

Effects of temperature and humidity on activity and microhabitat selection by *Littorina subrotundata*

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ABSTRACT: Animals living in intertidal habitats experience high temperatures and low humidity during emersion that represent extreme deviations from those experienced during immersion; some use behaviour to ameliorate these stressors. We made *in situ* observations of 3 behaviours displayed by the Pacific intertidal snail *Littorina subrotundata* on 3 exposed rocky intertidal shores in the northeast Pacific: microhabitat selection, activity level, and conspecific aggregation. We hypothesized that these behaviours might be altered in response to temperature and/or humidity at a particular time during tidal emersion. We used the Akaike information criterion to compare a set of models for each of the 3 behaviours that included combinations of substrate temperature (T_s), vapour pressure deficit (VPD) (which encompasses humidity), emersion time, snail shell width (Size), and study site (Site) as the independent variables. The best supported model of microhabitat selection in the summers of 2011 and 2012 used only the independent variables Site and Size. The best supported model of activity included both T_s and VPD in 2011 but included only T_s in 2012; increased T_s resulted in decreased activity. None of the models in the set explained much of the variance in conspecific aggregation. We conclude an alternate cue for microhabitat selection is likely in this system and suggest that biogenic refuges created by barnacles are a likely driver. Our findings also suggest that thermal stress during emersion is the primary cue that informs the snails to reduce their activity.

KEY WORDS: Habitat selection · Activity · Aggregation · Intertidal environment · Temperature · Vapour pressure deficit · Biogenic habitat · Akaike information criteria

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INTRODUCTION

Intertidal animals are known to alter their behaviour in response to a broad range of stressors including predation (Rochette & Dill 2000), water movement (Pardo & Johnson 2006), temperature (Judge et al. 2011), water chemistry (Bibby et al. 2007), and humidity (Rojas et al. 2013). Among these stressors, temperature and humidity have captured much attention due to the regularity of their occurrence (Chapman & Underwood 1996, Helmuth & Hofmann 2001). In rocky intertidal habitat it is highly beneficial for organisms to make condition-dependent

changes to their behaviour, as environmental variations on organism-level spatial scales result in a wide range of potential refuges from unfavourable temperature and humidity (Helmuth & Hofmann 2001, Helmuth et al. 2006, Harley 2006, Jackson 2010, Judge et al. 2011, Lathlean et al. 2013). A broad range of intertidal organisms have been observed to cope with this variability through behavioural change (Garrity 1984, Chapman & Underwood 1996, Bingham et al. 2011, Chapperon & Seuront 2011a).

Behavioural changes in response to abiotic stressors in marine intertidal animals can take on many different forms; some of the more common of these

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behaviours involve microhabitat use (Jones & Boulding 1999), altered activity level (Gray & Hodgson 2004, Bates & Hicks 2005, Judge et al. 2011), and the formation of aggregations (Garrity 1984, Chapman & Underwood 1996, Muñoz et al. 2008, Stafford et al. 2008).

There are a variety of different microhabitats available to snails such as crevices and cracks in the rock, as well as the 'biogenic habitats' that can be found between live or inside dead sessile invertebrates such as barnacles (Silva et al. 2015) and mussels (Cartwright & Williams 2012). On hot days some microhabitats are lower in temperature (Lathlean et al. 2013) and higher in humidity than the surrounding habitat (Jones & Boulding 1999) and provide refuges for animals that would otherwise be unable to persist due to their physiological desiccation thresholds (Newell 1979, Jackson 2010). Even though this may ultimately give only a small reprieve, if an animal is already at or near its physiological limits behavioural changes such as the above described could make the difference between survival and mortality (Newell 1979, Miller & Denny 2011).

In addition to altering microhabitat selection, many animals will lower their activity levels as temperature increases and humidity decreases (Garrity 1984, McMahon 1990, Iacarella & Helmuth 2011). Lowering activity level via withdrawing into a shell or otherwise sealing its body away from the air can allow an intertidal animal to reduce its water loss, or perhaps moderate it to regulate evaporative cooling (Iacarella & Helmuth 2011). It has also been shown that reducing the surface area of the body in contact with the underlying substrate can reduce body temperature through the reduction of conductive heat exchange (Vermeij 1971, Denny & Harley 2006, Miller & Denny 2011).

A third type of behaviour, aggregation with conspecifics, may also impart benefits to snails at low tide. Formation of aggregations during emersion has been shown to decrease water loss in intertidal snails (Vermeij 1971, McMahon 1990, Muñoz et al. 2008). Evidence from these studies is less clear on whether or not animals use high temperatures as a cue when aggregating (Chapman 1995). However, as low humidity is often correlated with high temperatures on temperate shores (Jones & Boulding 1999), it is reasonable to predict that substrate temperature (T_s) may be a suitable cue for aggregation in these locales.

