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Climate change effects on thermal tolerance plasticity and population dynamics in the eelgrass sea hare, *Phyllaplysia taylori*

By

Richelle Li Tanner

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requirements for the degree of

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in

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University of California, Berkeley

Committee in charge:

Professor Jonathon H. Stillman, Co-Chair
Professor Wayne P. Sousa, Co-Chair
Professor Caroline M. Williams
Professor Mark T. Stacey

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Abstract

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Professors Jonathon H. Stillman & Wayne P. Sousa, Co-Chairs

Understanding how climate change affects the physiology and population dynamics of grazers that can reduce epiphyte growth on seagrass is important for predicting the future dynamics of seagrass ecosystems. Seagrass habitats provide a number of important ecosystem services, including carbon sequestration, nutrient mitigation, sedimentation and erosion control, and providing nursery habitat for fishes and invertebrates. Climate change threatens seagrass communities as increased temperature promotes photosynthesis-inhibiting epiphytic growth. Grazers have the potential to mitigate this increased epiphytic growth if their physiological performance is maintained in future climatic regimes. *Phyllaplysia taylori* is a grazing sea hare with two generations per year in the *Zostera marina* seagrass habitat along the Pacific coast of North America. It exhibits direct embryonic development with crawl-away young, greatly limiting long-distance dispersal capacity and population connectivity. In Central CA, *P. taylori* is a bivoltine species with non-overlapping generations that span different seasonal regimes. As a grazer species inhabiting many thermally disparate environments throughout space and time, it provides an opportunity to look at how physiological plasticity plays a role in population persistence and how this can impact the seagrass ecosystem. In my dissertation, I examined the factors that influence *P. taylori's* geographic distribution at different spatial scales and variation in the plasticity of thermal physiology across life stages and among populations.

In my first chapter, I documented where *P. taylori* populations are found along the coast and investigated the environmental and/or ecological factors that account for their distribution. Although temperature was expected to be a major driver of population persistence, this was not the case. Nutrient-rich runoff from anthropogenically-modified land was the strongest predictor of *P. taylori* presence, as it may contribute to epiphyte growth resulting in increased sea hare food availability. I also found that seasonal fluctuations in eelgrass density and length, epiphytic coverage, and average temperature played the biggest roles in determining relative seasonal abundance of *P. taylori*. The presence/absence models applied to existing restoration sites within San Francisco Bay indicated a 53% success rate for *P. taylori* within currently planned restoration areas. These correlation models demonstrate that it is important to consider grazer communities when planning restoration efforts.

In my second chapter, I investigated the physiological differences among the populations examined in Chapter 1, testing for a relationship between phenotypes and thermal characteristics

of the source habitat. To address intraspecific differences in thermal tolerance plasticity across their geographic range, thermal sensitivity of metabolic rate and upper critical limits (CT_{max}), as well as the acclimation response of these metrics were assessed. Metabolic rate estimated by whole-organism respiration rate showed depression after acute heat stress in populations with warm thermal habitat histories. This trend was only observed in lab acclimation groups above 17ºC and indicates beneficial acclimation, whereby acclimation to warmer temperatures dampens the acute heat stress response, resulting in less energy expenditure with stress. Compared with other taxa, *P. taylori* had high intraspecific variation in CT_{max}. Short-term plasticity of CT_{max} with acclimation varied among collection sites, but contrary to my prediction, was not correlated with microhabitat temperature regimes. Instead, short-term plasticity elevated CT_{max} and its acclimation capacity well above habitat temperatures in no apparent pattern. Therefore, this study does not find sufficient evidence for positive selection acting upon the plasticity of CT_{max}. However, on the scale of seasons within a single population, I found significant seasonal acclimatization whereby individuals collected in the summer exhibited the highest CT_{max} and the greatest capacity for acclimation in this trait. These data provided relevant information on season-specific phenotypes, which were investigated in the next chapter.

In my third chapter, I used laboratory acclimation temperature treatments to induce seasonally-relevant phenotypes. Within these temperature treatments, I investigated how shifts in transgenerational and developmental plasticity impacted offspring success. I found that seasonally-driven differences in offspring success were correlated with egg size and total maternal investment per clutch (transgenerational plasticity), with winter temperatures corresponding to larger, fewer eggs. Future summer temperatures resulted in significantly decreased total maternal investment with more investment per egg. Developmental plasticity sufficiently mitigated differences in maternal investment in the current climatic regime to result in equal numbers of successful offspring in all seasonal scenarios, but acclimation capacity and offspring success decreased with acute and chronic heat stress in mothers and offspring. Transgenerational and developmental plasticity decrease with high temperatures, suggesting that these life stages may be more susceptible and subject to selection with increased habitat temperature.

These studies illustrate how the physiology of an important grazer in the eelgrass ecosystem will respond to climate change and outlines potential consequences at the population level. Although short-term plasticity sufficiently buffers adults from future climate shifts, maturation is made difficult by inadequate transgenerational and developmental plastic responses. While some populations may suffer due to increased mean and variation of habitat temperatures, responses across populations are highly variable. Therefore, in restoration efforts, selection of relevant source populations of *P. taylori* based on thermal physiology is highly advantageous. Understanding how the physiological plasticity of *P. taylori* is able to compensate for future climatic shifts is important for both eelgrass restoration and in determining how physiologically-plastic taxa cope with fluctuating environments.

Dedication

To my sister, Julia.

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I would like to thank my committee members Caroline Williams and Mark Stacey for broadening my horizons and guiding my ideas over the last three years. Taking Caroline's physiology course completely remodeled my framework of thinking for my dissertation, and it has been a pleasure learning from her. Every time I walk away from a meeting with her, I simultaneously feel inspired and like there was still something that pushed the boundaries of my understanding from our discussion. She motivates me to continue exploring new interpretations of my own interests, and I admire her multi-level approach to biological questions. Mark has been on all three of my committees and has served as the voice of reason in my (over)ambitious dissertation plans. I have greatly enjoyed learning from his perspective on estuarine flow dynamics and our discussions on their biological impacts. I look forward to working with him in the future and appreciate his involvement in my research, despite his seemingly tangential field of research. I would also like to thank my two qualifying exam committee members, Rauri Bowie and David Lindberg. Rauri has been instrumental in providing me the opportunity to try new techniques in sequencing and I am very grateful for his support in the population genetics project that will allow us to bolster the findings from the physiological studies outlined here. My conversations with David reminded me of what academia is supposed to be like – our exchanges were always so much fun and he always reminded me that everyone has something to learn.

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Chapter 1 | Predicting *Phyllaplysia taylori (Anaspidea: Aplysiidae)* presence from land use in Northeastern Pacific estuaries for use in the eelgrass restoration framework

Abstract

Estuaries are some of Earth's most productive ecosystems and are home to a diversity of flora and fauna – both on land and in the sea. This biodiversity is impacted by climate change and anthropogenic use due to the abundant exploitation of highly productive estuarine ecosystems. For these reasons, estuarine ecosystems are often the subject of major restoration efforts, especially submerged aquatic vegetation (SAV) such as eelgrass beds and their associated communities. This study investigated how abiotic, ecological, and landscape parameters predict the presence of an invertebrate within an SAV restoration area of interest in San Francisco Bay: *Phyllaplysia taylori*, the eelgrass sea hare. *Phyllaplysia taylori* is a grazer of epiphytes inhibiting eelgrass photosynthesis, providing substantial ecosystem services indirectly. By looking at nineteen eelgrass beds along the coast of the western United States, this study evaluated statistical models that explained the presence of *P. taylori* in eelgrass beds and seasonal patterns in population density. *P. taylori* presence was best predicted by a generalized linear mixed effects model including the positive impacts of nearshore irrigated land, vegetated land, and bare soil land. *P. taylori* abundance over time within one site was best described by a linear mixed effects model including eelgrass density, eelgrass length, epiphytic coverage, and average temperature. These models were used to predict habitat suitability for *P. taylori* in seventeen San Francisco Bay SAV restoration areas, indicating a 53% *P. taylori* success rate. Incorporating population persistence knowledge from the SAV-associated invertebrate perspective can greatly increase restoration success.

Introduction

Estuaries around the world host much of the nearshore marine and terrestrial biological diversity, regulate important biogeochemical processes, and are essential for ports and other water-based industries (Carter, 1988; Barbier et al., 2011). Biological diversity in nearshore estuarine ecosystems, like seagrasses and mudflats, is often influenced heavily by biotic, environmental, and anthropogenic stressors from terrestrial sources due to dense human occupation via industry use and cities (Coles et al., 2014; Bas Ventín et al., 2015) . Fluctuations in this biodiversity reflect on species-specific responses to environmental change and perturbations (Blake et al., 2014; Kelly et al., 2016). Of the taxa impacted by environmental change, mollusks have emerged as particularly persistent in population numbers, sometimes benefitting from anthropogenic inputs to the nearshore ecosystem (Goddard et al., 2011; Wilson et al., 2016). Kelly et al. 2016 have shown in nearshore eelgrass beds that while biological richness increases with local urbanization, this richness is mainly composed of molluscan taxa. Mollusks are successful invaders via ballast water and other shipping-related transport, so much of this species richness may be comprised of invasive species (Carlton et al., 1990; Carlton, 1999). This does not discount the major successes of native mollusks in estuaries (Coen et al., 2007; Schulte et al., 2009). Eelgrass beds are a fast-disappearing estuarine habitat due to increased runoff limiting photosynthetic scope with increasing algal growth, turbidity, and sedimentation (Orth et al., 2006; Burkholder et al., 2007; Carr et al., 2011). Mollusks have the capacity for maintenance of eelgrass health in a changing climate: they filter water, play an important role in the food web, and clear eelgrass blades of epiphytic material allowing for increased photosynthesis (Hoellein et al., 2015; Thormar et al., 2016).

The sea hare, *Phyllaplysia taylori* (Dall, 1900), is a grazer in *Zostera marina* eelgrass beds that feeds on encrusting epiphytes, which hinder eelgrass growth by light limitation (Beeman, 1963; Lewis and Boyer, 2014). It is also dispersal-limited with direct development and crawl-away young, a life history trait characteristic of many estuarine species (Beeman, 1966). Despite this, their reported range is from Vancouver B.C. to Baja California, inhabiting both subtidal and intertidal eelgrass beds (Beeman, 1963). There are no records of their strictly intertidal range in the literature, which was the focus of this study. Due to *P. taylori*'s camouflaged coloration, which closely resembles the color of eelgrass blades, and its dispersal limitations, incomplete historical records are inevitable. In contrast, the range for *Z. marina* extends from the tropics to the Arctic in both the Pacific and Atlantic Oceans (Short et al., 2010). By surveying habitats categorized as hosting *P. taylori* in historical records, this study examined how modern intertidal populations of *P. taylori* may have been impacted by past and ongoing modifications to their environment, including both abiotic and anthropogenic land use shifts.

This study aimed to identify which ecological, abiotic, and surrounding terrestrial landscape indicators best predict *P. taylori* presence and population density throughout seasonal fluctuations in suitable habitats along the western coast of the United States. In doing so, this work will inform submerged aquatic vegetation (SAV) restoration efforts in the wide latitudinal range that *P. taylori* occupies by identifying the key components of the environment that limit *P. taylori* populations. While *P. taylori* are by no means the only important eelgrass-associated invertebrate, understanding how a grazer species interacts with its environment can inform future community-scale investigations. Current restoration efforts in San Francisco Bay have made great strides towards understanding the needs of the eelgrass plant itself, but this study shows how the incorporation of important community players like *P. taylori* can be feasible. As an intertidal species occupying estuarine habitat, *P. taylori* was expected to have high tolerance of fluctuations in both abiotic variables and anthropogenic inputs. Across taxa, environmental temperature has been shown to be a major driver in population dynamics in intertidal invertebrates (Dahlhoff et al., 2002; Helmuth et al., 2002; Kroeker et al., 2016) and was expected to explain a significant portion of the variance in *P. taylori* abundance and presence. Ecological variables characterizing habitat structure and resource availability have long been a focus of marine habitat restoration and protection (Saarman and Carr, 2013; Burgess et al., 2014; Bas Ventín et al., 2015). Landscape variables play their largest role as indirect mediators of runoff and nutrient addition to these nearshore habitats (Jiang et al., 2014; Wang et al., 2013). As a primary consumer of epiphytic material, *P. taylori* plays a significant role in the maintenance of *Z. marina* beds yet its presence is likely dependent on a complex interaction of traits in its environment (DeLorenzo, 1999; Lewis and Boyer, 2014).

P. taylori presence was expected to depend heavily on water temperature and ecological characteristics of habitat structure. From this spatial model of *P. taylori* presence, existing SAV restoration sites within San Francisco Bay were evaluated for potential *P. taylori* population persistence to inform future eelgrass restoration efforts on incorporation of grazer communities. *P. taylori* abundance over time was expected to depend chiefly on measures of eelgrass health. In investigating both temporal and spatial models, we can gain a better understanding of how restoration efforts can take advantage of natural variation in potential seed populations. Additionally, modeling population persistence over space and time can inform expectations of restoration population establishment and maintenance. Determining the relationship between *P.*

taylori and its environment is a valuable resource in eelgrass conservation efforts within its geographic range, and as a model for important grazer communities in seagrasses worldwide.

Methods

Four datasets are represented in this study: training, test, predicted, and temporal. The training dataset was used to generate presence/absence models, the test dataset validated these models, and these validated models were implemented on the predicted dataset. The temporal dataset was used to generate models of *P. taylori* persistence throughout seasonal timescales at one site. Training sites (Table S1) consisted of nineteen field sites along the coast from the initial study. Test sites (Table S2) consisted of twelve field sites that also had known presence/absence of *P. taylori* with a similar geographic spread as the training data. Seventeen predicted sites were within San Francisco Bay (Table S3) and outlined in the San Francisco Subtidal Habitat Goals Project as ongoing and/or potential restoration sites for eelgrass. The temporal dataset consisted of 15 monthly measurements of ecological and abiotic parameters at one field site, Point Molate, SF Bay, CA starting in November 2015.

Ecological measurements in the field

The ecological factors investigated in this study were selected to provide a measure of eelgrass health, relating directly to the substrate and food source of *P. taylori*. To evaluate relationship between *P. taylori* and its surrounding environment, field measurements of eelgrass health and *P. taylori* abundance were taken at nineteen sites (Fig. 1, Table S1) at one time point during the season of expected *P. taylori* peak biomass, May 2016-September 2016, along the western coast of the United States (Beeman, 1963). To create a temporal model describing *P. taylori* presence through time, eelgrass health and *P. taylori* abundance were monitored once per month at Point Molate in San Francisco Bay, CA for two years (August 2015-July 2017). Eelgrass health was quantified by four measurements: eelgrass frequency, eelgrass density, eelgrass length, and epiphyte abundance along four 30 meter transects (standard diver method; (San Diego Unified Port District, 1979; Bernstein et al., 2011). These metrics of eelgrass health are strong correlates of the productivity of grazer communities in eelgrass beds (Duffy et al., 2001). Transects were chosen by starting the first transect at the eelgrass bed closest to the shore and running parallel to the shore. Each subsequent transect was placed five meters further from shore. If the eelgrass bed did not extend far enough for four transects to be placed, two transects were placed end to end along the shore instead (i.e., resulting in two 60 meter transects). Eelgrass frequency was measured by evaluating the substrate type at every meter on each of the four transects at a site, with each eelgrass measurement counting as one and any other substrate as zero. Eelgrass density was quantified using a 0.25 m^2 quadrat placed every ten meters on each transect starting with the meter zero mark, where the number of turions, or separate above-ground eelgrass shoots, was counted. Eelgrass length was measured within the same 0.25 m^2 quadrat as an average of all blade lengths within one quadrat (resolution = 5 cm). Epiphyte abundance was quantified as the percent coverage of all epiphytic growth (encrusting, algal, diatomaceous) on all blades, from stalk to tip, within each quadrat. This was approximated visually by the length of blade covered with brown or red epiphytic growth as a percentage of total blade length in all turions within a quadrat (resolution = 10%). Additionally, *P. taylori* abundance and *P. taylori* average length were measured on turions originating within the same quadrats (resolution = 0.5 cm).

P. taylori presence/absence

Three measures of presence/absence were obtained at each site: historical, present, and high density (>30 individuals). Historical records of presence were obtained from the primary literature and resources provided by the online community, iNaturalist (Table S1). All records of absence were documented by the author. If any note of the species (including written or photo) was found before 2015, it was counted as presence. Historical records for this species are lacking, so this may be an incomplete dataset. In each of our field surveys, the presence of any *P. taylori* individual or egg mass was counted as *P. taylori* presence. To account for spatial heterogeneity in populations within one eelgrass bed, high density presence was recorded during each survey and was designated by >30 individuals found in <1 hour (Schultz et al., 2011).

Abiotic data

The abiotic factors selected for use in this study reflect the importance of *P. taylori*'s thermal physiology in determining population persistence and the influence of water flow on benthic invertebrate population persistence. Water temperatures collected by weather stations near each field site for January 2015 through December 2015 were downloaded from the National Data Buoy Center and the National Estuarine Research Reserve (Table S4). If data were not available in the bay of the field site, the closest bay with similar physical structure and station placement was chosen. Data were summarized by 2015 average temperature and 2015 average daily variation (range between maximum and minimum temperature each day averaged over all days) (Fig. S1). At Point Molate, temperature was recorded at 30-minute intervals at two tidal heights using Maxim Integrated® iButton® thermochron loggers (San Jose, CA, USA) and Onset® UTBI-001 TidbiT® v2 loggers (Bourne, MA, USA) starting in November 2015. These data were used for the temporal model only. Data were summarized by monthly average temperature and monthly average daily variation.

Other geographical features at each field site were evaluated using Google Earth 7.3.0.3832 (64-bit), which included linear distance from the mouth of the estuary (i.e., where it meets the outer coast) to the midpoint of the transects and a categorical assessment of protection. The level of protection from currents was evaluated categorically, with 0 directly exposed to oceanic or estuary channel currents, 1 being within a small cove or lagoon, and 2 being directly behind the point of a small cove or lagoon (i.e. behind a jetty) (Fig. S2).

Land use satellite imagery

Landscape variables were incorporated into this study to reflect the importance of urbanization in shaping nearshore environments. Satellite imagery was downloaded from Google Earth 7.3.0.3832 (64-bit). Extent and type of urbanization was categorized used a 500x500 meter grid overlay using ImageJ 1.49v (Schneider, 2012) on the 500 meters of adjacent coastline of each field site extending 500 meters inland. This 500x500 meter square was centered on the Global Positioning System (GPS) coordinates (Table S1) of the first transect for each site, with the ocean side of the square aligning with the coastline as closely as possible. Selection of landscape bins was based on methods for detecting the urban heat island effect (Kalnay and Cai, 2003), most importantly including measures of water-impervious structure areas, vegetated areas, bare soil areas, and irrigated land. Categories included irrigated land, >50% water-impervious structures (primarily concrete, hereafter referred to by concrete) cover, <50% concrete cover, bare soil, and vegetated. Imagery was evaluated during wet seasons to account for full potential vegetation coverage. Each grid square was evaluated for the above criteria and percent cover of each land use type was totaled for each field site.

Statistical analyses

All candidate models tested were created using a generalized linear mixed effects ANOVA with one random effect. For a list of all parameters included in the full model, see Table S5. All metrics were centered so that the predictors have a mean of zero. Candidate generalized linear models were generated based on known ecological relationships, effects of water flow on population persistence, and assumed correlations between land use and runoff generation (Paule et al., 2014; Ongsomwang and Pimjai, 2015). All metrics were first evaluated for possible interactions. The two measures of concrete or building cover had high collinearity and an inverse relationship, thus only >50% concrete or building cover was used in generating models. Using R v. 3.1.1 (R Team Core, 2017) with packages "MuMIn", "lme4", "lmerTest", "nlme", and "effects", generalized linear mixed effects models with one random factor for field site were generated for the presence/absence models, high density models, and historical data models (Bates et al., 2015; Alexandra et al., 2017; Pinheiro et al., 2017; Barton, 2018). To test for a relationship between *P.* taylori and the environment over time, linear mixed effects models were generated with transect number and quadrat number as random effects and a time (month, year) autocorrelation structure established for transect number and quadrat number. Landscape variables were not used in the temporal model due to their static nature in considering only one site on a short timescale. Models were then compared using the Akaike Information Criterion (AIC) with correction for small sample sizes relative to number of explanatory variables (AICc) using the "AICcmodavg" package (Mazerolle, 2017).

Presence/absence (binomial) models were generated with training data with three response variable options: historical presence of *P. taylori* at each site as found in the primary literature and educational/citizen science networks, presence of *P. taylori* at each site in 2016 during surveying, and high-density presence of *P. taylori* at each site in 2016 during surveying (Table S1). Models were validated using the test dataset (Table S2) and later applied to restoration sites in San Francisco Bay (Table S3) using the "predict" function and the appropriate fit from the training data. Temporal models were generated accounting for temporal autocorrelation, a nested study design, and assumed unequal variances between collection dates.

Results

Predictive model selection: presence/absence

The presence of *P. taylori* on eelgrass was positively correlated to three landscape parameters. *Phyllaplysia taylori* presence at time of collection (2016) was best predicted by the positive impacts of irrigated land percentage, bare soil percentage, and vegetation percentage with a random effect for location (Tables 1 & 2, ΔAICc=0). No other model had an ΔAICc<2, which is the accepted cutoff for choosing the model with the most predictive power (Compton et al., 2002). All models attempting to predict *P. taylori* historical presence using all available parameters did not converge except one containing irrigated land percentage, bare soil percentage, and vegetation percentage, in which only irrigated land percentage was significant ($p<0.05$). An AICc could not be run with one converged model in which only one parameter was significant in only that configuration of parameters. *Phyllaplysia taylori* high density presence was best predicted by the positive impacts of the level of protection, average temperature, and bare soil percentage and the negative impacts of average daily variation (Tables 3 & 4, Fig. S3, ΔAICc=0). It was also well-explained by the same parameters minus bare soil percentage (Tables 3 & 4, ΔAICc=0.84). This response variable was the only model to incorporate measurements from all three categories: ecological, abiotic, and landscape.

