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Warm microhabitats drive both increased respiration and growth rates of intertidal consumers

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ABSTRACT: Rocky intertidal organisms are often exposed to broadly fluctuating temperatures as the tides rise and fall. Many mobile consumers living on the shore are immobile during low tide, and can be exposed to high temperatures on calm, warm days. Rising body temperatures can raise metabolic rates, induce stress responses, and potentially affect growth and survival, but the effects may differ among species with different microhabitat preferences. We measured aerial and aquatic respiration rates of 4 species of *Lottia* limpets from central California, and estimated critical thermal maxima. In a variety of microhabitats in the field, we tracked body temperatures and measured limpet growth rates on experimental plates colonized by natural microalgae. Limpet species found higher on the shore had lower peak respiration rates during high temperature aerial exposure, and had higher critical thermal maxima. Using our long-term records of field body temperatures, we estimated cumulative respiration to be 5 to 14% higher in warm microhabitats. Growth rates in the field appear to be driven by an interaction between available microalgal food resources, low tide temperature, and limpet species identity, with limpets from warmer microhabitats responding positively to higher food availability and higher low tide temperatures. Stressful conditions in warm microhabitats make up a small portion of the total lifetime of these limpets, but the greater proportion of time spent at non-stressful, but warm, body temperatures may result in enhanced growth compared to limpets living in cooler microhabitats.

KEY WORDS: Intertidal zone · Limpet · Microalgae · Shore height · Temperature stress · Thermotolerance

INTRODUCTION

Climate change research in a variety of aquatic systems has pointed to the potential for mildly increasing water temperatures to increase the metabolic and foraging rates of ectotherms, and the impacts of top-down control by consumers on resources (O'Connor 2009, O'Connor et al. 2009, Hoekman 2010, Kratina et al. 2012, O'Regan et al. 2014). As waters warm, the increasing speed of fundamental chemical reactions at the cellular level leads to increasing energy usage for maintenance metabolism and growth (Hochachka & Somero 2002) that must typically be balanced by increasing the rate of consumption, which in some cases can strengthen trophic cascades (Kratina et al. 2012). Provided there is time for acclimatization to a warmer temperature regime (Stillman 2003, Deutsch et al. 2008, Tewksbury et al. 2008), the rate of energy flow up through the trophic levels of the system could increase as species living below their optimum performance temperature move up the rising slope of their respective temperature performance curves (Fig. 1A; Huey & Stevenson 1979), potentially driving greater productivity (Angilletta et al. 2010).

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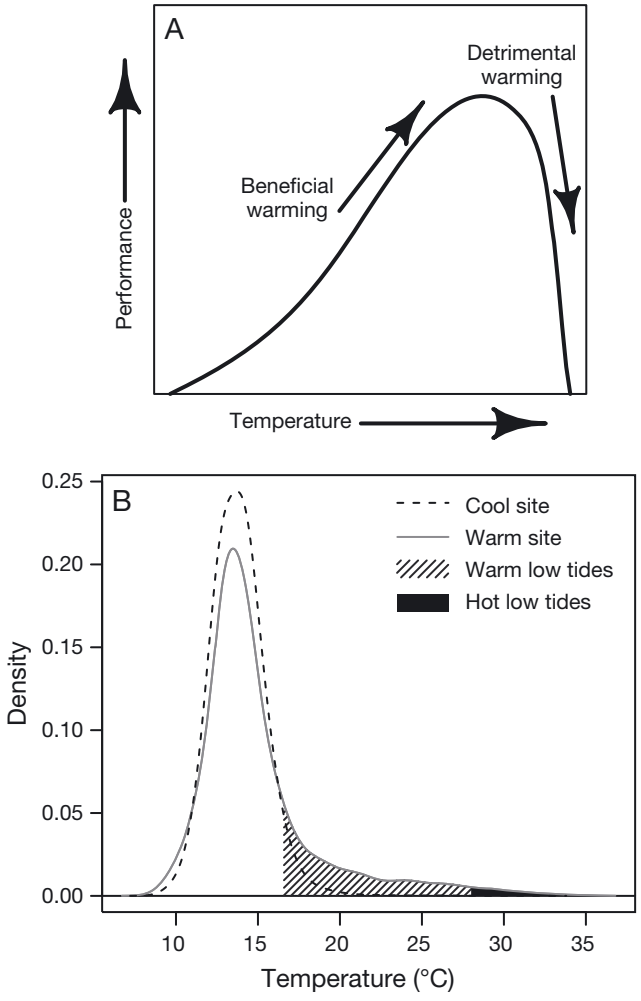

Fig. 1. (A) A hypothetical temperature–performance curve, where some metric of performance (grazing rate, growth rate, etc.) climbs with increasing temperature towards a peak or plateau, and then drops off quickly as temperatures increase further. (B) Kernel density estimates of the proportion of time limpets spent at various body temperatures between June and December 2013 at cool and warm microsites on the high shore at Hopkins Marine Station (1.7 m above Mean Lower Low Water). Gray hatched region: approximate proportion of time spent at temperatures between 16.6 and 28°C, which are above the warmest ocean temperature at the site, but below the range that typically induces a heat-shock response in *Lottia* limpets (Dong et al. 2008). Solid black region above 28°C: temperature range where most *Lottia* exhibit a heat-shock response. The samples include 3939 h of data collected at 12 min intervals at 2 sites, with the kernel bandwidth set at 0.6

In the rocky intertidal zone, the effects of benign water temperature fluctuations as seawater warms and cools during high tide have been suggested to potentially benefit some intertidal consumers. Sanford and collaborators have shown that foraging rate increases within a limited range of increasing water temperature for key intertidal species, including the keystone predator *Pisaster ochraceus*, which increases its per capita predation on intertidal mussels that are often the dominant competitors for space in the mid intertidal zone (Sanford 1999, Sanford & Menge 2001, Sanford 2002, Pincebourde et al. 2008). Warmer waters can also increase intertidal mussel growth (Phillips 2005, Kroeker et al. 2014) and speed up feeding rates in predatory snails (Largen 1967, Bayne & Scullard 1978, Yamane & Gilman 2009, Miller 2013). However, these are rare cases for which we know where on the thermal performance curve an intertidal organism sits relative to the range of varying temperatures experienced in its habitat. For other organisms, it is difficult to predict when or how often rising body temperatures could move the organism from the ascending slope of the curve, past its optimum temperature, and onto the descending slope. The distribution of temperatures experienced by intertidal organisms is dominated by the influence of water temperature at high tide, while aerial exposure during low tide can bring swings to either colder or warmer temperatures, as shown for high-intertidal-zone limpets from Monterey Bay, California (Fig. 1B). Most of these temperature fluctuations are mild enough to avoid temperature stress (Fig. 1B, hatched region, with the 28°C upper limit based on heat-shock protein expression data from Dong et al. 2008), but occasional hot weather conditions can drive body temperatures to extremes (Fig. 1B, black region), when low tides leave marine organisms high and dry for hours at a time (Helmuth 1999, Denny & Harley 2006, Denny et al. 2009). The temporal coincidence of warm water and air temperatures may also have complex interacting effects. Periods of cooler water temperatures at high tide have the potential to offset negative effects of warm low tide conditions by providing time to recover from stress, but periods with warm low tides and warm high tides occurring out of phase for several days could have strong negative effects by leaving little time to recover (Pincebourde et al. 2012).

Much of the climate change related research in intertidal systems has focused on the negative effects of increasing temperature, particularly of extreme aerial temperatures that induce heat stress and occasionally cause mortality events during low tide (Tomanek & Somero 1999, Stillman 2002, Tomanek 2002, Muñoz et al. 2005, Jones et al. 2009, Miller et al. 2009, Tomanek & Zuzow 2010, Miller et al. 2014). It is often assumed that low tide conditions, when animals and algae are exposed to air, can drive species past their optimal temperature range and down the

descending slope of the temperature performance curve. This potentially creates costly energetic consequences due to limited oxygen delivery or organ failure (Pörtner 2012) and the need to shunt energy into heat-shock responses to recover from high temperature insults (Feder & Hofmann 1999). In addition, due to the physiological need for available water, most algal photosynthesis and animal feeding occur at high tide when body temperatures are at equilibrium with the cool ocean. As the tide drops and the rocks dry, photosynthesis slows down (Hunt & Denny 2008) and nearly all feeding activities come to a stop (Craig 1968, Eaton 1968, Miller 1968). As a result, the potential benefits of a warming body and faster metabolism that can occur in fully aquatic systems are decoupled from the opportunity to feed or photosynthesize for the many sessile, or functionally sessile, organisms in the intertidal zone during low tide.

The cessation of feeding does not necessarily mark the end of energy acquisition, since digestion of a meal may take hours that can encompass warm daytime low tide conditions. In aquatic habitats, the temperature for optimal growth in ectotherms can increase when food is abundant (Elliott 1976, Elliott 1982, Stich & Lampert 1984, Pangle & Peacor 2010), and warmer temperatures can increase the rate of digestion (Brett & Higgs 1970, Diefenbach 1975, Bayne & Scullard 1978). However, in the intertidal zone where food availability or foraging time may be restricted, the scope for growth can be lower and there can be an expanded range of high temperatures where metabolic maintenance costs outstrip energy intake (Woodin et al. 2013, Iles 2014). Therefore, along the seashore, it remains uncertain whether warm, dry conditions during daytime low tides are a potential benefit or simply a cost for consumers and their algal resources, although some studies show that the effects of warm temperatures at low tide need not be solely negative (Gilman 2006, Blanchette et al. 2007).

