

The circatidal rhythm of the estuarine gastropod *Hydrobia ulvae* (Gastropoda: Hydrobiidae)

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Intertidal animals display a suite of cyclic behaviours that evolved as adaptations to the predictable cycle of inundation and exposure. In estuarine habitats, mud snails from the genus *Hydrobia* are among the most abundant grazers, and have received considerable attention with respect to the behavioural mechanisms mediating locomotion, dispersal, and feeding, although the nature of the control of these processes has remained elusive. In particular, it is not clear whether endogenous activity patterns are related to periodic changes of microphytobenthos biomass at the sediment surface, or whether they are timed to the tidal cycle at all. In the present study, we address the crawling activity of *Hydrobia ulvae* under constant conditions, as well as the effects of individual size and previous short-term exposure to tides of different range, by recording immersed individual snails under constant dark conditions. We show that the species displays an overt circatidal pattern of crawling, with activity peaks around high water, and that the start of inundation may act as an entrainment agent of the rhythm. Moreover, the results obtained indicate that smaller snails display higher levels of activity, although neither the size nor previous *in situ* influence of tidal range has an effect on the period and on the amplitude of the rhythm. These findings suggest that fluctuations of microphytobenthos biomass are not a sufficiently strong selective pressure to have shaped locomotor activity in *H. ulvae*. Moreover, feeding of *H. ulvae* should take place mostly during high water and be independent of periodic fluctuations of microphytobenthos biomass at the surface of the sediment. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, **100**, 439–450.

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INTRODUCTION

The predictable cycle of inundation and exposure of the intertidal zone brings about rapid changes in temperature, salinity, hydrostatic pressure, food, and predation pressure, exposing the inhabitants of this environment to a complex mixture of cyclic environmental stimuli. Therefore, tidally-timed rhythms regulating several aspects of their physiology and ecology, such as photosynthesis, respiration, feeding,

predator avoidance, desiccation avoidance, dispersal, and reproduction, have evolved in most intertidal organisms (Palmer, 1995). Mud snails of the genus *Hydrobia* are deposit feeders that form very large populations in estuaries and coastal lagoons of the north Atlantic, with densities that may reach 300 000 individuals m⁻² (Fretter & Graham, 1994). Such high densities, in conjunction with the usually high densities of many other invertebrate species in the rich and productive environment of intertidal mudflats, may pose considerable problems of competition for resources (Fenchel, Kofoed & Lappalainen, 1975;

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Levinton, 1979; Blanchard *et al.*, 2000; Barnes, 2001). The large biomass of *Hydrobia* populations also makes these snails a potentially important prey item in the diet of other invertebrates, as well as of fish and birds (Goss Custard, Jones & Newbery, 1977; Piersma *et al.*, 1993; Aarnio & Mattila, 2000). Given that feeding, resource allocation, and predator avoidance are connected to the locomotion and general dispersal mechanisms of individuals, behavioural reactions of *Hydrobia* have received considerable interest.

The locomotor and feeding activities of *Hydrobia* have been studied mostly in *Hydrobia ulvae* (Pennant, 1777). Several field and laboratory observations indicate that the periodic behaviour of *H. ulvae* is controlled by direct exposure to physical and biological conditions associated with the day and the tide. During low tide, these snails can be seen crawling over the surface of damp sediment, or standing still on boulders, as well as on the shoots of marsh plants and any structure protruding above the sediment. *Hydrobia ulvae* is more active during the night than during daylight hours as well as during high water than when exposed by the low tide. When out of water over dry surfaces *H. ulvae* does not move, withdrawing into the shell and sealing the aperture with mucus (Barnes, 1981a, b). Such behaviour that results in large numbers of *H. ulvae* being found during low tide sticking to boulders and shoots of plants is initiated when the snails are still covered with water and appears to be related to the normal foraging activity of the snails because they climb more often over objects that bear food than over those that are clean (Barnes, 1981a, b). Feeding activity under natural conditions in a population continuously submerged during spring tides was found to be related to the day cycle, peaking around noon (Barnes, 2003). This pattern of feeding is in opposite phase to the pattern of crawling activity and was attributed to an increase in the biomass and production of benthic microalgae during diurnal low tide, which in turn is related to the vertical migration of the microalgae along the first few millimetres of sediment, exposing them to appropriate light conditions for photosynthesis (Serôdio & Catarino, 2000; Consalvey, Paterson & Underwood, 2004; Serôdio *et al.*, 2006). Additionally, the presence of a natural day/night cycle entrained a clear daily rhythm in snails collected from a sand flat subjected to semidiurnal tides (tidal range of approximately 2 m), with activity peaks coincident with nighttime (Barnes, 1986).

