preferred temperatures (Martin & Huey 2008). Such risk-averse behavior is consistent with conceptual models that predict that species should not necessarily select for optimal temperatures but rather for temperatures that allow them to maintain performance while still offering protection against getting too close to their thermal limits (Martin & Huey 2008, Sinclair et al. 2016). Because their preferred temperatures are within a small range (3°C) of their thermal limits, chitons appear to be selecting these temperatures with a fair degree of precision.

Gumboot chiton microhabitat distribution also suggests that they are not selecting body temperatures to maximize grazing performance but rather to minimize risk of exposure to potentially harmful temper-

atures. We do note that without knowing their metabolic rates, it is possible chitons are maximizing energy efficiency at these body temperatures, particularly if the difference between food intake and metabolic demand is highest at these temperatures. Nevertheless, it is interesting that both SJI and CA chitons achieved body temperatures in the field that were within 1°C of each other but did so in very different ways. SJI chitons selected microhabitats that were sheltered from direct exposure to sunlight more often than CA chitons, which were found more frequently on the sides and tops of boulders. Also, SJI chitons were found lower on the shore than CA chitons, despite a similar proportion of tides during which chitons were emersed in both regions (SJI = 0.17, CA = 0.23; NOAA 2020). Thus, gumboot chitons appear to be selecting thermally protected habitats on SJI, where there is a higher likelihood of harmful temperatures, even if they are not achieving their preferred body temperatures. Such use of safer microhabitats has been observed in other intertidal invertebrates on SJI (e.g. sea stars [Monaco et al. 2016] and whelks [Hayford et al. 2018]). We should note that although chiton habitat selection was consistent with thermoregulating to avoid exposure to thermal extremes, chiton T_b values were not different between exposed and sheltered habitats in either region, suggesting that sheltered habitats were not providing thermal refugia for them during the time we sampled. However, the month that we surveyed chiton body temperatures on SJI was unseasonably cool and cloudy, and it is likely that examining chiton body temperatures over several months or across multiple years would reveal habitat-specific differences that we missed during our short sampling period.

Some ectotherms will select habitat to avoid not only temperature stress per se, but also desiccation stress (Kensler 1967, Jones & Boulding 1999, Allen et al. 2012). Gumboot chitons may be susceptible to desiccation stress as a result of their large surface area (Lowell 1984), so it is possible that they are both trying to stay below their thermal limits and minimize exposure to desiccation. The lack of difference in humidity between the sheltered and exposed habitats in our study area suggests that chitons are not selecting habitat based solely on humidity to reduce desiccation in either region, however. Wind may also play a role in exacerbating desiccation stress by increasing evaporative water loss (Miller et al. 2009). Even though wind speeds in CA were on average higher than those on SJI, CA chitons were not preferentially selecting for sheltered habitats, so sheltering from wind-driven evaporative water loss is also un-

likely to be driving gumboot chiton habitat selection. Nevertheless, organisms that seek refuge in sheltered habitats should experience fewer stressful days due to shading or retention of moisture (Gray & Hodgson 2004, Bazterrica et al. 2007), whereas organisms on exposed surfaces should be at much greater risk of extreme events (e.g. low wind and high solar radiation) on a given day to produce extreme body temperatures.

Behavioral thermoregulation (i.e. habitat selection) may not be the only mechanism by which chitons can avoid exceeding their thermal limits. We note 2 physiological mechanisms for thermoregulation that could be available to chitons. First, gumboot chitons are quite large, and their mass is close to that of the largest individuals of the intertidal sea star *Pisaster ochraceus*, a species that can adjust its thermal inertia in response to prior thermal exposure. *P. ochraceus* will intake sea water at high tide to slow the rate at which their body temperature increases in the subsequent low tide (Pincebourde et al. 2009). It may be possible that gumboot chitons have a similar thermoregulatory backup, but it is not currently known. Second, because their thermal limits are relatively low, gumboot chitons may rely on evaporative cooling to avoid reaching body temperatures $>20^{\circ}\text{C}$ at low tide (Helmuth 1998). For example, the California mussel *Mytilus californianus*, which lives in sympatry with gumboot chitons over much of their range, rely on evaporative cooling to keep their body temperatures from reaching detrimental temperatures during low tide (Helmuth 1998). These physiological mechanisms could also contribute to the difference between body temperatures in the lab (T_{set}) and those in the field (T_{b}). Therefore, careful consideration of how gumboot chitons adjust their thermal properties in ways other than behavior may further elucidate how well they cope with elevated air temperatures during low tide.

