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Original Citation:

Plasticity in the temporal organization of behaviour in the limpet Cellana grata / G. SANTINI; A. NGAN; G.A. WILLIAMS. - In: MARINE BIOLOGY. - ISSN 0025-3162. - STAMPA. - 158:(2011), pp. 1377-1386. [10.1007/s00227-011-1656-0]

Published version:

DOI: 10.1007/s00227-011-1656-0

Mar Biol (2011) 158:1377–1386
DOI 10.1007/s00227-011-1656-0

ORIGINAL PAPER

Plasticity in the temporal organization of behaviour in the limpet *Cellana grata*

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Received: 2 August 2010 / Accepted: 22 February 2011 / Published online: 13 March 2011

Abstract The behaviour of intertidal consumers is often tightly constrained to tidal movements, although activity patterns can vary within these constraints. Spatio-temporal variability in behaviour of a limpet, *Cellana grata*, was analysed over different tidal conditions (spring and neap tides) and during different times of the year (one summer and one winter) at sites in Hong Kong. Activity was generally dictated by tidal movements, being concentrated when animals were awash. Plasticity in behaviour was observed, with some limpets anticipating activity during the summer period and delaying activity during winter time. Limpets were active for a time equal, or slightly less, than time awash. As the time awash exceeded ~14–16 h, however, activity duration decreased. Within this general pattern, tidal variation as well as variation among times of the year was noted, with the lowest dependence on time awash being recorded during winter neap tides. Limpets showed a slight preference for being active during night-time, which was particularly evident when animals were emersed during the summer period. Although the basic activity in *C. grata* is constrained to a specific temporal window, this limpet is able to modulate its foraging strategies and resting height, according to local, daily changes in environmental conditions.

Communicated by F. Bulleri.

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Introduction

Rocky intertidal organisms are strongly influenced by fluctuations in stress levels due to natural variations in their environment (Branch 1981; Garrity 1984; McMahon 1990; Denny and Wethey 2001; Helmuth 2002). These fluctuations are usually cyclical and mobile organisms often exhibit behavioural patterns which are consistent with stress minimizing strategies, by matching activity bouts with periods of more favourable conditions (Little 1989; Chapman and Underwood 1992). Such favourable periods may be temporally consistent, resulting in rhythmicity in the animals' activities (Della Santina and Chelazzi 1991; Della Santina et al. 1994; Williams and Little 2007). Such rhythmic behaviour of many intertidal gastropod grazers is thought to be cued by zeitgebers such as daylight and/or tidal cycles (Hawkins and Hartnoll 1983; Little and Stirling 1985; Della Santina and Naylor 1993; Gray and Hodgson 1999).

The temporal consistency of activity patterns has been used to identify a species' Potential Activity Phase (i.e. the combination of diel and tidal phases where recorded activity of one species is maximal), which is usually considered to be rigid and species specific (Burrows et al. 2000). Even strongly predictable patterns may, however, exhibit temporal and/or spatial variation from the norm, due to variable external conditions (Chapman and Underwood 1992; Raffaelli and Hawkins 1996; Williams and Little 2007). On a short temporal scale, for example, daily changes in weather conditions, such as rainfall, wave action, wind or atmospheric pressure, are known to influence activity in some species (Little et al. 1991; Della Santina and Chelazzi 1991). On a longer temporal scale, seasonal influences are known to affect behaviour in limpets (Gray and Hodgson 1997; Santini et al. 2004) and chitons (Ng and Williams 2006), due to changes in abiotic (e.g. temperature, wave action) factors. Furthermore, intrinsic changes in energy metabolism such as gonad development (e.g. Blackmore 1969; Morais et al. 2003) or changes in food composition and availability (Hill and Hawkins 1991; Nagarkar and Williams 1999) have the potential to affect consumer behaviour.

Spatial variations in species' behavioural patterns have been documented among separate populations facing different sets of biotic and abiotic conditions (Hartnoll and Wright 1977; Branch 1985; Della Santina et al. 1994) and also among individuals of the same population experiencing different substratum slopes (Williams et al. 1999; Santini et al. 2004) or shore heights (Little and Stirling 1985; Little et al. 1988; Takada 2001; Ng and Williams 2006). Given this variability, intertidal animals represent ideal subjects for the study of behavioural plasticity, which is a specific component of phenotypic plasticity, involving rapid and reversible adaptive responses to environmental cues (Komers 1997; Brockmann 2001; Dingemanse et al. 2010; Mery and Burns 2010).

Cellana grata is a dominant grazer found on moderately exposed to exposed rocky shores throughout south-east Asia (Williams and Morritt 1995; Huang 2001). This non-homing limpet is active only when awash (Williams and Morritt 1995; Erlandsson et al. 1999; Davies et al. 2006) and its activity appears not to be synchronized to the light–dark cycle (Gray and Williams 2010). Individuals move up the shore with the rising tide, slow their movement during the peak of the tide and return to their resting height in the mid-high shore with the ebbing tide (Williams and Morritt 1995; Davies et al. 2006). Although the basic behavioural patterns of this species have been previously documented (Williams and Morritt 1995; Davies et al. 2006), comparatively little is known about how such patterns may change over temporal scales in response to long-term (seasonal) changes in favourable conditions. Furthermore, how daily environmental variations (related to weather conditions) or local habitat features (wave splash, etc.) may influence the behaviour of this species is also largely unexplored (but see Davies et al. 2006). To address these knowledge gaps, this paper elucidates temporal and spatial variabilities in the behaviour of *C. grata* over a combination of different times of the year (winter, summer) and tidal conditions (neap, spring). Investigating the behavioural patterns of *C. grata* over these temporal and spatial scales allows an evaluation of the repertoire of behavioural strategies available to this limpet to accommodate variation in environmental conditions.

Materials and methods

This study was carried out at Cape d'Aguilar (22°13′N, 114°12′E), Hong Kong (see Williams and Morritt 1995; Davies et al. 2006). Three moderately exposed sites (Sites 1–3, ~15–25 m in length) with different features (orientation, slope) were chosen over an area of ~90 m along the Cape d'Aguilar peninsula, to represent the variable conditions which *C. grata* would naturally experience (for further details see Ngan 2006).

