Orientation in a keystone grazer: interactions between habitat and individual identity drive patterns of resting behaviour

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ABSTRACT: Quantification of factors that modulate small-scale, individual patterns of location and behaviour is fundamental for ecology, as the behaviour of animals within habitats affects many components of fitness. At the smallest scale, animals show variations in orientation, which can interact with small-scale habitat variability and is probably modified by individual identity. Limpets on steeply-sloped substrata have a population-level downwards bias in orientation. We tested the hypotheses that (1) patterns of orientation are maintained by limpets orientating in the same direction when located within the same resting site, and (2) that the strength of this relationship depends on the identity of an individual. We showed that microhabitat level variation contributed strongly to orientation patterns. Different limpets occupying the same resting site orientated in the same direction, and this pattern was consistent at multiple temporal scales. In resting sites occupied by the same limpet, the strength of the association between habitat and orientation increased. The assumption that environmental factors drive patterns of orientation is widespread, but we have demonstrated that habitat properties may be equally as important. Ecologists must be conscious of habitat properties and individual identity when trying to understand the distribution and alignment of individuals.

KEY WORDS: Limpet · Vertical slopes · Rocky intertidal · Individual selection · Alignment

INTRODUCTION

Intra-individual variability in behaviour and patterns of habitat use occur amongst different habitats and microhabitats (e.g. movement, Underwood & Chapman 1989; predator avoidance, Stadler et al. 1994; nest site selection, De Santo et al. 2003). Innate differences between individuals also play a role in driving patterns of distribution and behaviour; that is, conspecifics are frequently distributed or behave differently from each other when located within the same habitat or microhabitat (e.g. diet selection, Burrows & Hughes 1991; antipredatory responses, Wahle 1992). Orientation, the fine-scale directional

position of an animal in space, can likewise vary greatly among individual organisms, and at different temporal and spatial scales (e.g. barnacles, Crisp & Barnes 1954; mussels, Garcia-March et al. 2007; limpets, Fraser et al. 2010); however, intrinsic differences among individuals do not always explain population levels biases in orientation (Fraser 2014a). Instead, the identity of the individual may interact with habitat variation to strongly modify patterns of orientation. Specifically, conspecifics of different age, sex, size, shape or with different prior experiences may orientate differently, and this can change depending on the habitat. Unlike research on behavioural or distributional patterns at larger spatial

scales, the role of habitat properties in driving patterns of orientation and how inter-individual differences among conspecifics may alter these associations has largely been ignored. This is despite several studies showing that each are separately capable of influencing patterns of orientation (Crisp & Barnes 1954, Webster et al. 2009, Bortolotti et al. 2011). The work presented here tests the idea that limpet orientation is determined by habitat/microhabitat properties, and that this relationship is dependent on the identity of the individual limpet.

Previous research on orientation has predominantly focused upon the role of environmental factors (such as the position of the sun or direction of waves) in affecting patterns of orientation, especially with respect to thermoregulation (e.g. herring gulls, Luskick et al. 1978; gastropods, Garrity 1984, Muñoz et al. 2005). In contrast, there are relatively few examples of the orientation of individuals being dependent upon the habitat or microhabitat context in which the animal is located (but see Crisp & Barnes 1954, Rutowski et al. 1991, Coelho 2001, Webster et al. 2009, Kang et al. 2012). In different habitats, conspecifics may also orientate using different stimuli (e.g. environmental vs. structural habitat cues) or for different biological goals (Rutowski et al. 1991). For example, when located on the ground, male desert hackberry butterflies orientate away from the sun (to thermoregulate), whereas when perched in trees their orientation is correlated with the direction into/away from the hackberry bush in which they are perched (to detect incoming females) (Rutowski et al. 1991, 1994). These examples demonstrate that structural habitat properties have the potential to influence patterns of orientation; however, such studies are very limited in number and the majority only demonstrate that a population bias in orientation exists, and subsequently assume (without presenting supporting evidence) that this bias is a behavioural response to the properties of their habitat.

When testing predictions on the links between habitat properties, an individual's orientation and identity, model organisms need to be easily monitored and individuals readily identifiable in the field. A species is required in which the individuals alter their orientation and location over relatively short periods of time, as changes in orientation can then easily be associated with changes in their habitat/microhabitat. Limpets are a suitable test organism to investigate the role of habitat properties in driving patterns of orientation as they fulfill all these criteria. The rocky intertidal is highly heterogeneous at very small spatial scales (Underwood 2000), and limpets may encounter a wide range of microhabitats during one tidal cycle. Intertidal limpets are widely studied, easily accessible and play an integral role in the ecology of their ecosystem (Branch 1981, Hawkins et al. 1992, Coleman et al. 2006). Grazing by limpets on temperate rocky shores exerts the strongest ecological effect of herbivory in any marine system (Poore et al. 2012), directly controlling the abundance and variation in algal assemblages and structuring rocky shore communities (see Coleman et al. 2006 for review).