Although the above observations indicate that behaviour of *H. ulvae* is controlled by exogenous stimuli, most intertidal organisms display periodic feeding and locomotion behaviours that are under endogenous control. *Hydrobia ulvae* collected in a sand flat from a coast subjected to semidiurnal tides (tidal

range of approximately 2 m) do display a faint endogenous diel rhythm of crawling when placed in the laboratory under constant dark conditions over a damp surface, with peak levels of activity that are 1.3- to 1.6-fold greater than the minimum levels (Barnes, 1986). However, this rhythm did not have a discernible relationship with the subjective day cycle. Moreover, it is surprising to note that this rhythm could not be related to the tidal cycle, given that most intertidal organisms display strong overt tidal rhythms controlled by endogenous clocks (Palmer, 1995).

In the present study, we address the crawling activity of *H. ulvae* under constant conditions. The study was designed to identify activity patterns displayed by individual snails, instead of by ensembles of individuals, aiming to assess intraspecific variability in rhythmicity. We hypothesize that these snails display a tidally-timed endogenous rhythm and, given that locomotor activity is usually associated with feeding in intertidal gastropods (Underwood, 1979), peak activity should be timed to low tide when the highest biomasses of microphytobenthos, their main food item (Fenchel *et al.*, 1975), are expected to occur at the surface of the sediment. Furthermore, we investigate the effects of individual size and previous short-term exposure to tides of different range on the amplitude and timing of the rhythm.

MATERIAL AND METHODS

Hydrobia ulvae were collected during low tide from three intertidal mudflats located in the Ria de Aveiro (Fig. 1). The Ria de Aveiro is a bar-built estuary on the northwest coast of Portugal where tides are semidiurnal. Tidal range at the inlet varies from approximately 1 m during neap tides to 3 m during spring tides, and the tidal wave is considerably lagged and attenuated as it progresses along the main channels of the Ria (Dias, Lopes & Dekeyser, 2000). Once in the laboratory, the snails were sieved from the sediment and measured under a stereoscopic microscope. All experiments were conducted from March to July of 2006.

To record the activity pattern, we used a digital video system in a laboratory maintained at constant temperature (18 °C). The system was composed of 6 Allied Vision Technologies GMBH AVT-D2 FireWire cameras connected to a computer and controlled by dedicated software. All experiments were run in constant dark conditions, using infrared light provided by 2 Derwent Miniflood 100 led projectors equipped with low-pass 850 nm filters. The movement of the subjects was constrained by placing individual snails in separate white plastic trays and covering them with an inverted bottom of a glass Petri dish with an internal diameter of 9.5 cm. Care was taken to fill the plastic tray so that the Petri dish was completely

