Vol. 627: 95–107, 2019
https://doi.org/10.3354/meps13058

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published September 26

The cost of emersion for the barnacle *Balanus glandula*

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ABSTRACT: Temperate intertidal species frequently experience broad temperature fluctuations during emersion. However, the metabolic cost of exposure to a particular emersion temperature is not known for most species. We quantified oxygen (O_2) consumption by the intertidal barnacle *Balanus glandula* over a combined 5 h emersion and a 6 h immersion period. Barnacles were exposed to air temperatures of 10, 15, 20, 25, 30, 35, or 38°C followed by a 10°C immersion. Respiration was monitored using a fluorometric O_2 system. Total O_2 consumption over the 11 h period by *B. glandula* increased with increasing emersion temperatures, reaching a maximum between 20 and 30°C, where consumption was significantly greater than that at 10°C. Aerial and aquatic phases showed similar patterns with temperature, but significant differences among temperatures were only detected in the aerial phase. We also found that respiration rates peaked during the first hour at temperature during emersion and the second hour of immersion. A separate analysis of barnacle behavior over a longer immersion period suggested that stressful emersion temperatures require recovery periods longer than 6 h; thus, our results may underestimate the full cost of thermal stress. When compared to previously published measurements of barnacle body temperatures in the field, our results suggest a large vertical gradient in thermal exposure costs, nearly doubling with a 1 m increase in shore height. We highlight both the difficulty and importance of generating accurate estimates of emersion costs. Such costs are likely to be critical in determining organismal and population responses to changing climate.

KEY WORDS: Intertidal barnacle · Respiration · Temperature · Emersion stress · Energetic cost

1. INTRODUCTION

Temperate rocky intertidal systems are characterized by high levels of both temporal and spatial thermal heterogeneity. Over the course of a daily tidal cycle, organisms may experience thermal fluctuations of as much as 30°C as they move from aquatic to terrestrial environments (Helmuth et al. 2006, Gilman et al. 2015). Moreover, organisms separated by as little as a meter may differ in body temperature by 10°C or more (due to differences in low tide duration, solar aspect, or other abiotic factors), and these factors can significantly influence thermal tolerance (Helmuth & Hofmann 2001, Seabra et al. 2011, Gilman et al. 2015, Stickle et al. 2016, 2017). Not surprisingly, thermal (and other abiotic) stresses of emersion have large ecological consequences for the species and communities that inhabit these shores. Chief among these is intertidal zonation, the tendency of intertidal organisms to occur in distinct vertical bands along the shore. Many species' upper vertical limits have been attributed to tolerance of thermal stress during emersion (Connell 1961, Somero 2002, Levinton 2017). Sublethal effects of thermal stress

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during emersion, such as reduced growth rates, changes in behavior, and changes in species interactions, have also been documented (Sutherland 1970, Gillmor 1982, Petes et al. 2008, Harley 2011, King & Sebens 2018).

Despite the clear importance of emersion stress for structuring intertidal communities, few studies have documented the energetic costs of emersion at different temperatures (but see Fly et al. 2012). Calculating these costs is also critical for predicting the consequences of climate change for both individual organisms and community interactions (Sarà et al. 2011, Dell et al. 2014, Matzelle et al. 2015). There is a large body of literature documenting the direct physiological consequences of temperature for organisms, including changes to enzyme function, induction of heat shock proteins, transcriptomic responses and others (Somero 2002, Sokolova et al. 2012, Somero et al. 2017). However, it is challenging to translate this information into specific ecological consequences for an organism, population, or community (Torossian et al. 2016, Gilman 2017). Energetic approaches, which focus on the aggregate energetic costs of thermal stress rather than the mechanisms underlying the costs, are a potential tool for quantifying the effects of both emersion and thermal stress on performance (Sokolova et al. 2012, Sarà et al. 2014). These are typically calculated from laboratory measurements of oxygen use under different abiotic conditions (Fly et al. 2012, Monaco et al. 2016). The approach has great potential both for modeling species' responses to climate change (Dillon et al. 2010, Sarà et al. 2011) and for calculating the energetic costs of low tide exposure (Fly et al. 2012, Monaco et al. 2016).

Measurements of the energetic costs that intertidal species incur at low tide can be challenging to obtain, for several reasons. First, measurements must be made in the appropriate medium. Temperate intertidal species generally experience the greatest range of temperatures during emersion, as water temperatures are much less variable than air temperatures (Helmuth et al. 2006, Seabra et al. 2011). Yet, as Bjelde & Todgham (2013) note, studies often measure thermal performance only in water. Species may show either greater or lesser rates of oxygen consumption in air than in water (Houlihan & Innes 1982, McMahon 1990, Bjelde & Todgham 2013), yet models often assume energetics during emersion either do not differ from immersion or do not matter (Kearney et al. 2010, Monaco et al. 2014). Second, measurements of aerial metabolism must be conducted in an ecologically relevant way. This means replicating a realistic tidal cycle in the lab, as intertidal animals can exhibit tidal rhythms in physiological processes (Hawkins et al. 1983, Connor & Gracey 2011). Similarly, measurements should be made over realistic emersion durations. Many studies have used short emersion periods of ~1 h (Fly et al. 2012, Miller et al. 2015), but longer measurements often reveal more complex patterns (McGaw et al. 2015, Yin et al. 2017). Finally, one must consider whether immediate oxygen use reflects the full energetic demand of exposure. Many (Ellington 1983), but not all (Castro et al. 2001), intertidal species show an oxygen debt after exposure to low tide, as evidenced by increased respiration rates upon re-immersion.

In this study, we quantify the energetic cost of low tide exposure over a range of temperatures for the intertidal barnacle *Balanus glandula*. This species is a common mid- to high-shore barnacle in the northeastern Pacific, acting as a foundation species on rocky shores (Glynn 1965), and has recently invaded the coasts of Asia, Africa, and South America (Geller et al. 2008, Simon-Blecher et al. 2008). We acclimated the animals to a laboratory tidal cycle and measured respiration in trials encompassing 5 h of emersion and 6 h of immersion. We exposed barnacles to a range of emersion temperatures from 10 to 40°C, based on previous field observations of barnacle body temperatures (Gilman et al. 2015). We use these data to address 4 specific questions. First, what is the overall energetic cost to *B. glandula* of low tide exposure at a specific temperature? Second, is there evidence of an oxygen debt at stressful emersion temperatures? Third, does the duration of measurement alter the estimate of energetic costs? Fourth, how do energetic costs vary across *B. glandula*'s vertical distribution on the shore?