All limpets >20 mm at the sites were marked ($\Sigma n = 102$) using Mollusc Tags (Hallprint, Australia) and a code written on the shell with permanent marker pens (Pilot, Japan). Tagging took place 2 months before the start of the observations, to allow for any possible disturbance effects. The average size of limpets (maximum length, ±0.1 mm) differed among sites ($F_{2,99} = 23.8$, $P < 0.0001$), limpets being smaller at Site 3 (34.4 ± SE 0.5 mm, $n = 58$) than at Sites 1 and 2 (38.9 ± 1.0 mm, $n = 25$ and 40.7 ± 0.7 mm, $n = 19$, respectively; Tukey test: $3 < [2 = 1]$). The activity patterns of *Cellana grata* at the three sites were recorded during summer (July–September 2003) and winter (December 2003–March 2004, see Kaehler and Williams (1996) for a description of Hong Kong climate). During each season, observations were replicated on three randomly chosen dates during neap and spring tides ($\Sigma n = 3$ sites × 2 seasons × 2 tidal conditions × 3 dates = 36). Predicted tidal amplitudes during neap and spring tides were 100–200 cm and 50–250 cm above Chart Datum (CD), respectively. During each observation period, the activity (active or inactive) and physical state (emersed, awash or submerged) of the limpets were recorded at 60-min intervals for up to 25 h. In practice, each limpet was observed for 15 consecutive seconds every hour. The order of sites and limpets observation was randomized each day. Activity was defined as any observable movement within a 15-s time frame. Active limpets lift their shell from the substratum, which is clearly recognizable even during submergence or splashing by waves. "Emersed active" described limpets found active above the wave splash zone, where wave splash did not reach the individual within the 15-s observation period. As such, this definition refers to a dry limpet, but can also include individuals that were infrequently splashed by waves. "Awash" refers to limpets continuously splashed by waves or successively covered by seawater and exposed to air within 15 s. "Submerged" refers to limpets continuously and completely covered by seawater during the 15-s time frame. Red light was used during nighttime observations to minimize possible disturbance (see Little and Stirling 1985; Williams and Morritt 1995). Finally, it was assumed that the activity and physical state observed for each limpet within the 15-s observation windows was maintained for the remaining hour (i.e., a limpet observed active for 15 s was scored as being active for the entire hour).

To assess variability in global activity patterns during tide, time of the year, day and sites, the following variables were measured:

1. total limpet activity (AT, hours), was computed as the number of hours during which a limpet was scored as "active" and could vary from 0 to 25 (total number of observation hours, within a "day");
2. resting height (RH, cm above CD), the height on the shore at which limpets were found at the beginning of each observation day;
3. start latency (LT, hours), the difference between the time of first wash received by a limpet during a day and the time of onset of activity. Negative LT values indicate that limpets started activity before being washed and positive values indicate a delay, whilst LT = 0 means exact coincidence. Only LT values in the range $-3 \leq \mathrm{LT} \leq 3$ h were considered as, for values greater than this, it is impossible to distinguish between a delay with respect to the current phase or an anticipation with respect to the next phase.

Variation in limpets' behaviour was analysed using linear mixed-effects models (LMM) following Pinheiro and Bates (2000) and Zuur et al. (2009). LMM allows direct modelling of the effect of repeated observations on the same limpet during different days, seasons and tides. Maximum likelihood solutions were used for estimating and testing, as ANOVA is known to be more sensitive to unbalanced data and outliers (McCulloch and Searle 2001). The model works by firstly specifying fixed and random components. All the fixed factors ("Season" and "Tide") are included in the first component, whereas the latter includes the factors "Day", "Site", "Limpet identity", plus any interaction between these and the fixed factors. A hierarchical structure was also specified in the random component, with the factor day being nested under Season and Tide. The factor Season was not replicated, as only one summer and one winter period were observed. Fitting started from a saturated model, which included all the possible factors in each of the fixed and random components of the model, plus their interactions. Model selection followed the protocol recommended by Zuur et al. (2009). Firstly, the optimal random structure of the model was determined. The contribution of each single random term was assessed by comparing the fit (obtained using restricted maximum likelihood estimation—REML) for models with, and without, the term. Comparisons were made through likelihood ratio tests and, to avoid problems associated with tests on the boundary, *P* values obtained from these tests were halved, as recommended by Pinheiro and Bates (2000) and Zuur et al. (2009). Non-significant terms were dropped from the model. Once the optimal random structure was determined, the significance of the fixed terms was then assessed using Markov Chains Monte Carlo simulations ($n = 50{,}000$ runs), as described by Baayen et al. (2008). Model assumptions for the within-group errors (independent and identical, normally distributed within-group errors, with mean zero and constant variance) as well as the distributional assumptions of the random effects (normally distributed with mean zero) were checked according to Zuur et al. (2009). Plots of residuals against fitted values, or against each explanatory variable, were inspected to ensure assumptions of the model were not violated.

To model the relationship between total activity of each limpet (AT, hours) and the duration of total wash received (TW, hours) during an observation day, generalized additive mixed-effect modelling (GAMM) was used (Woods 2006; Zuur et al. 2009). This allows modelling of non-linear dependence between the two variables, taking into account the repeated measures of taking records from the same limpet.

To quantify if limpets showed a preference for being active during the night or the day, selection ratios, which are usually employed in the context of resource selection studies, were calculated. In particular, Manly et al. (2002) showed how the best unbiased estimator to quantify resource preference when "use" (duration of activity) and "availability" (duration of day and night) are known for each individual *j* is:

$$\hat{S}_i = \frac{u_{i+}}{\sum_{j=1}^{n} \pi_{ij} \cdot u_{+j}}$$

where u_{i+} is total activity observed during the phase *i*, u_{+j} is total activity performed by individual *j* and π_{ij} is the proportional duration of phase *i* with respect to total time available, computed for the *j*-th limpet. Selection ratios thus weight the total use of a phase with its duration. If limpets show no preference for being active during a particular phase, they are expected to allocate their activity simply in proportion to the duration of each phase. A preference for a phase is said to occur when proportional use of the phase is higher than its proportional duration. Therefore, a $\hat{S}_{\mathrm{Night}} > 1$ indicates a preference for being active at night, $\hat{S}_{\mathrm{Night}} = 1$ no preference, and $\hat{S}_{\mathrm{Night}} < 1$ indicates an avoidance. The same reasoning applies to daytime activity. Statistical significance of selection ratios was assessed following Manly et al. (2002): an observed $\hat{S}$ value indicated a significant preference if its lower 95% confidence boundary was higher than 1, or an avoidance if its upper boundary was less than 1. To minimize the type-I error rate, confidence intervals were Bonferroni corrected for the number of comparisons made (Quinn and Keough 2002). All analyses were performed using R (ver. 2.8.1) statistical software (R Development Core Team 2007).