Presence/absence model testing and application: restoration implications

The most basic presence/absence model was selected for application to restoration areas because of the limited availability of test dataset parameters. Five sites with confirmed *P. taylori* presence in the year preceding the study (starting May 2015) and seven sites with confirmed *P. taylori* absence during the study were used to test the selected model (Table S2). Data were collected from these sites in the same manner as for the model itself, however, only landscape variables were obtained due to the exclusion of ecological and abiotic variables from the selected model (Fig. S4). Out of the five presence sites, four were validated by the model (80% success) and out of the seven absence sites, five were validated by the model (71.4% success) (Fig. 2). Seventeen sites in early through late stages of SAV eelgrass restoration in San Francisco Bay were evaluated using the model (Fig. S5) and eight sites were indicated as having potential *P. taylori* presence. Out of these sites, four were in early restoration stages (ten total; 40% predicted presence), three were in mid-stage (four total, 75% predicted presence), and one was in late-stage (three total, 33% predicted presence). Overall, 53% of active SAV eelgrass restoration sites are predicted to be habitable for *P. taylori* (Fig. 3). While environmental conditions are known to vary based on latitude within SF Bay due to large differences in environmental factors (temperature, salinity, flow rate) (Schraga and Cloern, 2017), no bias in location (north vs. south bay) was found, with two presence indications in the north bay, three in the mid-bay, and three in the south bay.

Predictive model selection: P. taylori abundance on a temporal scale

Phyllaplysia taylori abundance was best explained by a linear mixed effects model containing centered values of eelgrass density, eelgrass length, epiphytic coverage, and average environmental temperature (Tables 5 & 6, Fig. S6, ΔAICc=0). All models were investigated first using a mixed effects ANOVA with transect number and quadrat number within transect as random effects to check for significance. Only models with a $p<0.05$ significance for one or more parameter were included in the AICc. No models had significant collinearity between parameters. While *P. taylori* presence was best explained by the above model, the fit did not explain a significant portion of the variance resulting in a high residual and low R^2 value despite individual factors being significantly correlated with the response variable.

Discussion

As a direct developer with limited mobility, *P. taylori* population structure is expected to fluctuate according to its environment, including all ecological, abiotic, and landscape indicators. Population growth in an ideal environment in the absence of resource limitation would be high for large enough populations, while low densities of *P. taylori* would struggle to maintain population growth per the Alee effect, as low densities are a barrier in mating for benthic, nearly sessile organisms (Chambers, 1934; Dennis, 1989). Therefore, it follows that predicting abundant *P. taylori* would rely on many diverse factors that differ from finding a low density of *P. taylori*.

P. taylori presence model

It was unexpected that a presence/absence model for *P. taylori* irrespective of population density included only landscape factors, but this indicates that most likely localized terrestrial runoff, not ocean processes, plays the largest role in the presence of this sea hare. Since landscape factors did not necessarily correlate with the amount of human interaction with nearshore environments, there is not a consistent correlation with boat traffic, light pollution, or any other anthropogenic interference with nearshore eelgrass beds. Small-scale point sources of terrestrial runoff could play a large role in *P. taylori* presence for many reasons, but two distinct possibilities are (1) antagonistic, where the temporally-heterogeneous hydrodynamic stress of runoff prevents settling of *P. taylori* in chance establishment events (R. Tanner, unpublished data), and (2) runoff contains additional nutrients that promote epiphyte growth and therefore *P. taylori* population growth.

In considering possible antagonistic impacts of terrestrial runoff due to increased flow, *P. taylori* do not have means of establishing new populations in their life history characteristics. Population establishment is most likely due to chance, as the only likely method of large-scale transport is through rafting on eelgrass or using mucus strings. One individual likely starts a new population due to sperm storage (Chambers, 1934). These events are due to chance, as the only likely method of large-scale transport is through rafting on eelgrass or using mucus strings. Therefore, an area with high runoff facilitated by bare soil and irrigated land would dissuade settlement of drifting *P. taylori.* Since bare soil and irrigated land are negatively correlated with vegetation, runoff is most likely mitigated by vegetation. On the contrary, extra nutrients from runoff are known to cause eutrophication in some ecosystems (Burkholder et al., 2007) and provide additional elements that are typically limiting in an ecosystem. Eelgrass beds with an influx of nutrients typically have higher rates of eelgrass blade growth and epiphyte growth, especially of the algal variety (Williams and Ruckelshaus, 1993; Nelson, 2017). Since *P. taylori* rely on these epiphytes for food, population growth could be exponential if epiphyte growth rates remained high due to increased runoff. While locations with *P. taylori* represent a wide variety of landscape conditions, those without *P. taylori* are more limited in scope of predictor variables. High values of runoff-enhancing bare soil and irrigated land are never correlated with *P. taylori* absence; therefore, the second hypothesis regarding extra nutrients facilitating population growth is more likely. While land characteristics immediately surrounding an intertidal eelgrass bed may be highly correlated with *P. taylori* presence, this study acknowledges the shortcomings of assuming high impacts of small-scale terrestrial runoff. Future studies should investigate point sources of storm drain runoff, as many field sites included in this study corresponded with direct pipe outflow. Analyses of water content in these outflows would shed light on whether they positively contribute to eutrophication in the same way that purely land-based runoff does.

It is important to note that runoff in urban areas often has high concentrations of toxins from industrial and/or home waste products. Mollusks are often used as ecosystem indicators to judge the extent of toxicity and characterize the pollutants present (Byrne and O'halloran, 2001). Although there are some adverse effects of both biological and heavy metal toxins on mollusks, their physiological responses to sub-lethal doses characterize them as being less sensitive than other organisms (McLusky et al., 1986; Langston et al., 1998). If toxin-sensitivity in estuarine mollusks was high, an inverse relationship between human land use and *P. taylori* would be expected, however, the literature supports the idea that this group of organisms has relatively high toxin resistance, especially when looking at where mollusks succeed in population establishment (in marinas, next to power plants, etc.). While it would have been beneficial to consider the role that toxins play in shaping the effect of runoff, there were not sufficient spatially-explicit data to accomplish this. Therefore, it is most likely that high runoff facilitates extra nutrients and therefore accelerated epiphyte growth, supporting *P. taylori* population growth in turn.

P. taylori high-density model

This model included the most types of indicator parameters, highlighting the complexity of a grazer's interactions with the environment. Average temperature and average daily variation in temperature showed opposing correlations with *P. taylori* high density, which demonstrated that areas with high average temperature had little fluctuation and vice versa. This potentially has implications for the thermal tolerance and plasticity potential of sea hare thermal physiology for individual populations. Bare soil as an indicator landscape parameter carried over from the presence/absence model, which is indicative of the high level of nutrient runoff associated with it. The level of protection from currents as a parameter included in the model was not a primary candidate initially, but it is plausible that protection from currents would prevent existing *P. taylori* from washing away and allow for population growth. The findings from this specific model supported those of the presence/absence model, but did not build substantially upon them. The results from this model would be strengthened by further investigations of variation in *P. taylori* phenotypes across populations, especially in relation to thermal tolerance.

Temporal P. taylori abundance model

The combination of a temporal and spatial approach in this study provides perspective on not only where to find *P. taylori*, but what contributes to a successful population over time. Interestingly while presence/absence models relied heavily on land use parameters, predicting *P. taylori* over time is most successful when considering eelgrass structure and health metrics. Therefore, it is important to first select suitable spatial habitats when using *P. taylori* in restoration projects but also consider careful monitoring of eelgrass health until *P. taylori* population establishment. Since *P. taylori* contribute positively to eelgrass health, this early monitoring approach can have great returns in eelgrass bed restoration success after *P. taylori* population establishment.

Average monthly temperature at Point Molate, SF Bay, CA followed a predictable seasonal pattern with warming in the summer and cooling in the winter. It was unexpected that daily variation did not covary with average temperature or predict *P. taylori* abundance, as summer conditions generally coincided with lower variability and winter conditions were characterized by high variability. One explanation for this lack of pattern is that this population of *P. taylori*, among many other populations, experiences two generational cycles per year with peaks in maturity during the late summer and early spring (Beeman, 1966). This could result in high *P. taylori* abundance in asymmetrical thermal regimes. This also explains why average temperature, not daily variation, best explains *P. taylori* abundance, since average temperature at this temperate latitude during the mid-summer and early spring are within a few degrees of each other. This is in stark comparison to fall high temperatures and winter low temperatures.

A model biased by life history characteristics: the potential role of limited dispersal

Despite a survey of over a dozen parameters from three diverse sources (abiotic, ecological, and landscape), linear models selected as best fit by the AICc had high residual variance. This could be an indication of bad parameter selection in sampling design, but an inherent trait in *P. taylori* life history is more likely to be the cause. Extremely limited dispersal due to direct development in offspring and no swimming mechanism in adults is predicted to lead to high outbreeding depression and reduced connectivity between populations even in close spatial proximity to each other. Therefore, it is possible that the establishment of *P. taylori* populations is most dependent on chance events caused by shifts in flow regimes or ship traffic. Since these organisms are highly tolerant of a range of environmental characteristics and feed unselectively on algae, diatoms, and bryozoans (Beeman, 1966; DeLorenzo, 1999; Lewis and Boyer, 2014), even predicting the environment in which they flourish could have little to do with the environment itself. This study provides clear evidence for the need to investigate the population genetics of this species across the latitudinal and local spatial scales. Based on the results of this study, chance events of population establishment and no patterns in population relatedness on a spatial gradient are expected.

Other environmental factors including salinity, O_2 concentration, pH, and nutrient levels were omitted from this study due to field equipment constraints. Inclusion of these parameters would have strengthened the models, as estuarine systems fluctuate heavily in these factors on daily through yearly time scales. However at least for the models predicting *P. taylori* high density or temporal abundance, the inclusion of these factors may have had a negligible effect. A study by Smart et al., 2012 found little influence of many of these environmental indicators on reproductive phenology in estuarine polychaetes. The high amplitude and frequency of fluctuations in these parameters may be responsible for the evolution of extreme tolerance in estuarine animals, resulting in little predictive power of a population's growth (Elliott and Whitfield, 2011). When considering low density or population establishment of *P. taylori*, these factors may play a larger role.

Many of the sites selected for this study were in close proximity to ports and other avenues of high marine traffic. Since *P. taylori* does not have a large-scale dispersal mechanism in any life stage, transport via drift eelgrass blades in ballast water or even on the hulls of ships is a possibility. Looking at the volume and origin of marine traffic to an area around the *P. taylori* habitat may give clues to the probability of a population establishment and subsequent low density of *P. taylori* if in relatively early stages of population growth. More work needs to be done on how *P. taylori* is able to disperse and establish new populations. Within-bay estimations of dispersal can be accomplished using hydrodynamic models incorporating flow, salinity, and temperature, but latitudinal-scale dispersal is best informed by a survey of population genetics. Previous observations by field biologists have indicated many possible phenotypes of *P. taylori*, which is indicative of high levels of isolation between populations (Beeman, 1963).

P. taylori population persistence and expansion in the face of climate change

All ecological, landscape, and abiotic factors included in all models are subject to the effects of climate change and anthropogenic impacts. As the eelgrass substrate, *Z. marina*, is a widespread species, shifts in mean temperature with expected anthropogenic CO_2 inputs to the atmosphere are not expected to negatively impact the species. In fact, increased growth is expected with warming (Thom et al., 2014). If eelgrass growth is not outpaced by epiphytic algal growth, ecological factors measured in this study will not be negatively impacted. Epiphytic growth, however, is influenced by a myriad of factors including temperature, nutrients, and flow, to name a few (Williams and Ruckelshaus, 1993). It is unclear how climate change will impact epiphytic growth specifically, but it is generally accepted that it will increase with warming (Howard and Short, 1986). Land use and runoff rates will also change as coastal cities become more crowded and adequate land for agriculture will become sparse. The temperate climate of coastal shores makes them highly desirable for many human uses, so it is reasonable to expect significant coastal land use changes in watersheds feeding eelgrass beds. Additionally, water use – both with increasing infill and/or boat traffic – could play a large role in the existence of these eelgrass beds. All of these changes make a future for *P. taylori* populations uncertain; however, even with current levels of anthropogenic impacts on the climate and land and water use, *P. taylori* still persist at their current population abundance, barring extreme weather events. From this study, the effects of climate change were expected to act on a population-specific level, furthering the genetic and physiological divergence expected in existing populations. While some populations may flourish, others may face local extirpation. The predictive power of the models created here is not great enough to determine future impacts at the population level. Another thing to consider is the episodic nature of this sea hare species in San Francisco Bay, documented by K. Boyer (*personal communication*) and the author. Since it is unclear why populations are ephemeral and the geographic extent of this phenomenon, it could mean that the models described here are valid under an environmental regime that has not undergone catastrophic events, like flooding. These extreme events have the potential to further bottleneck populations and reduce available genetic variation to withstand future climatic shifts.

Conclusion

P. taylori is a significant primary consumer of epiphytic material in *Z. marina* beds in estuaries of the Northeastern Pacific Ocean (Beeman, 1963; Lewis and Boyer, 2014). Its role in the ecosystem is straightforward as a grazer on light-inhibiting epiphytes growing on eelgrass blades, but the prediction of where populations will be established and how they are maintained depends on a number of abiotic, ecological, and landscape parameters. It is important to consider both the spatial and temporal models in planning for *P. taylori* population establishment in restoration sites. Since the two models indicate very different parameters, they can inform appropriate efforts in different stages of restoration: site selection is important but so is maintenance of eelgrass health until *P. taylori* population establishment. These principles are applicable for many types of eelgrass-associated grazers, and future studies would benefit from investigating additional grazer-plant interactions in SAV restoration efforts.

Anthropogenic land use plays a large part in *P. taylori* presence, whether that is because of increased nutrients facilitating epiphytic growth or because of increased runoff inhibiting *P. taylori* settlement. Surprisingly, ecological characteristics of habitat structure and resources were least important in a survey of *P. taylori* presence at high and low densities. Ecological indicators are more important in a temporal analysis of abundance, and likely reflect seasonal fluctuations in natural population dynamics over time. The potential bias in the best fit models is a reflection of limited dispersal mechanisms in *P. taylori* and the possible role of random population establishment and resulting genetic drift or divergent selection between sites. This study provides a foundation for future modeling efforts investigating how estuarine invertebrates respond to many environmental factors and what this can mean for restoration efforts. Future models should include additional abiotic factors, especially when considering the impacts on organismal physiology. Other future studies should focus on relatedness between populations and divergence distance in evolutionary time to best explain *P. taylori* populations' distribution across the coast of the western United States. Even with random population establishment as a major unknown factor, predicting the persistence of *P. taylori* populations into the future will rely on a combination of all types of parameters within the eelgrass ecosystem. This study establishes that the spatial placement and health of eelgrass beds can influence the types of fauna that are able to establish. Additionally, only 53% of current restoration sites in San Francisco Bay were indicated to support this species if it were established in those locales. When modeling the presence of *P. taylori*, this study makes a necessary step towards successful incorporation of associated invertebrate fauna into eelgrass restoration efforts.

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Competing interests

The author declares no competing interests.

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Data Accessibility

Data will be uploaded to SEANOE (http://www.seanoe.org) upon publication.

Table 1. Results from the generalized linear mixed effects model of *P. taylori* presence predicted by irrigated land coverage, bare soil coverage, and vegetated land coverage with a random effect for location.

Fixed Effects	*Estimate*	*Standard Error*	*df*	*z value*	*Pr (>|z|)*
Generalized linear mixed effects model (AIC = 17.7)					
Irrigated Land	37.584	8.170	1	4.900	4.22e-06
Bare Soil	58.297	9.069	1	6.428	1.29e-10
Vegetated Land	32.088	7.323	1	4.382	1.18e-05

Table 2. AICc results for all candidate models for predicting *P. taylori* presence.

Model fixed effects	*K*	*AICc*	*ΔAICc*	*Akaike Weight*	*Cumulative Akaike Weight*	*Log likelihood*
Irrigated Land, Bare Soil, Vegetated Land	5	17.8980	0.0000	0.5321	0.5321	-3.8434
Vegetated Land, Bare Soil	4	20.2601	2.3621	0.1633	0.6954	-6.0598
Concrete over 50%, Bare Soil, Vegetated Land	5	20.7868	2.88 89	0.1255	0.8209	-5.2878
Average Daily Variation, Average Temperature, Concrete over 50%	5	20.8720	2.9740	0.1203	0.9412	-5.3303
Protection, Average Temperature, Average Daily Variation	5	25.2469	7.3489	0.0135	0.9547	-7.5178
Bare Soil	3	26.0181	8.1201	0.0092	0.9638	-9.9671
Protection	3	26.0379	8.1399	0.0091	0.9729	-9.9770
Irrigated Land	3	26.1366	8.2386	0.0086	0.9816	-10.0263
Average Temperature	3	26.1433	8.2453	0.0086	0.9902	-10.0297
Concrete over 50%	3	26.1447	8.2468	0.0086	0.9988	-10.0304
Eelgrass Frequency, Epiphyte Coverage, Concrete over 50%, Bare Soil	6	30.1037	12.2058	0.0012	1.0000	-8.9035

Table 3. Results from the generalized linear mixed effects model of *P. taylori* high density presence. Two accepted candidate models are shown below, both including the factors of level of protection, average temperature, and daily variation in temperature, and one including bare soil as well.

Fixed Effects	*Estimate*	*Standard Error*	*df*	*z value*	*Pr (>|z|)*
Generalized linear mixed effects model (AIC=23.4)					
Protection	230.559	1.508	1	152.94	<2e-16
Average Temperature	195.051	1.441	1	135.39	<2e-16
Average Daily Variation	-52.281	1.798	1	-29.08	<2e-16
Bare Soil	60.653	2.007	1	30.23	<2e-16
Generalized linear mixed effects model (AIC=24.3)					
Protection	63.742	2.876	1	22.162	<2e-16
Average Temperature	58.577	3.939	1	14.871	<2e-16
Average Daily Variation	-43.368	3.143	1	-13.796	<2e-16

Table 4. AICc results for all candidate models for predicted high density *P. taylori* presence.

Model fixed effects	*K*	*AICc*	*ΔAICc*	*Akaike Weight*	*Cumulative Akaike Weight*	*Log likelihood*
Protection, Average Temperature, Average Daily Variation, Bare Soil	6	23.6788	0.0000	0.3701	0.3701	-5.6910
Protection, Average Temperature, Average Daily Variation	5	24.5151	0.8363	0.2436	0.6137	-7.1519
Protection	3	26.0074	2.3286	0.1155	0.7293	-9.9617
Average Temperature, Bare Soil	5	26.1981	2.5193	0.1050	0.8343	-9.0289
Average Temperature, Average Daily Variation, Bare Soil	5	27.6951	4.0162	0.0497	0.8840	-8.7419
Bare Soil	3	28.1229	4.4440	0.0401	0.9241	-11.0195
Protection, Bare Soil	4	28.1316	4.4527	0.0399	0.9640	-9.9956
Vegetated Land, Bare Soil	4	29.8465	6.1676	0.0169	0.9810	-10.8531
Concrete over 50%, Bare Soil, Vegetated Land	5	31.7052	8.0264	0.0067	0.9877	-10.7470
Irrigated Land, Bare Soil, Vegetated Land	5	31.8853	8.2064	0.0061	0.9938	-10.8370
Average Temperature	3	32.5984	8.9195	0.0043	0.9981	-13.2572
Eelgrass Frequency, Epiphyte Coverage, Concrete over 50%, Bare Soil	6	34.1732	10.4944	0.0019	1.0000	-10.9382

Table 5. Results from the linear mixed effects model of trends in *P. taylori* abundance over time.

Fixed Effects	*Estimate*	*Standard Error*	*df*	*t value*	*p value*
			Linear mixed effects model (AIC = 593.14)		
Eelgrass Length	0.467	0.083	178	5.643	0.0000
Eelgrass Density	0.218	0.078	178	2.786	0.0059
Epiphyte coverage	-0.178	0.080	178	-2.224	0.0274
Average Temperature	-0.425	0.076	178	-5.564	0.0000

Table 6. AICc results for all candidate models for predicting temporal trends in *P. taylori* abundance.

Model fixed effects	*K*	*AICc*	*ΔAICc*	*Akaike Weight*	*Cumulative Akaike Weight*	*Log likelihood*
Eelgrass Length, Eelgrass Density, Epiphyte Coverage, Average Temperature	9	594.1006	0.0000	0.8033	0.8033	-287.5716
Eelgrass Length, Eelgrass Density, Average Temperature	8	596.9144	2.8138	0.1967	1.0000	-290.0762
Eelgrass Length, Eelgrass Density	7	621.7953	27.6948	0.0000	1.0000	-303.6029
Eelgrass Length	6	630.6019	36.5013	0.0000	1.0000	-309.0811
Average Temperature	6	630.8019	36.7014	0.0000	1.0000	-309.1811
Eelgrass Density	6	634.5862	40.4857	0.0000	1.0000	-311.0732
Eelgrass Frequency	6	638.9047	44.8041	0.0000	1.0000	-313.2324

Figure 1. All locations surveyed for training dataset (see Table S1), where black dots indicate presence and white dots indicate absence of *P. taylori*. The San Francisco Bay Area is enlarged on the right.

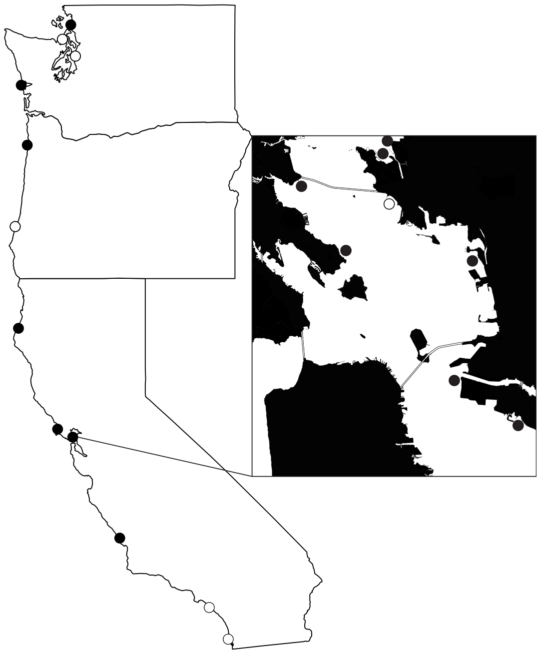

Figure 2. Test dataset results (see Table S2), where shapes indicate *P. taylori* presence as predicted by the model (triangle = present, circle = absent). Fill indicates observed *P. taylori* presence (filled = present, open = absent). Model and observation convergence results in filled triangles and open circles.