2. MATERIALS AND METHODS

2.1. Study site

Barnacles *Balanus glandula* were collected from the University of Washington's Friday Harbor Laboratories Biological Preserve (FHL) on San Juan Island in the Salish Sea of northern Washington and southern Canada (48° 32′ 44″ N, 123° 0′ 46″ W). The region is characterized by low wave action and mixed semidiurnal tides. Intertidal temperatures peak in the summer, as the lower low tide predominantly occurs in the daytime from March through September (Connell 1970). Gilman et al. (2015) reported summer body temperatures for *B. glandula* at FHL of 7 to

40°C with both a strong thermal gradient with shore height and a strong effect of microhabitat (Fig. 1, see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m627p095_supp.pdf). Water temperatures recorded at the FHL dock by a realtime sensor average ~10°C in the summer (www.nodc.noaa.gov/cwtg/all_meanT.html).

2.2. Animal collection and maintenance

We collected individual barnacles on PVC settlement tiles (15.24 × 15.24 × 0.16 cm) deployed intertidally on the main dock at FHL at least 12 mo prior to the study. We shipped tiles, wrapped in damp paper towels, on ice, overnight to the W. M. Keck Science Center (Claremont, CA, USA). Upon arrival, settlement tiles were submerged in a 200 l seawater tank at 10 ± 1°C for 2 d to recover from transport. After 2 d, we carefully cut individual barnacles from the tiles by cutting a circle of tile around each barnacle with a band saw. Individual barnacles were then labeled and suspended from short lengths of nylon monofilament line (5.4 kg test, attached using a hot melt adhesive) on a plastic egg crate rack (25 cm tall). We measured operculum length (Palmer 1980, Gilman et al. 2013) to the nearest 0.01 mm with digital Vernier calipers (model CD-6′CX, Mitutoyo America, Aurora, IL). Barnacles were then placed on a tidal cycle typical of summer neap tide conditions in the center of *B. glandula*'s vertical distribution at FHL, with 2 low tides from 09:30 to 14:30 h and 21:30 00:30 h and a 15 h light:9 h dark cycle from 05:30 to 21:30 h. Water temperatures were maintained at 10 ± 1°C and air temperatures during emersion at 19°C, typical of low to mid-shore temperatures during summer low tides in Friday Harbor, WA (Fig. 1, Fig. S1). Barnacles were given at least 1 wk to acclimate to the laboratory tidal cycle and to recover from any stress related to processing them. We fed barnacles twice weekly with *Artemia* spp. nauplii (INVE Aquaculture Nutrition, Salt Lake City, UT), hatched 48 to 72 h prior to feeding (Gilman et al. 2013). During feedings, barnacles were submerged in a separate 10 l tank for at least 5 h. This feeding regime has previously been shown to result in barnacle growth and weight gain (Gilman et al. 2013). We starved barnacles for 48 h prior to each trial.

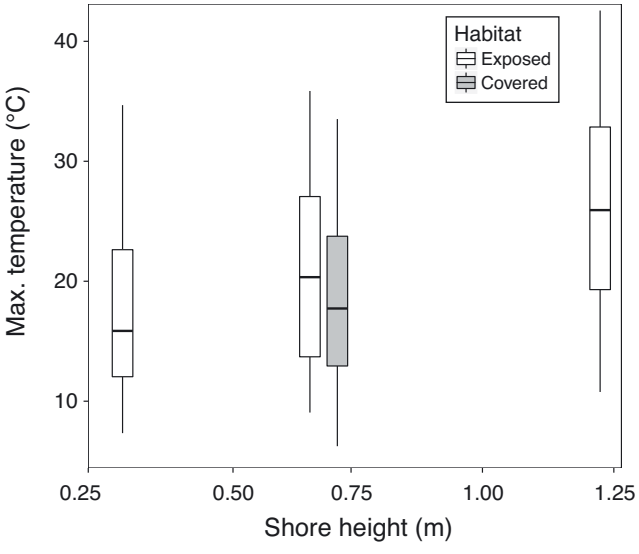

Fig. 1. Daily maximum summer body temperatures of *Balanus glandula* at Friday Harbor Laboratories Biological Preserve (FHL) by shore height and exposure, from data collected by Gilman et al. (2015). Horizontal lines represent medians, boxed by 25th and 75th percentiles, with whiskers representing the minimum and maximum

2.3. Respiration experiment

We tested barnacles using an incomplete block design, with 7 blocks of 3 barnacles. The blocks were tested at 3 randomly assigned emersion temperatures (10, 15, 20, 25, 30, 35, and 38°C). Barnacles were given at least 2 wk recovery time between repeated trials. The order of temperatures was partially randomized within blocks, with 35 and 38 always coming last. The first 38°C trial was actually run at 40°C, but 2 of 3 barnacles died shortly after the trial. Thus, 38°C was used for the rest of the experiment. We pooled the respiration data for 38 and 40°C trials, resulting in 9 replicates for all emersion temperatures. Trials consisted of 5 h of emersion and 6 h of immersion. Full details of experimental design and calculations are provided in the online supplement. A brief summary is given here.

Each trial started with 30 min of immersion at 10°C. Barnacles were placed into individual respiration chambers that were filled with seawater and were immersed in a 10°C water bath. After 30 min, the seawater was siphoned off, and once the oxygen sensors had stabilized, we began heating the water bath. We heated the bath at a rate of 10°C h^{-1} (Gilman et al. 2015, Miller et al. 2015) until the barnacles reached the experimental temperature and then held them at temperature for the remainder of the emersion period. They were then transferred to a second respirometry chamber filled with 0.22 µm filtered seawater, in a second 10°C water bath for the 6 h immersion period.