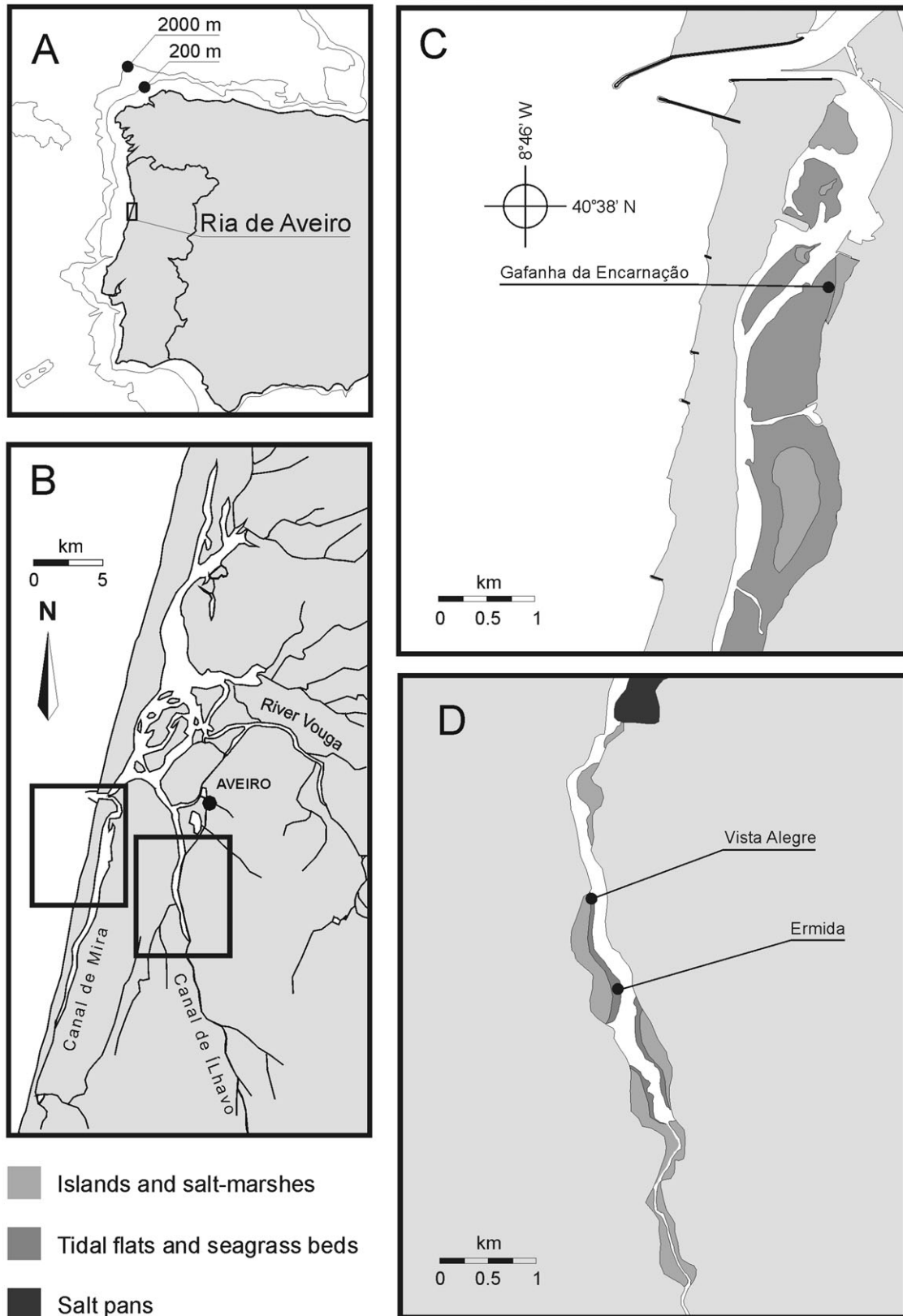


Figure 1. Map of the Iberian Peninsula (A) with the location of the Ria de Aveiro (B) as well as the sampling stations in Canal de Mira (C) and Canal de Ílhavo (D).