LMM and GAMM analyses were performed using the lme4 and gamm4 libraries, respectively.

Results

In general, activity conformed to a pattern dictated by tidal movements. The number of active individuals peaked during transitions from low to high tide and vice versa, when animals were awash on the flooding or ebbing tides (a typical set of examples is shown in Fig. 1). Duration of activity varied according to a complex interaction among the considered factors (Table 1). The final model retained the factors Site and Day and their interaction in the random component of the model, whilst in the fixed part a significant effect of Tide was detected (Table 1). In general, average activity duration was greater on neap than spring tides (Fig. 2). The greatest duration of activity was recorded on winter neap tides whilst the shortest duration was seen on summer spring tides.

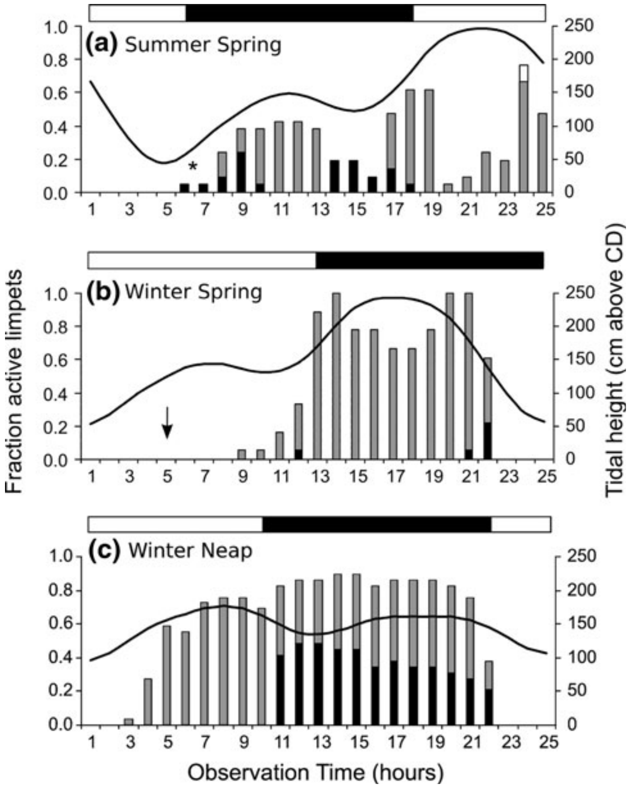

Fig. 1 Typical examples of *Cellana grata* activity patterns during different seasons and tidal conditions. Each figure shows results from one site during a single observation day. *White bars* represent the fraction of limpets active when submerged (only visible in the upper graph); *black bars* indicate those active when emersed and *grey bars* individuals active when washed by the tide. *Smooth line* is the predicted tidal pattern (Hong Kong Observatory). *Horizontal bar* indicates day (*white*) and night (*black*). The asterisk in (**a**) shows anticipation of activity with respect to incoming tide, whilst the *arrow* in (**b**) shows beginning of animals being washed by the tide. **a** Summer spring, day 2, Site 1. **b** Winter spring, day 1, Site 1. **c** Winter neap, day 3, Site 3

Table 1 Mixed model analysis to compare variation in activity (total activity duration), latency (time between the first awash and start of activity) and resting height (height which limpets were inactive). Only random factors that were included in the final model are shown. For fixed factors, coefficient estimates (±SE) are shown whereas variances are reported for random factors

Source	Activity	Latency	Resting height
Fixed factors			
Se	2.53 (1.64)	**0.85 (0.23)**	10.89 (10.54)
Ti	**−5.36 (1.64)**	−0.31 (0.19)	1.17 (3.12)
Se · Ti	−1.03 (2.32)	−0.27 (0.27)	**9.00 (4.74)**
Random factors			
Limpet	2.02	0.02	61.36
Da	3.55	0.06	*2.5×10^{-9}*
Si	*1.4×10^{-9}*	*2.0×10^{-3}*	*21.56*
Si · Da	1.08	0.06	33.81
Si · Se			149.80
Residual	9.93	0.61	358.33

Se season, *Ti* tide, *Limpet* individual limpet, *Da* day (nested in season and tide), *Si* site

Significant fixed factors ($P < 0.05$) are shown in bold. Italicized values represent components included into the final model because they were involved in higher-order interactions, although judged as non-significant on the basis of likelihood ratio tests

Resting height was also influenced by a Season × Tide interaction. The interactions Site × Season and Site × Day were also retained in the random part of the model (Table 1). On average, limpets rested higher on the shore during winter than summer (Fig. 3). During the winter, limpets rested higher on the shore during spring than neap tides. Variation in mean resting height was usually small, within ∼15 cm, between sampling days of the same tidal condition (Fig. 3).

Although activity generally started as soon as the limpets were washed by the incoming tides, delays and early initiation of activity were also observed. Again, the onset of activity was influenced by several factors (Table 1). In particular, an effect of Season was observed, with limpets generally anticipating activity during the observed summer period but, in contrast, showing more tendency to delay activity in winter. During the summer, the percentage of activity bouts starting before the incoming tide was 17% whilst 24% of bouts were delayed with respect to the tide. In the winter, however, 51% of foraging bouts were initiated 1–3 h after the limpet was washed by the tide, whilst only 5% were started prior to the incoming tide. The remaining 44% coincided with the beginning of animals being splashed by waves (see Fig. 1a, b which illustrates