Oxygen concentrations in both air and water were recorded every 10 s with a fluorometric oxygen system with non-invasive sensor spots (Fibox 4 with Pst3-NAU spots, PreSens Precision Sensing, Regensburg). Aerial measurements were made in units of air saturation (%a.s.), aquatic measurements in µmol l^{-1}. A control chamber was included in both phases. All aquatic chambers were flushed with fresh seawater any time oxygen concentrations in one chamber fell below 85% of the initial readings (Sokolova & Portner, 2001). Aerial chambers were never flushed as oxygen levels never dropped below 90% of starting values. In addition, the linear rates of oxygen consumption observed in both aerial and aquatic chambers suggest that hypoxic conditions were avoided.

All calculations were completed using the Presens Oxygen Calculator (v 2.2.6) and R (v 3.4.2). We divided all aerial observations by the corresponding control chamber value, to correct for the effect of pressure changes within the vial during heating. We then used the 10 s observations to calculate rates of oxygen consumption over each 5 min interval within the trial. For aquatic data, we first calculated the 5 min rates and then subtracted the value of the control chamber from each experimental chamber to control for backgrounds rates of oxygen consumption in the seawater. We converted measurements of concentration to µmol O_2 by multiplying by chamber volume.

We calculated the average 5 min respiration rates (µmol O_2 min^{-1}) for both aerial and aquatic trials over the total period and separately for each hour of the trial. We also calculated the total oxygen consumption (µmol O_2) for each phase and identified the 95th percentile of 5 min aquatic respiration rates for each barnacle. Finally, we calculated the energetic cost and oxygen debt of each temperature treatment relative to the observed oxygen consumption in the 10°C treatment.

2.4. Barnacle behavior during recovery

To observe recovery over a longer immersion period, we exposed additional blocks of 8 barnacles to each of the 8 emersion temperatures and followed their behavior over 13 h. These barnacles were placed into a separate aerial chamber and immersed in the water bath during 1 emersion trial at each temperature. After the emersion period, we transferred the barnacles to a 38 l glass aquarium with 10°C seawater and video-recorded their behavior for the next 13 h (Sony Handycam, HDR-XR260V). To compare the frequency of activity across temperature and time, we noted the number of barnacles actively extending their cirri within a 1 min period at 10 min intervals of the video.

2.5. Statistical analysis

Total oxygen consumption (µmol O_2) was compared across treatments using a mixed-model ANCOVA, with temperature as a fixed, categorical factor. We used a 2-way mixed-model ANCOVA to compare the effects of temperature and medium (air or water) on mean respiration rates (µmol O_2 min^{-1}). We compared respiration rates by hour and temperature using a separate mixed-model ANCOVA for each medium. Maximum (95^{th} percentile) aquatic respiration rates were compared using a mixed-model ANOVA. Finally, we compared the frequency of barnacle activity in the videos using a 2-way ANOVA with temperature and hour as fixed effects.

All statistics were performed using R Software (v. 3.4.2; packages: lme4 v. 1.1-17, lmerTest v. 2.0-33, multcomp v. 1.4-6). All models were tested for normality and homogeneity of variance and found to fit these assumptions. For all analyses (except barnacle behavior), block, individual barnacle ID, and trial date were initially added as random factors. Block and date were ultimately excluded, based on non-significant likelihood ratio tests. Operculum length was also included in these models as a covariate. Post hoc analyses were performed using either Dunnett's test, with 10°C as the control, or Tukey's HSD test.

2.6. Calculation of the cost of low tide exposure

To estimate the monthly costs of low tide at FHL, we fit our total cost data calculated from temperatures below 35°C with a Boltzmann-Arrhenius function (Gillooly et al. 2001, Vasseur & McCann 2005, Iles 2014):

$$\mathrm{Cost}_{\mathrm{mol}} = c\, e^{-E/kT}$$

Here, E (eV) is the activation energy, k is Boltzmann's constant (8.62310×10^{-5} eV K^{-1}), T is the temperature in Kelvin, and c is a normalization constant. The function excluded data for 35 and 38°C as these treatments likely underestimate costs (as discussed in Section 3.4). We then created 100 randomly sampled summer months, with a 'month' consisting of 30 daily maximum temperatures taken from the barnacle body temperature dataset of Gilman et al.

(2015) for a given height and habitat. We multiplied each temperature by the cost function, then summed across all days in the month to get a total cost for each sampled month. We converted from µmol O_2 to Joules (J) using 0.457 J µmol O_2^{-1} (Hill et al. 2008, Fly et al. 2012).

3. RESULTS

3.1. Total oxygen consumption

Total oxygen consumption of *Balanus glandula* over the 11 h trial was significantly affected by emersion temperature ($F_{8,47.1}$ = 5.34, p = 0.0003). Oxygen consumption increased with increasing emersion temperature between 10 and 20°C and remained high until 30°C, with a maximum mean observed oxygen consumption of 6.0 (±0.9 SE) µmol O_2 at 20°C (Fig. 2). A Dunnett's test revealed significantly elevated total oxygen consumption at all temperatures from 20 to 35°C, when compared to 10°C (Fig. 2). No other model terms were significant (operculum length: $F_{1,18.02}$ = 2.39, p = 0.14; barnacle identity: χ^2 = 2.05, p = 0.20).

3.2. Medium-specific respiration rates

Mean respiration rates in emersion and immersion trials followed a similar pattern to total oxygen consumption, with both aerial and aquatic rates increasing with increasing emersion temperature and peaking between 20 and 30°C (Fig. 3). Oxygen consumption varied significantly by temperature within both emersion and immersion phases (Table 1). We observed significantly elevated aerial oxygen consumption, up to 5-fold higher, at emersion temperatures of 20 to 38°C compared to that at 10°C, based on Dunnett's test. Conversely, aquatic respiration increases were modest (<25%), and the only significant difference detected from 10°C was a ~65% decrease at 38°C.