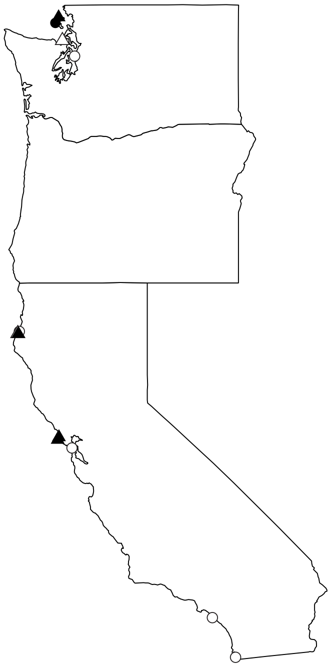

Figure 3. Predicted dataset results. Shapes indicate restoration stage of SAV restoration areas as notated in the San Francisco Bay Subtidal Habitat Goals Project (see Table S3) (circle = early, square = mid, triangle = late). Fill corresponds to predicted *P. taylori* presence (filled = present, open = absent).

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Supplemental Tables and Figures:

Table S1. Training dataset. All locations and dates surveyed used in initial model generation including presence/absence and quantity of *P. taylori* found. A maximum of 60 individuals were collected (quantity found) for each site.

Location	*Latitude, Longitude*	*Survey Date*	*Presence?*	*Quantity Found*
Albany, SF Bay, CA	37.887288, -122.313838	October 2016	Y	16
Bay Farm Island, SF Bay, CA	37.730752, -122.313838	September 2016	Y	60
Coos Bay, OR	43.338468, -124.328617	June 2016	Y	52
Crown Beach, SF Bay, CA	37.767329, -122.273053	September 2016	Y	60
Golden Gardens, WA	47.694463, -122.328617	July 2016	N	n/a
Grays Harbor, WA	46.951370, -124.128255	July 2016	Y	12
Humboldt Bay, CA	40.739294, -124.219544	May 2016	Y	60
Keller Beach, SF Bay, CA	37.922400, -122.386291	August 2016	N	n/a
Morro Bay, CA	35.377431, -120.854108	May 2016	Y	60
Netarts Bay, OR	45.420402, -123.927379	May 2016	N	n/a
Newport Bay, CA	33.603817, -117.881045	June 2016	N	n/a
Padilla Bay, WA	48.493725, -122.480585	June 2016	Y	2
Paradise Park, SF Bay, CA	37.893315, -122.455010	August 2016	Y	14
Point Molate, SF Bay, CA	37.957559, -122.415400	June 2016	Y	60
Point Orient, SF Bay, CA	37.957559, -122.420944	October 2016	Y	60
Port Townsend, WA	48.124789, -122.759617	July 2016	N	n/a
San Diego, CA	32.793338, -117.250047	June 2016	N	n/a
San Quentin, SF Bay, CA	37.943129, -122.484621	August	Y	1
Tomales Bay, CA	38.162204, -122.918881	September 2016	Y	60

Table S2. Test dataset. Locations used for testing the presence/absence model.

Location	*Observation Source & Date*	*Latitude, Longitude*	*Predicted Presence*	*Observed Presence*
Carkeek Park, WA	R.L. Tanner, July 2016	47.711006, -122.380629	N	N
Drakes Estero, CA	Amelia Ryan, iNaturalist.org, July 2016	38.068242, -122.933793	Y	Y
Eureka, CA	R.L. Tanner, June 2016	40.790912, -124.187660	N	N
Friday Harbor, WA	Brenna Green, iNaturalist.org, June 2016	48.545823, -123.010173	N	Y
Humboldt Bay, CA	Robin Agarwal, iNaturalist.org, June 2016	40.736447, -124.238566	Y	Y
Humboldt Bay, CA	R.L. Tanner, June 2016	40.726649, -124.220561	Y	N
Newport Bay, CA	R.L. Tanner, June 2016	33.596329, -117.878448	N	N
Orcas Island, WA	Sarka Martinez, iNaturalist.org, July 2016	48.688922, -122.903633	Y	Y
Port Townsend, WA	R.L. Tanner, July 2016	48.103600, -122.780453	Y	N
San Diego, CA	R.L. Tanner, June 2016	32.599481, -117.121473	N	N
Sausalito, CA	R.L. Tanner, August 2016	37.861615, -122.466100	N	N
Tomales Bay, CA	Bill Miller, iNaturalist.org, September 2016	38.132531, -122.893351	Y	Y

Table S3. Predicted dataset. Restoration locations as described in the San Francisco Bay Subtidal Habitat Goals Project interactive map (sfbaysubtidal.org/map_portal/subrest.html) and predicted *P. taylori* presence using the model. Restoration stages are defined as early (E, stage 1), mid (M, stage 2-3), and late (L, stage 4-5) using the legend on the interactive map (stages 1-5). All locations are within San Francisco Bay, CA.

Location	*Latitude, Longitude*	*Restoration Stage*	*Predicted Presence*
Carquinez Straight	38.066927, -122.207950	E	N
SE San Pablo Bay	38.027097, -122.279831	E	N
China Camp	38.003841, -122.468881	E	N
North Richmond Shoreline	37.970401, -122.395697	M	Y
San Rafael Bay	37.954685, -122.487834	L	Y
Corte Madera Bay	37.930763, -122.498799	L	N
Albany/Berkeley	37.887397, -122.318440	M	Y
Richardson Bay	37.889550, -122.489333	M	N
Horseshoe Cove	37.833546, -122.476058	E	Y
Emeryville	37.827829, -122.306509	M	Y
Oakland Middle Harbor	37.803625, -122.326611	L	N
Alameda Naval Air Station	37.770212, -122.299990	E	N
Pier 94-98 Region	37.735962, -122.373553	E	N
Hayward Regional Shoreline	37.689590, -122.183988	E	Y
Coyote Point North	37.591881, -122.326517	E	N
Coyote Point South	37.587528, -122.312670	E	Y
Eden Landing Ecological Reserve	37.588993, -122.148129	E	Y

Table S4. Source data for temperature records. NOAA NDBC is the National Oceanic and Atmospheric Administration's National Data Buoy Center (ndbc.noaa.gov), NERR CDMO is the National Estuarine Research Reserve's Centralized Data Management Office (http://cdmo.baruch.sc.edu/get/export.cfm), and SFBEAMS is the San Francisco Bay Environmental Assessment and Monitoring Station (sfbeams.sfsu.edu).

Field Site	*Station Name*	*Station Network*	*Station GPS Coordinates*	*Field Site GPS Coordinates*
Albany, CA	PPXC1 Point Potrero, CA	NOAA NDBC	37.906, -122.365	37.887, -122.313
Bay Farm Island, CA	AAMC1 Alameda, CA	NOAA NDBC	37.772, -122.300	37.730, -122.313
Coos Bay, OR	CWQO3 Charleston, OR	NERR CDMO via NOAA NDBC	43.338, -124.321	43.338, -124.328
Crown Beach, CA	AAMC1 Alameda, CA	NOAA NDBC	37.772, -122.300	37.767, -122.273
Golden Gardens, WA	46120 Pt Wells, WA	NOAA NDBC	47.761, -122.397	47.693, -122.406
Grays Harbor, WA	WPTW1 Westport, WA	NOAA NDBC	46.904, -124.105	46.951, -124.128

Humboldt Bay, CA	HBXC1 Humboldt, CA	NOAA NDBC	40.777, -124.197	40.739, -124.219
Keller Beach, CA	PPXC1 Point Potrero, CA	NOAA NDBC	37.906, -122.365	37.920, -122.386
Morro Bay, CA	MBXC1 Morro Bay, CA	NOAA NDBC	35.370, -120.858	35.377, -120.854
Netarts Bay, CA	TLBO3 Tillamook, OR	NOAA NDBC	45.555, -123.919	45.416, -123.937
Newport Bay, CA	OHBC1 Los Angeles, CA	NOAA NDBC	33.720, -118.273	33.602, -117.882
Padilla Bay, WA	BVQW1 Padilla Bay, WA	NERR CDMO via NOAA NDBC	48.496, -122.501	48.494, -122.492
Paradise Park, CA	Romberg Tiburon Center Pier Tiburon, CA	SFBEAMS	37.891, -122.447	37.893, -122.455
Point Molate, CA	RCMC1 Richmond, CA	NOAA NDBC	37.923, -122.410	37.957, -122.415
Point Orient, CA	DPXC1 San Pablo Bay, CA	NOAA NDBC	38.056, -122.264	37.957, -122.420
Port Townsend, WA	PTWW1 Port Townsend, WA	NOAA NDBC	48.111, -122.760	48.132, -122.761
San Diego, CA	SDBC1 San Diego, CA	NOAA NDBC	32.714, -117.174	32.785, -117.248
San Quentin, CA	SCQC1 China Camp, CA	NERR CDMO via NOAA NDBC	38.001, -122.460	37.942, -122.481
Tomales Bay, CA	BDXC1 Bodega, CA	NOAA NDBC	38.317, -123.071	38.162, -122.918

Table S5. All models utilized the same initial full model list of explanatory parameters. One quadrat area was 0.25 m^2 and one transect length was 30 meters, with quadrats placed every ten meters starting at zero.

Parameter	*Units*
Eelgrass Frequency	Number of meters with eelgrass presence in one transect
Eelgrass Length	cm (res. 5 cm) within the quadrat
Eelgrass Density	Turions/quadrat
Epiphyte Percent Coverage	% (res. 10%)
Average Temperature	ºC averaged over 2015*
Average Daily Variation	ºC (calc. by the difference between the maximum and minimum temperature on a given day) averaged over 2015*
Level of Protection	Categorical (0-2); unit-less
Irrigated Land	% of total land evaluated
>50% Water-Impervious Structures	% of total land evaluated (500mx500m)
<50% Water-Impervious Structures	% of total land evaluated (500mx500m)
Bare Soil	% of total land evaluated (500mx500m)
Vegetated	% of total land evaluated (500mx500m)

*For the temporal model, these parameters were averaged on a monthly, not yearly basis.

Figure S1. Average temperature and average daily variation in temperature across all field sites in the training dataset over the 2015 year.

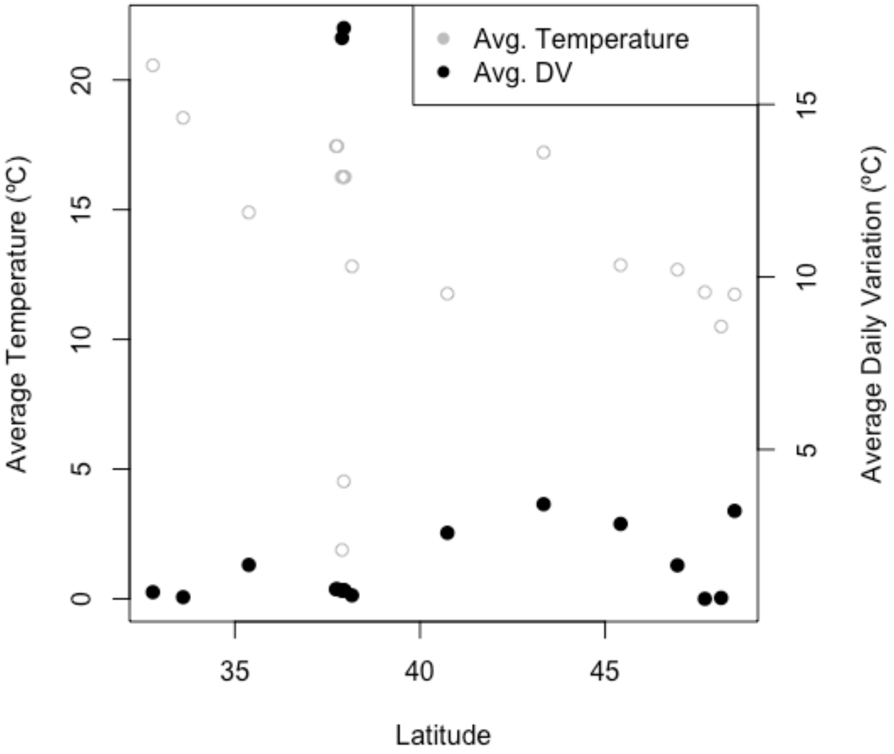


Figure S2. Categories for protection parameter illustrated in a simple graphic.

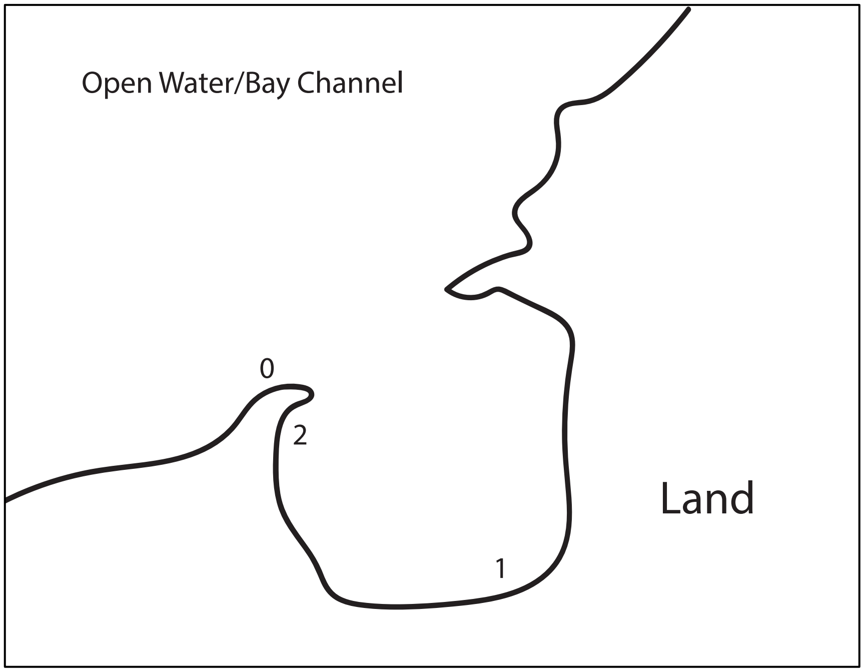


Figure S3. All land use parameters evaluated in the training dataset, expressed as a percentage of the total area surveyed. In order from darkest to lightest gray: irrigated land, <50% impervious structures, >50% impervious structures, bare soil, and vegetated land.

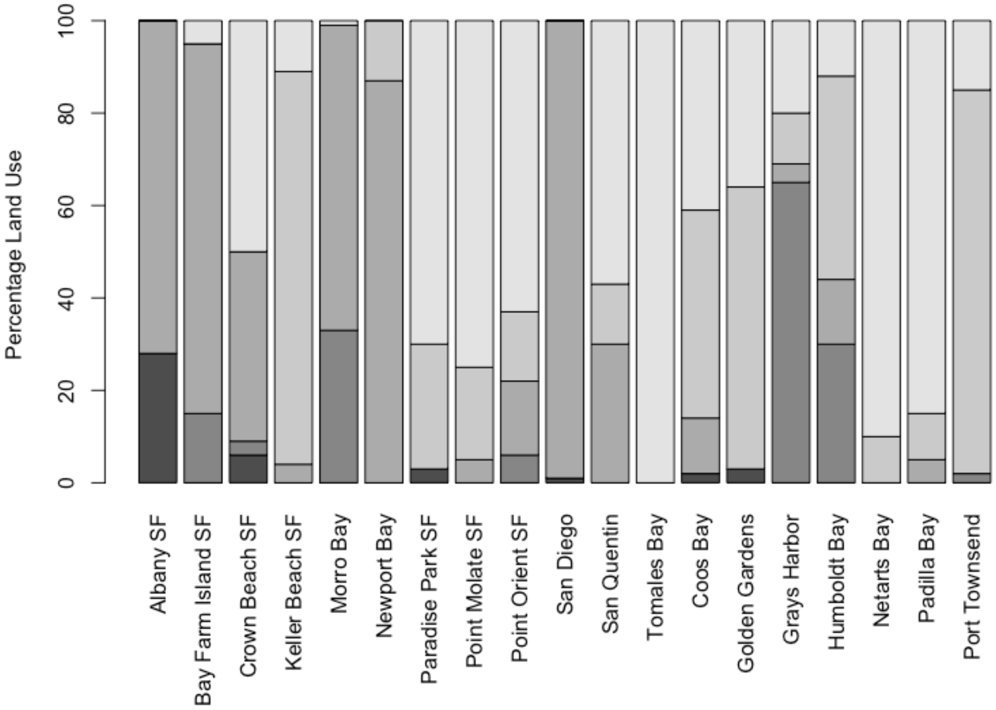


Figure S4. All land use parameters evaluated in the test dataset, expressed as a percentage of the total area surveyed. In order from darkest to lightest gray: irrigated land, <50% impervious structures, >50% impervious structures, bare soil, and vegetated land.

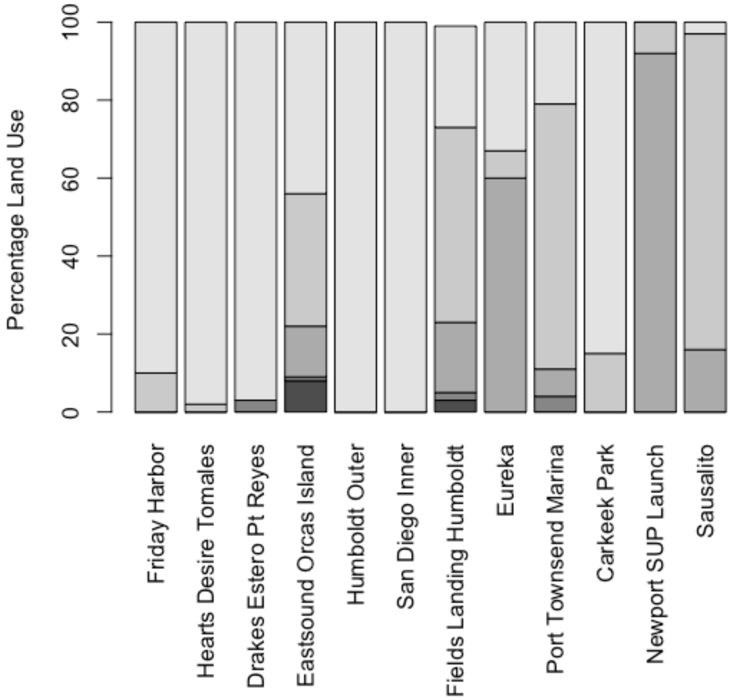


Figure S5. All land use parameters evaluated in the predicted dataset, expressed as a percentage of the total area surveyed. In order from darkest to lightest gray: irrigated land, <50% impervious structures, >50% impervious structures, bare soil, and vegetated land.

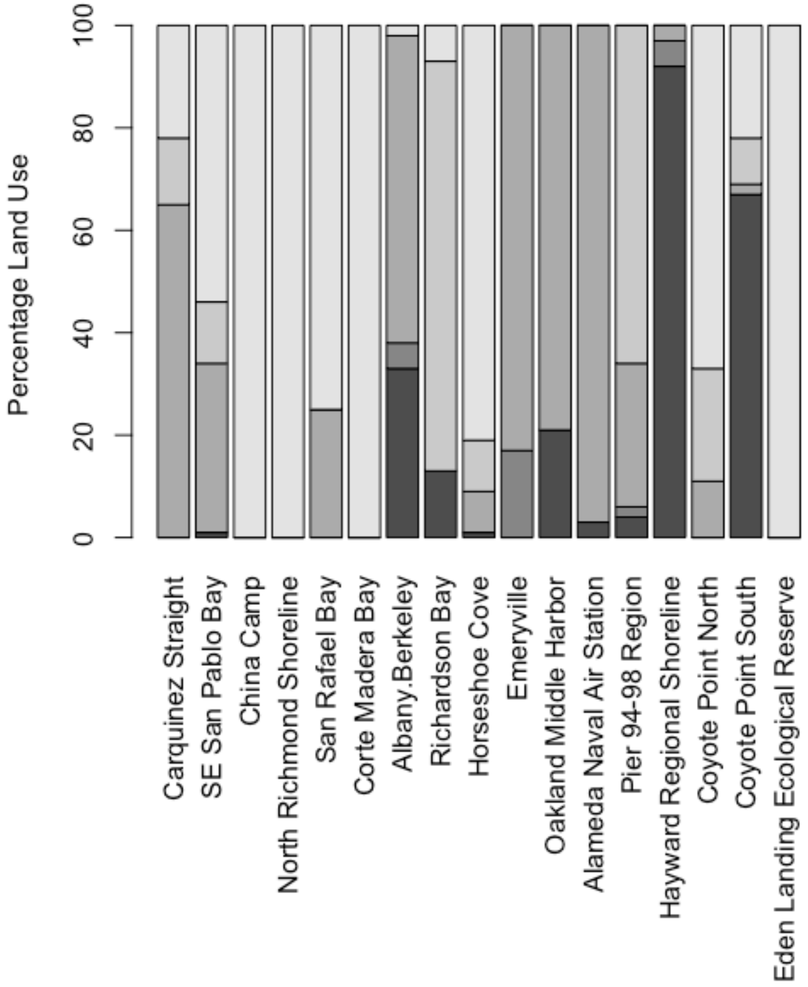
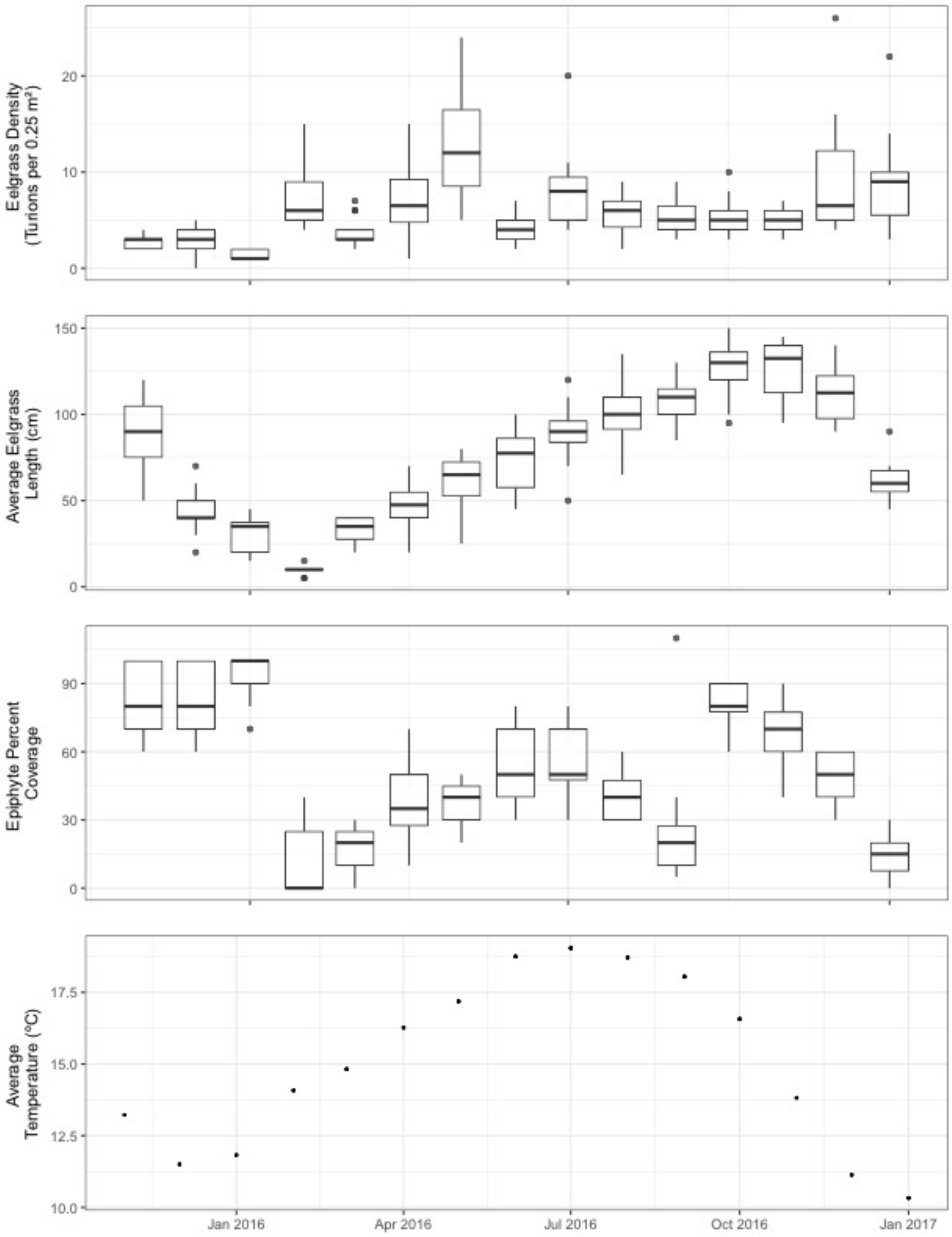


Figure S6. Parameters indicated in the best fit temporal model represented over time (November 2015 – January 2017). All data is from Point Molate, San Francisco Bay, CA.