All of these behaviours have been documented in many intertidal species, most notably littorinid snails, i.e. small marine snails in the genus *Littorina* (Raf-

faelli & Hughes 1978, Garrity 1984, Jones & Boulding 1999, Bates & Hicks 2005, Iacarella & Helmuth 2011, Judge et al. 2011). These snails typically live high in the intertidal zone and are regularly emersed (Emson & Faller-Fritsch 1976, Chapman & Underwood 1996, Reid 1996, Pardo & Johnson 2004). Though physiologically robust, several littorinid species change their behaviour in situations of high temperature and low humidity (Vermeij 1971, Garrity 1984, Chapman & Underwood 1996, Jones & Boulding 1999, Judge et al. 2011).

Most previous research into changes in littorinid behaviour in response to temperature and humidity focused on snails living in tropical intertidal habitats (Judge et al. 2011) and studied these behaviours singularly as opposed to together (Iacarella & Helmuth 2011, Judge et al. 2011). Furthermore, the metrics used to assess humidity were binary discrete values (such as 'wet' and 'dry') rather than a continuous metric that captures a range of values (Chapman & Underwood 1996). This study makes use of *Littorina subrotundata* (Carpenter 1864), an intertidal snail commonly found on the wave-exposed western coastline of North America. This study attempted to link behavioural changes with changes in T_s and vapour pressure deficit (VPD). VPD has not previously been used to quantify humidity in littorinid behavioural research; it is a measurement of how far the current level of water vapour in the air is below complete saturation (Grange & Hand 1987). When VPD is low the air will be close to saturation with water vapour, and therefore the snails would be expected to be under low desiccation stress. When considered in conjunction with the length of time since a snail was last inundated by the tide, VPD accurately captures the level of desiccation stress that a snail is experiencing.

This study addressed the following question: does *L. subrotundata* alter its behaviour in response to T_s and VPD? We initially noted 3 distinct behaviours in this snail species that may be altered in response to T_s and VPD: microhabitat selection, activity level, and formation of conspecific aggregations. We hypothesized that *L. subrotundata* snails would alter microhabitat selection, activity level, and aggregation propensity in response to T_s and VPD because complex microhabitats, lowered activity levels, and formation of aggregations provide a refuge from these stressors. Specifically, our hypothesis predicted that snails increase their use of complex microhabitats, lower their activity levels, and increase their propensity to aggregate as T_s and VPD values increase.

MATERIALS AND METHODS

Study sites

Field measurements and behavioural observations took place at 2 long-term study sites near the Bamfield Marine Sciences Centre (BMSC) on Vancouver Island, British Columbia, Canada, during May 2011 and August 2011 and mid-July to early August 2012 (Fig. S1A in Supplement 1 at www.int-res.com/articles/suppl/m537p163_supp.pdf). Nudibranch Point, (NP; 48.815° N, 125.176° W) and Prasiola Point, (PP; 48.817° N, 125.169° W) are 2 sites on the east and west sides of a sandy beach approximately 500 m apart with NP being less exposed because of an off-shore island. A third site, Cape Beale (CB; 48.786° N, 125.217° W) was added in 2012 after analysis of data from 2011. Of the 3 study sites, CB is by far the most exposed as it faces open ocean (Fig. S1B). The study sites were rocky beaches with well-defined intertidal zonation. Barnacle coverage (*Cthamalus dalli*, *Balanus glandula*, and *Semibalanus cariosus*) was heterogeneous, and there were several species of algae found in this zone (*Fucus* spp., *Mazzaella* spp.). At these sites there are an abundant number of littorinid snails. The focal species *Littorina subrotundata* was numerically dominant and can be distinguished by shell morphology (Reid 1996). Continuous Hobo-logger™ (Onset) temperature data going back to August 2004 suggest that T_s at the top of the high intertidal at these beaches can exceed the estimated upper thermal tolerance of *L. subrotundata*, which has been estimated at $34.8 \pm 2.8^\circ\text{C}$ (Lee 2008).

Field sampling design

Methods for recording environmental variables and behavioural observations were the same at each site. *L. subrotundata* is typically found in the barnacle zone on exposed or semi-exposed shores in the northeast Pacific (Reid 1996). *L. subrotundata* vertical distribution starts above the upper limits of the small acorn barnacles (*C. dalli* and *B. glandula*) and ends below the upper limit of the California mussel (*Mytilus californianus*). A 10 × 10 cm quadrat was randomly placed within the small acorn barnacle zone along one 20 m transect at each site that ran parallel to the water line. This method resulted in a random collection of snails. For each snail within the quadrat, the T_s , relative humidity (RH), dewpoint (t_{dp}), and air temperature (2012 only) of its habitat were recorded, its microhabitat and activity were

categorized, and its size (shell width in millimetres) was recorded. All snails were returned to within 10 cm of their original collection site. Quadrats were randomly placed and completed in this manner until the tidal level approached the area.

Measurement of variables

Substrate temperature. T_s was measured using an Omega brand (HH506RA) K-type wire thermocouple by touching the tip of the thermocouple wire (0.81 mm diameter) to the substrate as close to the snail as possible without physical contact (Fig. S2A). Care was taken not to contact the snail as this could induce the snail to retract into its shell prior to assessment of its activity level. The thermocouple was always in contact with the substrate that each snail's shell aperture was facing, as previous work has demonstrated that littorinid snail body temperature is heavily influenced by the temperature of the underlying substrate (Denny & Harley 2006, Chapperon & Seuront 2011a,b). The probe was held to the substrate with care taken to prevent direct insolation affecting temperature readings, and T_s was recorded when the reading stabilized.