We address this question using limpets of the genus *Lottia* found on the central coast of California. In rocky intertidal zones around the world, limpets represent an important class of herbivorous grazers that can structure the intertidal community by selectively removing algae and settling invertebrates (Jones 1948, Branch 1981, Hawkins & Hartnoll 1983). Limpets forage while rocks are awash during rising and falling high tides, and typically remain fixed in place on rocks during low tide when the sea recedes (some tropical species move with the tides; Williams & Morritt 1995). This foraging pattern often precludes shelter-seeking behavior during low tide when environmental conditions might generate temperature and desiccation stress. Unlike more mobile species that could shuttle between different thermal microhabitats to control body temperature near some optimum performance peak (Huey 1991, Hertz et al. 1993, Allen & Levinton 2014), limpets are functionally sessile at low tide and their body temperatures can exceed the optimum temperature range, inducing sublethal or lethal stress in some cases (Dong et al. 2008). While rocks are dry, there is no opportunity to graze microalgae, but there is some indication that digestion may continue during low tide periods (Walker 1968, L. Miller pers. obs.). Limpets are ideal for studies of temperature effects since their large foot keeps them tightly thermally coupled to the underlying substratum; hence, temperature measurements of the substratum can provide accurate proxies for limpet body temperature without disturbing the organism (Wolcott 1973, Denny & Harley 2006).

The 4 *Lottia* species utilized in this study differ in their preferred shore height and microhabitat (Fig. 2). The vertical distributions of the 4 species overlap to some extent, but they are often found in distinct microhabitats. *L. pelta* Rathke and *L. limatula* Carpenter are found in the low and mid intertidal zone, with *L. pelta* favoring wave-exposed walls or mussel beds where it consumes both microalgae and macroalgae, while *L. limatula* is often found on more sun-exposed horizontal surfaces and feeds primarily on microalgae (Craig 1968, Eaton 1968, Wolcott 1973). *L. scabra* Gould and *L. austrodigitalis* Murphy are found higher on the shore, above the *Mytilus californianus* mussel zone and often above the limits of the *Endocladia muricata* macroalgal zone (Wolcott 1973). Both high-zone species are found on vertical walls, but *L. scabra* is also found on horizontal, sun-exposed rocks where *L. austrodigitalis* is often absent (Collins 1976, Hahn & Denny 1989). *L. austrodigitalis* is the highest ranging limpet on the central California coast, and is often found >5 m above Mean Lower Low Water (MLLW) on wave-exposed rock walls (Miller 1968) in a region where the maximal still-water tidal range is ~2.5 m.

In Monterey Bay, *L. austrodigitalis* overlaps with a cryptic congener, *L. digitalis* Rathke, from which it can only reliably be distinguished via genetic methods; the 2 species share similar behaviors and occupy the same microhabitats (Murphy 1978, Crummett & Eernisse 2007). Recent work at our field site at Hopkins Marine Station (HMS hereafter, Pacific Grove, CA, 36.6217° N, 121.9043° W) has shown that *L. austrodigitalis* makes up the majority (88 to 89%) of the population of the cryptic species pair living on high-

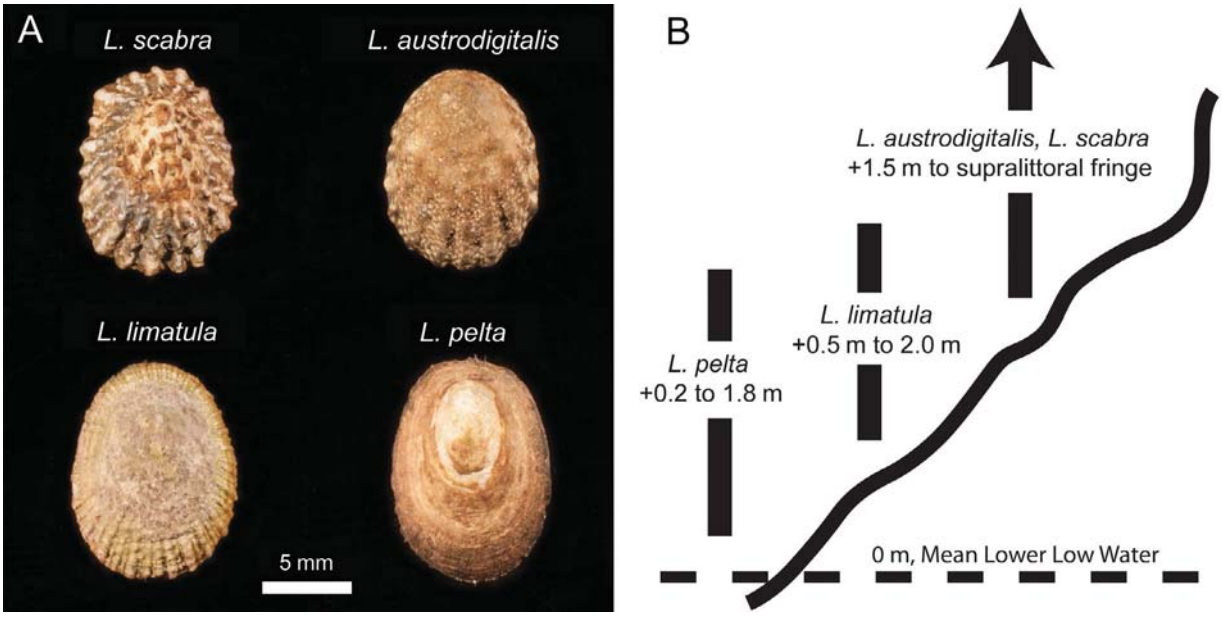

Fig. 2. (A) *Lottia* limpets from central California. (B) *L. scabra* and *L. austrodigitalis* are found in the high intertidal zone, while *L. limatula and L. pelta* are found in the low to middle intertidal zone

shore rock habitats where we sampled (Dong et al. 2008, Dong & Somero 2009). This work also indicates that the 2 species overlap in their median upper thermal tolerance limits, with *L. austrodigitalis* being marginally more tolerant and producing more thermally stable cytosolic malate dehydrogenase (Dong & Somero 2009). We refer to *L. austrodigitalis* hereafter in this study while acknowledging that a small fraction of our samples may include *L. digitalis.*

To explore the potential effects of sublethal temperature variation on intertidal limpets, we measured respiration rates across a range of temperatures under aquatic and aerial conditions in the laboratory and tracked growth in the field while measuring microhabitat temperature and microalgal food supply. We looked for evidence of physiological compensation for increasing temperatures via reductions in the Q_{10} response of respiration (Q_{10} is defined as the ratio of the rates of a physiological or biochemical process over a 10°C rise in temperature, where the common expectation is for a doubling of the rate, Q_{10} = 2; Hochachka & Somero 2002), and measured upper critical thermal maxima during aerial exposure. We expected to find increasing respiration rates with increasing body temperatures, such that field microhabitats with warmer low tide temperatures could either yield reduced limpet growth due to greater energetic demands, or increased growth if sufficient food was available to support higher metabolic rates. We hypothesized that high-shore and low-shore limpets would differ in their response to warmer low tide temperatures, with high-shore species being better adapted to cope with higher temperatures.

MATERIALS AND METHODS

Collections

We collected the 4 species of limpets — *Lottia scabra, L. austrodigitalis, L. limatula*, and *L. pelta* — from south- and east-facing rocks at HMS during September and October 2013. The individuals collected for the trials were representative of the range of sizes of sub-adult and small adult limpets found at HMS for each of the 4 species (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m522p127_supp.pdf). Batches of limpets were collected and held in a shaded seawater table for 2 to 7 d prior to use in the respiration trials. Temperature in the seawater table was monitored with an iButton temperature logger (DS1921G, Maxim Integrated) and remained at 15°C during the experimental period.

Respiration trials

The respiration chamber consisted of a custom-machined aluminum block with 15 wells of 15 ml vol-

ume each, and a bolt-on top plate that contained ports for purging the chambers and making oxygen measurements. The block was submerged in a digitally controlled water bath to maintain temperatures during trials. Oxygen measurements were taken using ruthenium sensor dots fixed to a glass port in the top plate of each well (aerial trials: RedEye patch, Ocean Optics; aquatic trials: SP-PSt3-NAU-D5-YOP, PreSens Precision Sensing) and read with a fiber optic fluorescence-based optode system (NeoFox, Ocean Optics). The chamber top plate contained machined recesses and an indexing pin to ensure that the optode was placed at the same height and incident angle relative to the sensor patch for every reading, since deviations in positioning will substantially alter the signal produced by the optode measuring system. Each oxygen sensor dot was recalibrated following each replicate trial using water-saturated normoxic air and pure CO_2 at the corresponding experimental temperature to make a 2 point calibration.

Aerial respiration

For aerial respiration trials, we reduced the volume of each chamber to 5 ml by inserting a 10 ml aluminum plug in the bottom of each well. Twelve limpets (3 per species) were run in individual wells along with 3 empty (blank) wells for each replicate trial. Each well also contained a 5 mm diameter piece of paper towel wetted with seawater to maintain 100% relative humidity during the trial. The aluminum block was initially held at 15°C for 20 min, and the temperature of the water bath and block was then raised or lowered to the target experimental temperature for each trial at a rate of 10°C h^{-1}. A total of 9 experimental temperatures were used in the aerial trials: 10, 15, 20, 25, 30, 32.5, 35, 37.5, and 40°C. The time during the ramp to lower and higher temperatures allowed limpets to acclimate to the chamber; for trial temperatures of 15°C, we waited 30 min (≈minimum acclimation period for the 10 and 20°C trials) before beginning the measurement process. The top plate of the chambers was bolted on and ports were sealed to begin a 2 h measurement period. During the sampling period, the fiber optic sensor for the optode system was moved to each chamber well in succession for a 15 s reading, and each well was sampled every 8 min. The 2 h exposure allowed sufficient time for limpets to consume a measurable amount of oxygen even at the lowest temperatures.