Chapter 2 | Intraspecific variation in thermal tolerance and temperature sensitivity of respiration rate in the eelgrass sea hare, *Phyllaplysia taylori*, along the Pacific coast of the United States

Abstract

Intertidal mollusks are subject to extreme temperature changes when low tides expose these nearly-sessile invertebrates to air on extremely hot or cold days. Local weather or microclimate causes variation in the frequency and severity of exposure to extreme temperatures across populations. Furthermore, climate change is expected to increase the magnitude and frequency of thermal extremes. Population and individual responses to variation in thermal means and extremes can be through long-term genetic or short-term plastic mechanisms. We investigated short-term plasticity in multiple populations of the direct-developing sea hare, *Phyllaplysia taylori,* to determine whether differences in microclimate influenced acclimation capacity. *P. taylori* were collected along the western US coast from Ocean Shores, WA to Morro Bay, CA and acclimated to winter, summer, and future summer temperatures. Populations from warmer locales exhibited metabolic suppression after heat stress when acclimated to high temperatures. Critical thermal maxima (CT_{max}), regardless of acclimation temperature, were well above average habitat temperatures across all habitats, even when considering average daily variation in habitat temperature (CT_{max} ranged from 24-35ºC, average=30.1±0.2ºC; average habitat temperature ranged from 12-20ºC, average=21±0.8ºC). Intraspecific variation in CT_{max} in this species was on the high end of values reported in the literature and was correlated with average habitat temperatures and the average daily variation in these temperatures experienced by populations, although the plasticity of this trait was not. This suggests that the plasticity of upper critical limits is not driven by habitat thermal history, and therefore may not be under positive selection.

Introduction

Warming is an ongoing threat to global ecosystems, with predicted average temperature increases of 4ºC, and even greater increases in the magnitude of extreme temperatures (IPCC, 2014). While the intertidal ecosystem regularly experiences great fluctuations in air and water temperature, an increase in the average habitat temperature and in the frequency of extreme temperatures will result in increased physiological stress for some intertidal invertebrates (Dahlhoff et al., 2002; Helmuth et al., 2002; Helmuth et al., 2006; Byrne and Przeslawski, 2013). Physiological stress can be characterized by aspects of performance over a range of temperatures (i.e. critical thermal limits, thermal optima, and thermal breadth). These measures of performance can be plastic, meaning they shift with acclimation or acclimatization to different temperature regimes. The upper critical thermal limit and its plasticity across acclimation temperatures is a useful metric for determining how organisms that experience highly fluctuating environments will fare with increases in average and extreme temperatures (Helmuth et al., 2002; Gunderson and Stillman, 2015; Faria et al., 2017). It is also important to understand the mechanism behind these limits, and whole-body metabolic rate can give an idea of the physiological strategy organisms are employing to achieve certain tolerance thresholds (Newell and Branch, 1980; Pörtner, 2002). Combined, critical limits and metabolic rate after recovery from these limits provide a picture of an organism's physiological strategy under thermal stress.

Since soft-bodied invertebrates lack morphological barriers to desiccation (i.e. hard exoskeletons and shells) and have a limited ability to behaviorally thermoregulate, those living in variable thermal environments have evolved high physiological plasticity in their critical thermal limits (Marshall et al., 2010; Marshall et al., 2011; Polgar et al., 2015). While thermal tolerance among populations may vary due to high plasticity in the trait, the underlying mechanism allowing for this plasticity may remain conserved. Understanding how ocean warming and an increase in extreme temperatures will impact this group of organisms will provide a deeper knowledge of how intertidal ecosystems will be affected by future change, both on the organismal and community scale.

Phyllaplysia taylori (Dall, 1900), an inter- and subtidal sea hare (Aplysiidae: Mollusca), was selected for this study because its habitat is characterized by a highly variable thermal environment, both in space and time. *P. taylori* historically occupies bay habitats along the western coast of North America from Vancouver, B.C. to Baja California (Beeman, 1963). It lives on *Zostera marina* eelgrass blades, mimicking the green color and stripes, and feeds on encrusting epiphytes composed of algae, diatoms, bacteria, sponges, and bryozoans growing on eelgrass blades (Beeman, 1963). They play a significant role as grazers in the eelgrass ecosystem and are an integral component in maintaining eelgrass health (Lewis and Boyer, 2014). *Phyllaplysia taylori* are also notable for their direct development, as they are the only species in their group of ~80 anaspideans that do not spawn pelagic larvae (Beeman, 1966). This reproductive tactic is common in other estuarine invertebrates, as suitable conditions are more likely close to parental habitats than within the farther reaches of the currents carrying waters to the open ocean (Johannesson, 1988; Smart et al., 2012). Limits on dispersal capacity may result in differences between populations' physiological traits due to differences in habitat characteristics.

This study investigated the correlation between habitat characteristics and thermal tolerance traits in *P. taylori* in populations along the western US coast. We also evaluated the relationship between habitat characteristics and thermal tolerance on two spatial scales, on the western US coast (latitudinal) and in San Francisco Bay (hereafter SFB) to examine whether populations within a single estuary have the same capacity for differentiation as those separated by hundreds of kilometers. We expected to see similar variation in thermal tolerance traits on these two spatial scales, demonstrating that thermal tolerance scales with differences in site microclimate. While many terrestrial species' thermal tolerance scales with latitude, the intertidal zone does not follow the same predictable cline in average habitat temperature. Even though air temperatures are lower at higher latitudes, low tide is closely aligned with high noon at higher latitudes, meaning that the range of temperatures experienced by an animal at higher latitudes is comparable to an animal at lower latitudes in the intertidal zone (Helmuth et al., 2002; Stillman, 2003; van Heerwaarden et al., 2014). Additionally, limited dispersal would contribute to isolation of populations in different bays, even possibly within the same bay, and therefore differentiation of physiological traits determined by habitat characteristics.

This study addressed thermal tolerance using foot muscle critical thermal maxima (CT_{max}) and its acclimation response, and temperature sensitivity of metabolic rate using respiration rate pre- and post-heat stress. Investigation of thermal tolerance in this group of organisms cannot be achieved using methods that require shell attachment, a common metric used in similar studies of shelled mollusks; instead, foot muscle function was assayed for the determination of critical limits. Whole-organism metabolic rate was estimated using respiration rate, since critical limits were also assayed at the scale of the whole individual. Physiological thermal tolerance traits were expected to correlate with habitat microclimate, with responses to stressful acclimation temperatures positively related to increasing habitat temperatures. We predicted that metabolic rate in animals acclimated to high temperatures in the lab would be lower after heat stress, and that this strategy would be conserved across populations. Investigating short-term plasticity in these populations of sea hares can shed light on how microclimate may drive acclimation capacity.

Methods

Sea hares were collected from twelve sites on two spatial scales (western US coast, SFB) and acclimated to three temperatures in the lab for a minimum of 14 days. Individuals were then assessed for routine respiration rate at their acclimation temperature, their upper critical thermal limit of foot muscle function, and then routine respiration rate at their acclimation temperature again after the critical limit heat shock.

Collection and thermal acclimation

Phyllaplysia taylori were collected from twelve intertidal sites along the western coast of the United States (Fig. 1, Table S1) between June 2016 and November 2016. Animals were transported to the San Francisco State University Romberg Tiburon Campus at the Estuary and Ocean Science Center in Tiburon, CA in coolers with ice packs and bubblers. The maximum transport time was 48 hours and temperature range in coolers was 11-15ºC. In the laboratory, specimens from each site were held separately in aerated 13 L aquaria held in a water table at 13ºC. Water and epiphyte feeding screens (Faye et al. *in prep*) were changed every three days. Epiphyte feeding screens were grown in outdoor flow-through tables fed by SFB water. Following two days, specimens were divided evenly between three temperature treatments (13ºC, 17ºC, and 21ºC) and held at those temperatures from 14 to 21 days. These temperatures were selected to represent one non-stressful temperature typical of a winter thermal profile in the current climatic scenario, one potentially stressful temperature typical of a summer thermal profile in the current climatic scenario, and one temperature representative of summer temperatures under future climatic conditions (data collected by R.L.T., presented in Faye et al. *in prep*).

Abiotic data for individual collection sites

Water temperature data from March 2016 through November 2016 were downloaded from the National Data Buoy Center and the National Estuarine Research Reserve databases at the closest available stations to each field site (Table S1). If data were not available at the field site, data from the closest bay with similar physical structure and station placement were chosen. If no data were available in that respect, the proximal nearshore oceanic buoys were used. Maximum habitat temperature, average habitat temperature, and average daily variation in temperature were calculated over the two months preceding collection.

Measuring metabolic rate and CT_{max} *of P. taylori*

Respiration rate at acclimation temperature as a proxy for routine metabolic rate was measured before and after determination of critical upper thermal limits using a thermal ramp. Prior to the experiment, 70 mL respirometry vials with planar optode spots (diameter 0.5 cm; PreSens Precision Sensing GmbH) were calibrated at each temperature treatment using a 2% sodium sulfite and deionized water solution for the 0% calibration and fully oxygenated seawater for the 100% calibration. On the day of measurement, specimens were photographed, placed into calibrated respirometry vials with and magnetic stir bars, and closed underwater to rid the vials of any air bubbles. Respirometry vials containing specimens were incubated at the acclimation temperature of each specimen for 30 minutes in a circulating water bath. Oxygen consumption was measured every 20 minutes for two hours using a Presens Fibox 3 (PreSens Precision Sensing GmbH; PST6v541 software). Vials with no sea hares (control "blanks") were run in tandem with each group.

Immediately following respirometry, specimens were placed on the glass walls of 250 mL beakers filled with continuously aerated seawater at the initial acclimation temperature. These beakers were placed in a circulating water bath set to the acclimation temperature for each specimen. After a 30-minute recovery, the temperature of the water bath was raised at a rate of 4ºC/hour (Stillman and Somero, 2000). The time and temperature at which specimens fell off the glass walls and did not respond to mechanical stimulation of their foot with a wooden probe was recorded as the maximal critical temperature (CT_{max}). This critical upper thermal limit is indicative of the temperature at which sea hares cannot perform their ecological roles as grazers since they cannot grasp their substrate and are likely to be washed away in flow. Specimens recovered at their acclimation temperature for one hour from the time that the last specimen fell (total time between first and last specimens to fall did not exceed 30 minutes). After recovery, specimens that were able to right themselves were returned to their respirometry vials and their respiration rates at their acclimation temperatures were recorded a second time. After two hours of respirometry, specimens were blotted to remove excess water, weighed, and frozen at -80ºC for future genetic analyses. Respiration rates were calculated as oxygen consumption over time corrected for body mass (it was assumed that body mass did not change between the first and second respirometry trials).

Statistical analyses

Respiration rates were analyzed among acclimation temperatures prior to the CT_{max} heat shock (HS) and as the difference between pre- and post-HS rates at each acclimation temperature (pre- subtracted from post-HS rates, where a negative number indicates downregulation of metabolic rate and a positive number indicates upregulation of metabolic rate). Thermal tolerance (CT_{max}) range was estimated by determining the difference between CT_{max} at 13ºC and 21ºC for each population. Acclimation response ratio (ARR) was estimated using the slope of the line relating CT_{max} at all three temperatures for each population (Gunderson and Stillman, 2015). Thermal sensitivity of mass-specific V_{O2} (Q_{10}) was calculated for each population as:

$$Q_{10} = \left(\frac{V_{O_{2(2)}}}{V_{O_{2(1)}}}\right)^{10 \div (T_2 - T_1)} \tag{1}$$

Where T_1 and T_2 were the outer bounds of the acclimation temperatures used in this experiment (13°C and 21°C) and V_{O2} was estimated by whole-organism respiration rate.

All analyses were conducted in R v. 3.4.1 (R Team Core, 2017) with the *lme4* and *nlme* packages (Bates et al., 2015; Pinheiro et al., 2017). Comparisons across latitudinal and SFB datasets were tested for statistically significant differences using the Welch two sample t-test assuming unequal variance for all physiological metrics: CT_{max}, CT_{max} range, ARR, and Q_{10}. Differences between populations were evaluated using the Kruskal-Wallis χ^2 test, due to kurtosis and heteroscedasticity. In analyzing the latitudinal dataset independently from the SFB dataset, Pt. Molate, SFB was used as the SFB representative.

Results

Thermal tolerance thresholds (CT_{max})

The amount of variability in CT_{max} in latitudinal and SFB datasets was comparable; latitudinal populations' CT_{max} in the 13ºC acclimation groups spanned 24-35ºC and SFB populations' CT_{max} in the same acclimation conditions spanned 26.2-34.4ºC (Fig. 2). CT_{max} across acclimation temperatures did not differ significantly between the latitudinal and SFB datasets (Welch two sample t-test: $t = -0.871$, $df = 5.39$, p-value = 0.42). The acclimation capacity of CT_{max} did not systematically change with latitude (regression slope = 0.210, $R^2 = 0.16$) and averaged 1.74ºC ± 0.45 (mean ± SE). Regardless of acclimation conditions, CT_{max} also did not systematically change with latitude (regression slopes = -0.0052, -0.012, -0.0041; $R^2 = 0.52, 0.63, 0.06$, respectively). Latitudinal and SFB datasets were combined, after transformation, to examine trends in the relationship between CT_{max} and habitat temperature irrespective of latitude. Since latitudinal specimens were collected within a 1-month period and all SFB samples were collected within a 1.5-month period, 3 months apart, data were standardized to account for differences (Welch two sample t-test: $t = 3.84$, $df = 7.99$, p-value < 0.01) in seasonal temperature and generation by dividing each point by the average of each dataset. CT_{max} was not correlated with average habitat temperature at any one acclimation condition, although there was a positive trend in the 13ºC acclimation group (regression slope = 0.35, $R^2 = 0.28$, p-value = 0.09). There was no relationship between CT_{max} in the 21ºC acclimation group and average daily variation in habitat temperature, contrary to our hypothesis. CT_{max} in the 13ºC acclimation group was significantly negatively correlated with average daily variation in habitat temperature (regression slope = -0.54, $R^2 = 0.43$, p-value = 0.03, see Fig. 4A). Average habitat temperature for each population was not always closely aligned with the coldest acclimation condition.

To make comparisons of CT_{max} relevant to habitat temperature (relevant CT_{max}), populations' habitat temperatures were binned into the 13ºC, 17ºC, and 21ºC groupings used in the lab acclimations. In averaging habitat temperatures over the two-month period preceding collection, we encompassed the entire non-embryonic lifespan of each sea hare population. Therefore, it is possible that developmental plasticity, not just reversible short-term plasticity, was influenced by acclimatization to average habitat temperature. If a population was acclimatized to a temperature close to 21ºC in the field, then CT_{max} measured in the 21ºC acclimation group was used for the comparison. There was a significant positive relationship between habitat temperature and the relevantly acclimated CT_{max} (regression slope = 0.57, $R^2 = 0.54$, p-value = 0.01, see Fig. 3A). Additionally, using CT_{max} estimated from a lab group acclimated to temperatures relevant to average habitat temperature provides a more accurate estimation of CT_{max} in the native environment. These data show that thermal tolerance limits closest to their habitat temperatures are correlated with habitat thermal characteristics, but not at acclimation temperatures independent of habitat temperature.

Acclimation capacity of CT_{max} (ARR)

Acclimation response ratios (ARRs) across populations differed significantly (Kruskal-Wallis $\chi^2 = 183.24$, $df = 9$, p-value < 0.0001). Populations from across the latitudinal range demonstrated similarly variable ARRs to those from SFB, though very few populations had linear ARRs through the three acclimation conditions. One population, Humboldt Bay, CA, displayed a near-perfect ARR across the entire range of acclimation temperatures (ARR = 0.45, $R^2 = 0.99$), which indicates that sea hares increased CT_{max} equally with each increase in acclimation temperature. ARRs ranged an order of magnitude across latitude from 0.04 in Morro Bay, CA to 0.45 in Humboldt Bay, CA (Fig. 2A). Similarly, ARRs also ranged an order of magnitude within SFB from 0.02 in Crown Beach, SFB, CA to 0.80 in Paradise Park, SFB, CA (Fig. 2B).

ARR did not significantly increase with latitude (regression slope = 0.02, $R^2 = 0.03$). ARR did not correlate with habitat temperature characteristics (average and average daily variation) (p-value > 0.05, Fig. 3B). All populations had the ability to increase their thermal limits with increasing acclimation temperature, indicated by a positive ARR across the entire 13ºC-21ºC range (Fig. 2A). This positive acclimation relationship was significant across all acclimation temperatures irrespective of population (linear mixed-effects model (REML) = 0.24, df = 175, p-value < 0.0001).

Thermal sensitivity of metabolism in pre- and post-stress scenarios

Average routine metabolic rate (MR) was measured before (pre-HS) and after (post-HS) the determination of upper critical limits, which constituted an acute heat shock. In the 17ºC and 21ºC acclimation groups, large shifts in MR post-stress were associated with low average habitat temperatures and vice versa (regression slopes = -1.21 and -0.99, $R^2 = 0.73$ and 0.66, respectively). The average change in MR pre- and post-stress in the 17ºC and 21ºC acclimation groups was significantly correlated with average habitat temperature (ANOVA, $F_{1,8} = 21.67$, p-value = 0.002 & ANOVA, $F_{1,6} = 11.4$, p-value = 0.02, respectively; see Fig. 5). Q10 of respiration rate, evaluated between the 13ºC and 21ºC acclimation groups, shifted within and across populations after heat stress, but not in a consistent pattern (see Table 1, ANOVA, $F_{1,6} = 0.03$, p-value = 0.87).

MR itself within populations was not significantly correlated with average habitat temperature at any acclimation temperature. Overall, MR averaged 1.08 ± 0.10 mg O_2 hr $^{-1}$ and the range was 0-13.57 mg O_2 hr $^{-1}$. While Q10 evaluated before HS differed between populations, it did not relate to ARR (ANOVA, $F_{1,6} = 0.006$, p-value = 0.94). The same non-relationship was found in Q10 values for respiration rates after HS (ANOVA, $F_{1,6} = 2.10$, p-value = 0.20). Q10 values ranged from 0.95 to 4.68, with Morro Bay after HS as an outlier at 44.30 (Table 1). We do note that the use of Q10 in the case of these data may be inappropriate, as there is not a consistent relationship between temperature and respiration rate (Fig. S1).

Post-metabolic strategies were not consistent across the *P. taylori* populations. Five populations showed downregulation of MR after heat stress at all acclimation temperatures (Table 2). Two populations, Humboldt Bay, CA and Tomales Bay, CA, showed upregulation of MR during the post-stress scenario across acclimation temperatures (Table 2). The largest change in MR with heat stress was in the Morro Bay, CA 21ºC group (12.79 ± 3.45 mg O_2 hr $^{-1}$) and the smallest change in MR with heat stress was in the Paradise Park, SFB, CA 17ºC group (-0.06 ± 0.41 mg O_2 hr $^{-1}$). Overall, the populations that consistently downregulated MR with heat stress had a much more canalized response than those with other strategies (average standard error of downregulated MR populations = 0.17, average standard error of other MR strategy populations = 0.72, see Fig. S1).

Discussion

Populations of *P. taylori* vary widely in thermal tolerance limits and metabolic strategies after heat stress. Animals that experience higher average habitat temperatures have high critical limits when acclimated to those temperatures, although the ability to be plastic is not correlated with these limits. Even though there is a correlation between habitat temperature and relevantly acclimated CT_{max}, the upper critical limits of all populations are still well within future projected climatic regimes in all locales. Populations at these higher average habitat temperatures are also more likely to downregulate metabolic rate after heat stress, demonstrating an increased beneficial response to warm extremes. Additionally, the variability of upper critical limits among *P. taylori* populations is on the high end of values reported in the literature for other intraspecific comparisons across latitude. The physiological traits reported here highlight the need for further genetic investigation into the differentiation between populations of *P. taylori*.