Several species of patellogastropod limpet display biases in head orientation during low tide on steeply sloped substrata (>60°) (Iwasaki 1993, Williams et al. 1999, Fraser et al. 2010, Fraser 2014b). In New South Wales (Australia), *Cellana tramoserica* (Holten, 1802) exhibit a downwards bias in head orientation during low tide when found on steeply sloped substrata (Fraser et al. 2010). Interestingly, the orientation of individuals can vary greatly at the scale of a single patch of rock or large boulder (0.25 to 1 m^2) (Fraser et al. 2010, also see the Appendix). A possible explanation for this variation may be that patterns of orientation in *C. tramoserica* are maintained over time by different limpets orientating in the same direction when located in the same resting site (their habitat in this context). Elsewhere in the ecological literature, the term site refers to a whole field site or large area within a study location, however in this study, 'site' is used only to refer to limpet-sized resting sites (approx. 10 × 10 mm in size). That is, if orientation is habitat dependent, different limpets should orientate in a similar way when found in the same habitat. For this to occur, individual limpets must also reoccupy previously occupied resting sites instead of other available areas of rock. We first aimed to test the predictions that on steeply sloped substrata, limpets would occupy resting sites previously occupied by other limpets instead of other areas of rock (Hypothesis 1), and that when found in previously occupied resting sites, limpets would orientate in the same direction as each other at both short (3 d; H2.1) and longer time scales (4 mo; H2.2).

In many species of limpet, individuals return to the same resting site at each low tide cycle (Branch 1981, Iwasaki 1992, Della Santina 1994, Aguilera & Navarrete 2011), a behaviour called 'homing' (Branch 1981, Coleman 2007). By homing to the same resting site, limpets can achieve a closer fit to the substratum, potentially reducing the risk of predation (Garrity & Levings 1983), dislodgment from waves (Orton 1929), and desiccation stress (Garrity 1984). Limpets vary in size and shell perimeter irregularities, and therefore

the orientation that allows for the best fit may vary between limpets within the same resting site. *C. tramoserica* does not consistently home to a fixed resting site and does not produce a home scar (Mackay & Underwood 1977); consequently, some resting sites will by occupied by the same limpet over time (a homing limpet) whereas other resting sites may be occupied by different limpets over the same time scale. This variation allowed us to evaluate if the potential relationship between habitat and orientation is dependent upon the identity of the limpet. We tested the hypothesis that in resting sites occupied by the same limpet, individuals will more frequently orientate in the same direction compared to in resting sites occupied by different limpets during each low tide at short (H3.1) and longer time scales (H3.2). Alternatively, identity may not be important, in which case all limpets found in the same resting site would be orientated in the same direction.

Although there is a population-level bias in orientation (Fraser et al. 2010) it can not be concluded that individual limpets are actively selecting to orientate in a downwards direction. Instead, limpets may select resting sites based on a property of the habitat that limits limpets to orientating downwards. If limpets do actively face downwards, they may choose to occupy resting sites that allow for this orientation i.e. a 'downwards facing resting site'. The final aim of the work reported here was to establish whether there are downwards facing resting sites (H4) and if limpets reoccupy these sites instead of other available resting sites (H5). If limpets are not specifically selecting downwards facing resting sites over others, they may still be selecting some sites over other known resting sites. This study also tested the hypothesis that some resting sites are occupied more frequently than other previously occupied resting sites (H6).

MATERIALS AND METHODS

This study was done at 2 locations around Sydney, Australia: Little Bay (33° 58′ S, 151° 15′ E) and Cape Banks Scientific Reserve (34° 59′ S, 151° 25′ E). Within these locations, experimental patches were haphazardly selected for all experiments. Each patch contained steeply sloped sandstone surfaces and held a minimum of 50 limpets. Patches were at a mid- to hightide height above mean low water and were moderately exposed to waves (Underwood et al. 1983). The aspect of surfaces within a patch varied 360° and were of mixed rugosity with little cover of

barnacles and algae. Two locations were used because of logistical constraints, but patterns of orientation were similar at each location (Fraser et al. 2010; C. M. L. Fraser unpubl. data).

Is orientation habitat-dependent and does limpet identity influence this relationship in the short term? (H1, H2.1 and H3.1)