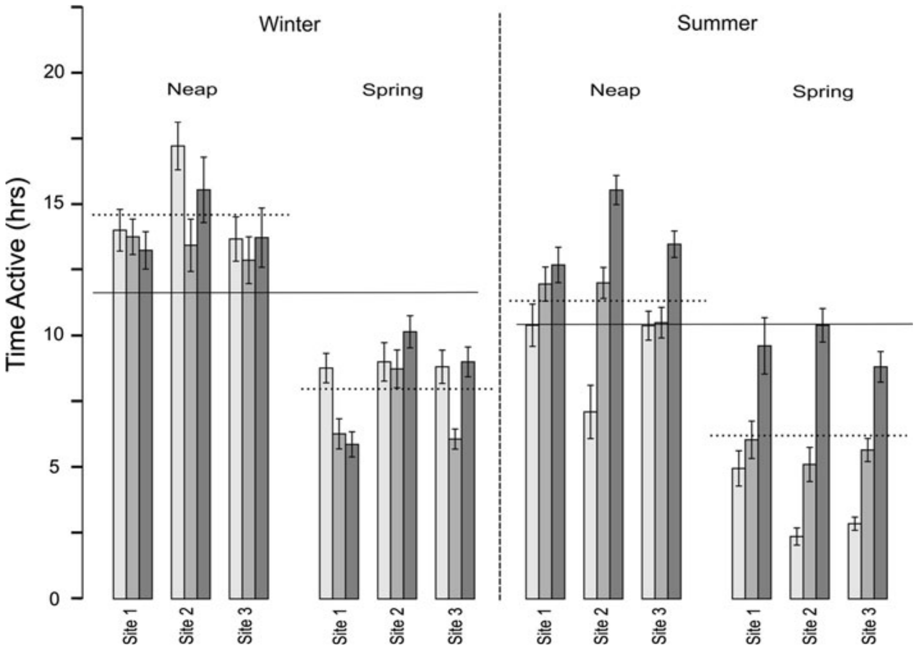

Fig. 2 Variation in activity (mean ± SE, hours) of individual *Cellana grata* at each site during each 25-h observation day (bars, days 1–3) under different tidal conditions (spring and neap) and season (summer and winter). *Horizontal dotted lines* mean of all sites for each tidal condition; *solid lines* mean for each season

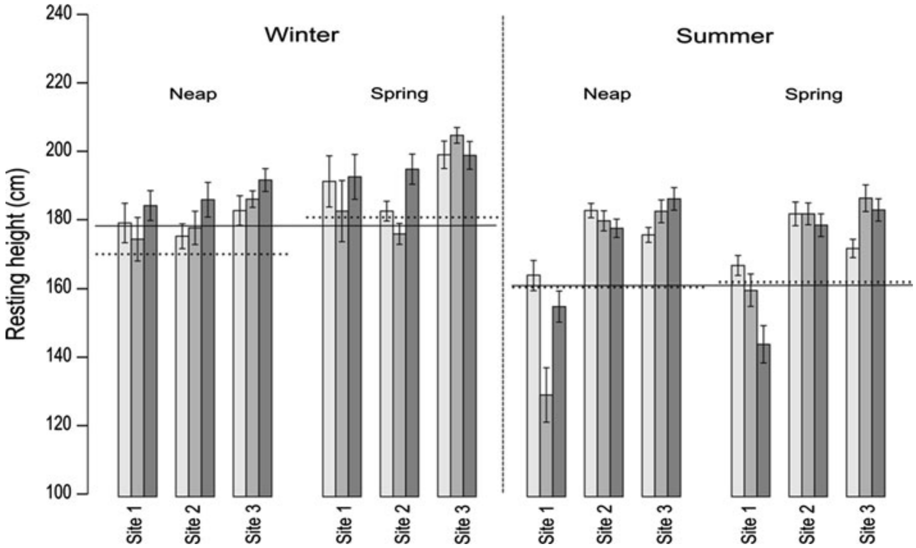

Fig. 3 Seasonal and tidal variations in resting heights (mean ± SE height above Chart Datum) of individual *Cellana grata* at each site during each day (bars, days 1–3) under different tidal conditions (spring and neap) and season (summer and winter). *Horizontal dotted lines* mean of all sites for each tidal condition; *solid lines* mean for each season

anticipation and delay of activity observed during a summer and a winter spring tide, respectively).

Total activity duration closely followed the time spent awash (Fig. 4). On average, limpets tended to be active for a time equal, or slightly less, than the number of hours awash. As the duration of time awash exceeded ~14–16 h, however, the number of hours animals were active did not increase as quickly and these two variables became less tightly linked. Within this general trend, there was also variation with season and tide (Fig. 5; Table 2) both due to the different amounts of wave wash received (being lower during spring than neap tides) and to the observed differences in resting heights and start latency. Resting height partly influenced the amount of wash received by each limpet, with higher tidal heights receiving less wave splash (Pearson's correlation $r = -0.24$, $t = -7.38$, $P < 0.0001$). During summer spring tides, activity increased proportionally to time awash (Fig. 5a) but, in general, observed duration of activity was slightly lower than time awash (values below the isocline). During summer neaps (Fig. 5b), limpets were active for a time equal to time awash, when this duration was less than 12–13 h. For longer durations of the awash period, activity duration was less than the time awash. A similar pattern was evident during winter springs (Fig. 5c). During this phase, however, limpets experienced a shorter duration of the potential activity phase, and activity started to deviate from perfect proportionality with time awash when this was ~8–9 h. The greatest deviation from the general trend was observed during winter neaps (Fig. 5d), when the activity of limpets appeared to be less dependent on time awash. When the period awash was less than 13–14 h, limpet activity generally exceeded time awash, being on average 12 h per day, although great variability was present. As time awash exceeded 15 h, limpet activity increased proportionally, although the full potential activity phase was not fully exploited (Fig. 5d).

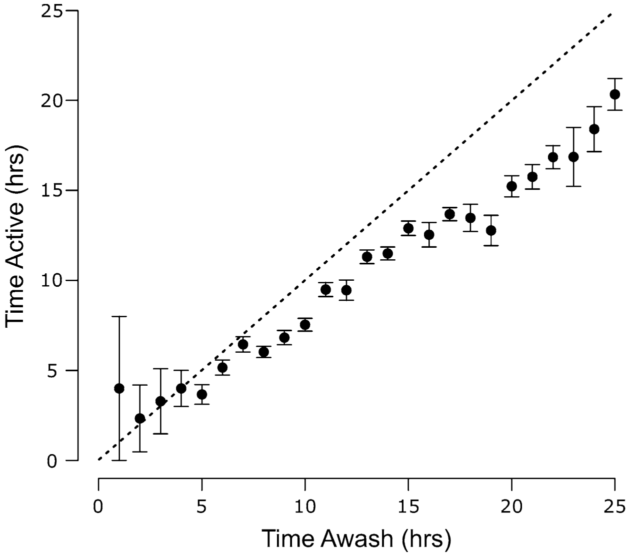

Fig. 4 Variation of time spent active of *Cellana grata* with hours awash. Each *dot* is the mean (±SE) activity for each possible time awash in hours. *Dotted line* isocline of perfect proportionality

Despite most activity taking place whilst awash, foraging sometimes occurred during emersion or submergence at high tide, the latter being less common. Of the total 8,308 limpet-hours of activity recorded, only 22 were recorded during submergence, mainly consisting of animals still moving for a short time after being submerged by the incoming tide. Activity whilst submerged was equally distributed among the four season/tide combinations ($\chi^2 = 6.36$, 3 *df*, $P > 0.09$). Activity during emersion was more frequent and unevenly distributed among season/tides ($\chi^2 = 253.92$, 3 *df*, $P < 0.00001$, after pooling all values). The greatest amount of activity during emersion was recorded during winter neaps (479 limpet-hours, corresponding to 19.1% of total recorded activity). Lower values were recorded during summer springs (294 limpet-hours, 19.0%) and summer neaps (266 limpet-hours, 9.3%), with a minimum during winter spring tides (100 limpet-hours, 7.0%).