Vapour pressure deficit. RH, t_{dp} and air temperature (2012 only) were measured by holding the tip of the probe connected to a Panther brand (I-999-HSI-HT100) hand held meter as close to the focal snail as possible (Fig. S2B). The readings were allowed to stabilize for 20 s then the values of RH and t_{dp} and air temperature (2012 only) were recorded. In 2011 we forgot to record air temperature, and so had to calculate it from RH and t_{dp} (Fig. S3 in Supplement 2 at www.int-res.com/articles/suppl/m537p163_supp.pdf). Air temperature was then substituted for T in Eq. (1) (Teten's equation) to calculate the saturation vapour pressure, e_s (kPa):

$$e_s = 611e^{\left(\frac{17.502 \times T}{T + 240.97}\right)} \quad (1)$$

Using this value of e_s and the measurement of RH, the air vapour pressure, e_a , was then calculated:

$$e_a = \frac{\text{RH}}{100} e_s \quad (2)$$

The difference between e_s and e_a is the VPD:

$$\text{VPD} = e_s - e_a \quad (3)$$

Emersion time. The time since a snail was last immersed (emersion time) was estimated so that it could be used for comparisons to models that made use of T_s and VPD. By comparing the effect of VPD

on snail behaviour to the effect of emersion time it is possible to determine if emersion time alone had a greater influence on snail behaviour than VPD. Emersion time was estimated using recorded tidal heights (for Bamfield, BC) and wave heights (from La Perouse Bank Buoy 46206; www.weatheroffice.gc.ca/marine/weatherConditions-currentConditions_e.html?mapID=02&siteID=06800&stationID=46206) obtained from the Canadian Hydrographic Service and temperature data obtained from Hobologger™ (OnSet) temperature loggers attached to rocky substrate in the barnacle zone using Z-spar marine epoxy. Temperature was recorded at 15 min intervals. In daylight hours a rapid increase in temperature could usually be observed when tide receded from the loggers. In situations where the temperature of the logger when exposed to air was similar to sea surface temperature (La Perouse Bank Buoy 46206), time of exposure was estimated from the combined tidal and wave height; if the combined height was less than 4 m, it was assumed that the study site was exposed. In all cases, estimates of the time of exposure were rounded to the nearest quarter hour. The value for time since last immersion was estimated by comparing the estimated time of last exposure to the time that a measurement was taken in the field.

Microhabitat categorization. The microhabitat occupied by a snail and whether or not it was aggregated was assessed immediately after T_s , t_{dp} , and RH had been recorded. Microhabitat was split into 5 distinct categories; 'bare rock and/or shallow non-biotic crevice' ('rock'; considered together due to rarity), 'bare rock next to a barnacle', 'inside a barnacle test/in a live barnacle shell', 'between barnacles', and 'other biotic association' (Table S1; Fig. 1). This classification into 5 categories allowed evaluation of the relative importance of microhabitat heterogeneity created by barnacle distribution from the perspective of an organism.

Activity level. After environmental and microhabitat data was recorded, the activity level of each snail was assessed. Activity level categories are 'moving', or 'foot out', or 'withdrawn' (Table S2). In some cases, the act of removing snails from crevices took several seconds; this delay may have resulted in the snail



Fig. 1. *Littorina subrotundata*. The 5 microhabitat classifications described in this study of Pacific intertidal snails (see also Table S1 in Supplement 1; www.int-res.com/articles/suppl/m537p163_supp.pdf). Green diamond: bare rock/crevice ('rock'); yellow circle: bare rock next to barnacle ('next'); light blue square: in wall plates of live or dead barnacle ('on'); magenta triangle: between barnacles ('btwn'); red pentagon: 'other' (in this case the snail is associated with a red alga). (Photo: E. N. Hay)

retracting its body into its shell before it was possible to observe its true activity level. For this reason, if it took longer than 5 s to remove a snail from the substrate, the snail was excluded from the activity level analysis.

Size. After a snail's activity level was assessed, the size of each snail was measured to the nearest millimetre; the shell was placed aperture down on a ruler and the widest point of the shell between the outermost edge of the aperture and opposite body whorl was measured perpendicular to the axis of coiling. The size class of snails between 0.5 and 1.4 mm was recorded as 1 mm, those between 1.5 and 2.4 mm was recorded as 2 mm, and so on. Snails in the 1 mm size class were not used because they are so small that they are easily overlooked. Snails in size class categories greater than 3 mm were not used because they were quite rare, and their use could have introduced the potential for spurious statistical results

(Hosmer & Lemeshow 2000). Therefore, the variable 'Size' had only 2 levels, representing the 2 and 3 mm size classes.

Aggregation classification. Aggregation was a simple binary response variable; if any part of a focal snail's body was in physical contact with one or more other littorinid snails (including the 3 littorinid species found in this area but not included in our study), then the focal snail was considered aggregated.

