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Temperature and desiccation do not affect aggregation behaviour in high shore littorinids in north-east England

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A large proportion of high shore littorinids are found in crevices or aggregations when emersed. It is generally considered that these behaviours are beneficial to littorinids by reducing desiccation stress, likelihood of dislodgement and threat of predation. Recent studies have shown that the selection of complex microhabitats, such as the formation of aggregations, is state-dependent in some species of high shore littorinids with more littorinids seeking complex microhabitats when conditions are warmer and more desiccating. This study, however, shows that the proportion of littorinids forming aggregations does not change with changes in temperature and evaporation rate. Differences between this and previous studies may be explained by littorinids in the current study living higher on the shore, with less frequent and less predictable foraging times.

Introduction

The high shore is an extremely stressful environment for organisms of marine origin (McMahon 1990). Physiological adaptations of littorinids to cope with this stress are common and include reduction of metabolic rate (Kronberg 1990), a sealable operculum (Garrity 1984) and an ability to live at levels of 70% dehydration (Thivakaran & Kasinathan 1990). Behavioural adaptations also occur, the most frequently studied being crevice occupation (Emson & Faller-Fritsch 1976, Raffaelli & Hughes 1978, Britton *et al*. 1991, Underwood & Chapman 1992) and aggregation formation (Raffaelli & Hughes 1978, Branch & Branch 1981, Chapman 1995, Chapman & Underwood 1996, Chapman 1998). Often aggregations are formed inside crevices (Raffaelli & Hughes 1978, Britton *et al*. 1991). The benefits of sheltering in aggregations and crevices have been well documented in terms of reduction of desiccation stress (e.g. Garrity 1984, Chapman 1995, Chapman & Underwood 1996). Recent work by Menge and Branch (2001) suggests that as levels of stress increase, greater importance is placed on the quality of the habitat or microhabitat. This suggests that littorinids should be more likely to aggregate during hotter and more desiccating conditions, as the microhabitat is of greater quality. Experimental work on *Littorina sitkana* supports this theory (Jones & Boulding 1999). Patches of high topographic complexity, or higher quality, were utilised by a greater proportion of the population during hotter conditions.

In this study we hypothesised that a greater proportion of high shore littorinids in north-east England would be found in aggregations during periods of higher temperature and higher evaporation than in cooler, less desiccating conditions. This hypothesis was tested by a laboratory experiment and a field study.

Materials and methods

The field study was conducted at two shores in north-east England: Brown's Bay, Cullercoats (55°02´13´´N, 1°25´48´´W) and Newbiggin-by-the-Sea (55°11´01´´N, 1°30´09´´W) from April to May 2001. Both shores are south facing and the high shore was vertical in slope. The shore at Cullercoats was man made from concrete blocks with a high number of small pits caused by erosion. The shore at Newbiggin was sandstone and again had numerous eroded pits and crevices. On both shores these crevices were commonly used by the littorinids as sites for aggregation. Ten randomly placed 0.25 m^2 quadrats were located along a 30 m transect at the level of mean high water springs (MHWS, 5.6 m + C.D.) and the proportion of littorinids in aggregations (defined as physical contact between a group of three or more individuals) was recorded. Two species of littorinid, *Melarhaphe neritoides* (L.) and *Littorina saxatilis* Olivi, were present at each site, with ~ 98% of individuals being *M. neritoides* at Cullercoats and ~ 75% at Newbiggin. At each shore the sampling procedure was repeated on six occasions. On three occasions, herein referred to as warm periods, the air temperature was > 22 °C (as measured 30 cm from the rock surface in any sunlight present), on the remaining three occasions, herein referred to as cool periods, the air temperature was ≤ 16 °C. These air temperatures corresponded to rock surface temperatures > 30 °C or ≤ 20 °C in all of the replicate sampling occasions. The sampling of the cool and warm periods was interspersed and the order in which they were sampled was determined randomly with breaks of at least 48 h between different sampling occasions. Sampling occurred during a falling tide, once the rock had dried sufficiently for the littorinids to stop moving. The preceding high tide wetted the littorinids for a period of > 1 h either through submersion, or more commonly through slight wave splash. Wetting of littorinids in this manner has been shown to stimulate movement in over 75% of individuals (Stafford 2002). At all sampling periods there was little wave action (waves < 0.5 m high) and no rain had fallen on the day of the study. The results were analysed using a nested ANOVA design for each shore with each replicate set of periods nested within the treatment (warm or cool period). Preliminary experiments at Cullercoats, to determine the variance of littorinids in aggregations, indicated the study would have a power of > 95% to detect a 10% difference in the number of littorinids in aggregations between replicated cool or warm periods with a significance value of 0.05. Evaporation rates were measured on one warm and one cool day at each shore. Ten balls of cotton wool were weighed, then soaked in water and reweighed. The balls were transported to the shore in sealed plastic bags and placed on the rock surface for 1 h. The balls were then reweighed and the percentage water loss calculated.