In terms of preference for activity during day or night-time, a slight, but significant, preference for being active during the night was observed during awash periods (Fig. 6a). The lower confidence intervals for the selection ratio for night were always >1, the value expected in cases

Fig. 5 Variation of activity duration (mean hours active ± SE, for each awash value) with hours awash for different seasonal and tidal combinations (*dots*). *Dotted line* represents the isocline of perfect proportionality. *Continuous line* is the fitted relationship, with estimated 95% confidence intervals (*dashed line*)

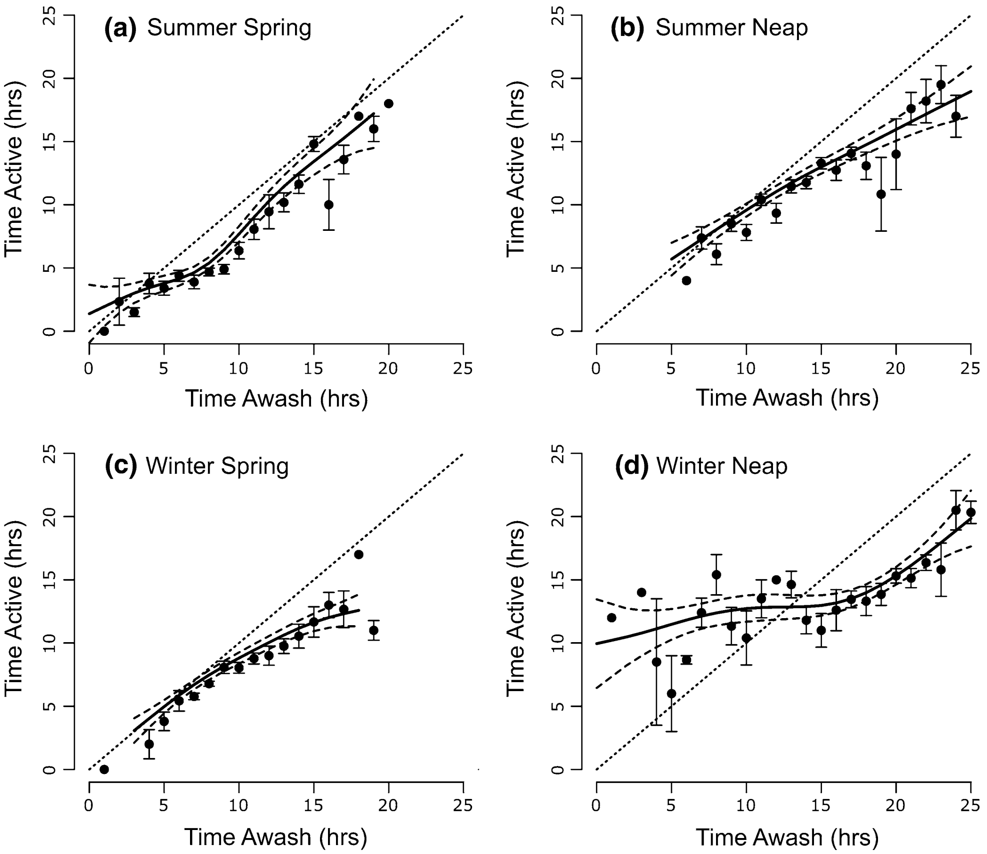

Table 2 Parameter estimates for the dependency of time active with time awash. Smooth term shows the estimated degrees of freedom of the smoothers (a smooth term = 1 represents a straight line)

Period	Smooth term	*F*	R^2
SS	3.92	92.4***	0.59
SN	1.93	101.5***	0.43
WS	2.92	92.2***	0.59
WN	3.60	18.5***	0.22

F is the approximate *F* test, R^2 adjusted *R* square. *** <0.001

SS summer spring tides, *SN* summer neap tides, *WS* winter spring tides, *WN* winter neap tides

of no preference (i.e. when a phase is used in proportion to its availability). The reverse was true for activity during the day: $\hat{S}_{\mathrm{Day}}$ values were always <1 and their 95% confidence intervals never crossed 1, indicating that activity during daytime was avoided. The greatest preference for nocturnal activity was during summer spring tides, whilst other sampling periods gave lower, comparable values. Activity during emersion was more prevalent during nighttime (Fig. 6b) than activity during awash. In this case, $\hat{S}_{\mathrm{Night}}$ values were >1.7, with the only exception on winter spring tides, when this value was ~1.2 and was not significantly different from 1. Conversely, selection ratios for activity during daytime were very low and close to 0, with the exception of winter springs (see Fig. 1c, where an example of activity during a day on winter neaps is shown). Here, the proportion of "limpets active" remained high throughout the 25-h observation period, irrespective of splashing by waves. Limpets were active when splashed during the day but continued activity during emersion at night, irrespective of their awash state.