Modelling

Model construction. Microhabitat selection was represented by a multinomial logistic model; the dependent variable was the microhabitat selected (one of 5 categories). In this model the designated reference category was 'non-complex'. Activity level was also a multinomial model, and the dependent variable was the categorized (3 levels) activity of a snail. The 'withdrawn' category was used as the reference category. Lastly, aggregation was modeled with a binary logistic model, the dependent variable being whether or not a snail was aggregated.

Model comparison with the Akaike information criterion (AIC). In this study, models were compared using values of corrected quasi-AIC (QAIC_c) as opposed to AIC_c, as recommended when data is overdispersed as in our dataset (Anderson 2008). A separate set of models was considered for each of the 3 dependent behaviour variables in relation to the 5 independent variables: T_s , VPD, Emersion time, Site, and Size. For each behaviour observed in this study (microhabitat use, activity level, and aggregation) a total of 9 working hypotheses were compared using the 2011 data, 4 of which were constructed with T_s and VPD to examine their effects on an observed behavior. One model contained only a constant to serve as a point of comparison for all models. The other 4 hypotheses were included after data collection but before analysis to compare the effect of T_s and VPD on other relevant environmental variables, in order to test their relative importance as cues (see Table S3 for detailed descriptions). Each working hypothesis was represented by a logistic regression model and made use of one or more of the following independent variables: T_s , VPD, Site (either PP or NP in 2011; in 2012 the third site of CB was added), Size, and (in 2011 only) emersion time. The number of multiple working hypotheses was reduced to 8 in 2012 to reflect findings from 2011. If a model had a Δ QAIC_c value less than 2.0, and if the only difference between that model and the highest ranked model

was one less predictor variable, the model with the smallest number of predictors was selected as the 'best' model in the set. Akaike weight (w) was also used to assess the best model (Anderson 2008).

Model estimation. All models were constructed in SYSTAT version 13.00.05. In order to calculate QAIC_c, log-likelihood values were obtained from each of the 8 models and input into a spreadsheet (Microsoft Office Excel 2007) that was programmed with equations for QAIC_c (Anderson 2008). Values of the overdispersion parameter (\hat{c}) were calculated (in Excel) from Model 5 (Table S3), with χ^2 and df values from the logistic regression analyses in SYSTAT 13. After completing the QAIC_c analysis, the most likely candidate model in each set was identified and then the effect of each parameter in the model was further assessed using SYSTAT. The effect of variables on probability of category membership was interpreted using odds ratios (OR).

Model fit. The fit of each of the 'best' models within a set was further assessed using the area under a Receiver Operating Characteristic (ROC) curve and classification success. Each model was checked for potential outliers using 3 different plots (ΔX^2 vs. $\hat{\pi}$, ΔD vs. $\hat{\pi}$, $\Delta \hat{\beta}$ vs. $\hat{\pi}$) with symbol size $\propto \Delta \hat{\beta}$ (Hosmer & Lemeshow 2000). These diagnostic plots did not indicate any outliers; therefore, no data points were excluded, (other than any VPD outliers previously removed from the models; Supplement 2).

RESULTS

Quadrats

A total of 106 quadrats were surveyed; 24 (11 at PP, 13 at NP) in May 2011, 31 (15 at PP, 16 at NP) in August 2011, and 51 (21 at PP, 18 at NP, 12 at CB) in 2012. Behavioural data for *Littorina subrotundata* of the 2 and 3 mm size classes that were accompanied by complete environmental data were included in the logistic regression analyses ($n_{2011} = 1483$ and $n_{2012} = 585$). The average number of snails in the 2 and 3 mm size classes per 100 cm² quadrat was 39.0 (range: 0 to 216). In both 2011 and 2012 NP had the highest mean (\pm SE) density of 2 and 3 mm snails (NP: 48.4 ± 8.45 ; PP: 32.0 ± 3.87 ; CB: 30.2 ± 8.86). The difference in snail density between the 2 sites used in both years was significant (2-way ANOVA: df = 1, 90; Year: $p = 0.830$; Site: $p = 0.042$ [NP > PP]; Interaction: $p = 0.552$) (Table S4). However, there was no difference in snail density among the 3 sites used in 2012 (ANOVA: df = 2, 48, $p = 0.188$).

Estimated percentage coverage of small acorn barnacles inside each quadrat ranged from 0 to 98%. NP had significantly lower mean (\pm SE) barnacle cover ($35.5 \pm 4.59\%$) than PP ($56.3 \pm 4.79\%$) in both 2011 and 2012 (2-way ANOVA: $df = 1,90$; Year: $p = 0.393$; Site: $p < 0.000$; Interaction: $p = 0.463$). NP also had a significantly lower barnacle density than the other 2 sites in 2012; however, there was no significant difference in barnacle density between PP and CB (ANOVA: $df = 2,48$; Site: $p < 0.001$. Tukey's test: NP < CB, $p = 0.024$; NP < PP, $p < 0.001$; PP = CB, $p = 0.625$).