The laboratory study was designed so that potential confounding factors present on the shore were eliminated and only temperature and desiccation rates were changed between experimental groups. Differences in species composition and size of littorinids was eliminated by using a single species, *Melarhaphe neritoides,* and using approximately identical sizes (the maximum length of the shell was 2.2 ± 0.2 mm in all cases). All specimens were collected from the shore on the same day in July 2001 and left in aerated seawater for 48 h before the experiment commenced. Temperatures in the laboratory study were slightly higher for both cool and warm treatments than the field experiment (cool treatments had temperatures of 22 °C and warm treatments had temperatures of 35 °C as taken on the plate surface). This range, however, is within the range of rock surface temperatures found on the high shore in the summer months (July–August) in north-east England, when desiccation levels may be at their highest (Stafford 2002). Comparative air temperatures to those used in the field study (30 cm from the substratum) could not be obtained due to the distance of the heat lamps from the plates and the directionality of the heat source. Marble tiles (herein referred to as plates) of 150 × 150 mm were used as an artificial substratum. Plates were drilled with 20 artificial crevices (4 mm diameter, 2 mm deep) in random positions on both sides of each plate. Approximately 20 *Melarhaphe neritoides* were placed haphazardly on each plate and sprayed with seawater until attached and crawling. The exact number of littorinids on the plates could not be determined in advance as many fell off the plates when the plates were immersed in seawater, *see* below (range between 17 and 22 individuals per plate). Four replicate plates were suspended by passing fishing line through small holes (< 1 mm ∅) in the plates. The plates were immersed vertically into freshly collected seawater for 30 min, during which the animals were active. They were then removed from the seawater and left to dry for 45 minutes (as initial studies had shown all movement had stopped after this time). The proportion of littorinids in aggregations (defined as three or more in contact with each other) was determined. Three treatments were applied. In the first treatment temperature was experimentally raised using heat lamps positioned 250 mm from the plates and angled at 45 ° to the plates (this raised the temperature of the plate surface to 35 °C). To reduce the effects of directional light from the heat lamps, four additional 100 W lamps were shone at the plates to flood the area with light. The second treatment was a procedural control for the heat lamp manipulation where conditions were identical to the treatment using the heat lamps except the heat lamps were turned off (plate surface temperature 24 °C). A control treatment was performed using a single 100 W lamp to light the area from above the plates (plate surface temperature 22 °C). Each treatment was conducted three times on each of the four replicate plates. This resulted in a nested design, *see* below, with a total of 12 replicate plates for each treatment. Plates were scrubbed between replicate trials to remove mucus trails and the order of treatments and trials was determined randomly. The results were analysed by a nested ANOVA, preliminary studies indicated the power of the test should be > 90% to detect a 10% difference (~ 2 littorinids) between the temperature treatments using a significance value of 0.1. Treatment was a fixed factor with three levels (control, heat lamp and procedural control). Trial was a random factor with three levels nested within treatment. For each treatment and trial, four individual replicate plates were used.

Results

Desiccation levels appeared to be lower on the cool days in the field experiment on both shores, (Cullercoats: mean = 31% weight loss h^{-1} on cool day, 55% h^{-1} on warm day; $t = 26.74$; d.f. = 16; $p < 0.001$. Newbiggin: mean = 33% h^{-1} on cool day, 54% h^{-1} on warm day; $t = 26.60$; d.f. = 16; $p < 0.001$). Despite this, no significant change was detected in the proportion of littorinids in aggregations on either shore (Tables 1 and 2) with a mean (± S.E.) value of 81.5% (± 1.13) of littorinids in aggregations at Newbiggin and 98.9 (± 0.08) at Cullercoats. The temperatures of the plates in the laboratory study differed between the treatments (mean values: Control = 22 °C; Heat Control = 24 °C; Heat lamp = 35 °C with maximum range of temperature < 2 °C between different plates in the same treatment). The proportion of littorinids in aggregations, however, did not differ significantly between the treatments (Table 3) with a mean (± S.E.) of 24.0% (± 2.56) of littorinids in aggregations across all treatments and trials.