Experiments were conducted during September 2011 within Cape Banks Scientific Reserve to test the prediction that (H1) limpets will reoccupy previously occupied areas of rock (resting sites) more frequently than expected by chance, and that (H2.1 and H3.1) irrespective of their identity, individuals will orientate in the same direction as previous occupants. A total of 3 patches were selected to test if patterns were spatially consistent, and in each patch 100 limpets were labelled *in situ* with shellfish tags (Hallprint). The head orientation of tagged limpets was measured (Fraser et al. 2010) and their resting sites labelled with waterproof paper and Selleys® Araldite epoxy adhesive. Labelling resting sites with waterproof paper and epoxy adhesive had no effect on the probability of a resting site being reoccupied or limpets orientating in the same direction within that site (see Supplement 1 at www.int-res.com/articles/suppl/m522p145_supp.pdf). Of the 100 limpets, 50 were removed from their resting site with a palette knife and relocated to nearby horizontal rock (slope less than 30°) within 30 cm of their original resting site in order to create vacant resting sites but minimise density-dependent effects. After 3 d (sufficient time for all limpets to leave their resting sites; C. M. L. Fraser unpubl. data), the identity and orientation of all limpet occupants for each previously occupied resting site were recorded. The null hypothesis that the frequency of previously occupied resting sites being reoccupied was no greater than expected by chance alone was tested using a replicated goodness-of-fit test (Sokal & Rohlf 1995). This test also allowed us to concurrently test the null hypothesis that the frequency of resting sites being reoccupied did not differ between experimentally vacated resting sites and control resting sites, or between different patches. The expected value was based on the ratio of occupied resting sites to the number of unoccupied but potential resting sites determined from photographs. To calculate the number of potential resting sites in a given area, we used a packing rule approach using the size (length: 22.28 ± 0.4 mm) and density (70.02 ± 4.67 limpets m^{-2}) of individuals

within the studied population of limpets. Subsequently, using sequential probability, we calculated the expected number of previously occupied resting sites that would be reoccupied after 3 d by chance alone (the expected value), based on the size, densities and arrangements of limpets on shallowly sloped surfaces (expected value = 0.002). These shallowly sloped substrata had very similar sized limpets and were of similar densities as the steeply sloped sites used here (see Tables S1 & S2, Figs. S2 & S3 in Supplement 2 at www.int-res.com/articles/suppl/m522 p145_supp.pdf). A replicated goodness-of fit-test (Sokal & Rohlf 1995) was also used to test the null hypothesis that the proportion of resting site occupants reorientating in the same direction as the original occupants was no different than expected by chance alone, and that there was no difference in this frequency between patches and treatments. A limpet was considered to be orientating in the same direction as a labelled resting site's previous occupant if it was within 10 degrees of the original limpet's orientation (Fraser et al. 2010). Whether or not a resting site occupant orientates in the same direction as the original occupant may be dependent on whether the resting site is occupied by the same or a different limpet at each sampling time. The Mantel-Haenszel procedure (Sokal & Rohlf 1995) was used to test the null hypothesis that the proportion of resting site occupants orientating in the same direction was independent of the identity of the limpet. Following rejection of this null hypothesis (i.e. consistency of orientation was dependent on limpet identity), we performed a second replicated goodness-of-fit test comparing the frequency of resting site occupants orientating in the same direction, where resting sites that contained the same limpet on each day were excluded (Type I error rates were adjusted to $\alpha' = 0.025$ using the Bonferroni method [Sokal & Rohlf 1995] because of 2 repeated tests).

Is orientation habitat-dependent and does limpet identity influence this relationship in the longer term? (H2.2 and H3.2)

Though the occupants of known resting sites may orientate in the same direction as each other after short time intervals, this may not be true at greater temporal scales. To test the prediction that (H2.2) limpet resting site occupants orientated in the same direction over a period of months, and (H3.2) that the frequency of limpets orientating in the same direction was dependent on whether the resting site was

occupied by the same or different limpets, the frequency of individuals orientating in the same direction was first compared between known resting sites occupied by putatively homing limpets and those occupied by different limpets over time. In patches across Cape Banks Scientific Reserve, 100 resting sites and their limpet occupants were labelled (as described above) between April and July 2012 (3 patches) and repeated between October 2012 and January 2013 (3 patches). Over 4 mo, the orientation and identity of any limpet occupant at each resting site was recorded every 2 wk. For a resting site to be included in the analysis, it had to be occupied a minimum of 5 times during the course of the experiment so as to collect a representative sample of the behaviour of limpets within each resting site. Resting sites that were occupied less frequently were excluded from the analysis. Due to the small number of resting sites that fitted this criteria (55 'putatively homing' vs. 40 'different limpets' resting sites), the resting sites from all patches and times were pooled together. In resting sites classed as occupied by putatively homing limpets, the same limpet had to be present at least 5 times over the 8 sampling occasions of the study. A 2-sample Kolmogorov-Smirnov test (Sokal & Rohlf 1995) was used to test the prediction that frequency of limpets orientating in the same direction was independent of whether the resting site was occupied by a homing limpet or by different limpets.

At any given point in time, a limpet has a 1 in 18 chance of orientating in the same direction (Fraser et al. 2010). This frequency was used to create a binomial frequency distribution of the number of resting site occupants that, by chance alone, will orientate in the same direction, which was then used as an expected distribution; this was the null hypothesis that was then compared against the observed frequency distribution of resting site occupants orientating in the same direction using a 1-sample Kolmogorov-Smirnov test.