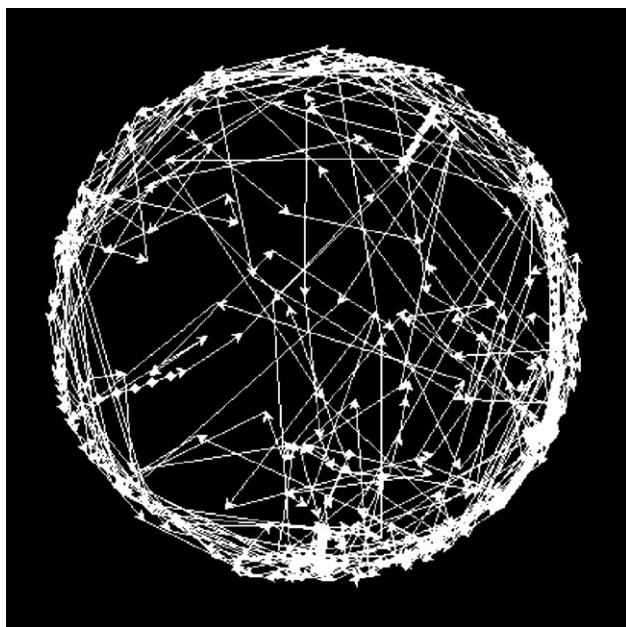


Figure 2. Track of a 2-mm long juvenile during 20 h. Movement was recorded by an automated digital video system set to an image frequency acquisition of 1 min^{-1} . The track was reconstructed using image analysis software.

covered with sea water and without air bubbles trapped inside. Typically, in each run, a batch of six snails from the same size class ($< 3 \text{ mm}$ or $> 3 \text{ mm}$ shell height) were recorded for periods of 5 or 10 days. In all recordings, the frequency of image acquisition was set to 1 min^{-1} . This frequency was chosen as a compromise between: (1) the disk space and processor velocity needed to store and analyse the bitmap files composing each sequence and (2) a correct representation of the distance crawled between successive frames. The snails tended to move more often along the perimeter of the Petri dish rather than across the dish (Fig. 2); therefore, the distance actually crawled is larger than the estimate, which was obtained from a straight line connecting the positions of the snail in two successive frames. However, because snail velocity rarely exceeded 4 cm min^{-1} , such a frequency ensured that they never crawled over more than one-seventh of the perimeter (28.3 cm) of the dish. Therefore, even if the snails would always crawl along the walls of the Petri dish at maximum speed, the distance moved between successive frames would never be underestimated by more than 3.3%.

The set of images obtained for each snail was analysed with IMAGE-PRO PLUS, version 5.1 (Media Cybernetics), using the automatic tracking option of the Sequence module, after differencing consecutive images. Spurious tracks were sometimes caused by

shadows of the snails. To detect them, the tracks generated by the automatic analysis were inspected and deleted as appropriate. Figure 2 shows a representative example of the track followed by one snail over 20 h.

To determine the presence and the period of an endogenous cyclic component, the time series of the average distance crawled by the snails were analysed with standard time series techniques (Chatfield, 1996). First, the series were filtered by averaging the data over 6-min bins to reduce noise and by removing the linear trend by least squares regression. The filtered series were then subjected to spectral and autocorrelation analyses. The strategy adopted was to perform an initial spectral analysis aiming to determine the dominating periods of oscillation. Because the time series do not correspond to perfect sinusoidal series, the spectral analysis often detected oscillations with periods equal to submultiples of the dominant periods. These periods were discarded whenever they failed to pass the 5% significance level criterion in the autocorrelation analysis (Levine *et al.*, 2002). Often, the snails stopped moving before the projected end of the experiment, and the time series were trimmed as appropriate before the analysis.