Habitat thermal history predicts acclimation capacity in metabolic rate, but not tolerance limits

While the ability to increase tolerance of heat stress was not tied to habitat thermal history, the ability to downregulate metabolic rate as a recovery response was correlated. Since we expected that metabolic rate shifts were potential mechanisms for high heat tolerance, it was surprising to find that acclimation capacity in only one of the two metrics was tied to habitat temperature. Our findings suggest that there are additional unstudied factors that determine foot muscle function under heat stress, and that whole-body respiration rate is determined by a number of physiological processes. Q10 of MR also followed no specific patterns with other physiological traits measured or habitat temperatures, and may have been difficult to interpret due to the non-linear relationship between temperature and MR. There was also not a consistent response across populations in either metabolic rate or foot muscle function acclimation capacity, perhaps indicating that these traits are either not conserved on the species level or are not under heavy selection. Further studies should investigate the genetic underpinnings of these traits to determine why there appears to be a break in the link between metabolic rate and food muscle function.

On the species level, this study supports the beneficial acclimation hypothesis where an increased trait value correlates with increased plasticity of that trait (Huey and Berrigan, 1996; Huey et al., 1999; Wilson and Franklin, 2002), but the drivers of variation between populations in thermal tolerance plasticity are unclear. While certain characteristics of *P. taylori*'s thermal tolerance traits are tied to habitat temperature, results do not indicate that acclimation capacity is directly influenced by habitat temperature. Since average habitat temperature influences critical maxima in the acclimation group nearest to source habitat temperature and average daily variation in habitat temperature influences critical maxima in the non-stressful (13ºC) acclimation group, there is no current evidence of thermal history driving the acclimation capacity of CT_{max} among populations. These sea hares are capable of beneficial acclimation in the laboratory to varying degrees based on source population, but this phenomenon is not easily explained by thermal history or ecological habitat characteristics alone.

It is possible that the lack of correlation between habitat temperature and thermal tolerance is caused by limited dispersal potential resulting in discontinuous populations even within continuous eelgrass beds. This limited dispersal potential provides the opportunity for both local adaption and genetic bottlenecks, which can both serve to fix certain genotypes (Hellberg, 1996; Hoskin, 1997). While the former drives positive selection, the latter may result in neutral variation among populations. This could explain why there is no correlation between upper critical limits or the plasticity of upper critical limits and habitat temperature.

Patterns in MR after heat stress showed some acclimation capacity for upper acclimation temperatures, where populations with warmer thermal histories were able to downregulate MR with warm acclimation. Almost all populations were able to downregulate MR with heat stress at the non-stressful acclimation temperature (13ºC). This indicates that the beneficial strategy is most likely downregulation of MR with heat stress, which is similar to strategies in other intertidal mollusks such as limpets, which are able to temporarily arrest heart rate with heat stress (Bjelde and Todgham, 2013). Other mollusks like snails and mussels also show metabolic depression as a strategy for dealing with thermal stress (Sokolova and Pörtner, 2001; Anestis et al., 2008). For a species that is nearly sessile, this strategy is beneficial with predictable daily cycles in temperature with tides. Populations from higher latitudes are exposed to greater variation in temperature during summer daytime low tides (Helmuth et al., 2006), but our dataset did not suggest greater metabolic depression with increased temperature from these populations. No trends in latitude or average daily variation in habitat temperature with MR after heat stress were found.

High variability in intraspecific CT$_{max}$

Intraspecific variability in critical thermal maxima was unusually high in comparison with prior studies across ectotherms. For example, terrestrial ectotherms' upper thermal limits (e.g. *Drosophila sp.*) are not as plastic as lower thermal limits across latitudes (Addo-Bediako et al., 2000; Overgaard et al., 2011; Hoffmann et al., 2013). A study by Sorte et al., 2011 showed that across multiple marine ectotherm epibenthic taxa, populations did not differ in upper lethal limits by more than 2.1ºC even without acclimation. Intraspecific variation in killifish only resulted in a ~1.5ºC difference between northern and southern populations irrespective of acclimation temperature (Fangue et al., 2006). When considering non-molluscan intertidal invertebrates, crustaceans displayed slightly more intraspecific variation in critical thermal maxima: *Carcinus maenas* (~5ºC, (Kelley et al., 2011), *Pachygrapsus marmoratus* (no sig. difference, (Madeira et al., 2012)), and *Petrolisthes sp.* (3.7 ± 0.7ºC, (Stillman and Somero, 2000). For comparison, the difference between the least and most tolerant individuals' critical thermal maxima in different populations in this study was 11ºC. This magnitude of difference between intraspecific critical thermal maxima was only matched by other molluscan taxa: *Doriopsilla albopunctata, Okenia rosacea* (8.4ºC & 7.3ºC, respectively; Armstrong et al. *in prep*), and *Lottia limatula* (7ºC, Wang et al. *in review*). This does not appear to be a phylum-specific phenomenon, as *Nucella canaliculata* only had a 2ºC spread in LT$_{50}$ values among populations (Kuo and Sanford, 2009). Critical limits can vary widely depending on the physiological trait being measured, therefore, it is possible that foot muscle function in this group of organisms is controlled by more than one physiological mechanism, leading to greater variation in performance. While datasets are not completely comparable depending on the metric used to estimate CT$_{max}$, the data presented in this study are outside of the intraspecific CT$_{max}$ ranges in the literature across multiple taxa and warrant further investigation into the evolutionary mechanisms and/or advantages of this phenomenon.

P. taylori populations are well within their tolerance limits with predicted climate change

When compared with other intertidal invertebrates, *P. taylori* do not live as close to their maximal thermal extremes (Somero, 2002; Tomanek, 2010). Even though some populations showed higher critical maxima, all were well above projected future habitat temperatures. This mismatch of upper critical limits and habitat temperatures has been described in other aquatic snails (Polgar et al., 2015), however, it is more likely that population differentiation due to random dispersal events results in neutral variation, with selection not strong enough to act upon critical thermal maxima. Cross-taxa studies have shown that critical thermal minima (CT$_{min}$)

enact more selective pressure on thermal tolerance than critical thermal maxima (Addo-Bediako et al., 2000; Hoffmann et al., 2013). Future studies should investigate the roles of other performance metrics on population differentiation to discern drivers of selection in thermal tolerance in *P. taylori*.

Since each population displayed a different combination of physiological characteristics in both MR and foot muscle thermal tolerance, each population has a unique physiological profile. Future studies should test whether this profile is adaptive for each population's local environment by characterizing habitat thermal profiles and corresponding thermal performance curves at both the individual and population levels for each population of sea hares. More fine-scale measurements within the eelgrass beds in which these organisms reside would serve to better inform accurate thermal histories of the populations. Additionally, investigating the same performance metrics in different systems within the organism (i.e. cellular processes, heart rate) could shed more light on whether a certain thermal stress response profile is driven by environmental conditions.

As a general rule, populations that experience a higher average habitat temperature have greater CT_{max} estimates, indicating that adaptation is possible if the time scale is slow enough. Since this sea hare inherently has a wide range of tolerance across many environmental conditions, it is unlikely that any of these populations will suffer great reductions in population size or genetic diversity in the near future. However, populations that show a decrease in CT_{max}, or a negative ARR slope, from 17-21ºC will likely face future threats to their population numbers. The most extreme example in this dataset is with Morro Bay, indicating that future populations will not be able to withstand extreme spikes in temperature when the average temperature nears 21ºC.

Conclusion

Thermal tolerance plasticity in foot muscle function and metabolic rate differed across populations in no consistent pattern, however, there were some individual traits tied to average habitat temperature and average daily variation in habitat temperature. This study demonstrates the need for population genetic analysis to quantify dispersal rates and the likelihood of local adaptation to environmental condition in this directly developing species. In a warming ocean, these differential responses to temperature across populations could play a role in maintaining this species in at least a portion, if not all, of its historical range. While future studies are needed to better describe the genetic connectivity of these individual populations, we present here physiological evidence for high variability in thermal tolerance traits in populations of the sea hare, *P. taylori*.

Ethics Declaration

Specimens were collected under permits California SCP 13357, Oregon SCP 20342, and Washington SCP 16-328 issued to R.L.T.

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Competing Interests
The author declares no competing interests.

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Data Availability
Data to be deposited in SEANOE (https://www.seanoe.org).

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Table 1. Q10 values calculated from respiration rates at 13ºC and 21ºC for each population before and after heat stress. Corresponding ARRs are shown, but there was no significant relationship between the two metrics. Three populations (Ocean Shores, WA, Albany, SFB, CA, and Paradise Park, SFB, CA) are not shown, as there were no individuals acclimated to 21ºC in the laboratory.

Source Population	*Q10, before HS*	*Q10, after HS*	*ARR*
Coos Bay, OR	2.69	2.17	0.20
Humboldt Bay, CA	0.77	0.95	0.46
Tomales, CA	0.68	1.03	0.30
Pt. Orient, SFB, CA	1.16	2.02	0.09
Pt. Molate, SFB, CA	3.77	1.28	0.38
Bay Farm Island, SFB, CA	1.35	4.65	0.25
Crown Beach, SFB, CA	1.95	4.68	0.02
Morro Bay, CA	1.55	44.30	0.04

Table 2. Post-stress metabolic strategies across populations as shown by a change in respiration rate from pre- and post-stress respirometry. Units for change in respiration rate are mg O_2 hr^{-1}. Upregulation is defined by a positive change, downregulation is defined by a negative change. Average and SE is estimated by population.

Source population	*Acclimation temperature (ºC)*	*Change in respiration rate*	*Strategy*
Ocean Shores, WA	13	-0.22±0.46	Downregulation
Coos Bay, OR	13	-0.57±0.52	Downregulation
Humboldt Bay, CA	13	3.08±3.47	Upregulation
Tomales Bay, CA	13	0.19±0.82	Upregulation
Pt. Orient, SFB, CA	13	-0.82±0.96	Downregulation
Pt. Molate, SFB, CA	13	-0.21±0.38	Downregulation
Albany, SFB, CA	13	N/A	N/A
Paradise Park, SFB, CA	13	-0.39±0.19	Downregulation
Bay Farm Island, SFB, CA	13	-0.59±0.23	Downregulation
Crown Beach, SFB, CA	13	-0.54±1.37	Downregulation
Morro Bay, CA	13	-1.19±0.76	Downregulation
Ocean Shores, WA	17	N/A	N/A
Coos Bay, OR	17	-0.92±0.83	Downregulation
Humboldt Bay, CA	17	10.51±13.39	Upregulation
Tomales Bay, CA	17	1.13±2.75	Upregulation
Pt. Orient, SFB, CA	17	-0.38±0.43	Downregulation
Pt. Molate, SFB, CA	17	-0.55±0.22	Downregulation
Albany, SFB, CA	17	0.19±0.74	Upregulation
Paradise Park, SFB, CA	17	-0.06±0.72	Downregulation
Bay Farm Island, SFB, CA	17	-0.60±0.59	Downregulation
Crown Beach, SFB, CA	17	0.08±0.33	Upregulation
Morro Bay, CA	17	6.54±9.17	Upregulation
Ocean Shores, WA	21	N/A	N/A
Coos Bay, OR	21	-1.34±1.20	Downregulation
Humboldt Bay, CA	21	2.81±3.03	Upregulation
Tomales Bay, CA	21	0.37±0.58	Upregulation
Pt. Orient, SFB, CA	21	-0.73±98	Downregulation
Pt. Molate, SFB, CA	21	-1.25±1.31	Downregulation
Albany, SFB, CA	21	N/A	N/A
Paradise Park, SFB, CA	21	N/A	N/A
Bay Farm Island, SFB, CA	21	-0.34±0.38	Downregulation
Crown Beach, SFB, CA	21	-0.22±1.24	Downregulation
Morro Bay, CA	21	9.58±7.48	Upregulation

Figure 1. Map of all field sites on the latitudinal scale (left) and in SFB (right). Colors correspond to Fig. 2.

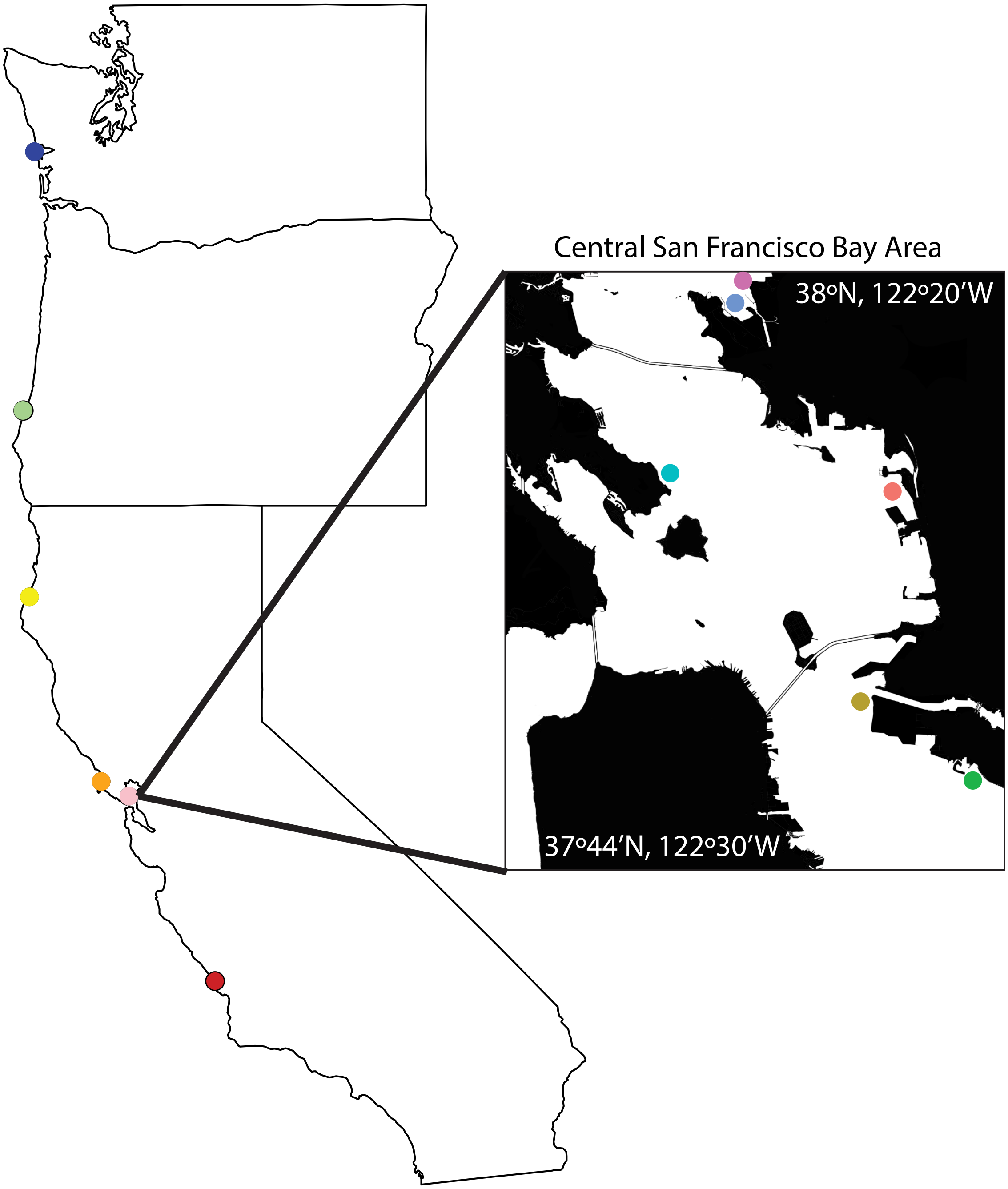


Figure 2. *P. taylori* CT_{max} for latitudinal (A) and SFB (B) populations over three acclimation temperatures encompass similar ranges regardless of spatial scale. In the latitudinal dataset, Pt. Molate, SFB was used as the SFB representative.

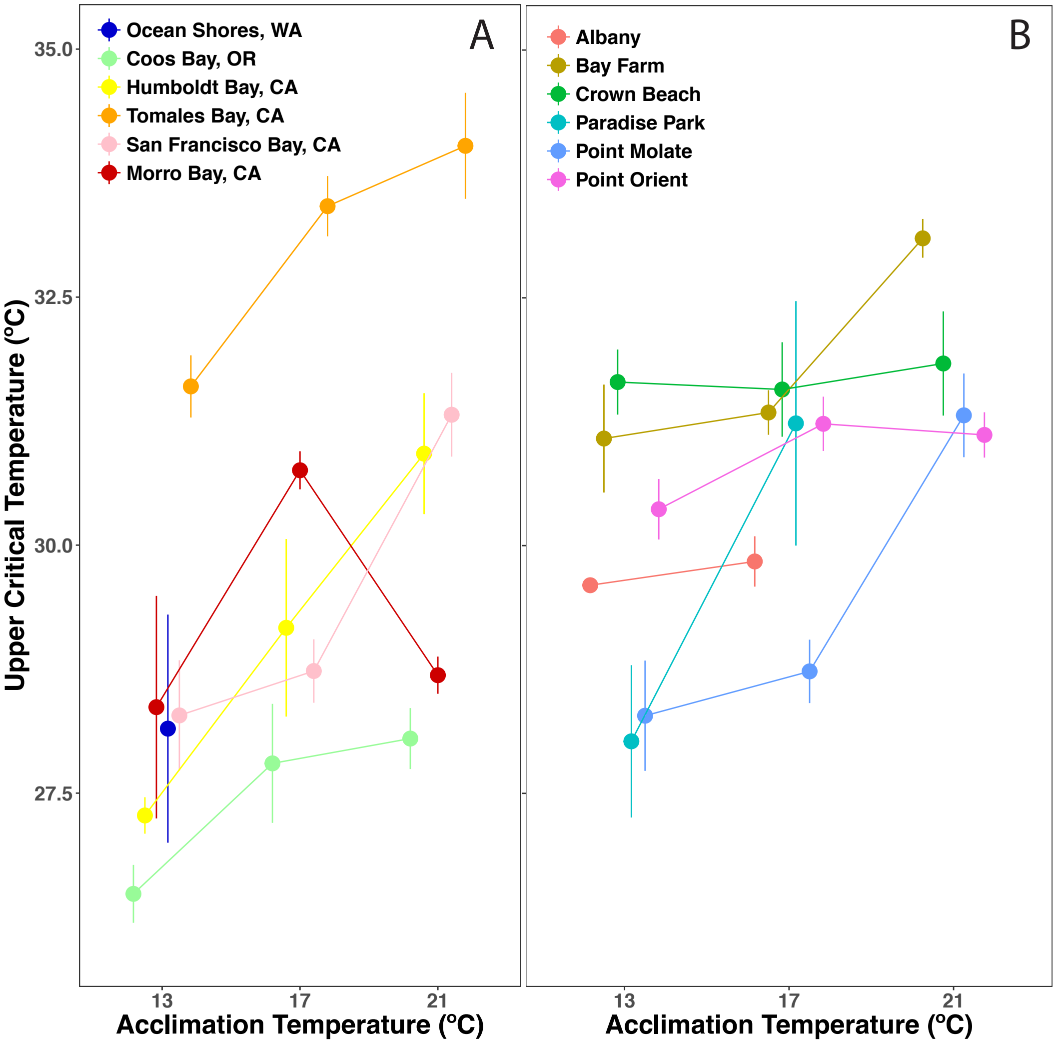


Figure 3. (A) A positive relationship between average habitat temperature in the two months preceding collection and *P. taylori* relevant $\mathrm{CT}_{\max}$ of each population. (B) No relationship between average habitat temperature and ARR of each population.

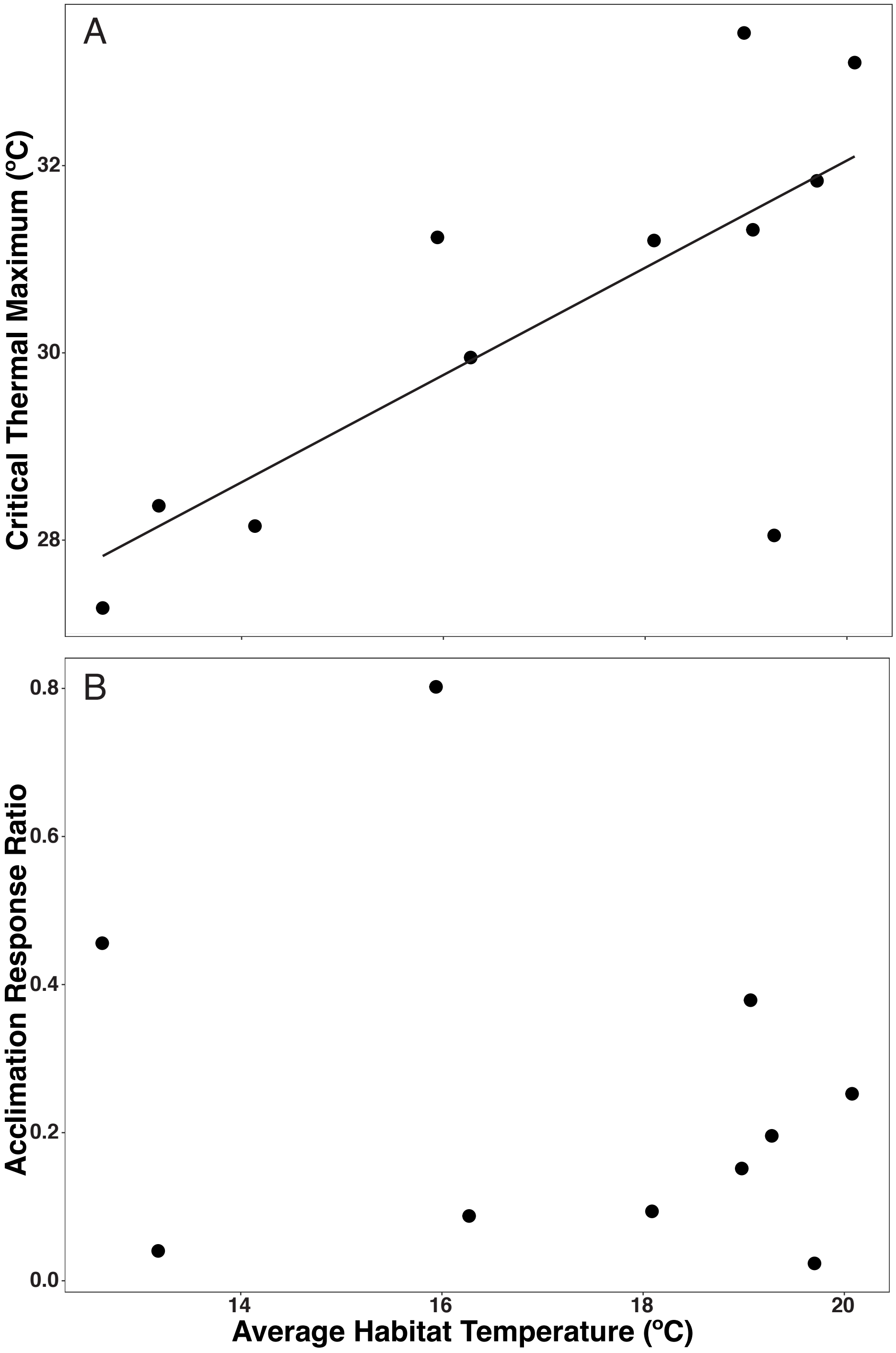


Figure 4. (A) A negative relationship between average daily variation in habitat temperature in the two months preceding collection and *P. taylori* CT_{max} of each population at the 13ºC acclimation temperature. (B) No relationship between average daily variation in habitat temperature and ARR of each population.