Our results and recent historical air temperature data suggest that gumboot chitons are currently exposed to aerial temperatures above their thermal limits ~10% of the time on SJI (Carrington 2017) and ~1% of the time in CA (Global Monitoring Laboratory 2002). In temporally variable environments like the intertidal zone, performance limits may occur at even lower temperatures than those derived from statically measured performance trials like the ones reported here (Dowd et al. 2015), suggesting that thermal limits may be breached even more often than projected here. Regardless, future temperature increases (Alley et al. 2007) will expose chitons to more thermally stressful temperatures even more

frequently. Our study therefore lays important groundwork for predicting how gumboot chitons will behaviorally modulate exposure to warming air temperatures, as increases in exposure to temperatures beyond their thermal performance limits, coupled with an inability to efficiently maximize grazing performance, make it likely that gumboot chitons will face a reduction in favorable habitat in the rocky intertidal zone in the future. Further studies quantifying how other environmental factors (e.g. desiccation stress [Jones & Boulding 1999]), thermoregulatory mechanisms (e.g. physiological [Pincebourde et al. 2009]), and species interactions (e.g. habitat modification [Burnaford 2004, Gilman et al. 2010]) are affecting gumboot chiton temperature selection, as well as how metabolic rates affect performance, will be needed to precisely predict how climate change-induced warming will affect this species.

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Appendix.

Table A1. Games-Howell post hoc test for water temperature effects on gumboot chiton *Cryptochiton stelleri* grazing rates. **Bold:** statistically significant differences at $\alpha = 0.05$

Comparison (°C)	df	<i>t</i>	<i>p</i>
11 vs. 13	4	0.06	1.00
11 vs. 15	4	0.94	0.88
11 vs. 18	4	2.52	0.14
11 vs. 20	4	3.25	0.04
13 vs. 15	4	1.03	0.838
13 vs. 18	4	2.56	0.13
13 vs. 20	4	3.36	0.04
15 vs. 18	4	3.66	0.02
15 vs. 20	4	4.46	0.01
18 vs. 20	4	1.05	0.83

Table A2. Mann-Whitney *U* post hoc tests for air temperature effects on gumboot chiton *Cryptochiton stelleri* grazing rates. **Bold:** statistically significant differences at $\alpha = 0.05$

Comparison (°C)	df	<i>U</i>	<i>p</i>
14 vs. 16	4	0.95	0.34
14 vs. 18	4	0.36	0.71
14 vs. 20	4	3.91	<0.001
14 vs. 22	4	2.59	<0.001
16 vs. 18	4	1.14	0.25
16 vs. 20	4	3.91	<0.001
16 vs. 22	4	1.67	0.09
18 vs. 20	4	3.91	<0.001
18 vs. 22	4	2.46	0.01
20 vs. 22	4	2.73	0.01

Table A3. Tukey's HSD post hoc test comparing the body temperatures (T_b values) of gumboot chitons *Cryptochiton stelleri* in different habitats between San Juan Island, WA (SJI) and California (CA). **Bold:** statistically significant differences at $\alpha = 0.10$

Comparison	Difference	<i>p</i>
CA exposed vs. SJI exposed	-1.13	<0.01
CA pool vs. SJI exposed	-2.01	<0.01
CA sheltered vs. SJI exposed	-1.30	<0.01
CA pool vs. SJI sheltered	-1.32	<0.01
CA pool vs. CA exposed	-0.88	0.03
CA sheltered vs. SJI sheltered	-0.61	0.19
CA sheltered vs. CA pool	0.71	0.26
SJI sheltered vs. SJI exposed	-0.70	0.29
CA exposed vs. SJI sheltered	-0.43	0.31
SJI pool vs. SJI exposed	-1.17	0.37
CA pool vs. SJI pool	-0.84	0.72
SJI sheltered vs. SJI pool	0.48	0.96
CA sheltered vs. CA exposed	-0.17	0.96
CA exposed vs. SJI pool	0.04	1.00
CA sheltered vs. SJI pool	-0.13	1.00

Editorial responsibility: Emily Carrington,
Friday Harbor, Washington, USA

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