Discussion

The results of this study indicate that, for high shore littorinids in north-east England, the frequency of occurrence of sheltering behaviour is not related to an increase in environmental stress caused by increased temperatures over the ranges we used. Many factors may play a role in causing sheltering behaviour in the field, for example, wave action, predation, spawning, crevice availability, and although the effects of wave action were controlled to some extent in the study, many other environmental and biological factors were not measured or controlled. The laboratory study was designed to eliminate these potentially confounding factors, including differences in size and species composition of the littorinids found on the shore, although it did result in littorinids being in a less natural environment, particularly lacking food and having a far lower topographic complexity than in the field. The similar results of the field and laboratory study give far more support to the acceptance of the hypothesis that sheltering behaviour of high shore littorinids is not affected by temperature than either of the studies conducted in isolation.

The absence of significant differences between the proportions of littorinids in aggregations at different temperatures and different evaporation rates is counter to the work of Jones and Boulding (1999) that used air temperatures almost identical to those in our work. The results of our field study, however, demonstrate that a large proportion of high shore littorinids are found in aggregations at all times. Personal observations also revealed that these aggregations were frequently associated with a crevice or pit in the rock (*see* also Raffaelli & Hughes 1978, Britton *et al.* 1991). It is clear that there is a large number of potential benefits of inhabiting crevices and forming aggregations (e.g. prevention of dislodgement, reduction in the risk of predation, mating in *Littorina saxatilis* as it is ovoviviparous) in addition to the reduction in desiccation stress (Raffaelli & Hughes 1978, Stafford 2002). In general, weather conditions in north-east England resulting in hot and desiccating conditions occur during periods of high pressure. This high pressure also tends to lead to calm seas with little wave action. Low pressure generally results in cooler, less desiccating conditions but will frequently lead to high wave action (King 1975). As such the benefits of crevices and aggregations may be clearly apparent in a wide range of weather conditions and temperature may not affect the behaviours of the littorinids used to find these habitats.

An important difference between this study and that of Jones and Boulding (1999) is the shore height occupied by the littorinids. *Littorina sitkana* was found amongst the highest barnacle and algal cover on the shore. Individuals of *Melarhaphe neritoides* and *Littorina saxatilis* used in this study were found above the level

Table 1. ANOVA to investigate the percentage of littorinids in aggregations of three or more individuals at sites at Cullercoats at two different temperatures (warm and cool – *see* text for details). Each site was surveyed three times at each temperature using 10 replicate quadrats at each time. Data have been arcsine transformed.

Source	d.f.	S.S.	M.S.	*F*	*p*
Temperature	1	0.000687	0.000687	0.21	0.672
Time (temperature)	4	0.013191	0.003298	1.27	0.292
Error	54	0.140032	0.002593		
Total	59	0.153910			

Cochran's test *C* = 0.222 (not significant).

Table 2. ANOVA to investigate the percentage of littorinids in aggregations of three or more individuals at sites at Newbiggin at two different temperatures (warm and cool – *see* text for details). Each site was surveyed three times at each temperature using 10 replicate quadrats at each time. Data have been arcsine transformed.

Source	d.f.	S.S.	M.S.	*F*	*p*
Temperature	1	0.0409	0.04085	1.41	0.300
Time (temperature)	4	0.1165	0.02891	0.78	0.542
Error	54	1.9966	0.03697		
Total	59	2.1531			

Cochran's test *C* = 0.235 (not significant).

Table 3. ANOVA to investigate the percentage of *Melarhaphe neritoides* in aggregations at three different temperature treatments on artificial plates (*see* text for details). Data have been arcsine transformed.