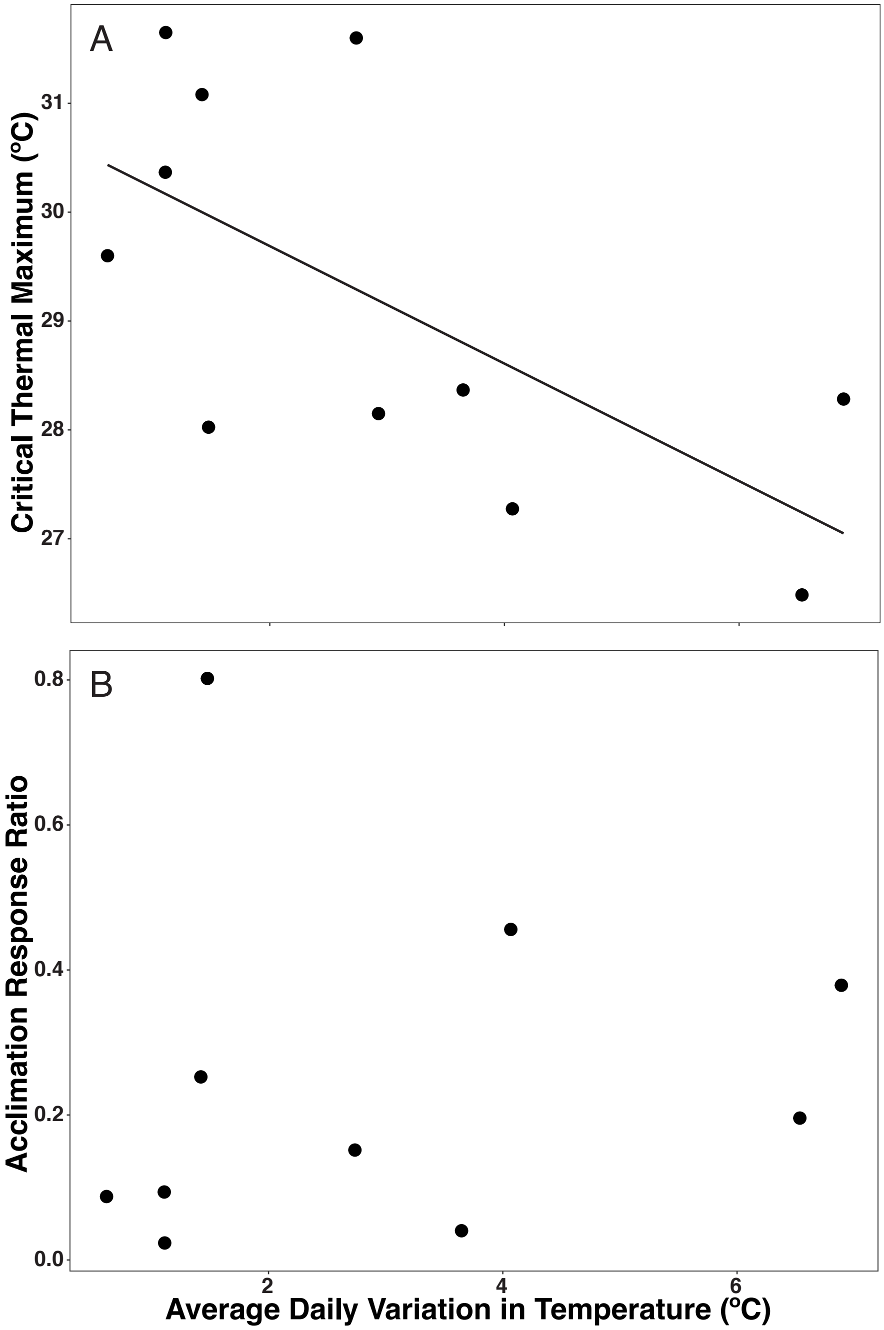


Figure 5. The shift in respiration rate (mg O_2 hr^{-1}) after heat stress has a negative relationship with average habitat temperature at the two upper acclimation temperatures, (B) 17ºC and (C) 21ºC, but not (A) 13ºC. The grey line indicates no change in respiration rate after heat stress, where all values below indicate downregulation of respiration rate and all values above indicate upregulation of respiration rate. Sample size for the 21ºC acclimation group was reduced by 2 (13ºC: n=10, 17ºC: n=10, 21ºC: n=8), resulting in a bias towards higher average habitat temperatures.

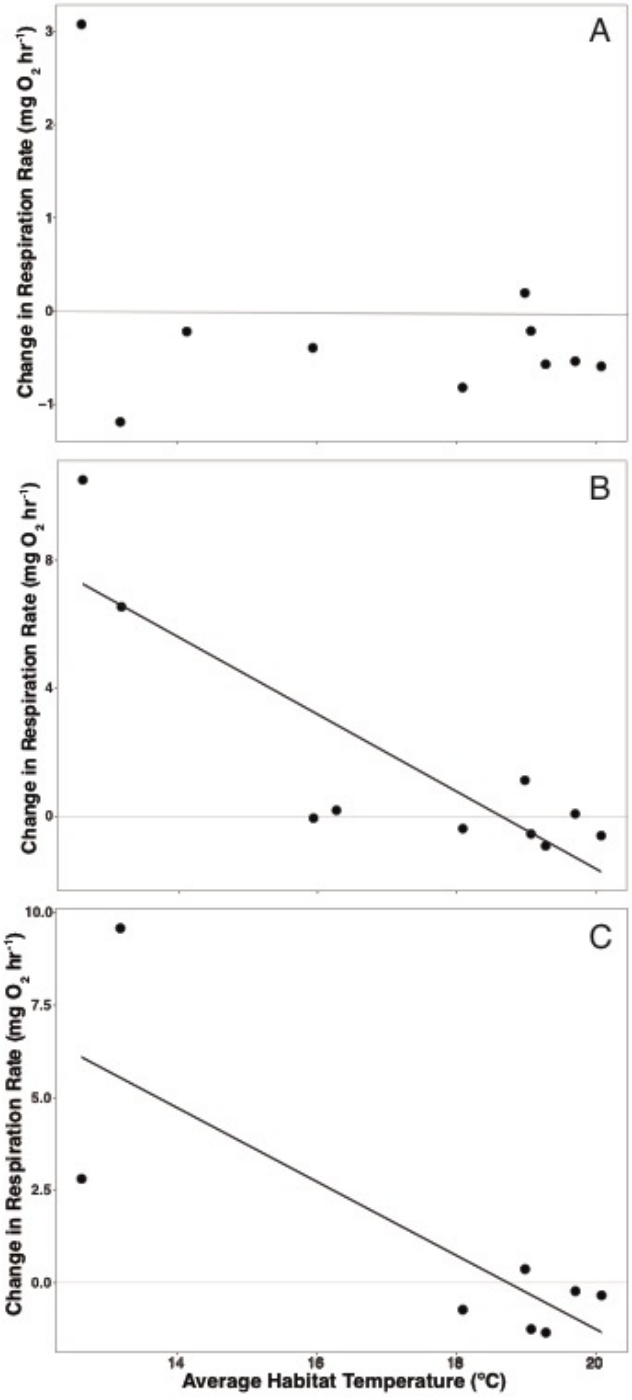
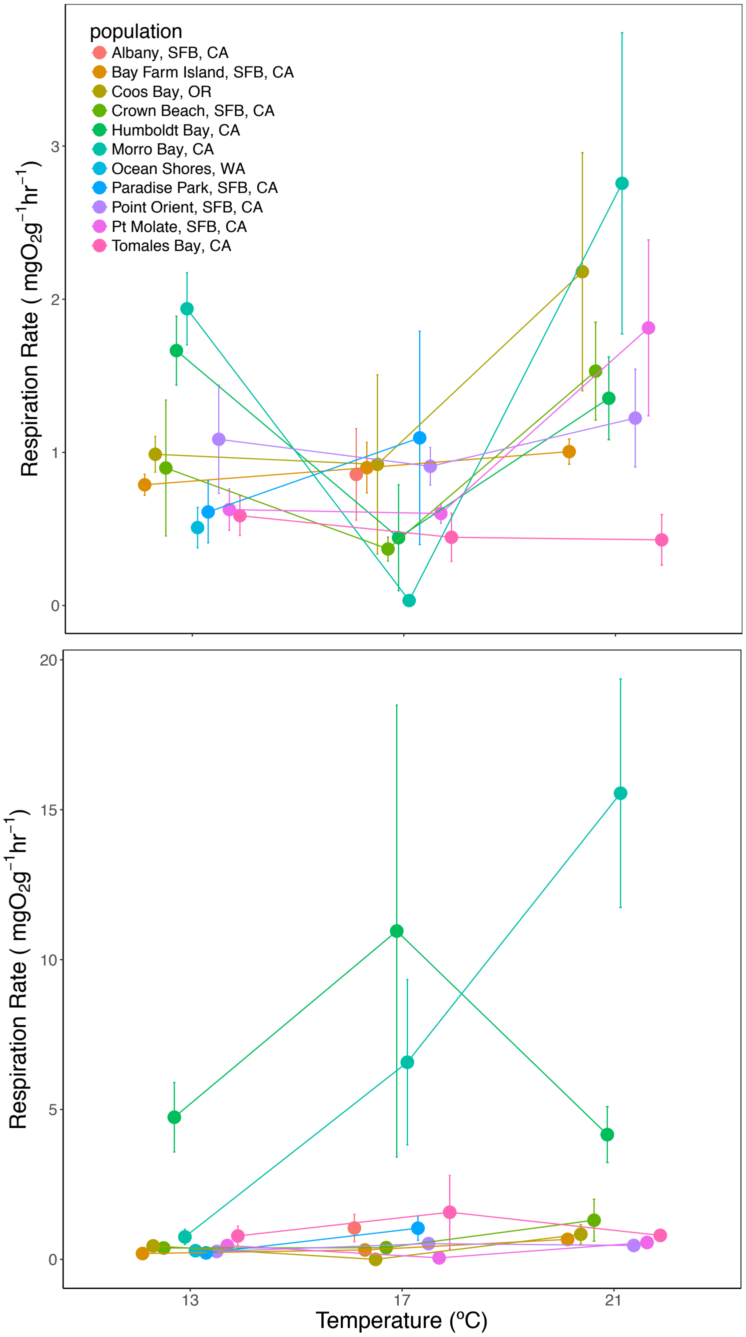


Table S1. GPS coordinates of field sites and associated temperature data buoy stations.

Field Site	*Station Name*	*Station Network*	*Station GPS Coordinates*	*Field Site GPS Coordinates*	*Seasonal Timeframe Used*
Albany, CA	PPXC1 Point Potrero, CA	NOAA NDBC	37.906, -122.365	37.887288, -122.313838	October-November
Bay Farm Island, CA	AAMC1 Alameda, CA	NOAA NDBC	37.772, -122.300	37.730752, -122.313838	June-July
Coos Bay, OR	CWQO3 Charleston, OR	NERR CDMO via NOAA NDBC	43.338, -124.321	43.338468, -124.328617	May-June
Crown Beach, CA	AAMC1 Alameda, CA	NOAA NDBC	37.772, -122.300	37.767329, -122.273053	August-September
Ocean Shores, WA	WPTW1 Westport, WA	NOAA NDBC	46.904, -124.105	46.951370, -124.128255	July-August
Humboldt Bay, CA	HBXC1 Humboldt, CA	NOAA NDBC	40.777, -124.197	40.739294, -124.219544	April-May
Morro Bay, CA	MBXC1 Morro Bay, CA	Cal Poly via CeNCOOS	35.370, -120.858	35.377431, -120.854108	April-May
Paradise Park, CA	Romberg Tiburon Center Pier Tiburon, CA	SFBEAMS	37.891, -122.447	37.893315, -122.455010	October-November
Point Molate, CA	Point Molate Intertidal	R. Tanner	37.957559, -122.415400	37.957559, -122.415400	June-July
Point Orient, CA	DPXC1 San Pablo Bay, CA	NOAA NDBC	38.056, -122.264	37.957559, -122.420944	August-September
Tomales Bay, CA	Hog Island Oyster Tomales Bay, CA	UC Davis via CeNCOOS	38.162, -122.8939	38.162204, -122.918881	July-August

Figure S1. Respiration rates across populations do not follow the same pattern with increasing temperature. Low variance in some populations indicate a canalization of response. Top panel is before heat stress and bottom panel is after heat stress.



Chapter 3 | Reduced transgenerational thermal tolerance plasticity in direct developer *Phyllaplysia taylori* with climate change

Abstract

Physiological plasticity has the potential to influence adaptation in organisms on timescales that do not allow for genetic change in a population. Transgenerational plasticity (e.g. maternal provisioning, shifts in offspring gene expression via methylation, and the resulting developmental acclimation capacity) allows parents to provision their offspring for rapid environmental shifts on the timescale of as little as one generation. Evolutionary strategies employed by direct-developing estuarine organisms can shed light on how transgenerational plasticity has evolved under predictable seasonal fluctuations in temperature. Using the direct-developing eelgrass sea hare, *Phyllaplysia taylori*, this study investigates how an increase in average temperature (chronic stress) and the likelihood of extreme heat days (acute stress) impact maternal provisioning and developmental plasticity. We found total maternal investment significantly decreased by 39% under future average warming and even further (78%) under combined acute and chronic temperature stress, while maternal investment per individual egg increased by 11% in this same treatment. Extreme heat days reduced hatching success by an average of 8% independent of timing. Developmental plasticity significantly decreased with parental exposure to both future climate stressors types. Total successful offspring is lower in these future scenarios, suggesting that population persistence of this important grazer in eelgrass beds will be threatened with climate change. It is important to understand how climate change impacts species accustomed to seasonal environmental fluctuations, and what this can tell us about the role of transgenerational plasticity in rapid adaptation to future climate change.

Introduction

Climate change continues to amplify existing environmental fluctuations for all organisms on earth. On a rapid timescale, genetic adaptation to these environmental shifts is not always feasible, especially in organisms with long generation times (Chevin et al., 2010; Somero, 2010; Franks and Hoffmann, 2012). Plastic responses to environmental stress have often been cited as weakening adaptive evolution (Wright, 1931; Ancel, 2000; Price et al., 2003), while other models find that plasticity can be a critical part of evolution (Ghalambor et al., 2007; Chevin et al., 2010; Ghalambor et al., 2015), such as with the Baldwin effect, described as the positive evolutionary effect of individual variation (Baldwin, 1902; Waddington, 1953). Plasticity varies depending on life stage and parental effects (Kingsolver et al., 2011; Del Giudice, 2015; Gerken et al., 2015; Kuijper and Hoyle, 2015). Through these within- and trans-generational plastic responses, adaptive capacity can be extended beyond the means of strictly genetically-based shifts in phenotype. Plasticity itself is an adaptive trait in habitats characterized by seasonally predictable environmental fluctuations, and the interaction between the types of plasticity within and across generations has recently been investigated as the primary means of adaptation to rapid climate change (Ghalambor et al., 2007; Chevin et al., 2010; Seebacher et al., 2015; Beaman et al., 2016).

Transgenerational plasticity (TGP) is broadly defined as the non-genetic effects observed in the offspring due to environmental exposure of the parental generation. These non-genetic effects due to environmental exposure in TGP can take several forms: epigenetic inheritance from mother or father (i.e. histone modification, DNA methylation) or maternal provisioning (i.e. against toxins or starvation) (Munday et al., 2013; Guillaume et al., 2016; Donelson et al., 2018).

For example, the embryos of annual killifish follow either a diapause or "escape" pathway, based on the temperature conditions experienced by the mother (Woll and Podrabsky, 2017). Recent reviews have narrowed the definition of TGP the interaction between the current and previous generation's environmental conditions, limiting it to just include direct parental effects on offspring success (Salinas et al., 2013; Donelson et al., 2018). Previous studies included developmental plasticity in the definition of TGP, as both parental provisioning and developmental plasticity occur in early life stages (Mousseau and Fox, 1998; Donelson et al., 2018). We argue that the line between these two definitions is blurred by the effects of TGP on the subsequent expression of developmental plasticity. An example of this crossover between timescales of plasticity can be found in *Daphnia* offspring, as they have armor dependent on predator cues in both the maternal and developmental environments (Dzialowski et al., 2003). In my study, the resulting developmental plasticity of the offspring will be considered as part of the TGP effect. Relationships between plasticity expressed in multiple life stages are difficult to parse, and the majority of studies addressing TGP only report resulting offspring phenotype. By crossing parental and developmental treatment conditions, this study investigates temperature-induced plasticity in maternal yolk provisioning and the resulting developmental plasticity in brood success.

In estuarine ecosystems, environmental factors (e.g. temperature, salinity, dissolved oxygen, and ocean carbonate chemistry) are highly variable yet occur on predictably fluctuating timescales (Kimmerer, 2004; Thom et al., 2014; Bible and Sanford, 2016). Predictable fluctuations in abiotic conditions within estuaries in the Eastern Pacific Ocean occur within diurnal tidal cycles, seasonal shifts, and large-scale patterns like the El Niño Southern Oscillation and the Pacific Decadal Oscillation. Unpredictable fluctuations within estuaries are expected to increase with climate change, as drought or rainstorms and extreme cold or hot events will become more frequent (Helmuth et al., 2002; AghaKouchak et al., 2014). Different timescales of fluctuations relate to different aspects of an organism's life history. Diurnal tidal cycles play a role in determining within-generation plasticity, while seasonal shifts are relevant to TGP and large-scale patterns may contribute to genetic changes in the population over time. Unpredictable fluctuations may have immediate consequences for population persistence, as they tend to be more extreme and not within the scope of available plasticity (Stearns, 1976; AghaKouchak et al., 2014). In predictable seasonally fluctuating environments, deterministic maternal effects are under positive selection for short-lived species due to the mother's ability to provision their offspring for a seasonal shift in environment, like lower salinity during winter conditions in an estuary (Proulx and Teotonio, 2017). Additionally, species endemic to estuarine habitats have larval forms and behaviors that keep offspring close to home, further suggesting that TGP plays a role in the success of these species despite the resulting restricted gene flow (Scheltema, 1975; Johannesson, 1988; Yamada, 1989; Teske et al., 2007).

This study uses a simultaneous hermaphrodite, *Phyllaplysia taylori* (Dall, 1900), the direct-developing eelgrass sea hare, to investigate how plasticity is expressed between generations in a predictable seasonally-fluctuating environment. Additionally, we ask whether this current relationship will be disrupted with climate warming and an increase in heat extremes. *Phyllaplysia taylori* has two non-overlapping generations per year, with maturity corresponding with late spring and early fall in Central California (Beeman, 1963). These time periods are characteristically different in both average temperature and frequency of extreme heat days (Fig. 1). It follows that acclimatory capacity and amount of plasticity differ between alternate generations (Fig. 2), meaning that mothers may differentially provision their offspring based on temperature. However, when temperatures deviate from historical trends, does this seasonally-matched provisioning break down? These questions will be specifically addressed by raising sea hare mating pairs through maturity in three temperature treatments reflecting current winter, current summer, and future summer conditions. From the resulting egg masses laid, we expect to see that embryos laid in future summer conditions will have decreased physiological plasticity in brood success across all environmental temperatures due to maternal and developmental thermal stress. Assessed using offspring hatching success, we also expect to see low survival with extreme heat days across all parental treatments. Understanding how a direct developing species with limited gene flow responds to rapid climatic shifts can shed light onto the role of TPG in adaptation.

Methods

Sea hare individuals (n=144) were collected during one low tide in August 2017 within a 50 m^2 area at Point Molate, San Francisco Bay, CA (37.9453, -122.4143). Individuals were assumed to be from the same genetic population and were collected before reproductive maturity at a size of 1-1.5 cm. Individuals were separated and raised in common garden conditions at 17ºC for one week and monitored for egg laying. Any individuals that displayed reproductive maturity by laying eggs or mating were removed from the experiment. After the common garden, individuals were weighed, photographed, and paired in flow-through cylinders. Cylinders were randomly placed in one of three temperature treatments of 13ºC, 17ºC, and 21ºC (n=24 cylinders/treatment). Pairs were fed every 3 days using biofilm growth screens (protocol from Faye et al. *in prep*) and cylinders were checked for egg masses daily.

In order to assess differences in maternal provisioning, egg number and egg size were evaluated from the parents in the three treatment temperatures before and after an acute heat shock of 30ºC. Upon egg laying, the first egg mass in each cylinder was noted but not removed until the second egg mass was laid. At this date, the first egg mass was frozen whole and the second was photographed, sectioned in six pieces, and three random selections from each piece were photographed under the microscope (hereafter referred to as the pre-HS egg mass). These six pieces were crossed among the three temperature treatments with three as control (no further manipulation) and three undergoing a one-hour heat shock of 30ºC <24 hours post-deposition. Adults were also subject to a 30ºC heat shock for one hour then returned to the cylinder and original temperature treatment for post-HS egg laying. This procedure was repeated for the third and fourth egg masses (hereafter referred to as the post-HS egg mass).

Egg mass pieces were placed in airtight plastic containers with 40 mL artificial seawater (32 ppt) with an air headspace maintained to minimize oxygen limitation between water changes. Water was changed every three days and egg masses were gently blotted to remove any algal growth. Egg mass pieces were maintained at their temperature treatments in the same tanks as the adults. Hatching success was assessed starting at ~25 days after egg deposition and subsequently every ~5 days after until all live embryos hatched. At this date, three random areas of the egg mass piece were photographed. Egg photographs were evaluated for embryo density (number of embryos per picture), embryo diameter (three samples per picture), and egg case diameter (three samples per picture) (source code from Chang, 2018, hosted by the Open Computing Facility at UC Berkeley). Size standard photos were taken every day of sampling and egg photographs were scaled accordingly. Any egg masses smaller than the photo frame were scaled in ImageJ.