Significant periods of activity related to the period of the tidal cycle were detected in most of the time series. To investigate the phase relationship of the rhythms regarding the ambient tide cycle, as well as the ability of immersion to act as a suitable *zeitgeber* capable of entraining the tidal rhythm, the following procedures were used. Water height series were first generated for the sampling sites and dates of the experiments using the harmonic coefficients reported by Dias (2001). A comparison of the estimated time series of water height against *in situ* observations made with an Anderaa 3230 CTD probe showed that the estimated time of high and low tides was within $\pm 1 \text{ h}$ of observed times, indicating that the predicted time series of water height are a fair representation of the tidal cycle at the collecting sites. Cross-correlation analyses were then performed between the series of crawling activity and water height. Because both series presented strong autocorrelations at a lag of 6 min, they were differenced at this lag before calculating the autocorrelations (Chatfield, 1996). If a significant cross-correlation was detected (at the 5% level), the lag corresponding to the highest positive correlation was used as a measure of phase synchrony between crawling activity and the expected tidal cycle. The phase response of individual snails was then calculated and plotted against time of start of immersion. Finally, a Rayleigh test (Zar, 1999) was applied to determine the average phase relationship between crawling activity and the expected tidal cycle. This test was calculated by converting the tidal

cycle period of 12.4 h to 360° and by assigning the expected time of high and low water to 0° and 180°, respectively. The time lags corresponding to the highest positive cross-correlations between the activity and the water height series were converted to a phase angle, and the mean angle and correlation coefficient were then calculated.

The effects of short-term *in situ* acclimation to tides of different range and of snail size on average velocity were analysed with a two-way orthogonal analysis of variance (ANOVA) with two levels for tidal range (spring and neap tides, respectively, with < 2 and > 2 m in range) and for size (small and large, respectively, with < 3 and > 3 mm in shell height), which was separately applied to the data obtained during the first day and during the entire length of each run. The effects of both factors on the period of the main rhythm were also investigated with a similar ANOVA. Cochran tests indicated that variances were heterogeneous in all cases, and this condition could not be corrected by any of the usual transformations. Therefore, the ANOVAs were performed on the ranks of the observations, *sensu* Zar 1999). This procedure homogenized variances and corresponds to a nonparametric ANOVA.

RESULTS

Continually submerged *H. ulvae* maintained under constant dark conditions alternated periods of crawling with periods of rest. Of the 48 snails recorded, four did not move at all. All others were active from the beginning of the trials, and all were intermittently active for at least 50 h. Of the 12 snails recorded for 120 h, ten were still active at the end of the recordings. Of the 36 that were recorded for 240 h, ten were still moving at the end of the experiments (see Appendix, Table A1).

Most of the snails showed an activity record with one clear periodic component, although several also showed secondary periodic components that passed the significance criteria adopted. Examples illustrating the range of variability detected during the study are given in Figures 3 and 4. Figure 3 shows one snail that expressed a locomotor activity record peaking during the expected high water, with a period of 13.0 h and no evidence of significant secondary components. Figure 4 shows a snail with primary activity centred around low tide, a main periodic component with a period of 12.6 h and a secondary component with a period of 19.7 h. Figures 3A and 4A also show that the level of crawling activity was very variable, with short periods of decreased activity or immobility within the main active phase or bursts of small amplitude crawling during the rest phase. The periods of the main cyclic component were in the range 6.8–

29.8 h in the 48 snails recorded (Fig. 5), with most periods concentrated in the range 12–16 h, which is related to the period of the tidal cycle. In some cases, there was an indication that a circadian component in activity was also present. For example, the record in Figure 3A indicates that the activity maximum during diurnal high water tended to be lower than during nocturnal high water. However, in most instances, the circadian component was not significant, and only one snail displayed a secondary component that could be clearly related to the period of the day cycle (Fig. 5). Therefore, most of the snails expressed a circatidal rhythm in crawling activity. The average period of the main component was 13.9 h, with a large standard error of 7.4 h reflecting the variability among the individual activity patterns.