Are there downwards and upwards facing resting sites? (H4)

If resting sites can be classified as 'downwards facing' (occupants orientate between 135 and 225°) and/or 'upwards facing' (occupants orientate between 315 and 45°), it would be predicted that if we categorise an occupant's orientation as downwards (or upwards) at a random point in time (Time A), the orientation of another occupant in the same resting site at a second random point in time (Time B) will also

fall into this category, irrespective of the identity of the occupant. To test if there are downwards and upwards facing resting sites, each reoccupied resting site was designated as a downward (n = 118 across all patches) or upward (n = 34) facing site based on the orientation of its occupants at Time A. Overall patterns of limpet orientation within each patch were similar to that previously found (Fraser et al. 2010). Resting sites that did not fall into either category or that were never reoccupied were excluded (n = 315). Each resting site was scored as to whether the occupant was facing the same direction at Time B as its original occupant, where Time B was a randomly selected time from 8 possible sampling occasions. If the resting site was not occupied at that time, another time was selected. Instances where a resting site was reoccupied by the same limpet were excluded to ensure that the results were not confounded by homing limpets orientating in the same direction. The null hypothesis that a resting site's orientation designation did not predict the orientation of future occupants was tested with a replicated goodness-of-fit test. As the ratios were not homogenous between samples, each sample was tested against the expected value individually (Sokal & Rohlf 1995). We adjusted α′ to 0.005 using the Bonferroni method to correct for 10 repeated tests.

Are downwards facing resting sites occupied more frequently than resting sites of other orientations? (H5)

We tested the hypothesis that the frequency at which downwards facing resting sites are reoccupied is greater than the frequency of other resting sites being reoccupied by monitoring the occupation of resting sites at Little Bay over a period of 4 mo. In November 2013, an equal number of putatively downwards facing resting sites (where the original resting site occupant was oriented downwards) and other non-downward facing resting sites were labelled (as described above) in each of 4 patches. Each resting site occupant was labelled and its orientation and length measured. Approximately every 2 wk, each patch was revisited and the occupation status of each resting site and the identity of the occupant were recorded. Resting sites may be reoccupied because limpets are selecting to occupy that site or purely because they are homing limpets. As a consequence, resting sites that were reoccupied by putatively homing limpets (at least 3 out of 5 occasions occupied by the same limpet; Mackay & Underwood

1977) were eliminated from the analysis. We tested the null hypothesis that the mean number of times downwards facing resting sites were reoccupied was no different from the mean number of times other resting sites were reoccupied using a 2-way ANOVA (fixed = downwards vs. other, random = patch). The assumption of homoscedasticity was tested using Cochran's C test and the analysis was done in WinG-mav 5 (EICC).

Among known resting sites, are some occupied more frequently than others? (H6)

Using the same experimental set up as in H2, the occupation state (i.e. whether occupied or not) of each resting site was monitored along with the orientation and identity of any limpet occupants over 4 mo. In this experiment, a resting site was defined as being reoccupied if a limpet was seen *in situ* at least once over the study period. Homing limpets were again removed from the data set (as described above) to avoid confounding the results. The frequency distribution of the proportion of occasions a resting site was occupied was compared against the expected binomial distribution of reoccupation frequencies if all resting sites had an equal chance of reoccupation at a given point in time. In contrast to the previous experiment where the expected values were based on the number of potential resting sites, in this experiment we compared rates of reoccupation between known resting sites. As a result, the expected distribution was based on the average proportion of resting sites occupied in each patch, not the total potential number of resting sites. The null hypothesis that the frequency distribution of resting sites being reoccupied was no different from the expected extrinsic distribution was tested using a 1-sample Kolmogorov-Smirnov test (α′ = 0.008 to correct for 6 repeated tests, Bonferroni method; Sokal & Rohlf 1995).

Resting sites may be reoccupied simply because individual limpets are either following their own or a conspecific's trail, and as a result finishing at the same location on the shore each tide cycle, or by responding to a cue left by the previous occupant (e.g. a mucus pad or trail). This could mean that once a resting site becomes vacant it is less likely to be occupied again if these cues fail to be renewed, and therefore whether a resting site is reoccupied at a given point in time is not independent of its reoccupation state at previous occasions. To test if the likelihood of a resting site being reoccupied was dependent on whether it was occupied the previous

time, at the time scale of this experiment, a runs test (Sokal & Rohlf 1995) was done for each resting site using the 2 elements 'occupied' and 'unoccupied'. A runs test determines if the number of groupings of similar and dissimilar elements is different from the number that would occur if the elements were randomly arranged. Critical values are not possible for all combinations of 'occupied' and 'unoccupied' (e.g. one time occupied, 7 times unoccupied), and therefore runs tests could only be done for resting sites that were occupied 3, 4 or 5 times out of a possible 8 (Sokal & Rohlf 1995). The number of resting sites in which the combination of 'occupied' and 'unoccupied' events diverged from random was compared against a binomial distribution, where the probability of a divergence event occurring (equivalent to α) was set at 0.05.