When pooled across all temperatures, aerial respiration rates were significantly greater than aquatic rates (Table 1). There was also a significant interaction between medium and emersion temperature. At cooler emersion temperatures (10 and 15°C), aquatic and aerial respiration rates were fairly similar (Fig. 3). At high emersion temperatures, aerial respiration rates were greater than aquatic rates, in some cases as much as double the rates recorded during immersion.

We also observed a significant effect of emersion temperature on maximum short-term aquatic respiration rates ($F_{6,61}$ = 4.23, p = 0.001). However, post hoc analysis of the 95th percentile respiration rates revealed similar rates across nearly all emersion temperatures, relative to the 10°C treatment (Table 2). The exception was the 38°C emersion treatment, which exhibited a 55% lower maximum respiration rate than the 10°C treatment.

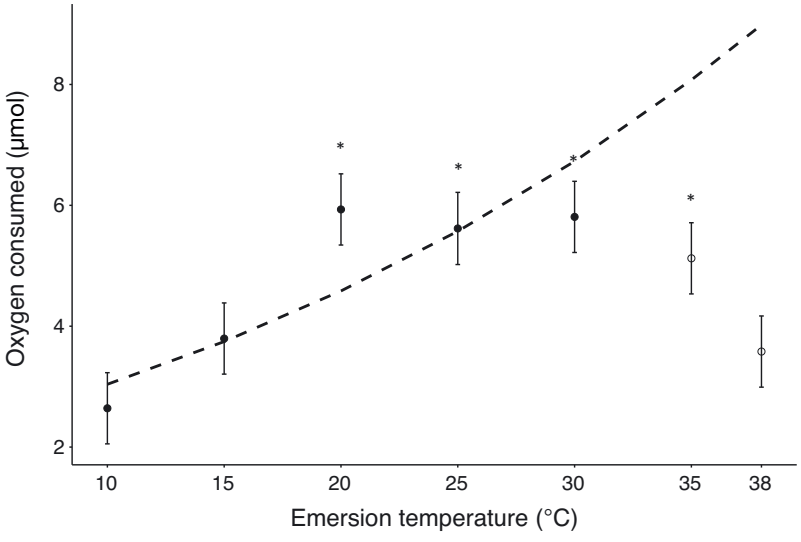

Fig. 2. Total oxygen consumption of a barnacle over the combined emersion and immersion phases. Data presented are least square means (LSM) ± SE for a 5.15 mm barnacle. N = 9 barnacles for all emersion temperatures. The dashed line reflects a Boltzmann-Arrhenius curve fit to data from 10–30°C only (black circles). *values significantly greater than that observed at 10°C, based on a Dunnett's test ($p < 0.05$)

3.3. Temporal patterns of oxygen consumption

The hourly aerial respiration rates were significantly affected by both temperature and the duration of time at emersion temperature, but their interaction was not significant (Table 3). At all emersion temperatures, respiration rates during the first hour at the treatment temperature were >20% greater than rates measured during subsequent hours (Fig. 4a). This difference was only statistically significant when comparing the first and third hours.

Similarly, both emersion temperature and time immersed significantly affected the hourly aquatic respiration rates, but their interaction was not significant (Table 3). Most emersion

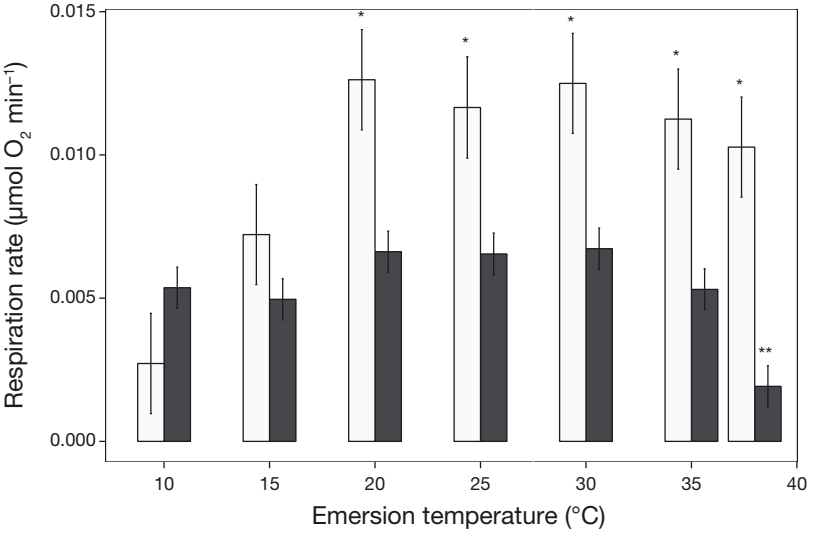

Fig. 3. Least squared mean respiration rates (±1 SE) during emersion (light gray bars) and immersion (dark gray bars), for a barnacle of 5.15 mm operculum diameter. Rates for emersion are calculated for only the hours spent at the treatment temperature, rates for immersion include the full 6 h period. Asterisks indicate values significantly different from that observed at 10°C, based on a Dunnett's test ($p < 0.05$) (*emersion, **immersion)

Table 1. Mixed-model ANCOVA for the effects of emersion temperature and medium on *B. glandula*'s respiration rate (µmol O_2 min^{-1}). Significant p-values are in **bold**

Fixed effects	df	SS	*F*	p
Temperature	6, 94.16	4.91×10^{-4}	5.57	**<0.001**
Medium	1, 93.21	6.10×10^{-4}	41.383	**<0.001**
Temp × Medium	6, 93.21	3.65×10^{-4}	4.15	**0.009**
Operculum	1, 18.67	3.20×10^{-5}	2.16	0.16
Random effects		**df**	**χ^2**	**p**
Barnacle		1	1.93	0.20

temperatures yielded the same general pattern of respiration over time, peaking in the second or third hour after immersion (Fig. 4b). However, after 38°C emersion, aquatic respiration rates increased continually throughout the 6 h of immersion. A Tukey's test revealed that, pooled across all temperatures, aquatic respiration rates during the first and sixth hours of immersion were significantly lower than the rates measured in the second and third hour of immersion.