The phase response diagram (Fig. 6) shows delays or advances of peak activity relative to high water, as a function of the time lag between start of immersion and high water. Shifts in the start of immersion relative to the predicted tidal cycle are able to reset the phasing of endogenous rhythm. On average, the snails advanced crawling (i.e. maximum activity occurred up to 6 h before expected high water) when immersion commenced before high water, whereas a delay was detected in the only batch of snails that was immersed after high water. However, considerable variation was observed in the magnitude and signal of the phase response. Individual snails in the same batch often showed opposite phase responses. Moreover, many of them were irresponsive to the time of the start of immersion, with 14 of 31 showing peaks in activity within ± 1 h of predicted high water, irrespective of time of immersion. On average, peak activity occurred 0.8 h before the expected high water at the collecting sites, and the distribution of phase angles relative to the tidal cycle differed significantly from a uniform distribution according to the Rayleigh test ($\bar{\alpha} = 336^\circ = 11.6$ h, $r = 0.39$, $N = 31$, $P < 0.01$; Fig. 7).

The results of the ANOVAs of the effects of size and previous *in situ* acclimation to tidal range indicated that smaller snails moved significantly more than large snails, although acclimation to tidal range had no effect on movement, both during the first day (size effect: Fisher $F_s = 9.680$, d.f. = 1, 39, $P < 0.01$; tidal range effect: $F_s = 0.765$, d.f. = 1, 39, $P > 0.50$) and during the whole duration of the trials (size effect: $F_s = 25.714$, d.f. = 1, 39, $P < 0.001$; tidal range effect: $F_s = 0.086$, d.f. = 1, 39, $P > 0.75$). The interaction between size and tidal range was not significant in either case (first day: $F_s = 2.719$, d.f. = 1, 39, $P > 0.10$; whole experiment: $F_s = 0.293$, d.f. = 1, 39, $P > 0.50$). Overall, mean \pm SE velocity of the small and large snails during the first day of the experiments was 0.53 ± 0.068 and 0.30 ± 0.047 cm min⁻¹, respectively, whereas values of 0.36 ± 0.049 and 0.08 ± 0.014 cm