Aquatic respiration

The full 15 ml volume of the respiration chamber wells was used for the aquatic trials. We used artificial seawater (Instant Ocean) mixed to a practical salinity of 33 to fill each chamber. Seawater was equilibrated to 15°C and aerated before filling the chambers. As in the aerial respiration trials, a single limpet was placed in each well, with 3 ind. of each of the 4 species filling 12 of the chamber wells, along with 3 empty (blank) wells. Aquatic temperature trials took place at 10, 12.5, 15, and 17.5°C, to cover the range of typical seawater temperatures found throughout the year at HMS. The temperature of the chambers was changed at a rate of 10°C h^{-1}, and a minimum acclimation period of 30 min was given for trials that took <30 min to reach the experimental target temperature (12.5, 15, and 17.5°C trials). We used the 10°C h^{-1} rate of water temperature change to harmonize our trial run times with those of the aerial respiration trials, and although our largest shift in water temperature did not exceed 5°C, it should be noted that this rate of water temperature change is faster than the rate of natural water temperature shifts at this field site. Immediately prior to closing the chambers, we flushed each chamber with aerated seawater that was pre-equilibrated to the experimental temperature. Prior to taking a reading in each chamber, the water was stirred manually for 20 s with a stir rod mounted in one of the top ports. Readings were taken for 15 s, and each chamber was sampled every 8 min for 1 h. We chose this shorter trial time to avoid oxygen depletion in the water.

Processing respiration data

Immediately following a respiration trial, we weighed each limpet to the nearest 0.1 mg, in air and submerged in seawater. The displaced mass of the live limpet in seawater was used to calculate the volume that the limpet occupied in a chamber well. The volume of air or seawater in the chamber (minus the volume of the limpet) was used to calculate the volume of oxygen present at each time point. For aquatic trials, the concentration of O_2 in seawater (mg l^{-1}) was calculated using the temperature and salinity values for the trial with the relationship from Benson & Krause (1984), and converted to µmol of O_2 using the volume of seawater in the chamber. We fit a linear regression to the µmol of O_2 through time to estimate the O_2 consumption rate. The values from the blank control chambers were averaged and used

to correct for any drift that occurred during a trial. We dissected the tissue from the shell of each limpet and dried it in a drying oven at 60°C for 48 h. The dry tissue mass was used to calculate the mass-specific oxygen consumption rate for each limpet. Each limpet was used in only one temperature trial, and a total of 12 replicate limpets were used at each of the experimental temperatures for each species. We estimated Arrhenius break temperatures for log-transformed respiration rates with a piecewise regression using the R package 'segmented' (Muggeo 2008).

We calculated Q_{10} values for aerial respiration rates across each successive pair of temperatures in the experiment as:

$$Q_{10} = \left(\frac{Rate_2}{Rate_1}\right)^{\frac{10}{Temp_2 - Temp_1}} \quad (1)$$

To calculate 95% CIs on this estimate, we used a bootstrap resampling procedure on each pair of 12 respiration values at 2 temperatures to produce a distribution of log-transformed Q_{10} estimates that better accounts for potential skew in the calculated values than a standard error estimate based on the assumption of normality (Davison & Hinkley 1997).

Heat coma temperatures in air

At the conclusion of each aerial respiration trial, we probed each limpet to determine if it was still adhered to the chamber wall. Any limpet that was poorly adhered and had also retracted the mantle tissue back from the edge of the shell was judged to be in heat coma. We fit a logit-link binomial generalized linear model to calculate the median heat coma temperature (also termed the critical thermal maximum, CT_{max}) for each species after 2 h at the experimental temperature.

Field growth experiment

In June 2013, we deployed a series of experimental plates in the rocky intertidal zone at HMS to track limpet growth in various thermal microhabitats. Each plate was made of aluminum, 10 cm in diameter and 12 mm thick, and topped with a layer of light gray rubber grip tape (Safety Walk Tape, 3M). A 20 mm tall stainless steel mesh fence with 5.5 mm square openings was attached around the perimeter of the plate to dissuade limpets from crawling off the plate. We machined a pocket into the underside of each aluminum plate to hold an individually calibrated, wax-coated, iButton temperature datalogger with a resolution of 0.5°C (DS1921G, Maxim Integrated). The high thermal conductivity of aluminum, the close proximity of the iButton to the upper surface of the plate, and the high conductive heat exchange between the substratum and the large foot of a limpet allowed us to use the iButton temperature as a direct proxy for the body temperature of the limpets attached to the plate without disturbing the organisms (Wolcott 1973, Denny & Harley 2006). The iButtons recorded temperatures in each plate every 12 min; we downloaded the data every 2 wk.

We attached the experimental plates to the granite bedrock at HMS using a single bolt through the center of the plate, and ensured good thermal contact with the underlying rock by installing a thin layer of concrete between the plate and rock surface to fill surface irregularities. Each plate held 4 ind. from one of the 4 species of *Lottia* described above, and we deployed additional plates without limpets to serve as grazer exclusion controls. The resulting density of 0.5 limpets cm^{-2} is similar to values measured for natural high-shore *L. scabra* populations and lower than densities of limpet populations lower on the shore (Sutherland 1970, Morelissen & Harley 2007). A total of 12 plates per species (48 plates with limpets + 12 grazer exclusion plates) were placed on sloped or vertical surfaces at 1.4 or 1.7 m above MLLW in horizontal transects at 6 sites. The sites included wave-exposed and wave-protected microhabitats that faced predominantly north, east, or west, encompassing much of the variety in microhabitats occupied by these species at HMS. The limpets were collected from surrounding rocks and individually tagged with numbered bee tags (The Bee Works) using cyanoacrylate glue. When limpets were lost from plates during the experiment, they were replaced to keep the total number of limpets on each plate at 4. Missing limpets typically crawled over the fences and reestablished on the surrounding rock face. The different species showed different propensities for escaping, with an average of 0.11 ± 0.15 (1 SD) *L. scabra*, 0.70 ± 0.42 *L. limatula*, 1.12 ± 0.85 *L. pelta*, and 1.31 ± 0.94 *L. austrodigitalis* leaving per plate per census period. It should be noted that *L. scabra* typically establishes a home 'scar' and grows the margin of the shell to fit the contours of the rock (Wolcott 1973). This tight fit was lost when we placed *L. scabra* on our plates, and it is possible that this may have affected desiccation rates and energy expenditures initially. We observed that *L. scabra* quickly established new home scars on the plates, and new shell growth matched the margins of the shell to the

flatter surface of the experimental plate by the next census date.

Following the initial deployment on June 17, the limpets on each plate were censused on July 10, August 6, September 6, October 6, November 6, and December 1, 2013 as tide cycles and wave conditions allowed. We tracked limpet growth using digital photographs taken from overhead on each plate with a framer designed to keep a constant height and orientation to the plate, so that we could measure the projected area of each limpet shell to 0.1 mm^2 using ImageJ (Rasband 1997–2014).

We used a PAM fluorometer (Diving-PAM, Walz) to track microalgal densities on the experimental plates. Microalgae were allowed to settle naturally from the ocean for 1 mo prior to the start of data collection. During nighttime low tides associated with each limpet census, we took 6 haphazardly arrayed readings of dark-adapted fluorescence (F_0) on each plate; F_0 serves as a non-destructive proxy for microalgal chlorophyll *a* density (Barranguet & Kromkamp 2000, Honeywill et al. 2002, Serôdio et al. 2008). The tip of the fiber optic measuring head of the fluorometer was fitted with a 10 mm spacer to maintain a fixed distance from the plate surface, and the opening covered an area of 53 mm^2. The tip was held in place at each measurement site until the F_0 value stabilized (typically 3–5 s) before recording a value, as recommended by the manufacturer. As the amount of surface moisture can affect F_0 values (Maggi et al. 2013), we restricted sampling to periods when the plates were moist, but not actively splashed or submerged by the tide.

We used a generalized least squares linear model from the R package 'nlme' (Pinheiro & Bates 2000) to assess the relationship between $\log_e$-transformed algal fluorescence (F_0) and average daily maximum temperature during each census period, with limpet species (or grazer exclusion plates) as a fixed factor. The temporal correlation of F_0 values on individual plates across the census periods was incorporated using an AR(1) autoregressive correlation structure (Pinheiro & Bates 2000). A fixed effect of shore level (1.4 m or 1.7 m) was initially included in the model, but was non-significant based on likelihood ratio tests, so it was removed from the final model, and plates from both shore heights were pooled. For the model of limpet growth rate (shell + tissue mass change relative to initial mass, mg d^{-1}) at each census period, we used a linear mixed effects model to evaluate the interacting fixed effects of average daily maximum temperature during a census period, our proxy for log-transformed algal density (F_0) at the start of each census period, and limpet species identity (n = 921 observations among 359 limpets across 6 census periods). The model included a random effect for plates and a random effect for individual limpets nested within plates to account for nesting and for repeated measures of individual limpets across census periods. Log-transformed F_0 from the start of each census period was also included as a random covariate to account for temporal autocorrelation, and the model included an AR(1) correlation structure for the random factors. While there are numerous ways to describe the temperature conditions in the field, we used the average daily maximum temperature during a census period to summarize the differences between plates deployed in different microhabitats. Model residuals were checked for normality and for evidence of heterogeneity of variances. All analyses were carried out in R 3.1.1 (R Development Core Team 2014).

Estimating cumulative respiration

Using the temperature records from a subset of experimental plates and the data from our respiration trials, we estimated the cumulative respired O_2 of an average sized limpet of each species on the coolest and warmest plates (2 plates per species) on which that species was present in the field experiment for the entire period from June to December 2013. We chose to use the single lowest variation and single highest variation plate for each species to encompass the full range of temperature variation the limpets might have experienced in the field experiment. Because the experimental plates were alternately submerged and emersed by the tides, we used NOAA tide records for Monterey, CA, to determine when plates were likely submerged at high tide, and used respiration rates from the aquatic respiration trials for those time periods. All other time periods used the aerial respiration data. The respiration rate at a given temperature (μmol O_2 h^{-1} g^{-1} dry tissue mass) was multiplied by the dry tissue mass of a representative average sized limpet of each species and assumed constant for a 12 min interval to estimate the respired μmol of O_2 for each time step. When the temperature for a time point fell between 2 of the respiration trial temperatures, we used linear interpolation between the 2 closest trial temperatures to estimate respiration at the intermediate temperature. For any temperatures that fell below the limits of our respiration trial temperatures, we used the respiration rate value for the lowest trial temperature.