Analyses were conducted in R (version 3.4.1) with the packages ggplot2 and nlme (Wickham, 2009; Pinheiro et al., 2017). The total number of eggs per clutch, or clutch size, was calculated as the product of mean embryo density per mm and total egg mass area (mm^2). The energy allocation per egg was calculated as the ratio between total number of eggs and average embryo diameter per clutch. Total maternal investment, or the total amount of egg material, was calculated as the average egg area (mm^2) multiplied by the number of eggs.

Results

Adult fecundity

Out of the 72 total mating pairs (n=24 pairs per temperature, n=144 individuals total), only 16 pairs produced egg masses overall, but even fewer produced egg masses after heat shock. In the 13ºC treatment, four of five pairs reproduced after heat shock. In the 17ºC treatment, four of six pairs reproduced after heat shock. In the 21ºC treatment, one of five pairs reproduced after heat shock. Possibly due to low sample size, no significant difference was found between treatments for the percentage of the reproductive population laying egg masses after heat shock (Pearson's χ^2 test, $p = 0.13$). While all individuals in all treatments began at the same size (328.4±14.8 mg), mass at initial egg laying was significantly different between treatment (Kruskal-Wallis $\chi^2 = 10.72$, df = 2, p-value < 0.005), with the 21ºC treatment parents as the smallest followed by the 13ºC then 17ºC parents (Table 1). Sample size was too small to detect an effect of mass at last egg laying; average mass gained was 779.3±125.8 mg.

Larger parents laid eggs earlier (slope = 0.03, $R^2 = 0.32$, Kruskal-Wallis $\chi^2 = 111.48$, df = 17, p-value = 0), had larger clutches (slope = 0.56, $R^2 = 0.11$, Kruskal-Wallis $\chi^2 = 112.36$, df = 19, p-value = 0), allocated less energy per egg (slope = -8.27×10^{-7}, $R^2 = 0.11$, Kruskal-Wallis $\chi^2 = 112.32$, df = 19, p-value < 0.0001), and invested more heavily in reproduction (slope = 0.009, $R^2 = 0.09$, Kruskal-Wallis $\chi^2 = 108.94$, df = 19, p-value < 0.0001).

Variation in total maternal investment per clutch

Clutches in the 13ºC treatment had 2.31±0.27 fewer eggs per mm^2 and 11% larger eggs regardless of parental origin than the 17ºC treatment. In the 21ºC treatment, there were eggs of intermediate size, but the number of eggs is not significantly different from the 13ºC treatment (Fig. 3, Table 2). There was no significant relationship between embryo diameter and clutch size irrespective of treatment temperature, but both metrics were significantly different per treatment temperature (embryo diameter: Kruskal-Wallis $\chi^2 = 64.516$, df = 2, p-value < 0.0001; eggs per clutch: Kruskal-Wallis $\chi^2 = 103.51$, df = 2, p-value < 0.0001). Earlier clutches had larger eggs (slope = -0.0004, Kruskal-Wallis $\chi^2 = 72.23$, df = 17, p-value < 0.0001), and this relationship held up within treatments (Table S1). Acute heat stress in parents did not influence embryo diameter (Kruskal-Wallis $\chi^2 = 0.33$, df = 1, p-value = 0.57). Parental acclimation temperature had no significant effect on total maternal investment (Table 2). Clutches with lower investment overall had higher investment per individual egg (slope = -6525.29), but this relationship was also independent of parental acclimation temperature (Fig. 4, Table 2). The highest investment per egg was in the 21ºC group, which had 3.2 times the amount of investment per egg when compared with the other two groups. Higher maternal investment was found in parent pairs that laid eggs later in the season (slope = 0.29, Kruskal-Wallis $\chi^2 = 97.48$, df = 16, p-value < 0.0001) and in colder temperatures (Table S2). Irrespective of treatment, clutch size and embryo diameter were strongly influenced by parental origin (clutch size: Kruskal-Wallis $\chi^2 = 283.05$, df = 12, p-value = 0; embryo diameter: Kruskal-Wallis $\chi^2 = 150.85$, df = 13, p-value < 0.0001) (Fig. 5).

By comparing the egg masses laid pre- and post-heat shock, we found that parental heat shock increased maternal egg provisioning in parents raised at ambient temperatures, but decreased maternal egg provisioning in parents raised at high stressful temperatures (Fig. 4, Table 2). In the ambient treatments, parental heat shock increased total maternal investment by 25% and in the stressful treatment parental heat shock decreased total maternal investment by 52%. Maternal investment was positively correlated with parental heat shock across treatments (slope = 2.04, Kruskal-Wallis $\chi^2 = 4.14$, df = 1, p-value = 0.04), but not within treatments (Table S2).

Hatching success

Accounting for clutch effects, hatching success was negatively impacted by acclimation of both parents and embryos to stressful temperatures (Table 3). Comparing stressful and non-stressful temperatures in either parental or developmental treatments, hatching success decreased between 9% and 33% (Fig. 6A). The 13ºC parental treatment had the highest survival overall at 13ºC and 17ºC developmental temperatures, while the 21ºC parental treatment had the highest survival only at the 21ºC developmental temperature (Fig. 6B; trend only, NS). Average hatching success at 21ºC was 18% lower than the average hatching success at 13ºC and 17ºC, the two non-stressful treatments. When considering total number of eggs per clutch, parental treatment did not predict the total number of individuals hatched, but the 21ºC treatment trended towards having the lowest success over all developmental temperatures (Fig. 6A, Table 3). Across parental treatments, development at 21ºC significantly decreased the total number of embryos hatched by an average of 32% (Table 3). Embryonic hatching success ranged from 0% to 100% of the clutch hatched. There was more variation in the 21ºC parental treatment with fewer parent pairs than the other two parental treatments. Irrespective of treatment, parental effects were strong in determining hatching success (Kruskal-Wallis $\chi^2 = 33.896$, df = 13, p-value = 0.001).

Both parental heat shock and embryonic heat shock negatively impacted the percentage of embryos hatched by an average of 7.6% and 9.3%, respectively (Fig. 7, Table 3). Parental and developmental treatments did not play a role in determining hatching success after heat shock.

Discussion

Chronic and acute thermal stress negatively affect *P. taylori* offspring, whether the stressors are experienced by the offspring themselves or during parental acclimation. Acute thermal stress decreased offspring success regardless of timing, while prolonged exposure to stressful average temperatures has the largest effect on reproductive output, possibly due to a lack of energy reserves under chronic stress. At the highest parental temperature exposure, smaller clutch size and lower hatching success across any developmental temperature resulted in the lowest overall successful offspring. Thermal stress in mothers may decrease the amount of energy allocated to reproduction, resulting in fewer successful offspring despite more provisioning per egg. Since the 13ºC and 17ºC treatments resulted in the same number of successful offspring, this may indicate that the seasonally predictable thermal regime maintains thermal tolerance plasticity, while deviations from these environmental patterns are detrimental to population persistence.

Maternal conditions alter clutch energy allocation strategy

Maternal thermal stress resulted in greater investment per egg, but total investment was reduced. Due to the short lifespan of *P. taylori,* life history tradeoffs may be characterized as a ‘fast-living’ strategy, where reproduction is promoted at the expense of survival under acute stress (Zera and Harshman, 2001). Egg size has long been recognized as a proxy for reproductive investment, corresponding to the assumed lipid, sugar, protein, and energy contents of the egg itself (Winkler and Wallin, 1987; Bernardo, 1996). While egg size and total investment, or reproductive effort, are often considered to be part of a two-step process and evolved independently (Winkler and Wallin, 1987), Caley et al. 2001 challenged this assumption. They found that these traits are linked across a wide clade of invertebrates, and that allocation towards total offspring and individual partitioning happens simultaneously. This study found that reproductive effort for the clutch was low at high temperatures, but within that effort, individual embryo energy allocation was proportionally high when compared with mothers that did not undergo thermal stress. Mothers that did not experience chronic thermal stress did not have limitations on their total maternal investment, and therefore the relationship between individual egg allocation and total reproductive effort was predictable. Therefore, our results support the proposed linkage of allocation in clutch and individuals by Caley and colleagues, but not under stressful conditions.

In addition to thermal stress, timing of reproduction was important for reproductive effort and egg size. Independent of temperature treatment, larger individuals laid large clutches with small eggs earlier. The egg size-egg laying date relationship is consistent with the results of the earlier studies that documented decreasing reproductive investment with age (Giron and Casas, 2003; Muller et al., 2017).

Temperature-dependent maternal provisioning strategies

Predictable seasonal shifts in average temperature represented by 13ºC (winter) and 17ºC (summer) in this study resulted in two types of provisioning tactics aligning with Thorson’s rule, which stipulates that egg size and egg number are inversely related with respect to temperature (Thorson, 1936; Thorson, 1950). Winter temperatures increased individual provisioning and decreased number of offspring, indicating that anticipatory, or deterministic, maternal provisioning maximized the percentage of successful offspring (Figs. 3, 4). Summer temperatures decreased individual provisioning and increased number of offspring, indicating that randomized maternal provisioning maximized the number of successful offspring (Figs. 3, 4). Both tactics resulted in the same number of successful offspring at both temperatures, showing that the best strategy – randomizing or deterministic – is dependent on the season (Dey et al, 2016). Within the current climatic regime, both strategies result in sufficient developmental plasticity to deal with current predictable fluctuating temperatures. Estuaries have both predictable and unpredictable fluctuations in temperature, and the unpredictable ones will only increase with climate change (AghaKouchak et al., 2014). The range of available developmental plasticity was severely limited when the mothers experienced conditions outside of the predictable seasonal fluctuations.

A parental environment of 21ºC, a temperature outside of the predictable seasonal range in average temperature, resulted in 18% lower survival of offspring overall, despite 3.2 times higher maternal provisioning per egg, when compared with the 13ºC and 17ºC groups. Low survival despite high provisioning per individual may indicate a breakdown of the relationship between temperature and maternal provisioning ability shown in the two non-stressful treatments. When parent and offspring environments, or maternally-predicted offspring environments, do not match, shifts in maternal provisioning are not adaptive (Marshall and Uller, 2007). In the 21ºC parental treatment, developmental plasticity was greatly reduced across all temperatures, showing that even increased provisioning per egg was not sufficient to ameliorate the effects of maternal exposure to high temperatures. While egg size may have been larger at this stressful temperature of 21ºC, resulting in increased energy stores, the epigenetic pathways underlying plastic responses during development may have broken down.

Future studies should investigate multi-generational responses to increased temperatures to see whether plasticity increases with multiple generations of exposure. Laboratory studies have shown that acclimatization to the appropriate plastic response can occur on relatively short timescales with predictable environmental cues (Bergland et al., 2014; Massamba-N'Siala et al., 2014; Dey et al., 2016;). The key word here is predictable: with an increase in unpredictable extreme heat events, this could be the barrier to the appropriate plastic responses of *P. taylori* in nature. Deterministic maternal effects have played a large role in the maintenance of species living through seasonal and otherwise predictably fluctuating environments, and climate change has the potential to disrupt current cues (Proulx and Teotonio, 2017). Further lab studies of this species should be done with a variable temperature regime acclimation, including increasing extreme frequency and duration.

Lasting effects of extreme heat days on offspring survival

Extreme heat days are expected to increase in frequency and duration with climate change (IPCC, 2014; Min et al., 2013; Seneviratne et al., 2012). The increase in the frequency and duration of extremes may be outside of the current window of environmental heterogeneity encompassed in an estuarine organism's plastic response, therefore making extreme temperature a bigger concern than increases in average temperature (Vasseur et al., 2014). Reduced hatching success across both parental and developmental heat shock treatments indicates that overall, extreme heat days are detrimental to population persistence (Fig. 7). The similarity between parental and developmental heat shock treatments is most likely due to the sperm storage strategy of these sea hares (Beeman, 1970). Since they are to store sperm for up to 42 days (R. Tanner, personal observation; previously 35 days in Chambers, 1934) and all parents laid eggs post-heat shock within that time window, the parental gametes stored within the acting mother experienced the same heat shock.

While we focused on maternal provisioning by measuring egg size, paternal effects have been found to match or even exceed maternal effects with temperature stress (Guillaume et al., 2016). Therefore, similar effects are expected to be expressed in resulting embryos that were heat shocked as gametic material in the mother and those that were heat shocked <24 hours post egg laying. Guillaume et al. 2016 found that thermal stress during gamete production prior to egg fertilization negatively impacted offspring success, with paternal gametes more susceptible to thermal stress than maternal gametes, which increased in viability with increased temperature. Reduced fertilization and reduced offspring success are reported with parental gamete heat shock at temperatures within the range used in this study, suggesting that addressing potential DNA damage with extreme heat days could shed light on reduced fecundity and offspring viability (Ericson et al., 2012; Binet and Doyle, 2013; Guillaume et al., 2016). We find in this study that extreme events are the limiting factor for evolving a reliable plastic response to future environmental change with deterministic maternal effects.

Impacts of ocean warming on population persistence

Four treatments in this experiment simulated future climatic stressors: parental and developmental exposure to 21ºC, and parental and developmental exposure to extreme heat days

(30ºC). Every treatment significantly reduced hatching success, and therefore reduced the total number of potential mating individuals in the next generation. This has significant consequences for future populations' genetic diversity, especially if mothers do not differentially provision their offspring (as in bet-hedging). An increase in average temperatures by 4ºC is highly likely, especially in the intertidal zone, where temperatures have already increased by 2ºC in the last two years at the source population's location for this experiment (Fig. 1). An increase in extreme heat days is also likely with climate change (Seneviratne et al., 2012), leading to an increased probability of these events occurring during developmentally sensitive times (early development or pre-egg deposition but post-coitus). This species is particularly vulnerable to climate change as a direct developer since escape into the water column is unlikely and gene flow is predicted to be low. This tactic common among estuarine species has the potential to result in disproportionately large effects of climate change on species accustomed to large fluctuations in environmental stressors. In *P. taylori*, effective population size will decrease with climate change barring any rapid adaptation to an increase in average temperature and extreme frequency.

Conclusion

An increase in extremes has the potential to disrupt the current seasonally-dependent fluctuations in temperature that shaped the current relationship between TGP and population persistence in species like *P. taylori*. Adverse responses to increased temperature and temperature variability in other estuarine organisms have been documented on the scale of a single generation, however, expanding to include the transgenerational effects of temperature in estuarine invertebrate responses is valuable and needed (Incze et al., 1980; Daufresne et al., 2004; Smart et al., 2012). This study finds that limitations on developmental plasticity stem from chronic stress in mothers and acute stress across all life stages. These limitations have the potential to decrease population persistence in a warming ocean and reflect the importance of TGP in rapid adaptation to climate change.

Ethics Declaration

Specimens were collected under California SCP 13357 issued to R.L.T.

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Competing Interests

The author declares no competing interests.

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Data Accessibility

Data to be deposited in SEANOE (https://www.seanoe.org).

Table 1. Mean parental weights across treatments, evaluated directly after egg mass laying for the experimental egg masses. Not all pairs of parents survived through treatment.

Parental HS	*13ºC*	*17ºC*	*21ºC*
No HS	931.0±72.7 (n=12)	1221.4±85.7 (n=8)	706.8±54.3 (n=6)
HS	1142.8±89.2 (n=9)	1274.0±141.8 (n=6)	892.6 (n=1)

Table 2. Statistical output for maternal effects linear mixed effects models (treatment contrasts). Treatment temperatures are 13ºC, 17ºC, and 21ºC (treated as factors) with 13ºC included in the intercept of the model. Random effects included were parental ID (all models) and the egg mass number (all models without parental heat shock).

Fixed Effects	*Effect Size*	*DF*	*p-value*
Embryo diameter ~ Treatment temperature			
17ºC treatment	-0.01482000	10	0.0450 *
21ºC treatment	-0.00084904	10	0.9194
Eggs per clutch ~ Treatment temperature			
17ºC treatment	443.6105	10	0.0935
21ºC treatment	-314.2703	10	0.3069
Total maternal investment ~ Treatment temperature * Parental HS			
Parental HS	6.794882	386	0.0000 ***
17ºC treatment	5.971694	10	0.1958
21ºC treatment	-3.277883	10	0.5261
HS x 17ºC treatment	-3.328103	386	0.0068 ***
HS x 21ºC treatment	-9.101909	386	0.0000 ***
Allocation per egg ~ Treatment temperature * Parental HS			
Parental HS	-0.0002007722	386	0.0000 ***
17ºC treatment	-0.0002957713	10	0.5782
21ºC treatment	0.0008641373	10	0.1767
HS x 17ºC treatment	0.0001815973	386	0.0005 ***
HS x 21ºC treatment	0.0004879505	386	0.0000 ***
Total maternal investment ~ Allocation per egg * Treatment temperature			
Allocation/egg	-6525.294	378	0.0008 ***
17ºC treatment	5.285	10	0.1713
21ºC treatment	-6.039	10	0.2056
Allocation/egg x 17ºC	-10178.120	378	0.0122 *
Allocation/egg x 21ºC	4303.613	378	0.0583
Embryo diameter ~ Treatment temperature + Parental HS			
17ºC treatment	-0.01371147	10	0.0369 *
21ºC treatment	0.00225191	10	0.7441
Parental HS	0.00399465	388	0.0208 *
Eggs per clutch ~ Treatment temperature + Parental HS			
17ºC treatment	485.8671	10	0.0705
21ºC treatment	-272.9619	10	0.3500
Parental HS	84.6011	388	0.0109 *

Table 3. Statistical output for hatching success linear mixed effects models (treatment contrasts). Treatment temperatures were analyzed as continuous variables. Random effects included were parental ID (all models) and the egg mass number (all models without parental heat shock).

Fixed effects	*Effect size*	*DF*	*p-value*
Hatching success ~ Parental temperature treatment * Developmental temperature treatment			
Parental temp	-25.92664	109	0.0034 **
Developmental temp	-22.07320	109	0.0010 ***
P x D temp	9.75360	109	0.0048 **
Hatching success ~ Parental HS + Developmental HS			
Parental HS	-5.13611	113	0.0397 *
Developmental HS	-9.33295	113	0.0407 *
Total embryos hatched ~ Parental temperature treatment + Developmental temperature treatment			
Par. temp. 17ºC	181.5224	11	0.1770
Par. temp. 21ºC	-139.4818	100	0.3256
Dev. temp. 17ºC	-17.5811	100	0.7518
Dev. temp. 21ºC	-134.3792	100	0.0134 *

Figure 1. Temperature records (November 2016-January 2018) from Point Molate, San Francisco Bay, CA. Average temperature and frequency of extreme temperatures have increased during the monitoring period.

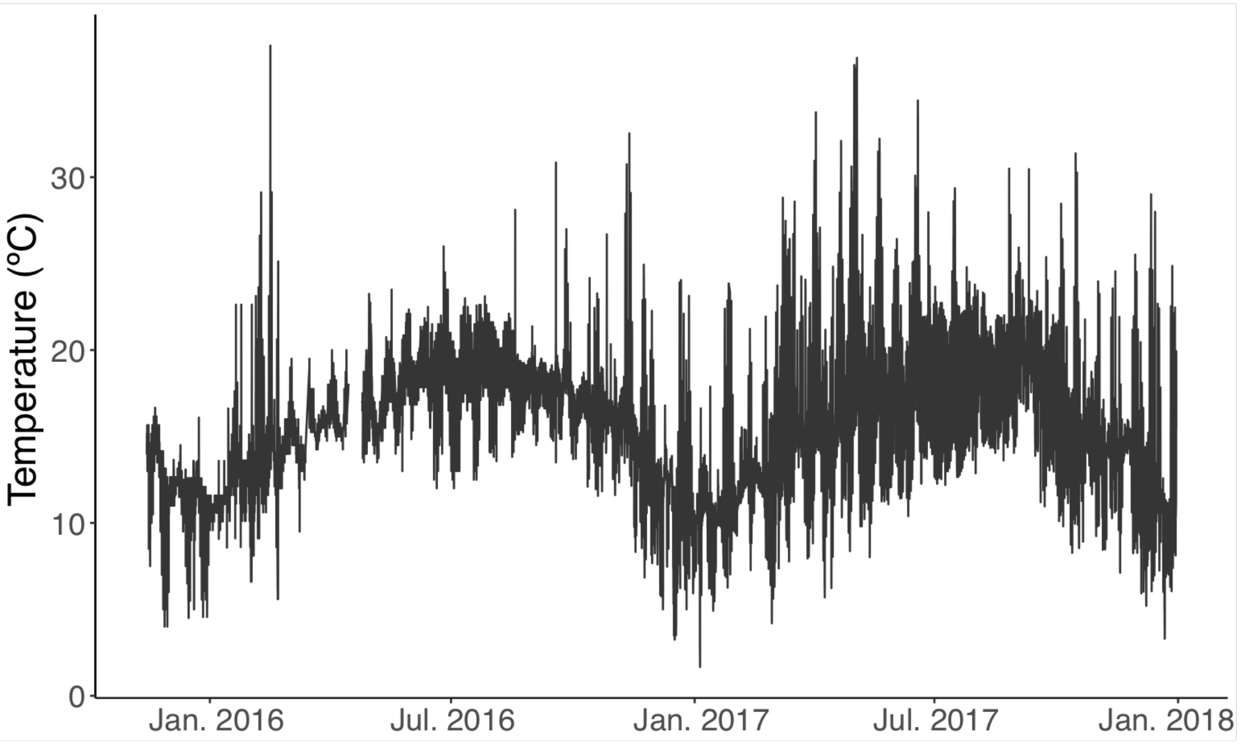

Figure 2. Upper critical temperature and acclimation response differ between two generations (winter population = triangles, summer population = circles) of the same population at Point Molate, San Francisco Bay, CA. Upper critical temperatures were measured using foot muscle function and a temperature ramp of 4ºC (see Chapter 2). Error bars are 95% CI.