Environmental variables

T_s showed considerable seasonal and diurnal variation (Table S5) and was significantly correlated with VPD (2011: $r = 0.673$, $df = 1,481$, $p < 0.001$; 2012: $r = 0.743$, $df = 1,734$, $p < 0.001$) and also with air temperature (2011: $r = 0.801$, $df = 1,481$, $p < 0.001$; 2012: $r = 0.729$, $df = 1,734$, $p < 0.001$; Table S6). In 2012 VPD showed the largest daily variation (CV = 0.398) followed by T_s (CV = 0.116) and then air temperature (CV = 0.144).

In May 2011, measured T_s ranged from 9.0 to 23.9°C (mean 16.0°C) at NP and 9.7 to 23.5°C (mean 14.3°C) at PP. VPD ranged from 0.160 to 2.190 kPa (mean 0.858 kPa) at NP and from 0.030 to 1.620 kPa (mean 0.552 kPa) at PP. August 2011 had measured T_s that ranged from 14.7 to 26.3°C (mean 20.2°C) at NP and from 14.3 to 28.2°C (mean 20.5°C) at PP. VPD ranged from 0.460 to 3.370 kPa (mean 1.641 kPa) at NP and from 0.290 to 3.000 kPa (mean 1.226 kPa) at PP. Analysis of the 2011 T_s showed that the interaction between Site and month was highly significant (ANOVA: $df = 1,1479$; interaction: $p < 0.001$; Table S5). Tukey's post hoc tests showed that T_s at both sites was significantly lower in May than in August, and that T_s at PP was significantly lower than NP in May ($p < 0.001$) but not in August ($p = 0.310$).

In 2012 T_s ranged from 14.2 to 26.9°C (mean 20.9°C) at NP, from 15.5 to 26.0°C (mean 20.0°C) at PP, and from 15.1 to 29.5°C (mean 20.7°C) at CB. VPD ranged from 0.235 to 1.194 kPa (mean 0.709 kPa) at NP, from 0.220 to 1.500 kPa (mean 0.626 kPa) at PP, and from 0.307 to 1.252 kPa (mean 0.706 kPa) at CB. In 2012 PP was significantly cooler than the other 2 sites (ANOVA: $df = 1,1838$; $p < 0.001$. Tukey's HSD: $p < 0.001$; PP < CB = NP). July to August T_s values

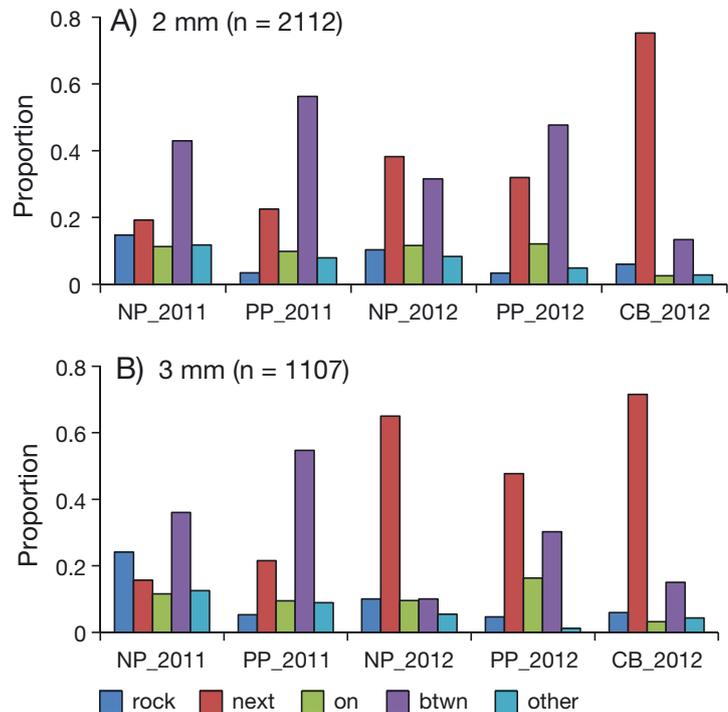


Fig. 2. *Littorina subrotundata*. Proportion of 2 and 3 mm snails found in each of 5 microhabitat categories at study sites on Vancouver Island (British Columbia, Canada) in 2011 (2 sites) and 2012 (3 sites). For descriptions of microhabitats see Fig. 1; for definitions of size classes see 'Materials and methods'. NP: Nudibranch Point; PP: Prasiola Point; CB: Cape Beale

were higher at NP in 2012 than in 2011 but were similar at PP in both years resulting in a significant interaction (ANOVA: $df = 1,1848$; $p < 0.001$; Table S5). VPD also differed among sites in 2012 (ANOVA: $df = 1,1838$; $p < 0.001$. Tukey HSD: $p < 0.001$; PP < NP = CB) but air temperature did not (ANOVA: $df = 1,1838$; $p = 0.19$).