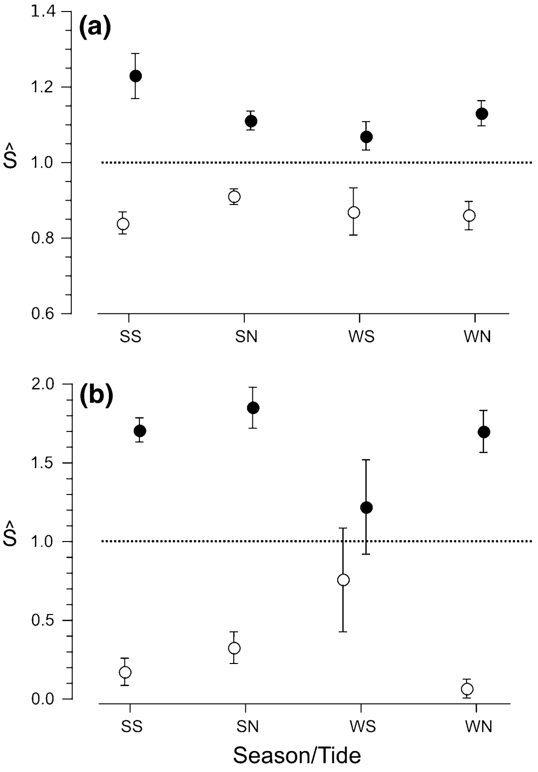

Fig. 6 Mean (±95% CI) selection ratios (S) for activity at night (*black dots*) and during daytime (*open dots*), when awash (**a**) or emersed (**b**). *Horizontal dashed line* is the value expected for a lack of preference for activity during night or day. *SS* summer spring tides; *SN* summer neap tides; *WS* winter spring tides; *WN* winter neap tides

Discussion

Important temporal variations in duration of activity, onset of activity and resting height, were found both on a large (season, tide) and small (day) temporal scale, suggesting a wide behavioural plasticity in the limpet, *Cellana grata*. Caution must, however, be exercised when interpreting the large-scale temporal variation in behaviour as a general season effect, as counts were not replicated at this temporal scale. Small-scale spatial effects (variation among sites) also influenced limpet behaviour.

Individual activity durations were longer during winter neap tides, reflecting an exploitation of longer periods of favourable conditions due to stronger wave action during the Hong Kong winter (Morton and Morton 1983; Williams and Morritt 1995). Continuation of activity during emersion mostly occurred between the two peaks of mixed semi-diurnal tides (i.e. during the high–low tide) and mostly at night. Conditions experienced whilst emersed during these periods are different from those characterizing the lowest low tides during daytime, as the substratum is already wet (Ngan pers. obs.), giving the advantage of a cooler rock and enhanced adherence and movement on mucus trails (Davies et al. 1990; Davies and Williams 1995). In contrast, activity during spring tides, especially in summer, was shorter in duration as a result of reduced time awash and greater periods of submersion resulting from the extreme high–high tides.

During the observed winter period, limpets tended to delay activity after receiving wave splash. Delaying of activity may be related to recovery after prolonged stressful conditions during emersion, which is known to cause detectable changes in physiology and metabolism (e.g. Coleman et al. 1995; Williams and Morritt 1995; Williams et al. 2005). Many limpets rehydrate their mantle cavities and defecate upon initiation of activity (pers. obs.). Such 'recovery' phases were, however, much less frequent during the summer, when the most stressful conditions during emersion occur. In summer, an opposite behaviour was observed, since 17% of foraging bouts were initiated ~1–2 h before becoming awash. This behaviour confirms that limpet activity is not simply triggered by a direct stimulus, such as continuous wave splash, but that other non-apparent cues, such as increased energy requirements or an internal clock may be involved (Della Santina and Naylor 1993; Gray and Hodgson, 1999; Ng and Williams 2006). *C. grata* is known to possess a circatidal endogenous clock, which may allow animals to anticipate immersion by the incoming tide (Gray and Williams 2010). These observations suggest that factors related to the triggering of activity should be considered in a wider perspective. Given that the potential activity phase (PAP) in *C. grata* is represented by periods of wave splash (Williams and Morritt 1995), a delay in activity onset may diminish the full utilization of the PAP and, as a consequence, diminish energy intake for individual limpets. Recovery may be traded-off in favour of a longer activity phase due to levels of energy deficit incurred by the animals (Houston and McNamara 1999). Delaying of activity is, therefore, to be expected when the duration of the PAP is longer (such as during neap tides) as the PAP will be long enough to accommodate recovery without impacting the net energy intake of the limpets. In contrast, early initiation of activity exposes the animals to higher costs and risks due to desiccation, heat stress and energetic costs due to mucus production (Williams and Morritt 1995; Davies and Williams 1995; Williams and McMahon 1998). However, such risk-prone behaviour may be potentially favoured when access to food is extremely time limited, such as during summer spring tides.

Resting height also proved to be highly variable and observed changes are in accordance with the seasonal differences found by Williams and Morritt (1995). Under normal climatic conditions, resting height strongly influences the amount of wave splash received, the duration of exposure to stressful conditions during emersion (Williams and Morritt 1995; Williams and McMahon 1998; Chelazzi et al. 1999) and the distance to be travelled to reach the feeding areas, which, at some sites, are more rich higher on the shore (Williams and Morritt 1995). Resting at an appropriate height is, therefore, an important contributor to the fitness of non-homing limpets. The decision to stop when moving downshore with the receding tide results from a trade-off between the need to stay low to avoid heat stress (as evident during the summer when resting height is lower than during winter) and the need to stay high and be able to reach richer, relatively unexploited feeding grounds (Wolcott 1973; Gray and Williams 2010).

Specific topographic features may add further complexity to this trade-off, since the factor Site seems to explain part of the variability in limpet behaviour. This effect implies the importance of topography and shore aspect in affecting limpet activity through changes in microclimate and exposure to waves or direct sunlight (Williams and Morritt 1995; Jones and Boulding 1999; Chelazzi et al. 1999; Helmuth and Hofmann 2001). Limpets are also known to avoid more complex rock surfaces whilst foraging (Erlandsson et al. 1999; Davies et al. 2006) and the slope of the substratum will also alter the topographic distance between tidal heights (see Hutchinson and Williams 2003).

The amount of wash received is the primary explanatory variable for the variation in duration of individual activity as, in general, limpets maintained themselves within the awash zone. These results are consistent with previous observations on *Cellana grata* (Williams and Morritt 1995; Erlandsson et al. 1999; Davies et al. 2006) and this temporal pattern of activity is similar to that described for other gastropods and chitons on Hong Kong shores (Hutchinson and Williams 2003; Ng and Williams 2006). Important variations in this general trend were, however, observed during the four season/tide combinations. During summer neaps and winter springs, activity closely followed the awash duration until an upper threshold was reached (different for each of the two tides), after which activity was lower than time awash. During summer springs, in contrast, no evidence of an upper bound to activity duration was found. The first part of this relationship can be interpreted as an energy maximization strategy (Stephens and Krebs 1986) with limpets saturating the available potential activity phase to fulfil their energy requirements (see Evans and Williams 1991, for a similar case in *Patella vulgata*). The reduction in activity observed for longer wave splash periods could be due to a combination of morpho-physiological constraints in gut capacity and processing times (Franz 1990; Burrows and Hughes 1991; Chelazzi et al. 1998; Burrows et al. 2000) and/or to a different evaluation of benefits and costs when opportunities for foraging are wide (Houston and McNamara 1999; Clark and Mangel 2000).