The video analysis of behavior revealed similar temporal patterns to the aquatic respiration measurements (Fig. 5). The average fraction active in each hour was significantly affected by both emersion temperature ($F_{7,95} = 155.1$, $p < 0.0001$), hour at immersion ($F_{11,95} = 11.99$, $p < 0.0001$), and their interaction ($F_{77,95} = 6.39$, $p < 0.0001$). We identified 3 distinct patterns of response. From 10 to 20°C, ~50% of the barnacles were active at any point in the first hour, declining to <25% by Hour 4 or 5. Few significant differences from 10°C were detected at 15 or 20°C (Fig. 5). Barnacles in the 25, 30 and 35°C treatments started out with >75% of individuals active during the first hour and declined gradually thereafter, dropping below 50% activity by Hour 7 in the 25 and 30°C treatments and Hour 10 in the 35°C treatment. At least 1 of those 3 treatments had significantly greater activity than 10°C for Hours 1 to 9. At 38 and 40°C, barnacles started with significantly

Table 2. Energetic costs of low tide exposure. The first 3 columns show the total and medium-specific costs, relative to the 10°C treatment. Total cost is based on all 11 h of aerial and aquatic observations, aerial cost is based on 5 h of data from the emersion trial, and aquatic cost is based on 6 h of data from the immersion trial. The final column is the maximum 5 min aquatic respiration rate observed at each emersion temperature. All values are least square means (±1 SE), calculated for a barnacle of 5.15 mm operculum diameter. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, relative to the control (10°C) treatment, Dunnett's test

Emersion temperature (°C)	Total cost (µmol O_2)	Aerial cost (µmol O_2)	Aquatic cost (µmol O_2)	95%ile Aquatic respiration rate (µmol O_2 min^{-1})
10	NA	NA	NA	0.014 ± 0.0015
15	1.154 ± 0.99	1.299 ± 0.81	−0.140 ± 0.44	0.011 ± 0.0015
20	3.290 ± 0.99***	2.833 ± 0.81***	0.462 ± 0.44	0.016 ± 0.0015
25	2.976 ± 0.99**	2.583 ± 0.81***	0.381 ± 0.44	0.012 ± 0.0016
30	3.168 ± 0.99***	2.711 ± 0.81***	0.480 ± 0.44	0.013 ± 0.0015
35	2.481 ± 0.99**	2.476 ± 0.81**	−0.014 ± 0.44	0.010 ± 0.0015
38	0.938 ± 0.99	2.165 ± 0.81**	−1.204 ± 0.44*	0.006 ± 0.0015**

Table 3. Mixed-model ANCOVAs for the effects of emersion temperature and hour on respiration rate during aerial and aquatic phases. Significant p-values in **bold**

Fixed factors	Aerial				Aquatic			
	df	SS	*F*	p	df	SS	*F*	p
Temperature	6, 151.2	2.90×10^{-3}	10.76	**<0.0001**	6, 329.2	9.02×10^{-4}	27.43	**<0.0001**
Hour	2, 140.2	3.10×10^{-4}	3.43	**0.035**	5, 316.0	1.41×10^{-4}	5.16	**0.0001**
Temperature × Hour	11, 140.2	2.60×10^{-4}	0.53	0.88	30, 316.0	1.57×10^{-4}	0.96	0.53
Operculum	1, 18.0	1.75×10^{-5}	0.40	0.54	1, 18.66	1.02×10^{-5}	1.87	0.19
Random factor		df	χ^2	p		df	χ^2	p
Barnacle		1	12.7	**<0.0001**		1	76.2	**<0.0001**

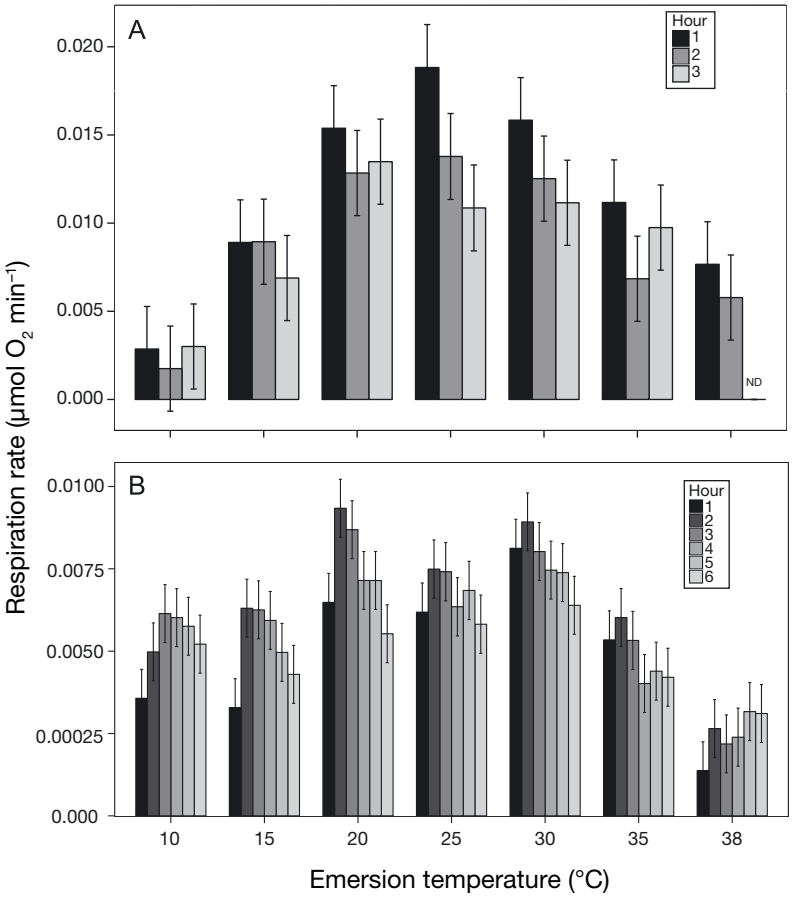

Fig. 4. Respiration rates by hour of (A) emersion and (B) immersion. Data are least square means (LSM) ± SE, calculated for a 5.15 mm barnacle. Rates for emersion are calculated for only the hours spent at the treatment temperature. Barnacles exposed to a 38°C emersion did not have a full third hour of data (ND) due to the long ramping time required for this temperature

lower percentages active than at 10°C, and activity increased gradually over time. Activity in the 38°C treatment ultimately exceeded levels in the 10°C (Fig. 5). The 40°C barnacles never exceeded 25% active in any hour. Many of the 40°C barnacles were initially open, but their cirri were inactive for the first several hours of immersion, and some died within 48 h of exposure. Mortality was not observed in any other treatment. Active barnacles at 35°C and above also appeared to move more slowly in the videos, although this behavior was not quantified.