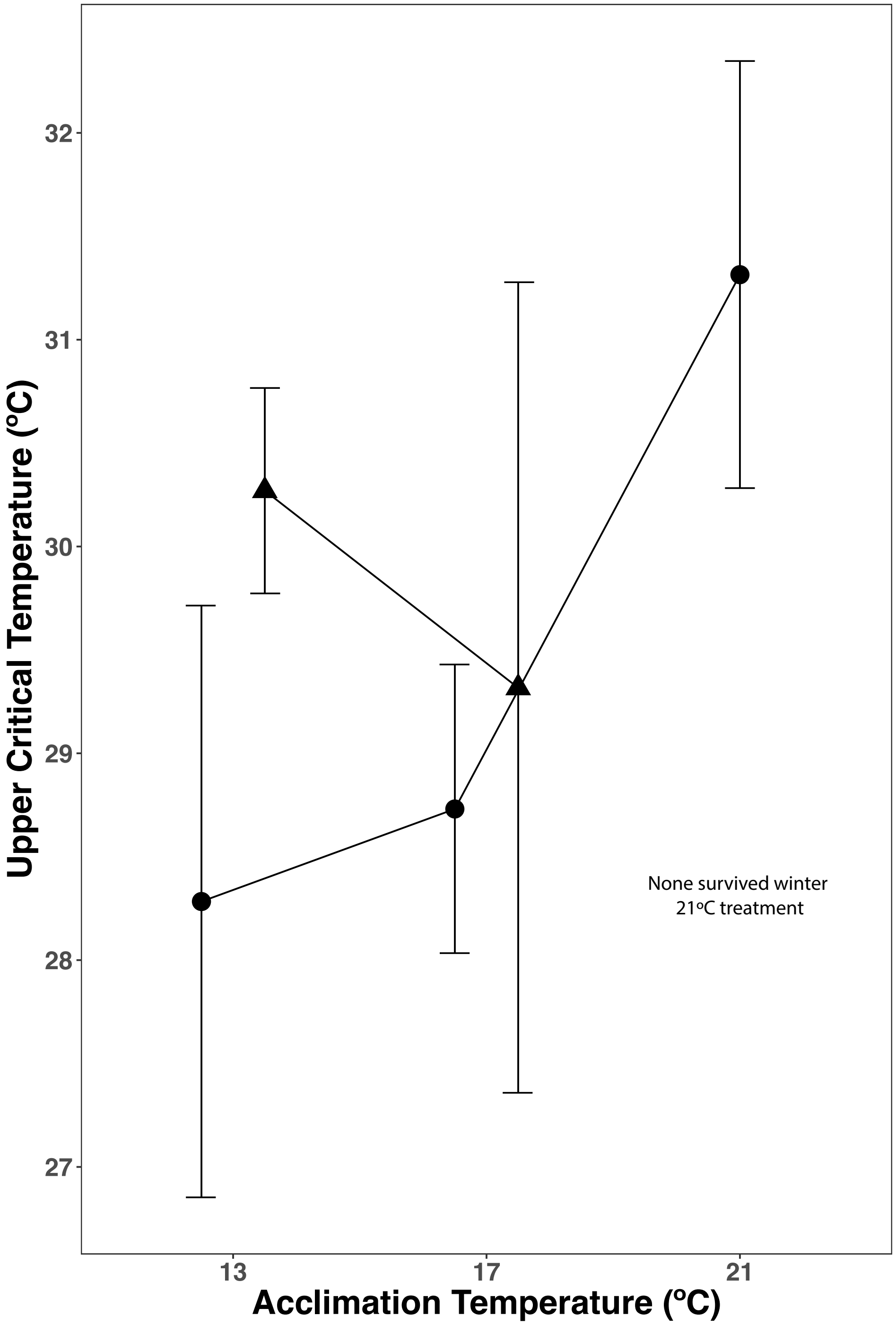

Figure 3. Egg diameter and number of eggs per clutch are inversely related across all clutches per Thorson's rule at current climate temperatures, but not at a future climate predicted temperature. Parental exposure temperatures are blue circles (13ºC), purple triangles (17ºC), and red squares (21ºC). Error bars are 95% CI.

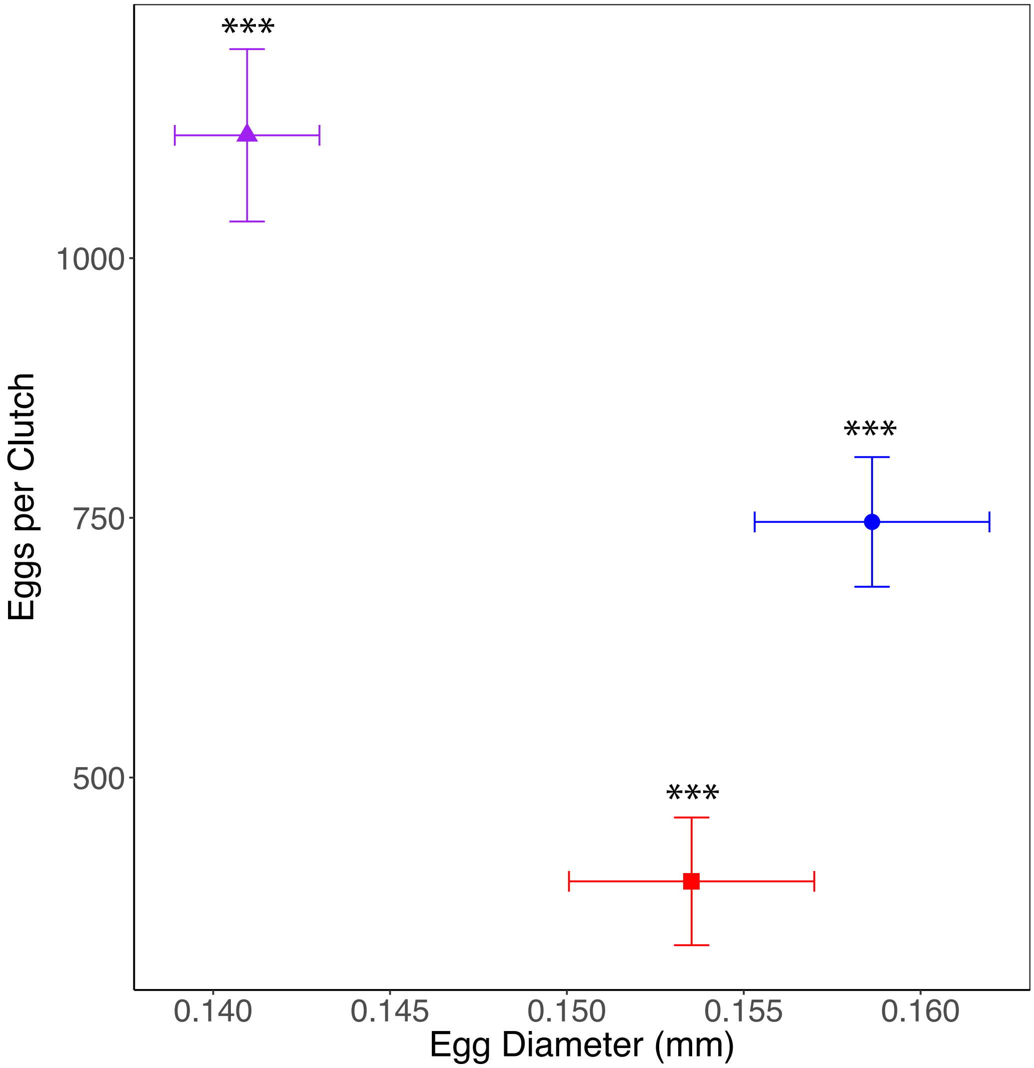

Figure 4. Investment per egg and total maternal investment are dependent on parental temperature. Parental exposure temperatures are blue circles (13ºC), purple triangles (17ºC), and red squares (21ºC). Open shapes indicate pre-HS treatment, filled circles indicate post-HS treatment. 21ºC reduces maternal investment overall, with more drastic effects after HS. HS shifts 13ºC group strategy towards that of the 17ºC group. All clutches are averaged from each treatment group (e.g. the open, red point is all egg clutches post-HS from parents exposed to 21ºC with a heat shock). Error bars are 95% CI. Investment per egg is expressed as a ratio, where 1 is the average investment per egg across treatments. See methods for units of total maternal investment.

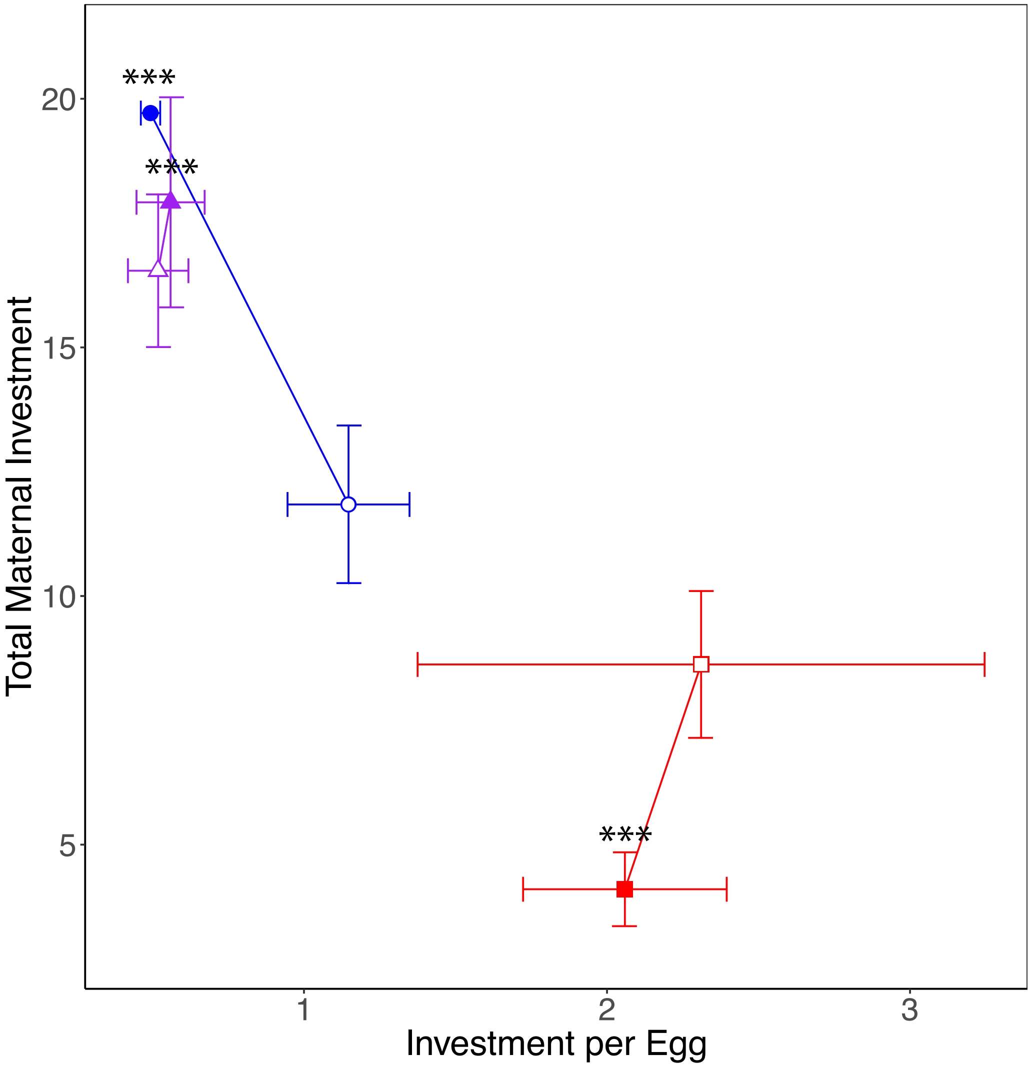

Figure 5. Parental effects on average clutch embryo diameter. Error bars are 95% CI.

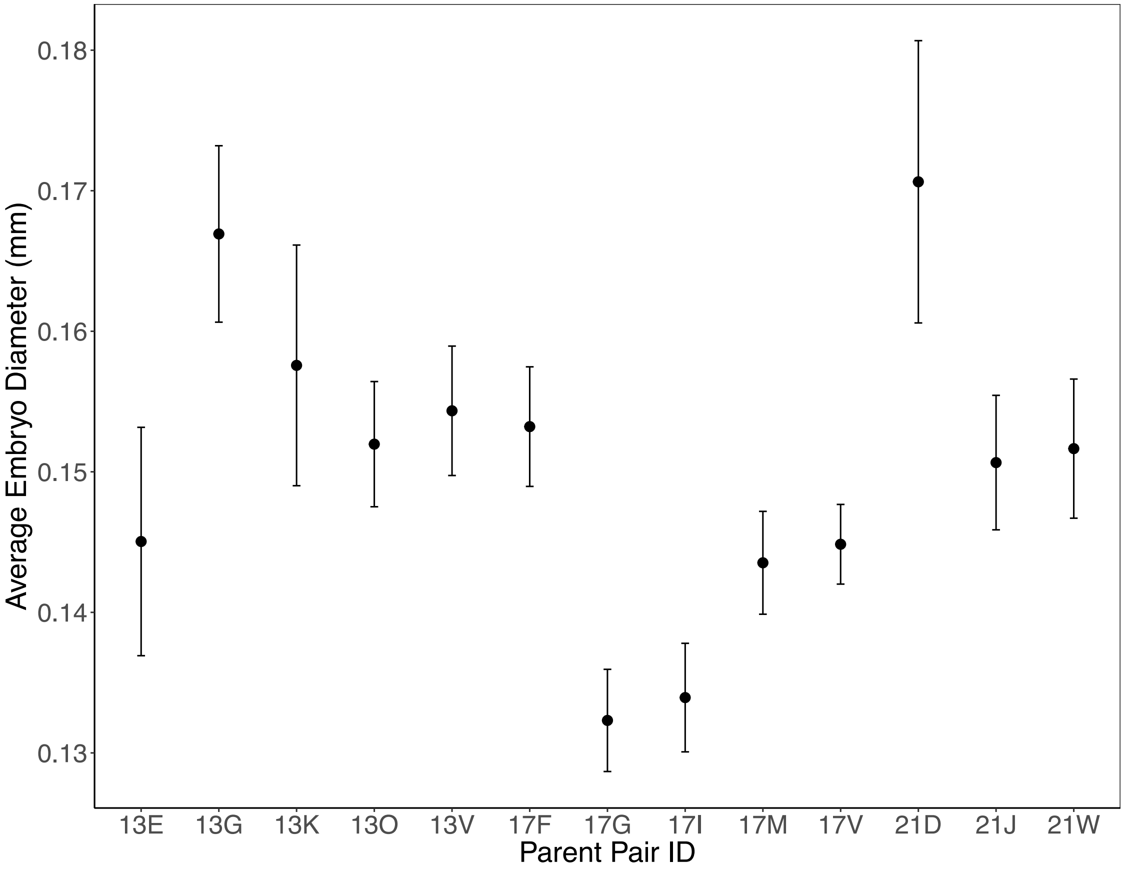

Figure 6. Parental exposure temperatures are blue circles (13ºC), purple triangles (17ºC), and red squares (21ºC). Current climate parental exposure temperatures result in a greater number of total successful offspring at current climate developmental temperatures (A) and the 13ºC group has the highest hatching success at current climate developmental temperatures (B). Error bars are 95% CI.

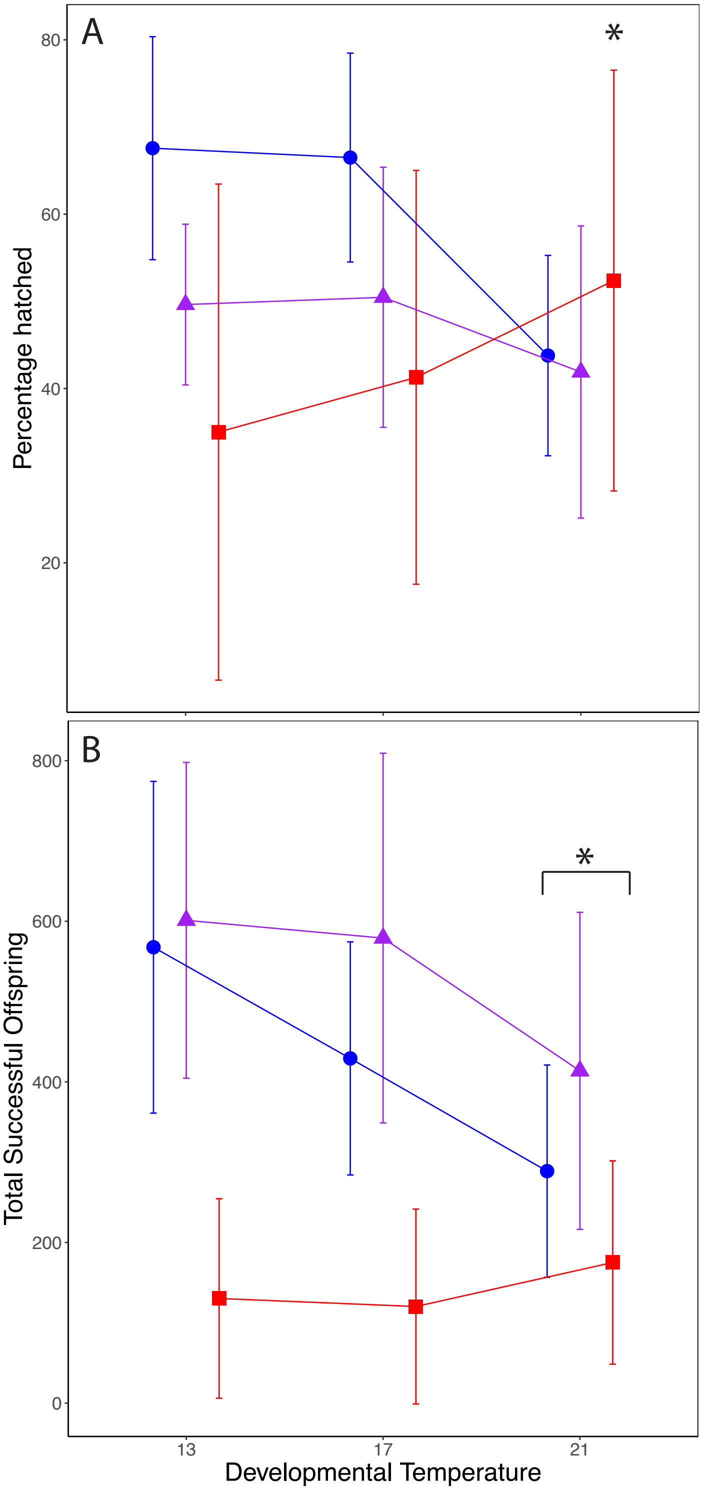

Fig. 7: Hatching success is decreased by 7.6% after parental HS (triangles) and 9.2% after embryonic HS (circles). Error bars are 95% CI.

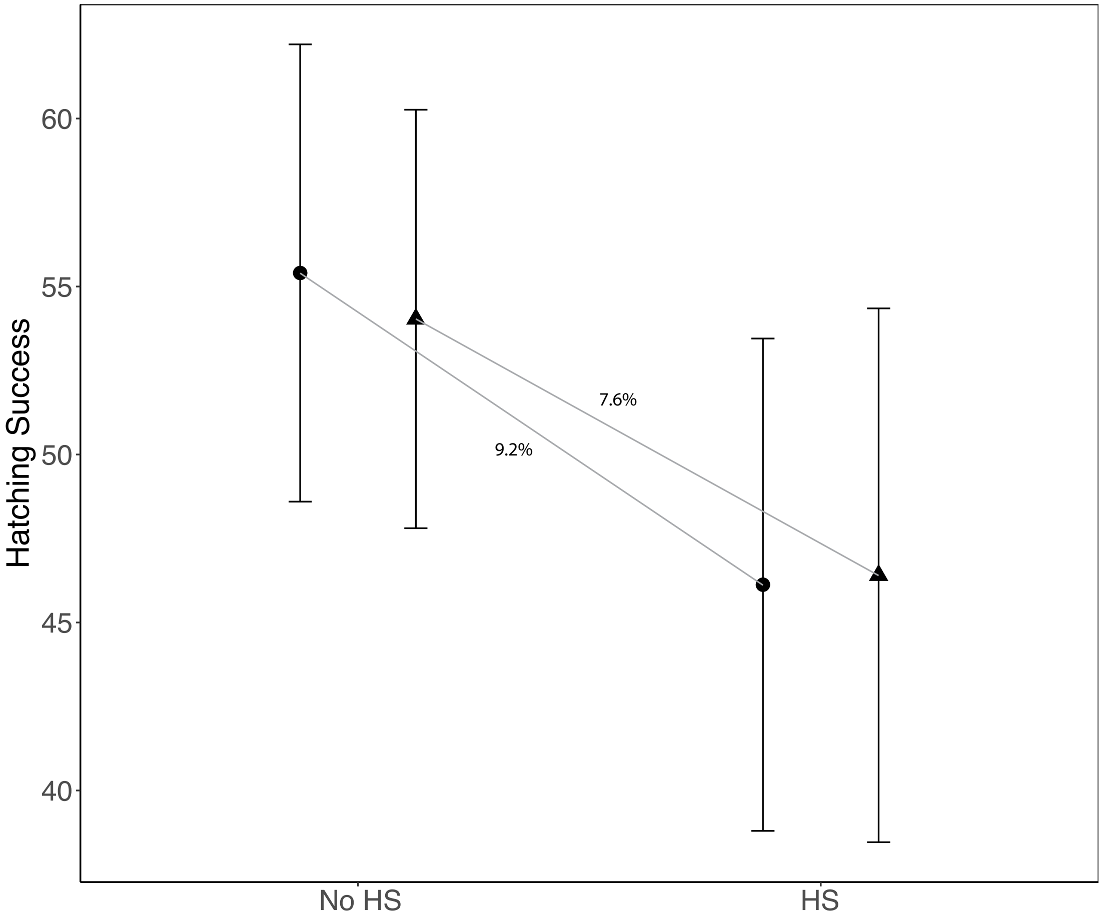

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Table S1. Egg laying date and embryo diameter are negatively correlated within parental treatment temperature. All tests are Kruskal-Wallis χ^2.

Parental temperature	χ^2	*DF*	*p-value*
			Embryo diameter ~ Egg laying date
13ºC	21.47	5	0.0007
17ºC	33.17	8	0
21ºC	10.18	4	0.04

Table S2. Egg laying date and maternal investment are positively correlated within parental treatment temperature. Parental heat shock and maternal investment are positively correlated within parental treatment temperature only in the 13ºC treatment. All tests are Kruskal-Wallis χ^2.

Parental Temperature	χ^2	*DF*	*p-value*
			Maternal investment ~ Egg laying date
13ºC	36.47	4	0
17ºC	42.61	8	0
21ºC	20.42	4	0.0004
			Maternal investment ~ Parental heat shock
13ºC	4.41	1	0.04
17ºC	1.43	1	0.23
21ºC	0.001	1	0.97