Source	d.f.	S.S.	M.S.	*F*	p
Temperature	2	0.0632	0.0316	2.31	0.180
Trial (temperature)	6	0.0821	0.0136	0.65	0.692
Error	27	0.571	0.0211		
Total	35	0.716			

Cochran's test *C* = 0.362 (not significant).

of barnacles and algae. These littorinids only received immersion during high spring tides and only received wave splash during high tides and periods of wave action. During neap tides or periods of calm water the littorinids could remain dry for periods of up to two weeks (personal observations). *L. sitkana* at a lower shore height, however, would be more likely to receive regular immersion or wave splash. This could allow individuals of *L. sitkana* either to seek a refuge or to continue foraging. If such an individual decides to maximise its foraging time at the expense of seeking a refuge, due to cooler, less desiccating conditions, it is likely that similar weather conditions (e.g. low temperature and desiccation) will prevail until the next immersion, during the next high tide period. The littorinids in the current study, however, are not faced by such predictable immersion times. Finding a crevice, therefore, may be important, even if there is a cool temperature and low desiccating conditions. These conditions may change drastically before the next immersion and the littorinid may increase its likelihood of survival by being in a crevice or aggregation and so reduce its exposure to stress during the vulnerable emersion period.

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References

Branch, G. M. & Branch, M. L. 1981: Experimental analysis of intraspecific competition in an intertidal gastropod, *Littorina unifasciata*. *Aust. J. Mar. Freshwater Res.* 32: 573–589.

Britton, J. C., McMahon, R. F. & Hart J. W. 1991: Relationships between topography, substratum composition and surface temperature, and the spatial distribution of intertidal fauna on rocky shores of south-western Australia. In: Wells, F. E., Walker, D. I., Kirkman, H. & Letherbridge, R. (eds.), *The marine flora and fauna of Albany, Western Australia*: 521–540. Western Australian Museum.

Chapman, M. G. 1995: Aggregation of the littorinid snail *Littorina unifasciata* in New South Wales, Australia. *Mar. Ecol. Prog. Ser.* 126: 191–202.

Chapman, M. G. 1998: Variability in trail-following and aggregation in *Nodilittorina unifasciata* Gray. *J. Exp. Mar. Bio. Ecol.* 224: 48–71.

Chapman, M. G. & Underwood, A. J. 1996: Influences of tidal conditions, temperature and desiccation on patterns of aggregation of the high-shore periwinkle *Littorina unifasciata* in New South Wales, Australia. *J. Exp. Mar. Bio. Ecol.* 19: 213–237.

Emson, R. H. & Faller-Fritsch, R. J. 1976: An experimental investigation into the effect of crevice availability on abundance and size structure in a population of *Littorina rudis*: Gastropoda; Prosobranchia. *J. Exp. Marine Bio. Ecol.* 23: 285–297.

Garrity, S. D. 1984: Some adaptations of gastropods to physical stress on a tropical rocky shore. *Ecology* 65: 559–574.

Jones, K. M. M. & Boulding, E. G. 1999: State dependent habitat selection by an intertidal snail: the cost of selecting a physically stressful microhabitat. *J. Exp. Mar. Bio. Ecol.* 242: 149–177.

King, C. A. M. 1975: *Introduction to physical and biological oceanography*. Edward Arnold, London.

Kronberg, I. 1990: Heat production in *Littorina saxatilis* (Olivi) and *Littorina neritoides* (L.) (Gastropoda: Prosobranchia) during experimental exposure to air. *Helgolander Meeresuntersuchungen* 44: 125–134.

McMahon, R. F. 1990: Thermal tolerance, evaporative water loss, air water oxygen consumption and zonation of intertidal prosobranchs: a new synthesis. *Hydrobiologia* 193: 241–260.

Menge, B. A. & Branch, G. M. 2001: Rocky intertidal communities. In: Gaines S. D. & Hay M. E. (eds.), *Marine community ecology*: 162–166. Sinauer Associates, USA.

Raffaelli, D. G. & Hughes, R. N. 1978: The effects of crevice size and availability on populations *of Littorina rudis* and *Littorina neritoides*. *J. Anim. Ecol.* 47: 71–83.

Stafford, R. 2002: *The role of environmental stress and physical and biological interactions on the ecology of high shore littorinids in a temperate and a tropical region.* Ph.D. thesis, University of Sunderland, UK.

Thivakaran, G. A. & Kasinathan, R. 1990: Salinity, temperature and desiccation tolerance of intertidal gastropods *Littorina quadricentus* and *Nodilittorina pyramidalis*. *Indian J. Mar. Sci.* 19: 57–60.

Underwood, A. J. & Chapman, M. G. 1992: Experiments on topographical influences on density and dispersion of *Littorina unifasciata* in New South Wales. In: Grahame J., Mill P. J. & Reid D. G. (eds.), *Proceedings of the Third International Symposium on Littorinid Biology*: 181–195. Malagological Society of London, London.