Microhabitat selection

In 2011 very few 2 mm size class snails were found in 'non-complex' microhabitat at PP (3.4%) but at NP 14.7% of the 2 mm size class were in this microhabitat (Fig. 2A). In 2012 the 3 mm *L. subrotundata* at NP were equally likely to be found on 'non-complex' (10.1%) and 'between barnacles' microhabitats (10.1%; Fig. 2B). The 2 mm size class was found most often 'between barnacles' in 2011; in 2012 at NP and CB it was more often found 'next to barnacles' (Fig. 2A). For the 3 mm size class the most common microhabitat occupied at NP and at PP was 'between barnacles' but this changed to 'next to barnacles' at all 3 sites in 2012 (Fig. 2B).

Table 1. *Littorina subrotundata*. Akaike information criterion (AIC) rankings of 9 models representing working hypotheses used to assess microhabitat selection by Pacific intertidal snails in 2011 and 2012 (for details of models see Table S3 in Supplement 1). Models have been organized by their w values. w : Akaike weight; VPD: vapour pressure deficit; n/a: not applicable (because 'time since emersion' was not a hypothesis that was evaluated in 2012). Corrected quasi-QAIC (QAIC_c) and Δ QAIC_c values are shown in Table S7

Model	w_{2011}	w_{2012}
Site + Size (mm)	0.968	0.993
Site	0.019	0.007
Temperature (°C) + VPD (kPa) + Temperature*VPD	0.006	0.000
Size (mm)	0.004	0.000
Temperature (°C) + VPD (kPa)	0.001	0.000
Temperature (°C)	0.000	0.000
Emersion time (hh:mm)	0.000	n/a
VPD (kPa)	0.000	0.000
Constant only	0.000	0.000

Table 2. *Littorina subrotundata*. AIC rankings of 9 models representing working hypotheses used to assess activity level of Pacific intertidal snails in 2011 and 2012. Models have been organized by their w values from 2011. For abbreviations see Table 1 legend. QAIC_c and Δ QAIC_c values are shown in Table S8

Model	w_{2011}	w_{2012}
Temperature (°C) + VPD (kPa) Temperature*VPD	0.372	0.303
Temperature (°C) + VPD (kPa)	0.312	0.290
Emersion time (hh:mm)	0.103	n/a
Temperature (°C)	0.094	0.241
Site + Size (mm)	0.031	0.034
Size (mm)	0.028	0.018
Site	0.022	0.027
VPD (kPa)	0.019	0.072
Constant only	0.019	0.015

Site and were the best predictors of its multinomial microhabitat choice ($w_{2011} = 0.968$; $w_{2012} = 0.993$; Table 1, Table S7). Models that incorporated T_s and/or VPD were not highly ranked ($w_{2011} = 0.006$; $w_{2012} < 0.001$; Table 1). Snails were more likely to be found in complex microhabitats at PP as opposed to NP, though the magnitude of this effect varied based upon the type of microhabitat (OR range 2.536 to 4.731; Table S8). Similarly, snails were more likely to be found in complex microhabitats at PP as opposed to CB (OR range 1.074 to 4.285; Table S8) and were almost always less likely to be associated with complex microhabitat at NP as opposed to CB (OR range 0.902 to 1.776, with 1.776 being the only value above 1.000; Table S8). The effects of both Site and Size were consistent, but further examination of model fit, i.e. classification success (CS), suggested that the model could not accurately predict choice for all 5

individual microhabitats (Table S8). For example, these models did not demonstrate an acceptable ability to predict whether a snail will be occupying a barnacle shell (CS = 0.672 in 2011 but 0.701 in 2012) or whether a snail will fall into the 'other' category (CS = 0.650 in 2011 and 0.637 in 2012).

Activity

The majority of snails observed in this study were inactive and withdrawn into their shells (2011: 85.3% at PP, 78.8% at NP; 2012: 78.1% at PP, 81.2% at NP, and 76.2% at CB). In 2011, a greater proportion of snails had their foot extended at NP (17.4%) compared to PP (11.7%), but each site had nearly the same proportion of moving snails (2011: 3.5% at PP, 3.8% at NP; 2012 4.2% at PP, 2.0% at NP, and 0.6% at CB). Despite the difference in activity level between the 2 sites, it was found that T_s , VPD, and the interaction between the two ranked highest in the QAIC_c analysis; however, there was only a small difference (Δ QAIC_c < 2.0) between this complex model and the more parsimonious model that excluded the interaction term. Therefore, in 2011 T_s and VPD were shown to be the best predictors of a snail's activity level. In 2012 VPD was not a suitable predictor, though T_s was still incorporated into the best model

(Table 2, Table S9). In 2011 increased T_s resulted in an overall lowering of a snail's activity level (OR_{1,2} = 0.674 [activity level 2 compared to reference level 1], OR_{1,3} = 0.640), whereas an increase in VPD resulted in a snail increasing its activity (OR_{1,2} = 3.931, OR_{1,3} = 4.704; Table S10). The 2011 results support the hypothesis that snails use T_s and VPD as cues to alter their activity levels, yet the effect of VPD was opposite to that initially predicted. In 2012 increased T_s resulted in a lower activity level (OR_{1,2} = 0.709, OR_{1,3} = 0.602), but the model was not improved by VPD.