RESULTS

Is orientation habitat-dependent and does limpet identity influence this relationship in the short term? (H1, H2.1 and H3.1)

Originally occupied resting sites were reoccupied disproportionally more frequently compared to other previously unoccupied but potential resting sites, and this pattern was spatially consistent across patches. The frequency of previously occupied resting sites being reoccupied was 8 times greater than predicted by the available number of potential resting sites ($G_{Pooled} = 2664.01$, df = 1, p < 0.05; Fig. 1A) and there was no difference in the frequency of reoccupation of these resting sites between different patches or treatments ($G_{Heterogeneity} = 9.56$, df = 5, ns); hereafter G_{Pooled} and $G_{Heterogeneity}$ will be represented by G_P and G_H. When located in the same resting site, limpets orientated in the same direction (within 10° of each other) (Fig. 1B). The number of resting sites where there was no difference between the orientation of the original occupant of a resting site and its occupant after 3 d was greater than predicted by chance alone (1/18) ($G_P = 658.88$, df = 1, p < 0.05) and there was no difference in the frequency of this occurring between patches or treatments ($G_H = 1.04$, df = 5, ns). When resting sites that contained the same limpet on each day were excluded, similar results were found, as the frequency of new occupants orientating in the same direction as previous occupants was significantly greater than expected ($G_P = 270.55$, df = 1, p < 0.05). In resting sites that were reoccupied by the same limpet, individuals were 2.8
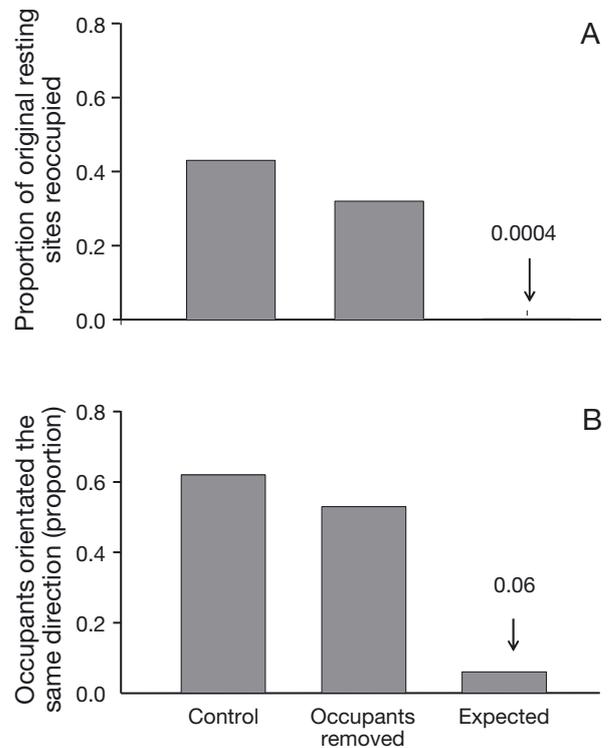

Fig. 1. Observed and expected proportion of (A) original resting sites reoccupied at locations where the original occupants were untouched (control) or removed from the resting site (creating vacant resting sites), and (B) resting sites where new occupants orientated in the same direction as previous occupants, at sites where the original occupants were untouched or removed

times more likely to orientate in the same direction compared with those resting sites occupied by a new limpet ($X^2_{Mantel-Haenzel} = 7.56$, df = 1, p < 0.05), and this was consistent across sites ($X^2_{Heterogeneity} = 2.57$, df = 2, ns).

Is orientation habitat-dependent and does limpet identity influence this relationship in the longer term? (H2.2 and H3.2)

The frequency of an occupant of a known resting site orientating in the same direction was not dependent on whether the resting site was occupied by the same limpet (putatively homing limpets) or different limpets over time. The frequency distribution of the number of occasions a limpet orientated in the same direction in resting sites occupied by the same limpet on each sampling occasion was no different from the frequency distribution of resting sites occupied by different limpets (2-sample Kolmo-
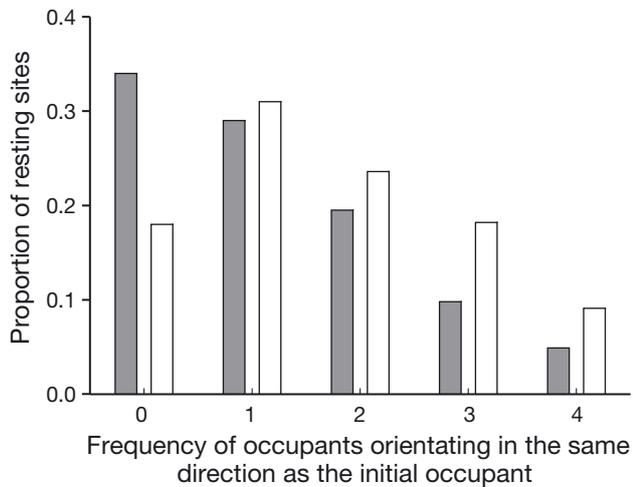
Fig. 2. Distributions of the number of times resting site occupants orientated in the same direction as the original site occupant, when resting sites were reoccupied by putatively homing limpets (white bars) or different limpets (grey bars). A site had to be reoccupied at least 4 times during the 8 census periods to be included in the analysis
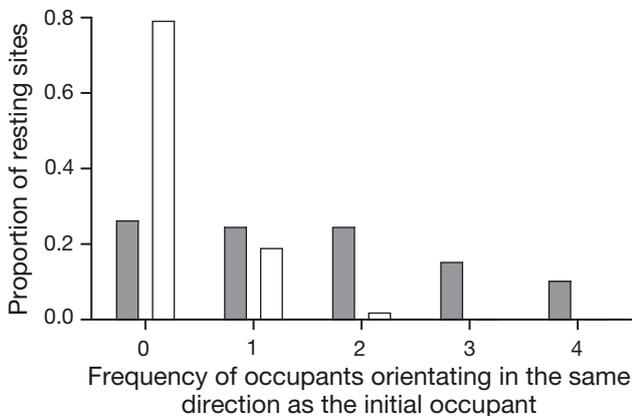