RESULTS

Respiration

All 4 species of *Lottia* limpets showed an increase in aerial respiration rates as temperatures rose until reaching a peak temperature after which respiration dropped as limpets entered heat coma (Fig. 3A, closed symbols). *L. scabra*, the high-shore species often found in sun-exposed horizontal microhabitats, had the highest temperature of peak respiration at 37.5°C. The high-shore, vertical-wall-favoring species *L. austrodigitalis* and the low-shore sun-exposed *L. limatula* both had a peak respiration rate near 35°C. The low-shore species *L. pelta*, which favors cooler wave-exposed vertical walls, had the lowest peak respiration temperature at 32.5°C. By 40°C, all of the species exhibited a decline in respiration rate, which is likely indicative of heart failure and heat coma (Bjelde & Todgham 2013). Our range of trial temperatures and the high peak temperature of respiration for *L. scabra* did not allow for a proper estimation of a break temperature for that species, but the break temperatures of the other species were lower than the likely break point for *L. scabra* near 37.5°C (Table 1). All 4 species exhibited their highest Q_{10} values at temperatures between 10 and 20°C (4.3 for *L. scabra*, 2.3 for *L. austrodigitalis*, 2.9 for *L. limatula*, and 2.2 for *L. pelta*; Fig. 3B). Each species showed a relaxation in Q_{10} to the 1.1–1.5 range at

Table 1. Estimated respiration break point and median heat coma (critical thermal maximum, CT_{max}) temperatures for limpets held in air, with temperatures raised from 15°C to a target temperature at a rate of 10°C h^{-1} and held for 2 h. NA: not applicable due to the occurrence of peak respiration for *Lottia scabra* at 37.5°C, which precluded estimation of a break point via piecewise regression from the single temperature above the peak

Species	Respiration break point temperature (°C ± 1 SE)	CT_{max} (°C ± 1 SE)
L. scabra	NA	39.6 ± 0.9
L. austrodigitalis	34.2 ± 1.0	38.8 ± 0.5
L. limatula	36.5 ± 0.4	36.9 ± 0.5
L. pelta	34.4 ± 0.4	34.6 ± 0.4

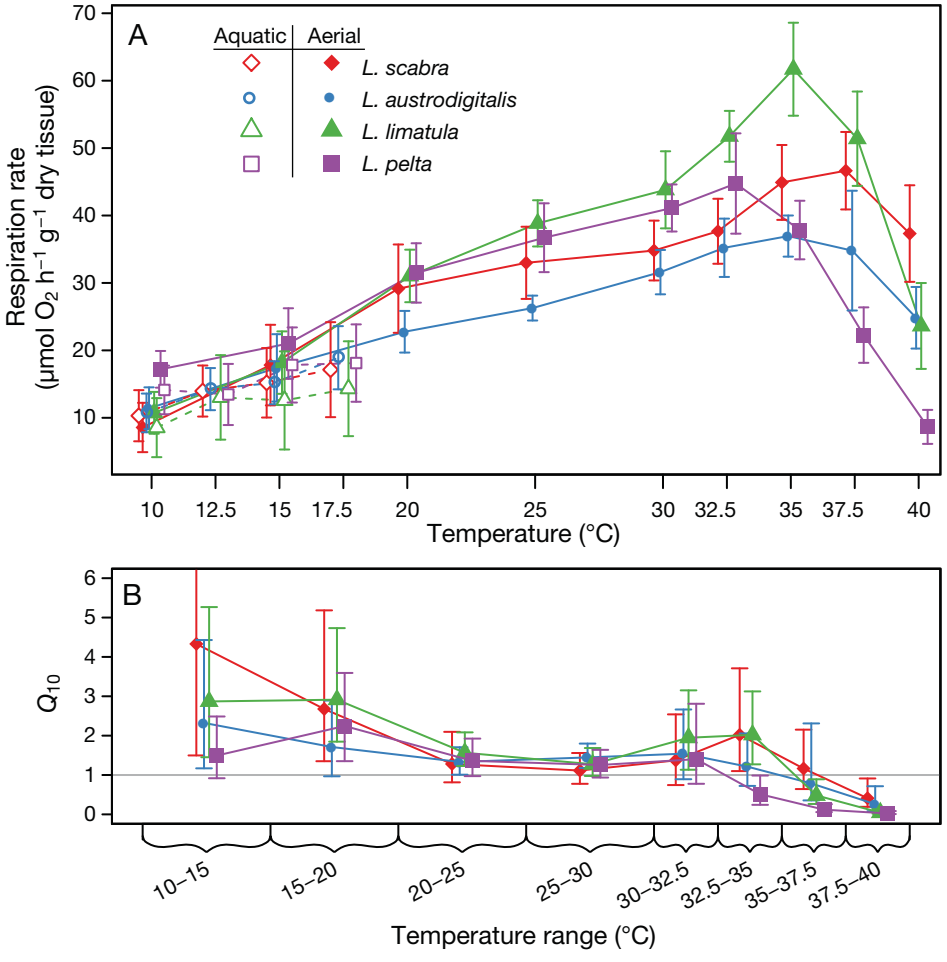

Fig. 3. (A) *Lottia* limpet mass-specific aerial respiration (closed symbols) and aquatic respiration (open symbols) rates with 95% CIs. The horizontal positions of the points have been staggered, but trials occurred at the temperatures indicated on the horizontal axis (n = 12 limpets per temperature). (B) Aerial respiration Q_{10} values for each temperature range, with bootstrapped 95% CIs back-transformed from log-transformed samples. The upper confidence limit for *L. scabra* in the 10–15°C range (=10.3) is cut off for clarity of the plotted values

temperatures between 20 and 30°C, with a brief increase in Q_{10} prior to the peak respiration temperature.

For the narrower range of water temperatures used in the aquatic trials, changes in respiration rate were much smaller than in the aerial trials (Fig. 3A, open symbols), with overlapping 95% CIs at all temperatures from 10 to 17.5°C. Three of the species, *L. scabra*, *L. austrodigitalis*, and *L. limatula*, had aquatic respiration Q_{10} values between 2.0 and 2.2 over the 10 to 17.5°C range, while *L. pelta* had a lower Q_{10} of 1.4.

Heat coma temperatures

Each of the 4 limpet species exhibited symptoms of heat coma at the highest aerial respiration trial temperatures (Table 1), and there were significant differences in median CT_{max} between species (Analysis of deviance for temperature $\chi^2 = 218$, df = 1, $p < 0.001$; species $\chi^2 = 43.9$, df = 3, $p < 0.001$). The 2 high-shore species, *L. scabra* and *L. austrodigitalis*, had significantly higher median CT_{max} values than the low-shore species (*L. scabra* = *L. austrodigitalis* > *L. limatula* > *L. pelta*; Tukey post-hoc tests, $p < 0.05$). All *L. limatula* and *L. pelta* had entered heat coma at 40°C, while some *L. scabra* and *L. austrodigitalis* remained adhered and responsive at the conclusion of the 2 h exposure even at the highest temperature in the experiment.

Field growth experiment

The ANCOVA analysis of log-transformed dark-adapted fluorescence F_0, our proxy for algal density, showed a non-significant interaction between average daily maximum temperature and limpet species identity ($F_{4,350} = 1.89$, $p = 0.111$), but there was a significant main effect of limpet species identity ($F_{4,350} = 80.8$, $p < 0.001$) and of the average daily maximum temperature covariate ($F_{1,350} = 104.3$, $p < 0.001$; see Table S2 in the Supplement at www.int-res.com/articles/suppl/m522p127_supp.pdf). Coefficient estimates for the model are given in Table S3. Tukey post-hoc tests of the main effect of limpet species (including grazer exclusions) showed that the intercepts for all 4 limpet species treatments were significantly lower than those for the grazer exclusion plates; *L. pelta* and *L. scabra* were not significantly different from each other, nor were *L. limatula* and *L. austrodigitalis*. Limpet grazing reduced the amount of algae on plates relative to grazer exclusions, but did not change the slope of the negative relationship between algal density and average daily maximum temperature found on all plates (Fig. 4).

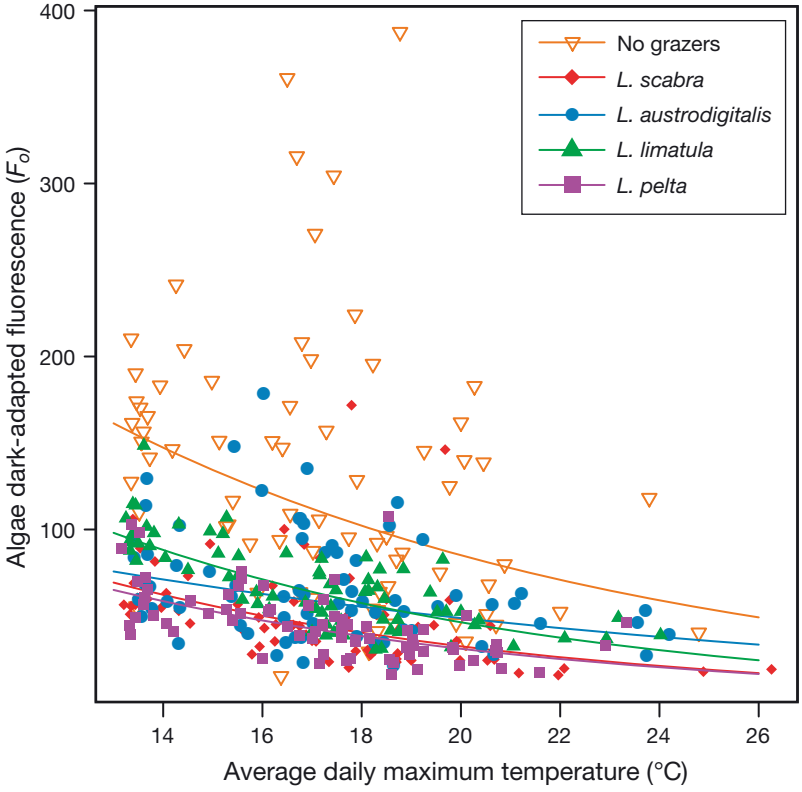

Fig. 4. Algae dark-adapted fluorescence, F_0 (a proxy for algal density), on experimental plates at different *Lottia* limpet species treatments and average daily maximum temperatures prior to each of 6 census dates between June and December 2013. Fitted lines are back-transformed estimates from models fitted with log-transformed F_0 values