Aggregation

In both 2011 and 2012 the variables Site (PP or NP; CB added in 2012) and Size were the 2 predictors in the highest ranked model to assess snail aggregation

($w_{2011} = 0.501$; $w_{2012} = 0.381$), yet the simpler model that incorporated only Site was ranked nearly as high ($w_{2011} = 0.400$, $\Delta\text{QAIC}_c = 0.446$; $w_{2012} = 0.281$, $\Delta\text{QAIC}_c = 0.605$; Table S11). Therefore, the model that used only site to predict whether or not a snail is aggregated was the best model in this set (Table S11). It was found that snails were less likely to be aggregated at PP than at NP ($\text{OR}_{2011} = 0.479$). Although this model was highly ranked, it displayed poor discrimination (<0.60) and CS in 2011 ($\text{ROC} < 0.65$), which suggested that the model was not an accurate predictor. As this highest ranked model incorporated only the site variable as opposed to the T_s and VPD variables, selection of this model did not allow us to support or reject the hypothesis that T_s and VPD were cues that affected aggregation behaviour during this study.

DISCUSSION

Microhabitat selection

T_s and VPD were not relevant factors for predicting microhabitat use. Rather, Site and Size were the factors that best predicted the microhabitat use. These results indicate that microhabitat selection behaviours in littorinid snails that have been reported to be associated with temperature (Cartwright & Williams 2012) and humidity cues (Chapman & Underwood 1996) were primarily driven by other variables at our sites.

The 'shelter' found in these microhabitats from T_s and VPD stress may not be beneficial enough to act as a primary driver that alters the behavioural patterns of these snails. Ultimately, if the microhabitats do not ameliorate the stress then there is no advantage for the animal to use T_s and VPD as a cue for when to inhabit them. Based upon a heat budget model developed by Miller & Denny (2011) for littorinid snails, the main energy inputs affecting a snail's body temperature are solar radiation, heat exchange with the air, heat exchange with the substrate, and long-wave radiation. Only by reducing at least one of these inputs will a microhabitat reduce the temperature stress on the snails during maximum summer temperatures.

Similarly, a microhabitat must retain water better than the surrounding rock before snails there gain an advantage in withstanding desiccation stress. It has been demonstrated that complex microhabitats can retain more water than surrounding bare rock (Jackson 2010). Yet in this study VPD was found to be

highly variable, suggesting that in this environment VPD is not a relevant factor influencing behavioural change in *Littorina subrotundata*.

The lack of support for any of models that incorporated T_s and VPD suggests that thermal and desiccation stress were not the primary cues used by *L. subrotundata* to select a microhabitat. Nevertheless, there seems to be an alternate environmental cue driving microhabitat selection in our system because snails were found more often in complex microhabitats. Our results show that models incorporating site and size best predicted which microhabitat a snail was found in; this suggests that an environmental variable that changes between sites and differentially affects snails of different size is influencing microhabitat selection.

The most noticeable difference among our 3 study sites that might differentially affect snails of different sizes was a highly significant difference in the percent cover of barnacles (Table S4). PP had a significantly higher barnacle density than NP in both years but NP had a significantly higher snail density (Table S4). Barnacle densities well below 100% cover can result in a higher density of biogenic habitats for 2 to 3 mm snails; the number of snail-sized pockets between barnacles is at a maximum when an intermediate number of barnacles are present within a quadrat (Fig. 1 in Boulding & Harper 1998). Increasing the number of empty barnacle tests at a particular intertidal site, by killing live barnacles with forceps, immediately increases the local density of the common periwinkle; the snails living inside the barnacle tests are significantly smaller than those outside the tests (Silva et al. 2015).

Closely spaced barnacles create topographically complex microhabitats within which flow velocities are greatly reduced in wave-swept environments; these refuges been identified as beneficial to intertidal animals small enough to utilize them (Vogel 1981, Thomason et al. 1998, Denny & Wetthey 2001, Silva et al. 2015). Within barnacle microhabitat the forces exerted by waves on snail shells can be greatly reduced and allow snails to persist in an environment that would otherwise be too physically stressful for them to cling to the substrate (Wright & Boxshall 1999). Although it has been shown that these snails can survive after being swept off of the rocks (Miller et al. 2007) there can be great cost associated with dislodgement, as it exposes snails to heavy predation from subtidal species (Rochette & Dill 2000, Boulding et al. 2001). Consequently, we believe that the highly ranked models of microhabitat selection that incorporated site and size can best be explained by the

highly significant difference in barnacle cover between sites.

Activity level

In 2011 *L. subrotundata* altered its activity level in response to thermal and desiccation cues (highly ranked models with T_s and VPD), consistent with previous studies (Garrity 1984, Bates & Hicks 2005, Iacarella & Helmuth 2011). Yet, in contrast to previous work, in this study *L. subrotundata* increased activity levels as humidity decreased ($OR > 1.0$). Furthermore, in 2012 the most highly ranked model made use of T_s alone, as opposed to T_s and VPD. Increased T_s resulted in decreased activity ($OR < 1.0$) in 2011 and 2012 (Table S10).