Fig. 3. Distributions of the number of times that resting site occupants orientated in the same direction (grey bars) and the expected distribution if selection of orientation was random (white bars). A site had to be reoccupied at least 4 times during the 8 census periods to be included in the analysis. Expected distribution is based on a limpet having a 1 in 18 chance of orientating in the same direction at any given time

gorov-Smirnov test, $d = 0.168$, $D_{0.05} = 0.28$, ns; Fig. 2). As there was no difference in frequency, all resting sites were pooled together for the second analysis. Occupants of known resting sites orientated in the same direction as each other more frequently than would be predicted if the selection of orientation was random (1-sample extrinsic hypothesis Kolmogorov-Smirnov test, $g_{max} = 0.538$, $g_{0.05} = 0.120$, $p < 0.05$; Fig. 3).
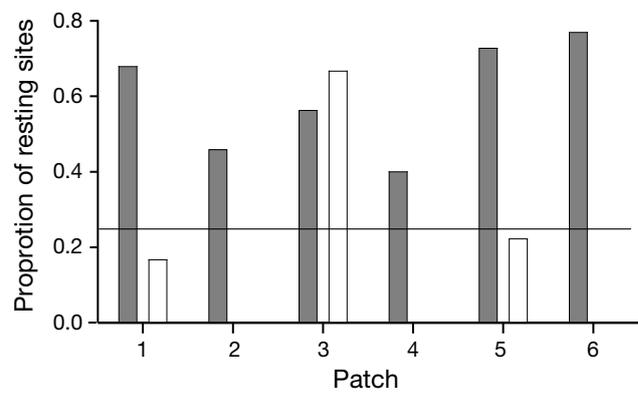

Fig. 4. Proportion of resting sites where the orientation of the original occupant (either up, white bars or down, grey bars) predicts the orientation of another occupant at a random point in time. Horizontal line: expected value if by chance alone (1/4). In patch 2 and 3, the proportion value for potentially 'upwards resting sites' was based on only 3 limpets each and were not included in the analysis. ($\alpha' = 0.005$)

Table 1. (a) Replicated goodness-of-fit (G) test (Sokal & Rohlf 1995) of frequency of resting sites where the orientation of the original occupant predicts the orientation at a random point in time, and (b) partitioning of G_{Total} into individual contributions. $\alpha' = 0.005$. (−) sample size too small for analysis

(a) Tests	df	G	p
Pooled (G_P)	1	44.23	<0.05
Heterogeneity (G_H)	9	37.21	<0.05
Total	10	81.44	<0.05
(b) Site/original direction		G	p
Patch 1 Down		22.69	<0.004
Patch 1 Up		0.24	>0.004
Patch 2 Down		4.97	>0.004
Patch 2 Up		−	−
Patch 3 Down		7.05	>0.004
Patch 3 Up		−	−
Patch 4 Down		1.62	>0.004
Patch 4 Up		2.88	>0.004
Patch 5 Down		22.03	<0.004
Patch 5 Up		0.04	>0.004
Patch 6 Down		15.41	<0.004
Patch 6 Up		4.60	>0.004

Are there downwards and upwards facing resting sites? (H4)

Some resting sites can be designated as a downwards facing resting site. Those sites that were originally occupied by a downwards facing limpet were more frequently than predicted reoccupied by a limpet also facing downwards (Fig. 4, Table 1). This pattern was consistent across sampling patches and

time, although it differed in magnitude. This was not true for resting sites originally occupied by an upwards facing limpet.

Are downwards facing resting sites occupied more frequently than resting sites of other orientations? (H5)

Downwards facing resting sites were not reoccupied more frequently than other resting sites ($F_{1,3}$ = 0.009, ns; see Table S3 in the Supplement at www.int-res.com/articles/suppl/m522p145_supp.pdf), as the average number of times a resting site was reoccupied was equal between downwards facing (1.7 times out of a possible 6 times) and other resting sites (1.9 times out of a possible 6 times). There was no significant interaction between patch and site orientation ($F_{3,192}$ = 1.83, ns) but the average number of times a resting site was reoccupied did differ between patches ($F_{3,193}$ = 3.36, p < 0.05). As patch was a random factor, further interpretation of the difference between patches is inappropriate.