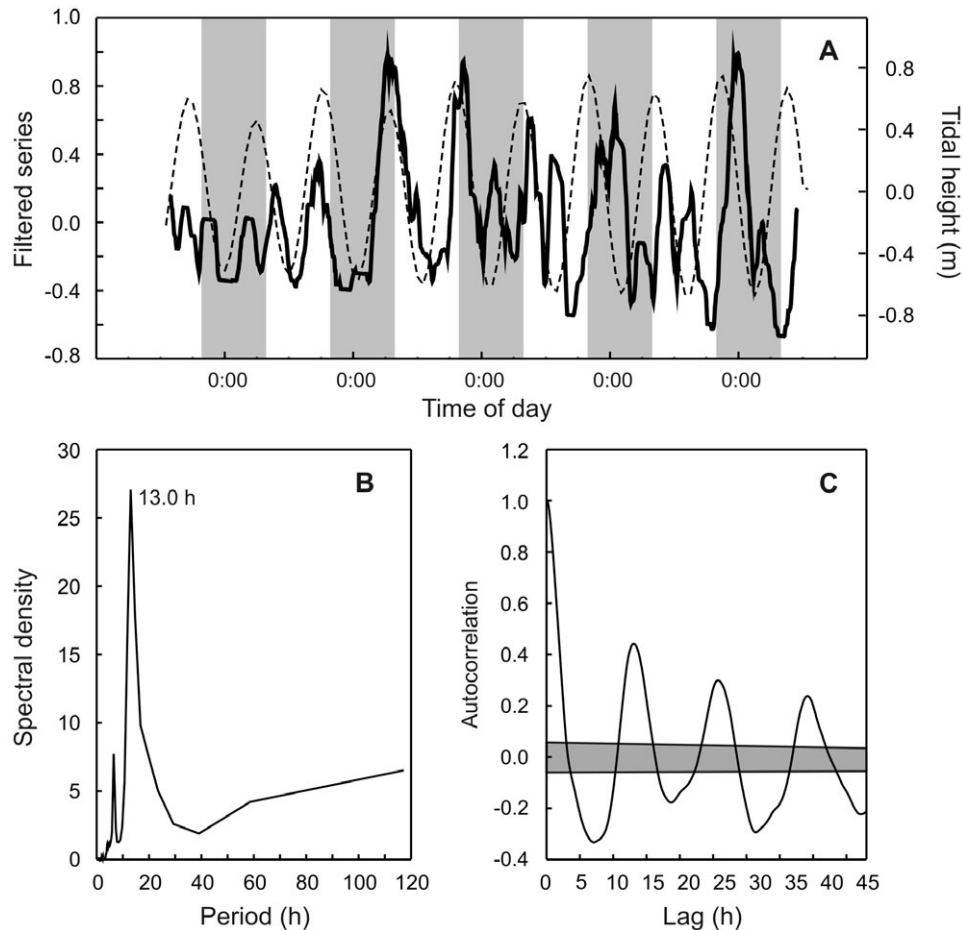


Figure 3. Filtered series of crawling activity (thick line) under constant dark conditions of one individual collected during spring tides at Vista Alegre and tidal height (thin dashed line) at the collecting site (A), periodogram (B) and autocorrelation (C) of the time series in (A). The shaded band in (C) represents nonsignificant values.

min^{-1} were estimated for the full length of the experiments, indicating a decline of activity levels with time. Size and previous acclimation to tidal range did not have any statistically significant effect on the period of the main cyclic component of crawling activity (size effect: $F_s = 0.840$, d.f. = 1, 39, $P > 0.35$; tidal range effect: $F_s = 0.521$, d.f. = 1, 39, $P > 0.45$; interaction: $F_s = 0.024$, d.f. = 1, 39, $P > 0.85$).

DISCUSSION

Individual *H. ulvae* maintained under constant conditions of immersion and darkness displayed free-running cyclic patterns of crawling activity, which, in most cases, had a circatidal periodicity with activity maximum during expected high water. These patterns were intrinsically variable: some snails did not crawl at all or stopped crawling after various lengths of time, the period of the main rhythm was frequently considerably shorter or longer than the period of the

tidal cycle, and several individuals showed secondary activity rhythms that were either of shorter or longer period than the tidal period. Such variability in tide-related rhythms of activity is common in intertidal organisms, where inter-individual differences in period length, spontaneous changes in period in the same individual, and average period lengths exceeding the average interval of the tides are commonly reported (Barnwell, 1966; Palmer, 1989; Williams, 1991). Intra- and inter-individual variability of circatidal rhythms has been attributed to the tide-to-tide deviations from the average tidal period, which is much greater than that found in the daily and seasonal cycles, making the tide a comparatively weaker selective pressure. Although fine tuning to a precisely timed environmental cycle decreases mortality from a hierarchy of biotic and abiotic factors associated with the cycle, a poorly timed cycle may favour the persistence of genetic (and phenotypic) variability that increases adaptability in the long term. The ecological