3.4. Cost of low tide

We detected a significant cost of low tide exposure, relative to 10°C, at all temperatures examined except 15 and 38°C (Table 2). The bulk of the increased oxygen consumption occurred during emersion, with elevated aerial respiration composing anywhere from 85 to 100% of the total cost (Table 2). We observed only a modest increase in aquatic oxygen (0.38 to 0.48 µmol) for temperatures between 20 and 30°C (Table 2), indicative of an oxygen debt; however, these values were not significantly different from 10°C.

Total oxygen consumption declined at temperatures above 30°C, which is suggestive of functional impairment. Thus, our recorded total costs likely underestimate the true cost of low tide at these temperatures. Our fitted Boltzmann-Arrhenius equation for temperatures below 35°C was $\text{Cost}_{\text{mol}} = 0.518 \times e^{-0.294/kT}$ (Fig. 2). From this equation, we predict the full cost of exposure to 35 and 38°C to be 8.1 and 9.0 µmol, respectively.

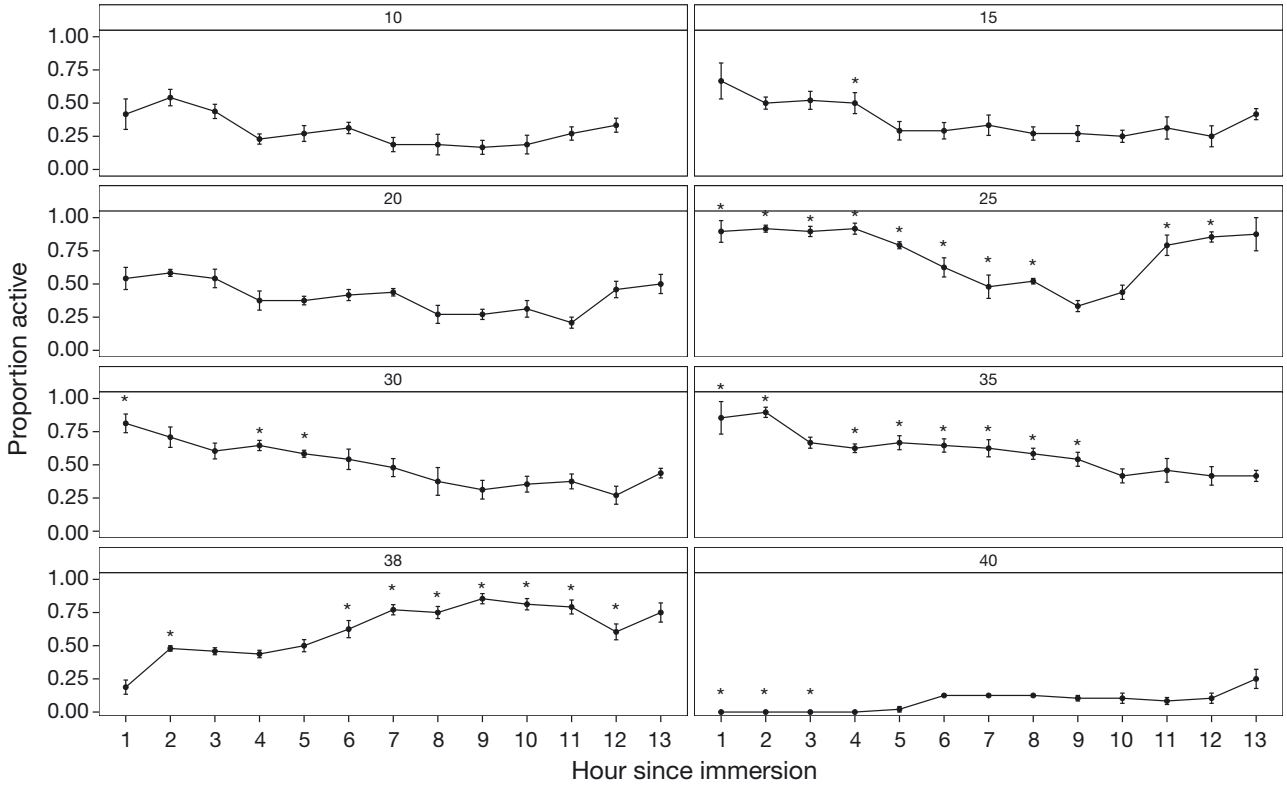

Fig. 5. The mean (±1 SE) fraction of barnacles active within each hour of immersion, by emersion temperature. N = 8 barnacles for each temperature. *value differs significantly from the 10°C treatment for the same hour (Dunnett's test, $p < 0.05$)

Our calculations suggest that the monthly energetic cost of low tide exposure during the summer at FHL nearly doubles between the lowest part of *B. glandula*'s vertical distribution and the highest (Fig. 6). Microhabitat also influenced cost, as the shaded mid-shore habitat was intermediate in cost between the sun-exposed habitats on the mid and low shore. Energy costs in the shaded mid-shore habitat were roughly 92% of those in the sun-exposed habitat, whereas moving 0.39 m lower on the shore but staying fully sun-exposed reduced energy costs to 88% of the mid-shore.