Among known resting sites, are some occupied more frequently than others? (H6)

Overall, no resting sites were disproportionately reoccupied more frequently than other available known resting sites, but patterns of resting site reoccupation differed between patches. At some patches, the observed frequency distribution of resting site reoccupations was different from the expected distribution (Table 2), but in other patches there was no significant difference. In patches where resting site reoccupation differed from random, a large proportion of the resting sites were only reoccupied once, but the proportion of resting sites that were consistently reoccupied was small.

The likelihood of a resting site being reoccupied was independent of the occupation history of the resting site, at the time scale of 14 d, at 2 of the 3 patches where it was possible to conduct runs tests. In one patch, 1 of 22 resting sites had significantly fewer number of runs than would occur if the arrangement of data points (occupied or unoccupied) was random. In another patch, no resting sites had patterns of occupation that diverged from random arrangement (out of 11). The frequency of a significant divergence from random arrangement occurring was no greater than would take place by chance alone in both patches. In a third patch, reoccupation

Table 2. Kolmogorov-Smirnov test of distributions of frequency of reoccupation of known resting sites by limpets compared against an extrinsic expected distribution (based upon the average proportion of resting sites occupied). α' adjusted to 0.008 to correct for repeated tests

Patch	g_{max}	p
Time 1 patch 1	0.270	<0.008
Time 1 patch 2	0.202	>0.008
Time 1 patch 3	0.313	<0.008
Time 2 patch 4	0.270	<0.008
Time 2 patch 5	0.228	>0.008
Time 2 patch 6	0.343	<0.008

was dependent on if the resting site was occupied during the previously sampling time (4 of 16 resting sites diverged from random arrangement, p < 0.05).

DISCUSSION

Habitat-dependent orientation

The orientation of *Cellana tramoserica* was habitat-dependent since different limpets found in the same resting site orientated in the same direction at short (after 3 d) and longer (over 4 mo) temporal scales. This was also true for resting sites sequentially occupied by the same limpet. These results support our original predictions (H2.1 and H2.2), and are similar to findings from research done on barnacles, where cyprids orientated in different directions in different microhabitats (Crisp & Barnes 1954). The next step towards understanding the link between orientation and habitat is to determine the habitat property to which limpets are responding. Resting sites can vary in their topographic complexity, algal growth, humidity and time shaded, though many of these factors obviously co-vary with topography and aspect (Helmuth & Hofmann 2001, Jackson 2010, Fraser 2014a). Surface topography has previously been found to be important in determining cyprid orientation (Crisp & Barnes 1954), and at a larger spatial scale there are indications that topography is important for limpet orientation (Fraser et al. 2014). Animal behaviour (movement, Underwood & Chapman 1989; competition, Petren & Case 1998; feeding, McCormick & Lonnstedt 2013) and distribution (e.g. Kohn & Leviten 1976, McCormick 1994, Lemire & Bourget 1996) also frequently varies with topographic complexity across different taxa. Differences in the abundance, distribution and behaviour of intertidal organisms are often associated with specific topographic

features, such as crevices and pits, and overall changes in complexity (Underwood & Chapman 1989, Beck 1998, Johnson et al. 2003). During low tide, the topography of a limpet's resting site and surrounding substrata can influence how closely the limpet's shell fits to the substrata. For example, fine scale differences in topography have been suggested to influence the friction limpets can produce between their shell and the substrata when clamping to evade dislodgment (Ellem et al. 2002). Limpets orientated in different ways within the same resting site may fit differently to the surrounding substrata, and this may in turn alter their ability to withstand dislodgement from waves and potential predators. If topography is driving orientation, it would be predicted that on surfaces with smooth topography the proportion of downwards facing limpets will be different from that found on more complex substrata.

Patterns of orientation may be influenced by some property of the resting site itself, as discussed above, or alternatively, limpets may be responding to the presence of the previous occupant. The orientation of limpets has previously been shown to vary with barnacle cover (Fraser et al. 2014), but the role of conspecifics in driving orientation is unknown. Previous studies have found differences in the orientation of individual animals when in the presence of conspecifics (McBride et al. 1963, Spurr 1975, Brumm & Todt 2003). These interactions have been based on visual (McBride et al. 1963, Spurr 1975) and auditory cues (Brumm & Todt 2003, Brumm et al. 2011). Animals also often display behavioural responses to chemical cues produced by conspecifics and heterospecific organisms (Chivers & Smith 1998, Kats & Dill 1998, Galef & Giraldeau 2001, Hay 2009). For marine organisms, chemical cues are often more or equally important and are very common in marine systems (Chivers & Smith 1998, Hay 2009). In gastropods, mucus trails are laid whilst foraging, and during low tide a mucus pad is created between their foot and shell and the substratum. Mucus pads have the potential to act as a form of public information (Davies & Hawkins 1998), potentially signalling a suitable resting site and the optimal orientation to adopt within that site, as well as providing protection from desiccation (Wolcott 1973), predation and dislodgment from waves (Smith 1991, 1992). Limpet pedal mucus has been shown to persist in the field for up to 6 d in the congeneric *Cellana grata* (Davies & Williams 1995) and over 40 d in *Patella vulgata* (Davies et al. 1992). Although at the temporal scale of 2 wk, occupation history does not influence resting reoccupation, this does not preclude the influence of

mucus pads on patterns of occupation after only days, as the persistence of mucus in this species is unknown and it is uncertain how stationary mucus performs in the field. When mucus pads are removed from resting sites on steeply sloped (C. M. L. Fraser et al. unpubl. data) and shallowly sloped substrata (R. A. Coleman et al. unpubl. data), limpets reoccupy resting sites less frequently than resting sites with intact mucus pads.