The observed response to VPD in these snails is counterintuitive; why would a snail expose more of its tissue as desiccation stress increases? The mostly likely explanation for the effect of VPD appearing to be in the opposite direction to that hypothesized is the high correlation between the independent variables T_s and VPD (0.673 in 2011) (Table S6).

Another possibility is that when a snail is in a stressful environment, it increases its movement (i.e. activity level) in an attempt to get away from the stressful location rather than simply retracting. Withdrawn snails will expose less of their body and therefore slow water loss from their tissue, but this measure can only go so far. The operculum is not impermeable, and in a highly desiccating environment a withdrawn snail could still lose a critical amount of water.

In light of these counterintuitive results, caution should be taken when extrapolating the effect of VPD observed in this study to broader conclusions until more work has been completed. There are at least 2 potential sources of error in the environmental measurements: first, the probe used was often larger than the microhabitat inhabited by a snail, meaning that the probe may not have been measuring the exact conditions that a snail was subjected to. This is problematic; however, it is a common issue. Additionally, mixing of the air from convective currents likely minimized any potential error. Second, in 2011 we neglected to make a concurrent measurement of air temperature with the humidity probe and therefore air temperature had to be calculated from t_{dp} and RH (Supplement 2). Small measurement errors in t_{dp} or RH could have resulted in large errors in calculated air temperature due to the exponential relationship between e_s and air temperature. We estimated this error using the 2012 dataset and showed that val-

ues of VPD using air temperature calculated from t_{dp} and RH were slightly smaller but highly correlated with those calculated using measurements of measured air temperature (Supplement 2). In 2012 air temperature was measured concurrently with RH, presumably resulting in a more accurate calculation of VPD. However in 2012 the model containing VPD was not notably better than the model that contained T_s alone (Table 2). These results must be interpreted cautiously until further work can be completed and sources of error are fully considered.

The activity response of snails to T_s was as predicted, and is in agreement with the body of published literature (Garrity 1984, Bates & Hicks 2005, Muñoz et al. 2005, Iacarella & Helmuth 2011, Rickards 2012). Our 2012 data showed that in summer T_s was significantly higher than the air temperature. By lifting its foot, a snail will decrease the amount of surface area of its body in contact with the substrate, thereby reducing heat exchange. Previous modeling work has demonstrated that the simple act of withdrawing into the shell can reduce body temperature by up to 4°C (Miller & Denny 2011). If these snails can adequately compensate for high thermal stress simply by withdrawing into their shell, then it would not be necessary for them to seek out thermally beneficial microhabitats, and there would be no benefit to altering microhabitat selection in response to increased T_s .

Aggregation

Aggregation has been shown in other species to provide a benefit to snails during times of high thermal and desiccation stress (Chapman & Underwood 1996); however, this study found no change in aggregation behaviour in response to T_s and VPD. Analysis of aggregation propensity in 2011 gave a poor model fit, suggesting that all of the variables in this study are poor predictors of whether or not a snail will aggregate at these sites; therefore, further analysis of aggregation was not carried out in 2012. The best candidate model was the one that incorporated site as a variable, yet despite its higher rank over other models (including a constant only model) this model displayed poor discrimination and poor classification success (Table S12); this suggests models considered here are not useful for predicting whether or not a snail is aggregated. Considering the microtopography of the sites studied, this is not entirely surprising. A modeling study by Stafford et al. (2008) found that aggregation behaviour was selected against at

shores that had a high crevice density. There was an exceptionally high density of barnacles at our sites, which created 'biogenic habitats' between closely packed conspecifics, and this microhabitat was the most utilized by snails at each site (Fig. 1). Furthermore, it is possible that for this species, aggregation is a simple by-product of mating activity. *L. subrotundata* are not broadcast spawners; they must come into physical contact to copulate (Reid 1996). Littorinid snails are capable of following mucous trails left by conspecifics and are able to locate other snails at the densities found in this study (Davies & Blackwell 2007, Johannesson et al. 2008).

CONCLUSIONS

This study examined 3 behaviours (microhabitat use, activity level, and aggregation propensity) that have been shown in other species to change with temperature and humidity, but it appears in this system that the only behavioural response to these variables was altered activity level. Though this study did not find support for the hypotheses that T_s and VPD were responsible for differential microhabitat selection, it did find that site was important. Future work examining microhabitat selection in this species should focus on alternative environmental variables such as wave exposure or barnacle density that may affect the availability of biogenic refuges at a particular site. Activity level was shown to decrease with increasing T_s . The effect of VPD on activity was less clear because its direction was contrary to predictions. Problems associated with the measurement of VPD in this study suggest that further testing of its possible role is needed before firm conclusions can be made. Similar to microhabitat, the models constructed in this study for aggregation that included T_s or VPD were such a poor fit that it is highly likely that other variables are responsible for this behaviour.

In conclusion, *Littorina subrotundata* primarily makes use of altered activity in response to changes in T_s . This simple behaviour has been demonstrated to be beneficial to snails in other studies (Vermeij 1971, Garrity 1984, Miller & Denny 2011) and could impart a large fitness benefit to snails on days when T_s approach lethal limits.

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