4. DISCUSSION

Temperature greatly influences the physiology and fitness of organisms, particularly ectotherms, and thermal stress at low tide has long been identified as an important factor structuring patterns of intertidal zonation (Connell 1961, Sutherland 1970, Somero 2002), yet few studies have attempted to estimate the metabolic costs of low tide exposure (Fly et al. 2012). We set out to quantify these energetic costs for the barnacle *Balanus glandula* by measuring both aerial and aquatic respiration over a continuously monitored 5 h emersion and 6 h immersion period. We expected that barnacle respiration rates and total oxygen consumption would increase with increasing temperatures, reflecting a combination of metabolic effects at all temperatures and increased physiological costs at stressful temperatures. We found that barnacles incur a substantial cost to even moderate temperatures at low tide. Total oxygen consumption roughly doubled in barnacles exposed to emersion temperatures of 20 to 30°C, relative to 10°C. While over 80% of this observed increase occurred during the low tide period, we also found some evidence of an oxygen debt, in the form of elevated respiration and greater frequency of activity for up to 9 h post immersion. At temperatures over 30°C, barnacles failed to recover full aquatic respiration even after 6 h, suggesting physiological damage. We also found that the duration and medium of measurement strongly affected estimates of energetic costs.

Most importantly, our results provide one of the first estimates of how energetic costs vary with shore

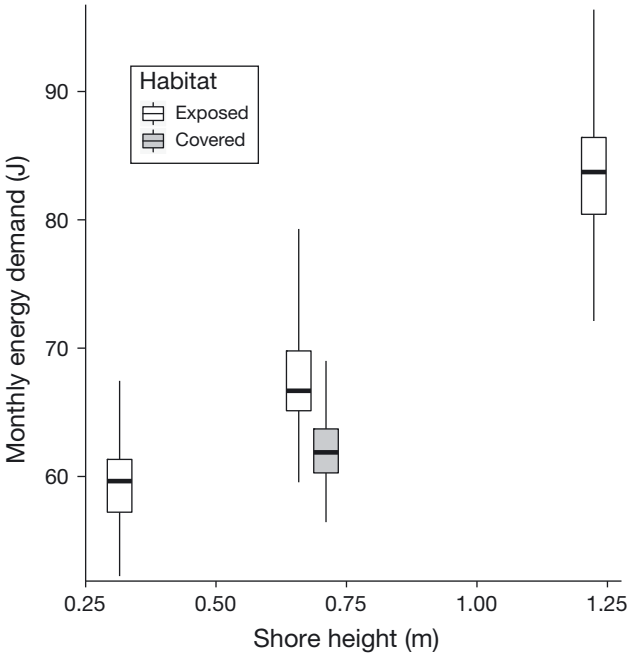

Fig. 6. Monthly summer energy demand among shore heights and habitats for *B. glandula* at FHL. Horizontal lines are medians, boxed by 25th and 75th percentiles, with whiskers representing the minimum and maximum energy demand calculated from 100 randomly sampled months

level. We found an increased cost during summer months of ~24 J mo^{-1} for *B. glandula* near their upper vertical limit when compared to a location 0.9 m lower on the shore. This is a ~40% increase in metabolic demand, suggesting that there are large metabolic costs to living high in the intertidal. Such costs have long been suspected to be important drivers of ecological patterns in the rocky intertidal (Connell 1961, Somero 2002, Levinton 2017) but have rarely been quantified. Notably, this cost estimate assumes that *B. glandula* on the high and low shore are genetically identical. We could also be overestimating the energetic costs for high-shore barnacles if *B. glandula* shows local adaptation to shore height. Such adaptation has been reported for other temperate intertidal barnacles (Schmidt et al. 2000) and for one of *B. glandula*'s predators, *Nucella ostrina* (Stickle et al. 2016, 2017).

We could only find one other published comparison of energetic costs across intertidal shore heights. Fly et al. (2012) reported increased costs of ≤35% in the seastar *Pisaster ochraceous* over a 0.5 m vertical range. Their estimates are much smaller than ours, even considering the smaller vertical range they used. The difference appears to derive from the more modest difference in body temperature across shore heights reported by Fly et al. (2012), as they actually reported a greater effect of temperature on energy demand for *P. ochraceous* than we observed for *B. glandula.* The difference in these 2 studies highlights the importance of measuring organism-specific temperatures (Broitman et al. 2009).

The interpretation of our energetic cost estimates depends on the accuracy and completeness of our lab measurements at each temperature. We observed substantial increases in oxygen consumption at warm temperatures, particularly during emersion. Total oxygen consumption more than doubled for barnacles exposed to 20 to 35°C, relative to a 10°C emersion. Aerial respiration rates in those treatments were also 50% greater, on average, than those measured in water. While these differences appear dramatic, they are comparable to other published measurements for *B. glandula*. For example, using allometric equations from Spivey (1989), we calculate that our aerial respiration measurements at 20°C are equivalent to 0.07 µmol O_2 h^{-1} mg^{-1} of somatic tissue. This is comparable to the 0.11 µmol O_2 mg^{-1} h^{-1} reported by Wu & Levings (1978) for a population of *B. glandula* from Vancouver, Canada. Our aquatic respiration measurements at 10°C translate to approximately 0.026 to 0.028 µmol O_2 h^{-1} mg^{-1}, which is lower than Wu & Levings (1978) value of 0.063 µmol O_2 h^{-1} mg^{-1}, but close to the value of 0.022 µmol O_2 h^{-1} mg^{-1} reported for FHL *B. glandula* by Nishizaki & Carrington (2014).

Even with the high aerial respiration rates we observed, we also found some evidence for an oxygen debt at temperatures at or above 20°C. At these temperatures, total aquatic oxygen consumption increased by ~25% and barnacles were significantly more active for up to 9 h post immersion, relative to the 10°C treatment. Because they rely on cirral beating to generate a current that brings water into the mantle cavity (Anderson 1994), immersed barnacles can only access oxygen when cirri are active. Thus, the behavioral results suggest that we may have underestimated aquatic oxygen consumption at some temperatures, as elevated respiration may have persisted beyond the 6 h of immersion for which we conducted aquatic respirometry.

The aquatic respiration data also showed a consistent pattern of low initial oxygen consumption upon reimmersion, peaking in the second or third hour. At some temperatures, this was associated with delayed opening upon reimmersion. However, the low initial rates may also reflect the fact that barnacles are generally poor at circulating water

efficiently through their mantle cavity. For example, Davenport & Irwin (2003) found that a similar high-shore barnacle, *Eliminius modestus,* could take as much as 2 h to fully oxygenate its mantle cavity upon immersion.