Interaction between identity and habitat

The role of habitat in determining orientation is partially dependent on the identity of the limpet, though this only appears to be true at short temporal scales. One explanation for this difference is that the orientation limpets adopt is linked to the direction an individual takes on departure or when returning from their foraging bout, and over a few days this direction is similar each low tide cycle, but over a period of months this direction is less consistent and more variable. In *P. vulgata*, the leaving direction of consecutive foraging excursions by an individual was identical in 40% of sampled excursions within a population, but over longer periods of time individuals did not consistently forage or depart in the same direction (Chelazzi et al. 1998). This difference in consistency of foraging direction at different temporal scales is not true for all species of patellogastropod (e.g. *P. rustica*, Della Santina 1994), and caution should be taken in extrapolating findings to other species without further experimental evidence (Fraser 2014b). Differences in foraging direction with respect to limpet identity and time may also interact seasonally with the supply of microalgae (Jenkins et al. 2001).

In resting sites occupied by the same limpet, individuals orientated more frequently in the same direction than those occupied by different limpets after 3 d; however in all resting sites, occupants (irrespective of their identity) orientated in the same direction more frequently than random. These results suggest that the identity of the limpet only modifies the strength of the association between an individual's orientation and the habitat or microhabitat in which they are located. No interaction has been found in other organisms between an individual's orientation, habitat and identity; however in some animals, associations between orientation and environmental factors (e.g. position and location of the sun) are dependent on the identity of the individual (Bóhorquez-Alonso et al. 2011, Bortolotti et al. 2011). In the lizard

Gallotia galloti for example, no consistent differences in orientation between individuals occurred under most environmental conditions, but when first encountered in sunny conditions, males exhibited different patterns of orientation to females (Bóhorquez-Alonso et al. 2011). Habitat-dependent foraging, escape responses and activity patterns can also vary with individual identity (e.g. Vesakoski et al. 2008, Griffiths et al. 2014). Male European minnows were found to be more active than females when located downstream of a pool, whereas in upstream locations, activity per hour was equal between sexes (or females were more active; Griffiths et al. 2014).

Microhabitat use

Limpets reoccupy previously occupied resting sites disproportionately more than previously unoccupied patches of rock. Homing by limpets has been extensively examined (Mackay & Underwood 1977, Branch 1981, Della Santina 1994), but our findings show that occupancy of a given resting site is independent of limpet identity. It is widely acknowledged that habitat selection and patterns of animal distribution occur at multiple spatial scales (Mayor et al. 2009) and our results provide further evidence that habitat selection does occur at very small spatial scales (i.e. cm).

One mechanism by which a downwards bias in orientation within populations may be maintained through time is via limpets selecting resting sites that allow them to orientate downwards. Across the patches studied, there are resting sites that can be classified as 'downwards facing resting sites', but limpets do not actively select to occupy these resting sites over other known available resting sites. Our findings suggests that the areas of rock used by limpets as their resting site during low tide are being selected for some other factor and that facing downwards is either not important for limpets or is secondary to other factors. Accordingly, the population-level bias in orientation is not being driven by a selection of resting sites that allow limpets to orientate downwards. Differences in desiccation and thermal stress associated with variations in orientation are other mechanisms which may explain biases in limpet orientation (Gallien 1985, Williams et al. 1999, Fraser et al. 2010). On the other hand, a downwards head orientation may allow for greater flushing of the nuchal cavity to remove waste products (Williams et al. 1999). All these models need to be explicitly tested.

CONCLUSIONS

For *Cellana tramoserica*, orientation is habitat-dependent and this relationship is influenced by the identity of the limpet at short temporal scales. Our findings provide evidence that small-scale habitat properties may be equally as important in driving orientation as environmental factors. The results clearly demonstrate that ecologists should consider previous distributions of organisms when trying to understand how current patterns of spatial organisation are maintained. We also demonstrate that multiple factors can interact to modify patterns of orientation; consequently, possible drivers should not be studied in isolation. This study illustrates the importance of examining patterns of orientation at multiple temporal scales and times, to generate a greater level of understanding of how orientation interacts with small-scale patterns of distribution.

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Appendix. *Cellana tramoserica* orientating in different directions with the proportion of limpets orientating downwards (135–225°, a) similar to the proportion of downwards facing limpets found at the population level