Using the regression values for limpet mass vs. projected area in the census photographs (see Table S4), we were able to non-invasively track limpet growth on the experimental field plates throughout the experiment. There was a significant 3-way interaction between average daily maximum temperature during a census period, log-transformed F_0 at the start of each census period, and limpet species identity (Table 2; $F_{3,671} = 8.12$, $p < 0.001$). Partial effects plots for the 3-way ANCOVA (Fox & Weisberg 2011) revealed that predicted limpet growth rate remained flat or increased with

Table 2. Linear mixed effects model summary for limpet growth rate (mg d^{-1}). Average daily maximum temperature during a census period, algal density (log-transformed dark-adapted fluorescence, F_0) at the start of a census period, and limpet species were treated as fixed factors. Random effects included log-transformed algal density, experimental plates, and individual limpets nested within experimental plates in order to account for nesting and repeated measures through time. The model accounts for first order autocorrelation among repeated measures using an AR(1) autoregressive structure

Treatment	df_{num}	df_{den}	F	p
Average (avg.) daily maximum (max.)(°C)	1	671	20.36	<0.001
Algae density, Log (F_0)	1	671	17.20	<0.001
Species	3	44	1.63	0.196
Avg. daily max. × Algae density	1	671	37.50	<0.001
Avg. daily max. × Species	3	671	4.84	0.002
Algae density × Species	3	671	3.16	0.024
Avg. daily max. × Algae density × Species	3	671	8.12	<0.001

increasing average daily maximum temperature across a range of representative F_0 values, but that the slope of the relationship differed among limpet species and F_0 levels (Fig. 5; coefficient estimates are given in Table S5).

Estimated respiration in the field

The experimental plates deployed in the field at HMS showed a 3-fold variation in average daily temperature range from the coolest to the warmest plate for each species, and a

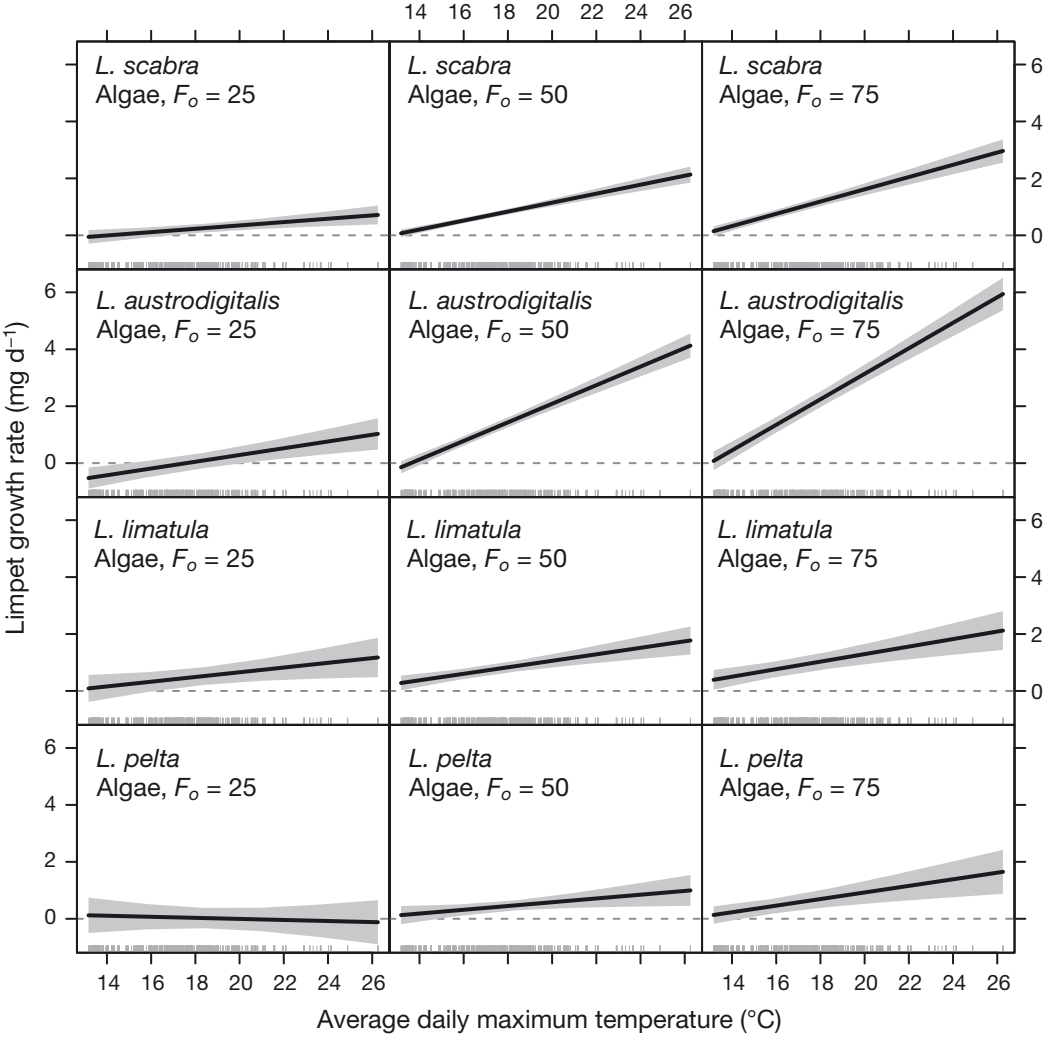

Fig. 5. Partial regression slopes (±95% CIs shown in gray) from the linear model of *Lottia* limpet growth rate illustrating the fitted relationship between average daily maximum temperature (°C) and limpet growth rate (mg d^{-1}) for the different limpet species during a census period, at different starting algal densities (log F_0) of 25 (left column), 50 (middle column) and 75 (right column). The rug of points on the *x*-axis represents the distribution of average daily maximum temperatures in the dataset

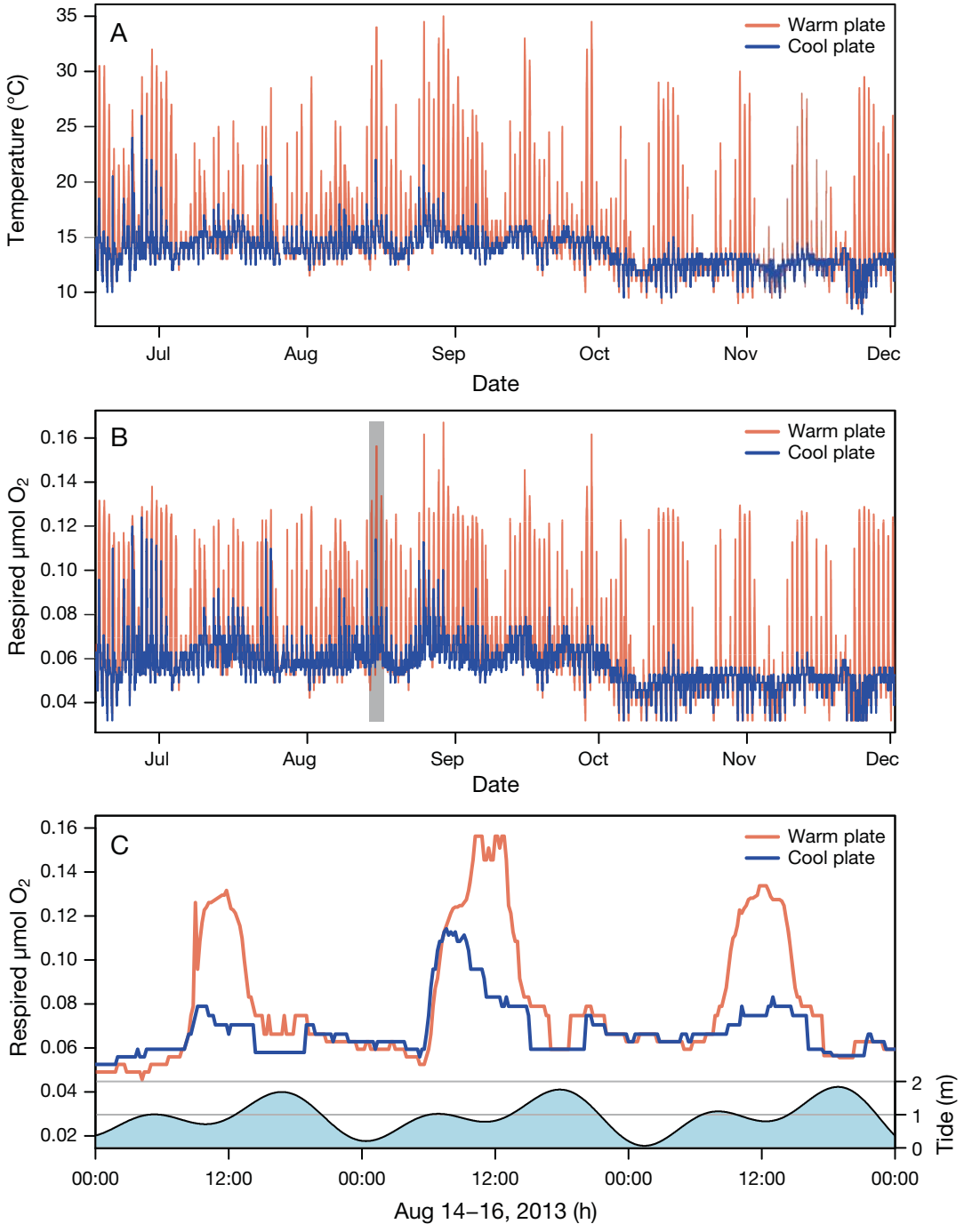

Fig. 6. (A) Temperature records, and (B) estimated respired oxygen per 12 min time step for an average sized *Lottia scabra* (18.5 mg dry tissue mass) living on the single warmest (red) and single coolest (blue) plate in the field experiment. A close up view of the 3-day time period represented by the gray box in (B) is shown in (C), along with the corresponding tides

9 to 13°C difference in maximum temperatures (Fig. 6A, Table 3). Using the temperature data from the coolest and the warmest plates for each species, we predicted a 5 to 14% increase in cumulative respired O_2 for average sized limpets living on the warmest plates (Fig. 6B,C; Table 3) over the entire experimental period, relative to the coolest plate. None of the plates exceeded the estimated CT_{max} thresholds for any of the species during the 24 wk of the experiment.