Conversely, the fact that limpet activity during winter neaps greatly exceeded the awash phase shows that during this period, the potential activity phase is wider than may be predicted and was not limited to wave splash alone. As previously described, during winter neaps, the combination of lower temperatures, timing of low tide (night) and limited tidal amplitude extends the potential activity phase to the higher of the mixed semi-diurnal low tides, since the rock substratum remains wet and cool even when exposed to air. Finally, it is worth stressing that not all activity happened whilst limpets were awash. Although activity during submergence was negligible, activity during emersion was not and ranged from a minimum of 7% of total activity during winter springs to a maximum of 19% during winter neaps. Secondly, although patterns of activity are mainly driven by tidal components and wave action, evidence for the influence of the day–night cycle was also found. During awash conditions a slight, but significant, preference for activity at night was observed, which reached a maximum during summer spring tides, when extreme stressful conditions are experienced during daytime (Williams and Morritt 1995; Williams et al. 2005). A stronger preference for nocturnal activity was observed when limpets remained active during emersion periods, suggesting a clear avoidance of stressful environmental conditions.

Although the basic pattern of activity in *Cellana grata* does not show the level of variability described in other species, such as *Patella vulgata* (Little and Stirling 1985; Williams et al. 1999; Santini et al. 2004), observed changes in organization and duration of activity suggest that this limpet is able to modulate its foraging strategies according to energy requirements, local environmental constraints and daily changes in environmental conditions. A similar degree of plasticity has been described in the chiton, *Acanthopleura japonica,* living on the same shores (Ng and Williams 2006). As such, these results show that although the behaviour of tropical intertidal species appears tightly constrained, a certain degree of plasticity is available and may confer selective advantages to individuals. Further experimental and theoretical work is, however, needed to disentangle the role of the different environmental factors involved to explain the observed variability in behaviour and to put these results in the wider context of behavioural plasticity theory (e.g. Dingemanse et al. 2010; Mery and Burns 2010).

Acknowledgments We are grateful to the Agriculture, Fisheries and Conservation Department, The Government of the Hong Kong SAR for permission to work in the Cape d'Aguilar Marine Reserve. We are indebted to the many willing volunteers who helped with fieldwork and Ms. Cecily Law who provided excellent technical assistance. GS stay in Hong Kong was supported by The Department of Ecology & Biodiversity, The University of Hong Kong. F. Bulleri and two anonymous referees provided useful suggestions which greatly improved the manuscript.

References

Baayen RH, Davidson DJ, Bates DM (2008) Mixed-effects modelling with crossed random effects for subjects and items. J Mem Lang 59:390–412

Blackmore DT (1969) Studies of *Patella vulgata* L.: I. Growth, reproduction and zonal distribution. J Exp Mar Biol Ecol 3:200–213

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

Branch GM (1985) Limpets: their role in littoral and sublittoral community dynamics. In: Moore G, Seed R (eds) The ecology of rocky coasts. Hodder & Staughton, Sydney, pp 97–116

Brockmann JH (2001) The evolution of alternative strategies and tactics. Adv Stud Behav 30:1–51

Burrows MT, Hughes RN (1991) Optimal foraging decision by dogwhelks, *Nucella lapillus* (L.): influences of mortality risk and rate-constrained digestion. Funct Ecol 5:461–475

Burrows MT, Santini G, Chelazzi G (2000) A state-dependent model of activity patterns in homing limpets: balancing energy returns and mortality risks under constraints on digestion. J Anim Ecol 69:290–300

Chapman MG, Underwood AJ (1992) Foraging behaviour of marine benthic grazers. In: John DM, Hawkins SJ, Price JH (eds) Plant-animal interactions in the marine benthos. Clarendon Press, Oxford, pp 289–317

Chelazzi G, Parpagnoli D, Santini G (1998) A satiation model for the temporal organization of grazing in limpets. Funct Ecol 12:203–210

Chelazzi G, Williams GA, Gray DR (1999) Field and laboratory measurement of heart rate in a tropical limpet, *Cellana grata*. J Mar Biol Ass UK 79:749–751

Clark CW, Mangel M (2000) Dynamic state variable models in ecology: methods and applications. Oxford University Press, New York

Coleman JS, Heckathorn SA, Hallberg RL (1995) Heat-shock proteins and thermotolerance: linking molecular and ecological perspectives. Trends Ecol Evol 10:305–306

Davies MS, Williams GA (1995) Pedal mucus of a tropical limpet, *Cellana grata* (Gould): energetics, production and fate. J Exp Mar Biol Ecol 186:77–87

Davies MS, Hawkins SJ, Jones HD (1990) Mucus production and physiological energetics in *Patella vulgata* L. J Moll Stud 56:499–503

Davies MS, Williams GA, Edwards M (2006) Movement patterns of the limpet *Cellana grata* (Gould) observed over a continuous period through a changing tidal regime. Mar Biol 149:775–787

Della Santina P, Chelazzi G (1991) Temporal organization of two Mediterranean limpets, *Patella rustica* and *P. coerulea*. J Exp Mar Biol Ecol 153:75–85

Della Santina P, Naylor E (1993) Endogenous rhythms in the homing behaviour of the limpet *Patella vulgata* Linnaeus. J Moll Stud 59:87–91

Della Santina P, Naylor E, Chelazzi G (1994) Long term field actography to assess the timing of foraging excursions in the limpet *Patella vulgata* L. J Exp Mar Biol Ecol 178:193–203

Denny M, Wethey D (2001) Physical processes that generate patterns in marine communities. In: Bertness MD, Gaines SD, Hay ME (eds) Marine community ecology. Sinauer Associates, Sunderland, pp 3–35

Dingemanse NJ, Kazem AJN, Reale D, Wright J (2010) Behavioural reaction norms: animal personality meets individual plasticity. Trends Ecol Evol 25:81–89

Erlandsson J, Kostylev V, Williams GA (1999) A field technique for estimating the influence of surface complexity on movement tortuosity in the tropical limpet *Cellana grata* Gould. Ophelia 50:215–224

Evans MR, Williams GA (1991) Time partitioning of foraging in the limpet, *Patella vulgata* (L.). Anim Ecol 60:563–575