Our results suggest that the types of short-term respiration measurements that are typically used to calculated energy demand (e.g. Fly et al. 2012, Bjelde & Todgham 2013, Miller et al. 2015) could either underestimate and/or overestimate the energy demands of prolonged aerial exposure in intertidal invertebrates such as *B. glandula*. Overestimation would occur if the pattern that we observed of reduced aerial respiration with increasing duration of low tide is common to other intertidal invertebrates. Averaged across all temperatures, *B. glandula's* aerial respiration rates in the first hour exceeded those in the second and third hours by ≥20%. Thus, aerial measurements of ≤1 h could be overestimating energy costs by as much as 20%. This effect was more pronounced at ≥20°C, suggesting it may reflect a response to thermal stress, rather than directly to low tide. A similar pattern has been reported in *Drosophila* (Hoekstra & Montooth 2013) and was interpreted as a spike in energy use from the initiation of the heat shock response. There are few studies of temporal variation in the oxygen consumption of intertidal invertebrates during emersion, but both Tagliarolo et al. (2012) and McGaw et al. (2015) found temporal variation in aerial respiration rate during emersion in a bivalve and an echinoderm, respectively. However, neither pattern matches the one we report here. Clearly, more research is needed to understand how and why respiration rates might vary over the duration of a low tide exposure.

Conversely, short-term aerial respiration measurements may underestimate metabolic costs in species that show an oxygen debt upon immersion. We observed a modest, but not significant, increase in aquatic oxygen consumption at emersion temperatures as low as 20°C. However, these experiments were conducted under high humidity, as there were always a few drops of water in the chambers during emersion. Many intertidal animals maintain high levels of aerobic respiration under humid emersion (Marshall & McQuaid 1991), and thus, *B. glandula* might show a more pronounced oxygen debt under a lower-humidity emersion regime. Oxygen debts have been reported in nearly all intertidal species studied (Ellington 1983), including some (Vial et al. 1999), but not all barnacles (Castro et al. 2001). If widespread, they suggest that aerial measurements alone could underestimate the full energetic costs of low tide exposure.

It is important to consider that the high metabolic costs we observed at temperatures over 20°C need not lead to reduced fitness, in terms of growth or reproductive rates. In fact, King & Sebens (2018) report a 47% increase in growth for FHL *B. glandula* routinely exposed to 27°C low tides, relative to 13°C. Growth depends on the balance of energy intake and energy consumption (Sanford 2002) and can increase whenever intake rates exceed energy demand at high temperatures. Emersion temperatures influence feeding rates in many intertidal species (Stickle et al. 1985, Pincebourde et al. 2008, King & Sebens 2018), and this may be true for *B. glandula* as well (G. T. Ober et al. unpubl. data).

While our respiration measurements are comparable to previous studies, they might not accurately reflect actual metabolic costs in the field for several reasons. First, we chose a feeding regime which included only intermittent feeding and 48 h of starvation prior to each trial. This was intended to minimize digestive contributions to metabolism, but also resulted in a pattern of food availability quite different from that in the field. While previous studies with this feeding regimen did not limit barnacle growth (Gilman et al. 2013) and barnacles can be food-limited in the field (Qiu & Qian 1997), the limited food access likely reduced our estimates of metabolic rate. Second, we used barnacles that had grown on mid-intertidal settlement plates hanging under a shaded dock, and it is possible that they were field-acclimated to cooler emersion temperatures than barnacles living on sun-exposed rocky shores. This would lead to an overestimation of metabolic cost. But that seems unlikely given that all barnacles were laboratory-acclimated to ecologically relevant conditions for periods of 1 wk to 2 mo prior to use. Conversely, the laboratory acclimation itself might have influenced costs estimates. The laboratory acclimation was much more stable than natural intertidal environments (Helmuth & Hofmann 2001, Seabra et al. 2011), and the timing and duration of thermal events has been shown to alter the physiology of many intertidal organisms (Widdows & Bayne 1971, Stenseng et al. 2005). Thus, our results are imperfect estimates of metabolic cost.

Additionally, because barnacles showed evidence of impairment at temperatures above 30°C, our measurements likely underestimated the full costs at these temperatures. Estimating the full costs of exposure to these temperatures is critical because as

much as 25% of the summer daily maxima experienced by high-shore *B. glandula* at FHL exceeded 30°C (Gilman et al. 2015). This result contrasts with other studies that have assumed that exposure to extreme temperatures (above the 'thermal optimum') are rare and therefore their metabolic costs can be ignored (e.g. Gillooly et al. 2001, Iles 2014). We chose to extrapolate from a Boltzmann-Arrhenius curve of the calculated costs for temperatures above 35°C. Many studies have used this function to predict the effects of temperature on performance (Vasseur & McCann 2005, Dillon et al. 2010), but not all species, or all processes within a species, fit this model (Kingsolver & Woods 1997, Dell et al. 2011, Iles 2014, Schulte 2015). For example, Iles (2014) found that incorporating polynomial terms into the Boltzman-Ahrenius model provided better estimates of metabolic rates for some intertidal invertebrates. A complete model of *B. glandula*'s thermal costs of exposure to high temperature will require more detailed estimates of metabolic costs at stressful temperatures than we provide here. Measurements of costs at these temperatures will be critical to predicting the consequences of climate change for intertidal communities.

Acknowledgements. We thank J. Brice-Horowitz, E. Hazelton, E. Kim, S. Lim, and O. Wei for their help monitoring trials and maintaining barnacles. We thank 3 anonymous reviewers whose helpful comments strengthened this manuscript. This work was supported by the National Science Foundation (grant IOS-1351445 to S.E.G.) with additional support from the Keck Science Department of Claremont McKenna, Pitzer, and Scripps Colleges.

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

Submitted: August 21, 2018; Accepted: July 3, 2019
Proofs received from author(s): September 23, 2019