Table 3. Temperature statistics for the single coolest and single warmest plate containing each *Lottia* limpet species in the field, and estimated cumulative respiration on the coolest and warmest plate over the course of the experiment from June to December 2013

Species	Average daily temperature range (°C)		Maximum temperature (°C)		Estimated cumulative respired oxygen (µmol)		Estimated respiration increase
	Cool	Warm	Cool	Warm	Cool	Warm	(%)
L. scabra	3.3	10.6	26.0	35.0	1150	1314	14.3
L. austrodigitalis	2.9	8.5	23.0	36.5	1383	1456	5.3
L. limatula	2.4	7.8	21.5	34.5	2520	2732	8.4
L. pelta	2.9	8.2	24.5	33.5	2148	2363	10.0

DISCUSSION

All 4 species of *Lottia* limpets exhibit a large increase in respiration rate with increasing temperature while aerially exposed. In seawater temperatures within the normal yearly range for HMS (10 – 17.5°C), respiration rates are typically in the range of 8 to 18 µmol O_2 h^{-1} g^{-1} dry tissue mass, but when limpets are exposed to air temperatures within the range of extremes found at low tide, their peak respiration rates range from 30 to 60 µmol O_2 h^{-1} g^{-1}. At low temperatures, there was substantial overlap in aerial and aquatic respiration rates, although the trend for increasing respiration rate in water appears lower than in air. A lower overall respiration rate in water at higher temperatures has been observed in *L. digitalis* (Bjelde & Todgham 2013), but other intertidal species such as *Pisaster* seastars show the opposite pattern, with higher aquatic respiration rates than aerial respiration rates at the same temperature (Fly et al. 2012).

We see some evidence for differential respiration responses and susceptibility to heat stress while emersed between the low-shore species (*L. pelta* and *L. limatula*) and the high-shore species (*L. austrodigitalis* and *L. scabra*). Both high-shore species have higher median CT_{max} values and maintain slightly lower aerial respiration rates at temperatures ranging from 25 to 32.5°C than the low-shore species. *L. pelta*, the low-shore species that is typically found on vertical faces, in wave-exposed microhabitats, or hiding under algal cover, is the least tolerant of prolonged aerial exposure at high temperatures and had the lowest CT_{max} and lowest temperature of peak respiration, although it also interestingly maintains a relatively high aerial respiration rate at temperatures common in its preferred cool microhabitats. *L. pelta* may be particularly adapted to maximizing metabolism and growth in cool microhabitats, at the cost of reduced tolerance to higher temperatures. The second low-shore species, *L. limatula,* which inhabits microhabitats that are similar to those of *L. pelta* but is also often found on horizontal sun-exposed rocks in the low and mid shore zone, shows the most drastic increase (6-fold) in aerial respiration rate over the 10 to 35°C range. Both low-shore species had estimated respiration break points within 1°C of their median CT_{max} values, so that the range of temperatures where maximum respiration rate occurs are just below temperatures that cause the onset of heat coma. In contrast, *L. austrodigitalis*, which is the limpet species living highest on the shore at HMS, limits its respiration rate increase to half that of *L. limatula* across the same temperature range, perhaps reflecting a need to limit energy expenditure during the frequent prolonged aerial exposure periods that come with living high on the shore and the reduced availability of algal food resources to support a high metabolic rate. The CT_{max} for *L. austrodigitalis* was 4°C higher than the estimated respiration break point temperature, indicating that this high-shore species can maintain attachment to rocks and avoid signs of heat coma longer after its respiration has begun to falter. The other high-shore species, *L. scabra*, is typically found in warmer microhabitats than *L. austrodigitalis*, living on horizontal sun-exposed rocks that occasionally reach the highest intertidal temperatures at HMS. While *L. scabra* exhibits a slightly higher respiration rate than *L. austrodigitalis*, the peak rate of the former occurs at a slightly higher temperature, and is accompanied by a slightly higher CT_{max}, indicating greater thermotolerance.

Each of the limpet species shows some evidence of metabolic rate control as they move through the 20 to 30°C temperature range, which is the most common range of warm, but not extreme, daytime low tide rock temperatures at this site (Denny et al. 2006, Miller et al. 2009). Q_{10} values for this temperature range typically remain below 1.5, which is lower than the expected value of 2 to 3 for many temperature-dependent metabolic processes (Hochachka & Somero 2002). There is a growing number of examples of intertidal organisms showing some level of metabolic rate control or depression during warm temperature exposures, including the limpet *L. digitalis* (Bjelde & Todgham 2013), which has an overlapping range

with *L. austrodigitalis* in Monterey Bay. Limpets from South Africa (Marshall & McQuaid 1991) and some intertidal snails also exhibit metabolic rate control (McMahon & Russell-Hunter 1977, Sokolova et al. 2000, Marshall et al. 2011) in the range of warm daytime low tide temperatures, although the response is not universal for intertidal gastropods (McMahon & Russell-Hunter 1977, McMahon et al. 1995). Among the tropical species that exhibit metabolic rate depression, the magnitude of this depression appears to be greater than that shown here by the temperate limpets, and it is hypothesized that the more frequent and severe exposure to high temperatures in the tropics may accentuate the need to control energy expenditures during prolonged emersion (Marshall et al. 2011). Even when there is evidence for metabolic rate control at moderately warm temperatures in intertidal molluscs, Q_{10} values still tend to increase at the extreme limits of thermotolerance (Marshall et al. 2011), as seen in all 4 limpet species studied here.

In the field, we observed a strong negative relationship between average daily maximum temperature and algal fluorescence (F_0) on plates in different thermal microhabitats. The effect of limpet grazing lowered algal density compared to grazer exclusion plates, but did not change the negative relationship with increasing temperature. The greater amount of variability in F_0 values on 'No grazer' plates may be due to a combination of factors related to plate location on the shore, including exposure to sun or shading, wave splash, and the presence of small opportunistic grazers such as *Littorina* snails that may have crawled through the mesh fence and grazed the plates at some sites. Additionally, the consistent feeding of limpets on the grazed plates may serve to mute the inherent variability in microalgal density along the shore. For the limpets growing on these plates, the interacting effects of algal availability and temperature across plates led to flat or slightly positive growth rates with increasing maximum temperature and algal resources.

The observed increase in growth rates on warmer experimental plates, at least in the presence of higher algal densities for *L. austrodigitalis* and *L. scabra*, supports the possibility of a potential benefit to mild increases in low tide temperature above the predominant sea surface temperature range. On the warmest plates measured here, limpets spent only 10% of the total time at temperatures above 20°C during the 24 wk field experiment (Fig. 1B shows representative data for 2 plates), and never exceeded the estimated CT_{max} limits for any of the species. The predictions of increases in cumulative respired O_2 on the warmest plates ranged from 5 to 14%, but the increased respiration did not manifest as significant decreases in growth rate. The positive or neutral interactive effects of algal density and temperature on limpet growth rates seem to outweigh the negative effects of increasing temperature alone. In fully aquatic habitats, growth rates often increase if there is sufficient food to support higher metabolic rates (O'Connor 2009, Pangle & Peacor 2010, O'Regan et al. 2014). The combination of warmer waters and warmer low tide conditions between sites on the California coast has also been implicated in faster growth rates in mussels and other intertidal consumers (Phillips 2005, Blanchette et al. 2007). In the current experiment where ocean temperature is consistent across all of our microsites, we see evidence for positive effects of warmer low tide conditions alone. However, it is important to reiterate that these low tide temperature conditions were primarily non-stressful, and that low tide temperatures approaching the CT_{max} values of the limpet species did not occur. Conditions on our temperate shoreline are only rarely stressful enough to reach critical thermal maxima, in contrast to tropical sites where limpets and other high-shore grazers may routinely experience near-lethal temperatures (Williams & Morritt 1995, Williams et al. 2005, Marshall et al. 2010, Dong et al. 2014); thus, further warming in more stressful tropical regions will likely have predominantly negative effects.

There are several caveats to this general conclusion of beneficial effects of increased microalgal densities and warmer temperatures on limpet growth rates. All 4 limpet species graze microalgae and cyanobacteria from the substratum, but the different thermal environments in our field experiment may drive differences in algal growth rates and species composition that could change the available energy for limpet growth (Castenholz 1961). Although we observed a general decline in algal density on plates with increasing temperature, we lack information on the species composition of the microalgal communities on the different plates, or their nutritional value. A second caveat is that our estimates of cumulative respiration are based only on rates for limpets at rest during a single acute exposure to air or seawater. Particularly following a high-temperature aerial exposure, the post-exposure period during the next high tide may bring prolonged increased respiration rates to accommodate increased metabolic demands of the heat-shock response that drives repair or degradation of damaged proteins (Dong et al. 2008, Bjelde & Todgham 2013). There could also be a need

to recover from anaerobic metabolism (Ellington 1983), although there was no evidence of anaerobic metabolic end product accumulation in *L. digitalis* from central California following aerial exposure (Bjelde & Todgham 2013). Due to these potential additional metabolic demands, our long-term estimates of cumulative respiration may underestimate the respired O_2, particularly on days when temperatures reach stressful levels during low tide, although this would make the pattern of increased growth rate in warmer microsites all the more surprising. Finally, it should be noted that our experiments utilized subadult and small adult limpets, but we have no performance information for smaller limpets. Smaller limpets should have higher mass-specific metabolic rates and reduced energy stores relative to larger limpets; these factors may enhance the impacts of emersion temperature stress on newly recruited individuals (Spencer Davies 1966, Kiørboe & Hirst 2014).