Franz CJ (1990) Feeding patterns of *Fissurella* species on Isla de Margarita, Venezuela: use of radulae and food passage rates. J Moll Stud 56:23–35

Garrity SD (1984) Some adaptations of gastropods to physical stress on a tropical rocky shore. Ecology 65:559–574

Gray DR, Hodgson AN (1997) Temporal variation in foraging behaviour of *Patella granularis* (Patellogastropoda) and *Siphonaria concinna* (Basommatophora) on a South African shore. J Moll Stud 63:121–130

Gray DR, Hodgson AN (1999) Endogenous rhythms of locomotor activity in the high-shore limpet, *Helcion pectunculus* (Patellogastropoda). Anim Behav 57:387–391

Gray DR, Williams GA (2010) Knowing when to stop: rhythms of locomotor activity in the high-shore limpet, *Cellana grata* Gould. J Exp Mar Biol Ecol 391:125–130

Hartnoll RG, Wright JR (1977) Foraging movements and homing in the limpet *Patella vulgata* L. Anim Behav 25:806–810

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

Helmuth BST (2002) How do we measure the environment? Linking intertidal thermal physiology and ecology through biophysics. Integr Comp Biol 42:837–845

Helmuth BST, Hofmann GE (2001) Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone. Biol Bull 201:374–384

Hill AS, Hawkins SJ (1991) Seasonal and spatial variation of epilithic microalgal distribution and abundance and its ingestion by *Patella vulgata* on a moderately exposed rocky shore. J Mar Biol Ass UK 71:403–423

Houston AI, McNamara JM (1999) Models of adaptive behaviour: an approach based on state. Cambridge University Press, Cambridge

Huang R (2001) Spatial variation in *Cellana grata* populations: the interplay of population dynamics and food availability. PhD thesis, The University of Hong Kong, Hong Kong

Hutchinson N, Williams GA (2003) An assessment of variation in molluscan grazing pressure on Hong Kong rocky shores. Mar Biol 142:495–507

Jones KMM, Boulding EG (1999) State-dependent habitat selection by an intertidal snail: the costs of selecting a physically stressful microhabitat. J Exp Mar Biol Ecol 242:149–177

Kaehler S, Williams GA (1996) Distribution of algae on tropical rocky shores: spatial and temporal patterns of non-coralline encrusting algae in Hong Kong. Mar Biol 125:177–187

Komers PE (1997) Behavioural plasticity in variable environments. Can J Zool 75:161–169

Little C (1989) Factors governing patterns of foraging activity in littoral marine herbivorous molluscs. J Moll Stud 55:273–284

Little C, Stirling P (1985) Patterns of foraging activity in the limpet *Patella vulgata* L.—a preliminary study. J Exp Mar Biol Ecol 89:283–296

Little C, Williams GA, Morritt D, Perrins JM, Stirling P (1988) Foraging behaviour of *Patella vulgata* L. in an Irish sea-lough. J Exp Mar Biol Ecol 120:1–21

Little C, Partridge JC, Teagle L (1991) Foraging activity of limpets in normal and abnormal tidal regimes. J Mar Biol Ass UK 71:537–554

Manly BFJ, McDonald LL, Thomas DL, McDonald TL, Erickson WP (2002) Resource selection by animals: statistical design and analysis for field studies, 2nd edn. Kluwer Academic Publishers, Boston

McCulloch CE, Searle SR (2001) Generalized, linear and mixed models. Wiley-Interscience, New York

McMahon RF (1990) Thermal tolerance, evaporative water loss, air-water oxygen consumption and zonation of intertidal prosobranchs: a new synthesis. Hydrobiologia 193:241–260

Mery F, Burns JG (2010) Behavioural plasticity: an interaction between evolution and experience. Evol Ecol 24:571–583

Morais S, Boaventura D, Narciso L, Ré P, Hawkins SJ (2003) Gonad development and fatty acid composition of *Patella depressa* Pennant (Gastropoda: Prosobranchia) populations with different patterns of spatial distribution, in exposed and sheltered sites. J Exp Mar Biol Ecol 294:61–80

Morton BS, Morton JE (1983) The seashore ecology of Hong Kong. Hong Kong University Press, Hong Kong

Nagarkar S, Williams GA (1999) Spatial and temporal variation of cyanobacteria-dominated epilithic communities on a tropical shore in Hong Kong. Phycologia 38:385–393

Ng SSJ, Williams GA (2006) Intraspecific variation in foraging behaviour: influence of shore height on temporal organization of activity in the chiton *Acanthopleura japonica*. Mar Ecol Prog Ser 321:183–192

Ngan A (2006) Environmental stress and its implications for behavioural plasticity of foraging in *Cellana grata*. PhD thesis, The University of Hong Kong, Hong Kong

Pinheiro JC, Bates DM (2000) Mixed-effects models in S and S-PLUS. Springer, New York

Quinn G, Keough M (2002) Experimental design and data analysis for biologists. Cambridge University Press, Cambridge

R Development Core Team (2007) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Raffaelli D, Hawkins SJ (1996) Intertidal ecology. Chapman & Hall, London

Santini G, Thompson RC, Tendi C, Hawkins SJ, Hartnoll RG, Chelazzi G (2004) Intra-specific variability in the temporal organisation of foraging activity in the limpet *Patella vulgata*. Mar Biol 144:1165–1172

Stephens DW, Krebs JR (1986) Foraging theory. Princeton University Press, Princeton

Takada Y (2001) Comparison of the activity patterns of nine molluscan grazers on a boulder shore at Amakusa, Japan. Venus 60:157–172

Williams GA, Little C (2007) Foraging behaviour. In: Denny MW, Gaines SD (eds) Encyclopedia of tidepools and rocky shores. University of California Press, Berkeley, pp 239–242

Williams GA, McMahon BR (1998) Haemolymph pH and oxygen levels in a naturally stressed tropical limpet, *Cellana grata*. In: Morton BS (ed) The Marine biology of the South China Sea. Proceedings of the third international conference on the marine biology of the South China Sea. Hong Kong University Press, Hong Kong, pp 239–245

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

Williams GA, Little C, Morritt D, Stirling P, Teagle L, Miles A, Pilling G, Consalvey M (1999) Foraging in the limpet *Patella vulgata*; the influence of rock slope on the timing of activity. J Mar Biol Ass UK 79:881–889

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

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

Woods SN (2006) Generalized additive models: an introduction with R. Chapman and Hall/CRC, Boca Raton

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer, New York