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Knowing when to stop: Rhythms of locomotor activity in the high-shore limpet, *Cellana grata* Gould

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ABSTRACT

The high shore limpet, *Cellana grata*, forages whilst awash, moving upshore with the rising tide and retreating downshore on the ebbing tide to become inactive in refuges. Spraying inactive, emersed individuals with seawater at low tide invokes a locomotory response, with limpets moving up the shore. Controlled laboratory experiments under continuous white or red light (to simulate light or dark periods respectively) and continuous emersion, immersion or seawater spray showed that *C. grata* possesses a free-running endogenous rhythm of locomotor activity. This rhythm was maintained over 30 days in continuous seawater spray and white light. Maximum entropy spectral analysis (MESA) revealed two major components to this rhythm, at 7.2 h and 12.4 h. The 12.4 h component is of a circatidal nature and appears to initiate activity, allowing individuals to anticipate immersion by the incoming tide, although this clock can be over-ridden by strong wave splash or spraying vigorously with seawater. The 7.2 h period, however, was the most significant component and is suggested to act as a stopwatch enabling the limpet to assess the duration of each foraging excursion in order to prevent being stranded at the wrong height on the shore. The environmental stimulus for both components of the endogenous rhythm in *C. grata* appears to be the time of first exposure to wave wash from the incoming tide. *C. grata*, therefore, has behavioural rhythms entrained to initiate and also terminate activity, which play a role in the limpet maintaining a fixed vertical level on the shore when inactive.

1. Introduction

Many intertidal animals exhibit rhythmic behavioural patterns, synchronising their activity with external environmental variables such as tidal cycles, day–night cycles, monthly spring-neap cycles and even annual seasonal changes (reviewed by Hawkins & Hartnoll, 1983; Naylor, 1988; Little, 1989; Morgan, 2007). Many of these patterns are primarily driven by changes in external conditions, whilst some species continue to exhibit free-running activity rhythms under constant conditions. The circatidal and circadian rhythmicity of the behaviour and physiology of many mobile intertidal animals is well documented (e.g. Naylor, 1958; Zann, 1973) and the temporal synchronization of foraging behaviour with cyclical environmental variables has been suggested to ensure optimal resource utilization and the avoidance of stressful situations (Naylor, 1988, 1989; Williams and Little, 2007).

Slow-moving, intertidal herbivorous gastropods, such as limpets, exhibit rhythmic foraging excursions away from their home scars or refuge areas, to which they often return upon completion of their feeding cycle (Orton, 1929; Branch, 1981; Little, 1989; Gray and Hodgson, 1997). Apart from some equivocal experiments (Funke, 1968) only a few studies have indicated that the on-shore rhythmic foraging activity of limpets is endogenously controlled (Della Santina and Naylor, 1993; Gray and Hodgson, 1999). Clear endogenous rhythms have only been reported in a few species of intertidal molluscs including bivalves (Beentjes and Williams, 1986; Akumfi and Naylor, 1987), gastropods (Zann, 1973, Petpiroon and Morgan, 1983) and chitons (Ng and Williams, 2006), possibly due to the difficulties involved with maintaining these animals under laboratory conditions.

Cellana grata Gould is abundant in the mid-high shore of moderately exposed to exposed rocky shores in Hong Kong (Morton and Morton, 1983; Williams, 1993), and forages whilst awash, moving up shore with the rising tide and downshore on the ebb, rarely being completely submerged (Williams and Morritt, 1995; Davies et al., 2006). This limpet appears to be an opportunistic forager, as individuals are active during periods of high wave action, when they are splashed by seawater, even at the time of predicted low water (e.g., during typhoons, Williams and Morritt, 1995). Individuals do not, however, follow the tide to its lowest point but take refuge in habitats that offer shelter from high temperatures at ~1.75–2.0 m

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above Chart Datum (C.D.). If *C. grata* fails to retreat to a suitable refuge after foraging, especially in the transition period between the cool/dry and hot/wet monsoon (see Kaehler and Williams, 1996 for description of Hong Kong climate) when it migrates lower down the shore, animals can suffer thermal and desiccation stress and subsequent mortality (Williams, 1994; Williams and Morritt, 1995; Ngan, 2006). The maintenance of a rhythmic locomotry behaviour and subsequent habitat selection would, therefore, appear to have a selective function for this limpet. The present study, therefore, aimed to establish whether the rhythms of locomotor activity exhibited by *C. grata* on the shore have an endogenous component and, if so, what those components may be.

2. Materials and methods

2.1. On-shore observations and collections

All experiments were conducted at The Swire Institute of Marine Science, Hong Kong (22° 17′N, 114° 09′E) and on the shores within the Cape d'Aguilar Marine Reserve. Hong Kong tides can be broadly described as mixed semi-diurnal, with tidal range ~2.5 m (see Williams, 1994 and Morton et al., 1996) and spring tides of unequal amplitude. The normal resting height of *C. grata* on the shores around Cape d'Aguilar is ~1.75–2.0 m above C.D.

To determine whether wave action stimulates locomotor activity in *C. grata*, limpets were sprayed with seawater using hoses. During low tide, when animals were inactive, small groups of limpets were sprayed via hosepipes with unfiltered seawater from a large holding tank. Due to storage and transfer time, the seawater sprayed had a temperature ~1.5 °C higher than ambient seawater temperatures (28–29.5 °C).