The thermal response curves measured here should be contrasted with the types of curves typically reported for organisms such as lizards or insects (Angilletta 2009). The peak in respiration we observed for limpet temperatures approaching 40°C may have a different interpretation than a thermal performance curve representing other metrics such as feeding rate, locomotion speed, growth rate, or fecundity. For these other metrics, temperatures at the peak of the performance curve might well be the most desirable place to spend time from the standpoint of individual or population growth. In contrast, our measure of respiration rate, as a metric of metabolic rate and calorie consumption, is somewhat removed from true measures of organismal fitness. While we observed a peak in respiration at temperatures in the 32.5 to 37.5°C range, it is not clear whether these temperatures necessarily represent a true 'performance peak' or 'optimum', particularly as temperatures in this range are known to induce a heat-shock response in limpets (Dong et al. 2008, Bjelde & Todgham 2013). Instead, for whole-organism fitness, limpet body temperatures slightly below the range of peak respiration rates may be closer to an optimum (Martin & Huey 2008), particularly if they reduce the risk and associated cost of a heat-shock response but allow for faster catabolic and anabolic rates.

Lottia limpets at HMS showed a clear rise in respiration rates in response to rising body temperatures during aerial exposure, and a quick decline in respiration as they reached extreme high temperatures that induce heat coma. The 2 high-shore species, *L. scabra* and *L. austrodigitalis*, maintained slightly lower respiration rates than their low-shore counterparts during intermediate temperatures in the 20 to 30°C range; they also had a higher CT_{max}, in line with the expectation that the frequency and severity of high temperature exposures should be higher in the upper littoral zone. In tracking growth over several months in the field, we saw little evidence for decreased growth in microhabitats with higher temperature variability and attendant higher peak temperatures, despite estimated respiration demands being at least 5 to 14% higher. In some cases we even observed increased growth rates at warmer sites when food was abundant. The relatively short amount of total time encompassed by warm low tide exposures may have a small impact on growth rates, but when the majority of these exposures are mild enough to avoid stressful extreme temperatures, warmer microhabitats may be beneficial for intertidal consumers. Faster metabolic rates among limpets could drive increased grazing effort at high tide to support greater metabolic demand and increase growth rates, strengthening top-down control of microalgal density on the shore. However, given the negative relationship between warmer low tide temperatures and microalgal density observed in our grazer exclusion treatments, there is a possibility for negative feedback on limpet growth if algal growth rates cannot support increased grazing pressure from limpets. Ultimately, the impacts of climate warming on energy transfer and growth rates in intertidal habitats will be determined by this interaction between rising temperatures and species' individual temperature responses that are likely optimized for intermediate temperature ranges. The present-day variation in temperature over small spatial scales in the intertidal (Denny et al. 2011) encompasses conditions that could increase growth rates in some instances, but continuing warming of low tide aerial temperatures could begin to push organisms past their performance optima.

Data Accessibility. Data related to this paper are deposited in the Stanford University Libraries Digital Repository at http://purl.stanford.edu/mz343tz6255. The project is registered with the NSF Biological and Chemical Oceanography Data Management Office (BCO-DMO; http://www.bco-dmo.org/) under grant numbers OCE-1131038 and OCE-1130095.

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LITERATURE CITED

Allen BJ, Levinton JS (2014) Sexual selection and the physiological consequences of habitat choice by a fiddler crab. Oecologia 176:25–34

Angilletta MJ (2009) Thermal adaptation: a theoretical and empirical synthesis. Oxford University Press, Oxford

Angilletta MJ, Huey RB, Frazier MR (2010) Thermodynamic effects on organismal performance: Is hotter better? Physiol Biochem Zool 83:197–206

Barranguet C, Kromkamp J (2000) Estimating primary production rates from photosynthetic electron transport in estuarine microphytobenthos. Mar Ecol Prog Ser 204: 39–52

Bayne BL, Scullard C (1978) Rates of feeding by *Thais* (*Nucella*) *lapillus* (L.). J Exp Mar Biol Ecol 32:113–129

Benson BB, Krause D Jr (1984) The concentration and isotopic fractionation of oxygen dissolved in freshwater and seawater in equilibrium with the atmosphere. Limnol Oceanogr 29:620–632

Bjelde BE, Todgham AE (2013) Thermal physiology of the fingered limpet *Lottia digitalis* under emersion and immersion. J Exp Biol 216:2858–2869

Blanchette CA, Helmuth BST, Gaines SD (2007) Spatial patterns of growth in the mussel, *Mytilus californianus*, across a major oceanographic and biogeographic boundary at Point Conception, California, USA. J Exp Mar Biol Ecol 340:126–148

Branch GM (1981) The biology of limpets: physical factors, energy flow, and ecological interactions. Oceanogr Mar Biol Annu Rev 19:235–380

Brett JR, Higgs DA (1970) Effect of temperature on the rate of gastric digestion in fingerling sockeye salmon, *Oncorhynchus nerka*. J Fish Res Board Can 27:1767–1779

Castenholz RW (1961) Effect of grazing on marine littoral diatom populations. Ecology 42:783–794

Collins LS (1976) Abundance, substrate angle, and desiccation resistance in two sympatric species of limpets. Veliger 19:199–203

Craig PC (1968) The activity pattern and food habits of the limpet *Acmaea pelta*. Veliger 11:13–19

Crummett L, Eernisse D (2007) Genetic evidence for the cryptic species pair, *Lottia digitalis* and *Lottia austrodigitalis* and microhabitat partitioning in sympatry. Mar Biol 152:1–13

Davison AC, Hinkley DV (1997) Bootstrap methods and their application. Cambridge University Press, Cambridge

Denny MW, Harley CDG (2006) Hot limpets: predicting body temperature in a conductance-mediated thermal system. J Exp Biol 209:2409–2419

Denny MW, Miller LP, Harley CDG (2006) Thermal stress on intertidal limpets: long-term hindcasts and lethal limits. J Exp Biol 209:2420–2431

Denny MW, Hunt LJH, Miller LP, Harley CDG (2009) On the prediction of extreme ecological events. Ecol Monogr 79: 397–421

Denny MW, Dowd WW, Bilir L, Mach KJ (2011) Spreading the risk: small-scale body temperature variation among intertidal organisms and its implications for species persistence. J Exp Mar Biol Ecol 400:175–190

Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR (2008) Impacts of climate warming on terrestrial ectotherms across latitude. Proc Natl Acad Sci USA 105:6668–6672

Diefenbach COdC (1975) Gastric function in *Caiman crocodilus* (Crocodylia: Reptilia) — I. Rate of gastric digestion and gastric motility as a function of temperature. Comp Biochem Physiol A 51:259–265

Dong Y, Somero GN (2009) Temperature adaptation of cytosolic malate dehydrogenase of limpets (genus *Lottia*): Differences in stability and function due to minor changes in sequence correlate with biogeographic and vertical distributions. J Exp Biol 212:169–177

Dong Y, Miller LP, Sanders JG, Somero GN (2008) Heat-shock protein 70 (Hsp70) expression in four limpets of the genus *Lottia*: Interspecific variation in constitutive and inducible synthesis correlates with *in situ* exposure to heat stress. Biol Bull 215:173–181

Dong Y, Han G, Huang X (2014) Stress modulation of cellular metabolic sensors: interaction of stress from temperature and rainfall on the intertidal limpet *Cellana toreuma*. Mol Ecol 23:4541–4554

Eaton CM (1968) The activity and food of the file limpet *Acmaea limatula*. Veliger 11:5–12

Ellington WR (1983) The recovery from anaerobic metabolism in invertebrates. J Exp Zool 228:431–444

Elliott JM (1976) The energetics of feeding, metabolism and growth of brown trout (*Salmo trutta* L.) in relation to body weight, water temperature and ration size. J Anim Ecol 45:923–948

Elliott JM (1982) The effects of temperature and ration size on the growth and energetics of salmonids in captivity. Comp Biochem Physiol B 73:81–91

Feder ME, Hofmann GE (1999) Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu Rev Physiol 61:243–282

Fly EK, Monaco CJ, Pincebourde S, Tullis A (2012) The influence of intertidal location and temperature on the metabolic cost of emersion in *Pisaster ochraceus*. J Exp Mar Biol Ecol 422-423:20–28

Fox J, Weisberg S (2011) An R companion to applied regression. SAGE Publications, Thousand Oaks, CA

Gilman SE (2006) Life at the edge: an experimental study of a poleward range boundary. Oecologia 148:270–279

Hahn T, Denny M (1989) Tenacity-mediated selective predation by oystercatchers on intertidal limpets and its role in maintaining habitat partitioning by *Collisella scabra* and *Lottia digitalis*. Mar Ecol Prog Ser 53:1–10

Hawkins SJ, Hartnoll RG (1983) Grazing of intertidal algae by marine invertebrates. Oceanogr Mar Biol Annu Rev 21:195–282

Helmuth B (1999) Thermal biology of rocky intertidal mussels: quantifying body temperatures using climatological data. Ecology 80:15–34

Hertz PE, Huey RB, Stevenson R (1993) Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. Am Nat 142:796–818

Hochachka PW, Somero GN (2002) Biochemical adaptation: mechanism and process in physiological evolution. Oxford University Press, New York, NY

Hoekman D (2010) Turning up the heat: temperature influences the relative importance of top-down and bottom-up effects. Ecology 91:2819–2825

Honeywill C, Paterson D, Hagerthey S (2002) Determination of microphytobenthic biomass using pulse-amplitude modulated minimum fluorescence. Eur J Phycol 37: 485–492

Huey RB (1991) Physiological consequences of habitat selection. Am Nat 137:S91–S115

Huey RB, Stevenson RD (1979) Integrating thermal physio-

logy and ecology of ectotherms: a discussion of approaches. Am Zool 19:357–366
Hunt LJH, Denny MW (2008) Desiccation protection and disruption: a trade-off for an intertidal marine alga. J Phycol 44:1164–1170
Iles AC (2014) Towards predicting community level effects of climate: relative temperature scaling of metabolic and ingestion rates. Ecology 95:2657–2668
Jones NS (1948) Observations and experiments on the biology of *Patella vulgata* at Port St. Mary, Isle of Man. Proc Trans Liverpool Biol Soc 56:60–77
Jones SJ, Mieszkowska N, Wethey DS (2009) Linking thermal tolerances and biogeography: *Mytilus edulis* (L.) at its southern limit on the east coast of the United States. Biol Bull 217: 73–85
Kiørboe T, Hirst AG (2014) Shifts in mass scaling of respiration, feeding, and growth rates across life-form transitions in marine pelagic organisms. Am Nat 183: E118–E130
Kratina P, Greig HS, Thompson PL, Carvalho-Pereira TSA, Shurin JB (2012) Warming modifies trophic cascades and eutrophication in experimental freshwater communities. Ecology 93:1421–1430
Kroeker KJ, Gaylord B, Hill TM, Hosfelt JD, Miller SH, Sanford E (2014) The role of temperature in determining species' vulnerability to ocean acidification: a case study using *Mytilus galloprovincialis.* PLoS ONE 9:e100353
Largen MJ (1967) The influence of water temperature upon the life of the dog-whelk *Thais lapillus* (Gastropoda: Prosobranchia). J Anim Ecol 36:207–214
Maggi E, Jackson AC, Tolhurst T, Underwood AJ, Chapman MG (2013) Changes in microphytobenthos fluorescence over a tidal cycle: implications for sampling designs. Hydrobiologia 701:301–312
Marshall DJ, McQuaid CD (1991) Metabolic rate depression in a marine pulmonate snail: pre-adaptation for a terrestrial existence? Oecologia 88:274–276
Marshall DJ, McQuaid CD, Williams GA (2010) Non-climatic thermal adaptation: implications for species' responses to climate warming. Biol Lett 6:669–673
Marshall DJ, Dong Yw, McQuaid CD, Williams GA (2011) Thermal adaptation in the intertidal snail *Echinolittorina malaccana* contradicts current theory by revealing the crucial roles of resting metabolism. J Exp Biol 214: 3649–3657
Martin TL, Huey RB (2008) Why 'suboptimal' is optimal: Jensen's Inequality and ectotherm thermal preferences. Am Nat 171:E102–E118
McMahon RF, Russell-Hunter WD (1977) Temperature relations of aerial and aquatic respiration in six littoral snails in relation to their vertical zonation. Biol Bull 152:182–198
McMahon RF, Russell-Hunter WD, Aldridge DW (1995) Lack of metabolic temperature compensation in the intertidal gastropods, *Littorina saxatilis* (Olivi) and *L. obtusata* (L.). Hydrobiologia 309:89–100
Miller AC (1968) Orientation and movement of the limpet *Acmaea digitalis* on vertical rock surfaces. Veliger 11: 30–44
Miller LP (2013) The effect of water temperature on drilling and ingestion rates of the dogwhelk *Nucella lapillus* feeding on *Mytilus edulis* mussels in the laboratory. Mar Biol 160:1489–1496
Miller LP, Harley CDG, Denny MW (2009) The role of temperature and desiccation stress in limiting the local-scale distribution of the owl limpet, *Lottia gigantea.* Funct Ecol 23:756–767
Miller LP, Matassa CM, Trussell GC (2014) Climate change enhances the negative effects of predation risk on an intermediate consumer. Glob Change Biol 20:3834–3844
Morelissen B, Harley CDG (2007) The effects of temperature on producers, consumers, and plant-herbivore interactions in an intertidal community. J Exp Mar Biol Ecol 348:162–173
Muggeo VMR (2008) Segmented: an R package to fit regression models with broken-line relationships. R News 8: 20–25
Muñoz JLP, Finke GR, Camus PA, Bozinovic F (2005) Thermoregulatory behavior, heat gain, and thermal tolerance in the periwinkle *Echinolittorina peruviana* in central Chile. Comp Biochem Physiol A Mol Integr Physiol 142:92–98
Murphy PC (1978) *Collisella austrodigitalis* sp. nov.: a sibling species of limpet (Acmaeidae) discovered by electrophoresis. Biol Bull 155:193–206
O'Connor MI (2009) Warming strengthens an herbivore–plant interaction. Ecology 90:388–398
O'Connor MI, Piehler MF, Leech DM, Anton A, Bruno JF (2009) Warming and resource availability shift food web structure and metabolism. PLoS Biol 7:e1000178
O'Regan SM, Palen WJ, Anderson SC (2014) Climate warming mediates negative impacts of rapid pond drying for three amphibian species. Ecology 95:845–855
Pangle KL, Peacor SD (2010) Temperature gradients, not food resource gradients, affect growth rate of migrating *Daphnia mendotae* in Lake Michigan. J Gt Lakes Res 36: 345–350
Phillips NE (2005) Growth of filter-feeding benthic invertebrates from a region with variable upwelling intensity. Mar Ecol Prog Ser 295:79–89
Pincebourde S, Sanford E, Helmuth B (2008) Body temperature during low tide alters the feeding performance of a top intertidal predator. Limnol Oceanogr 53:1562–1573
Pincebourde S, Sanford E, Casas J, Helmuth B (2012) Temporal coincidence of environmental stress events modulates predation rates. Ecol Lett 15:680–688
Pinheiro JC, Bates DM (2000) Mixed-effects models in S and S-PLUS. Springer Verlag, NY
Pörtner HO (2012) Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes. Mar Ecol Prog Ser 470:273–290
R Development Core Team (2014) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria
Rasband WS (1997–2014) ImageJ, U. S. National Institutes of Health, Bethesda, MD, USA. http://rsb.info.nih.gov/ij/
Sanford E (1999) Regulation of keystone predation by small changes in ocean temperature. Science 283:2095–2097
Sanford E (2002) Water temperature, predation, and the neglected role of physiological rate effects in rocky intertidal communities. Integr Comp Biol 42:881–891
Sanford E, Menge BA (2001) Spatial and temporal variation in barnacle growth in a coastal upwelling system. Mar Ecol Prog Ser 209:143–157
Serôdio J, Vieira S, Cruz S (2008) Photosynthetic activity, photoprotection and photoinhibition in intertidal microphytobenthos as studied *in situ* using variable chlorophyll fluorescence. Cont Shelf Res 28:1363–1375
Sokolova IM, Granovitch AI, Berger VJ, Johannesson K (2000) Intraspecific physiological variability of the

gastropod *Littorina saxatilis* related to the vertical shore gradient in the White and North Seas. Mar Biol 137: 297–308

Spencer Davies P (1966) Physiological ecology of *Patella*. I. The effect of body size and temperature on metabolic rate. J Mar Biol Assoc UK 46:647–658

Stich HB, Lampert W (1984) Growth and reproduction of migrating and non-migrating *Daphnia* species under simulated food and temperature conditions of diurnal vertical migration. Oecologia 61:192–196

Stillman JH (2002) Causes and consequences of thermal tolerance limits in rocky intertidal porcelain crabs, genus *Petrolisthes.* Integr Comp Biol 42:790–796

Stillman JH (2003) Acclimation capacity underlies susceptibility to climate change. Science 301:65

Sutherland JP (1970) Dynamics of high and low populations of the limpet, *Acmaea scabra* (Gould). Ecol Monogr 40: 169–188

Tewksbury JJ, Huey RB, Deutsch CA (2008) Putting the heat on tropical animals. Science 320:1296–1297

Tomanek L (2002) The heat-shock response: its variation, regulation, and ecological importance in intertidal gastropods (genus *Tegula*). Integr Comp Biol 42:797–807

Tomanek L, Somero GN (1999) Evolutionary and acclimation-induced variation in the heat-shock responses of congeneric marine snails (Genus *Tegula*) from different thermal habitats: implications for limits of thermotolerance and biogeography. J Exp Biol 202:2925–2936

Tomanek L, Zuzow MJ (2010) The proteomic response of the mussel congeners *Mytilus galloprovincialis* and *M. trossulus* to acute heat stress: implications for thermal tolerance limits and metabolic costs of thermal stress. J Exp Biol 213:3559–3574

Walker CG (1968) Studies on the jaw, digestive system, and coelomic derivatives in representatives of the genus *Acmaea.* Veliger 11:88–97

Williams GA, Morritt D (1995) Habitat partitioning and thermal tolerance in a tropical limpet, *Cellana grata.* Mar Ecol Prog Ser 124:89–103

Williams GA, De Pirro M, Leun KMY, Morritt D (2005) Physiological responses to heat stress on a tropical shore: the benefits of mushrooming behaviour in the limpet *Cellana grata.* Mar Ecol Prog Ser 292:213–224

Wolcott TG (1973) Physiological ecology and intertidal zonation in limpets (*Acmaea*): a critical look at limiting factors. Biol Bull 145:389–422

Woodin SA, Hilbish TJ, Helmuth B, Jones SJ, Wethey DS (2013) Climate change, species distribution models, and physiological performance metrics: predicting when biogeographic models are likely to fail. Ecol Evol 3: 3334–3346

Yamane L, Gilman SE (2009) Opposite responses by an intertidal predator to increasing aquatic and aerial temperatures. Mar Ecol Prog Ser 393:27–36

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