On two separate day-time low tides in July 1994, groups of limpets were sprayed and not sprayed (as a control) at different sites (Cape Wall and Cape Slope, see Williams and Morritt, 1995). Four groups of limpets were monitored on 07/07/1994 (90 min prior to low tide, two groups of 14 limpets sprayed and two groups of 9 and 14 not sprayed) and 18/07/1994 (at the time of low tide, two groups of 18 and 29 sprayed animals; and two groups of 30 individuals not sprayed). Animals were scored at ~20–30 min intervals as either inactive (inert, firmly attached to the rock surface within a 15 s observation period); active, but no movement (lifting off the rock but stationary, pallial tentacles visible) or moving (movement seen within 15 s). On 18/07/1994, the height relative to C.D. of the animals was also recorded at the Cape Wall site, as this site is a vertical wall where tidal heights can be readily estimated (Williams and Morritt, 1995).

2.2. Laboratory experiments to test for endogenous rhythms

To determine whether locomotor activity in *Cellana grata* is endogenously controlled, limpets were collected from a 150 m stretch of south-facing shore line at Cape d'Aguilar for laboratory experiments in September–October 1997. Easily removable animals (28.5–37 mm, from the same cohort) were collected by hand whilst active and awash by the tide. Once collected, the limpets were immediately returned to the laboratory (a distance of approximately 400 m), placed in 40-l experimental tanks and allowed to attach to one side of the tank. This side of the tank was fenced to prevent limpets moving off this surface and, therefore, out of the field of view. The animals were then immediately transferred to an experimental area (3 m × 2 m × 2 m), which was separated from the main aquarium with blackout curtains and monitored using a video camera (Sony, CCD — TR3E) connected to a time lapse video recorder (Panasonic, AG — 6730) outside the aquarium. In this way the limpets could be observed without any disturbance. Within the experimental area, red or white light could be used and photoperiod varied whereas temperature and relative humidity were constant. At the end of the experiments limpets were returned to an area of the shore from which further collections were not made.

Ten limpets were placed in each of three 40-l experimental tanks ($\Sigma n = 3 \times 10 = 30$; data was not pooled) and different treatments applied to each tank to simulate three stages of the tidal cycle. All treatments experienced relatively constant temperature (25.5–27.5 °C) and relative humidity (>90%). Limpets were kept either permanently immersed; constantly emersed or constantly sprayed by seawater (to simulate awash conditions). The experiment was repeated once in red light (80 W Phillips Par 38 Flood) for a period of 96 h and once in white light (120 W Phillips Par 38 Flood) for 48 h. Movement of *Cellana grata* is not inhibited by red light (Williams and Morritt, 1995) as is also the case for *Patella vulgata* (Little and Stirling, 1985) and *Helcion pectunculus* (Gray, 1997). The red light was, therefore, an attempt to simulate darkness for the limpets and yet be able to maintain observations. Many limpets were dead at the end of the red light experiment in both the emersed and immersed tanks, therefore, the duration of the white light experiment was reduced to 48 h to minimize mortality. In order to explore potential variations in any rhythms observed over the full spring-neap tidal cycle, 20 *Cellana grata* were maintained for 30 days (i.e., two full spring-neap cycles) in a 150-l experimental tank under constant white light and seawater spray.

Video tapes were viewed on fast-forward and the observations divided into 15 time-lapsed minute intervals or “bins”. To standardize scoring, the same person scored all the tapes. The number of limpets active within the 15-minute bin was recorded along with the number of limpets visible. If a limpet moved off the side of the tank, it was excluded from records until it returned. Activity was defined as turning movements as well as actual displacement and calculated as the percentage of limpets active of the individuals visible. To determine the presence of rhythms, Maximum Entropy Spectral Analysis (MESA; Dowse and Ringo, 1989) employed in conjunction with autocorrelation (e.g., Statgraphics 1991, as advised by Palmer, 1995) were used on the month-long observations.

3. Results

3.1. On-shore observations

In both experiments on the shore, non-sprayed limpets did not show signs of being active in any of the control groups, except at the end of the experiment on 18/07/1994 when waves from the incoming tide reached the lowest animals, which then started to move. Limpets sprayed with seawater to simulate wave action, however, became active within 30 min of the initiation of spraying (Fig. 1). Limpets initially raised their shells off the substratum, extended their pallial tentacles, and then started moving. Within 1 h, between 60 and 80% of the limpets in both groups were moving (Fig. 1). In general, animals were stimulated to move by strong jets of water. On 07/07/1994 spray was terminated after 1 h, after which most animals stopped moving, and all became inactive within 1 h as the rock surface started to dry (Fig. 1a).

On 18/07/1994 animals were sprayed continuously for 2.5 h (after which waves from the incoming tide wetted the experimental population). Sprayed limpets, as in the previous experiment, rapidly became active and within 30 min, >60% of the two groups were moving, and continued to move when the animals were washed by the incoming tide (Fig. 1b). Sprayed limpets moved up-shore by ~40 cm from their resting tidal height after 2 h (Fig. 2).

3.2. Laboratory experiments to test for endogenous rhythms

In the laboratory, activity of *Cellana grata* was found to be rhythmical throughout both the red and white light experiments, under all experimental conditions (Fig. 3). Most limpets were active