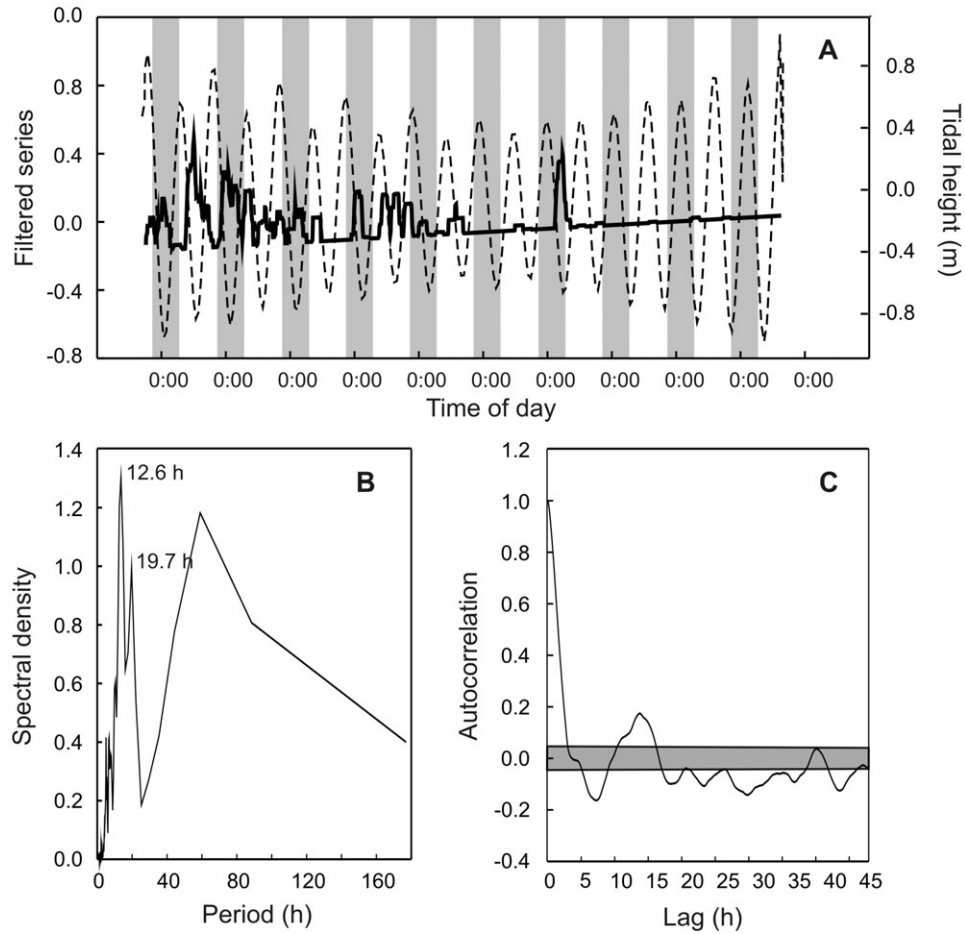


Figure 4. Filtered series of crawling activity (thick line) under constant dark conditions of one individual collected during spring tides at Gafanha da Encarnação and tidal height (thin dashed line) at the collecting site (A), periodogram (B) and autocorrelation (C) of the time series in (A). The shaded band in (C) represents nonsignificant values.

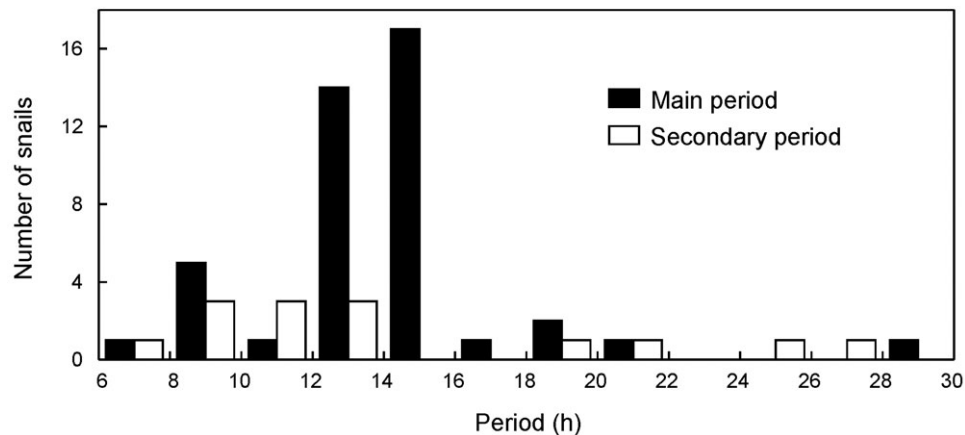


Figure 5. Frequency distribution of main and secondary periods lengths detected in 43 individual snails.

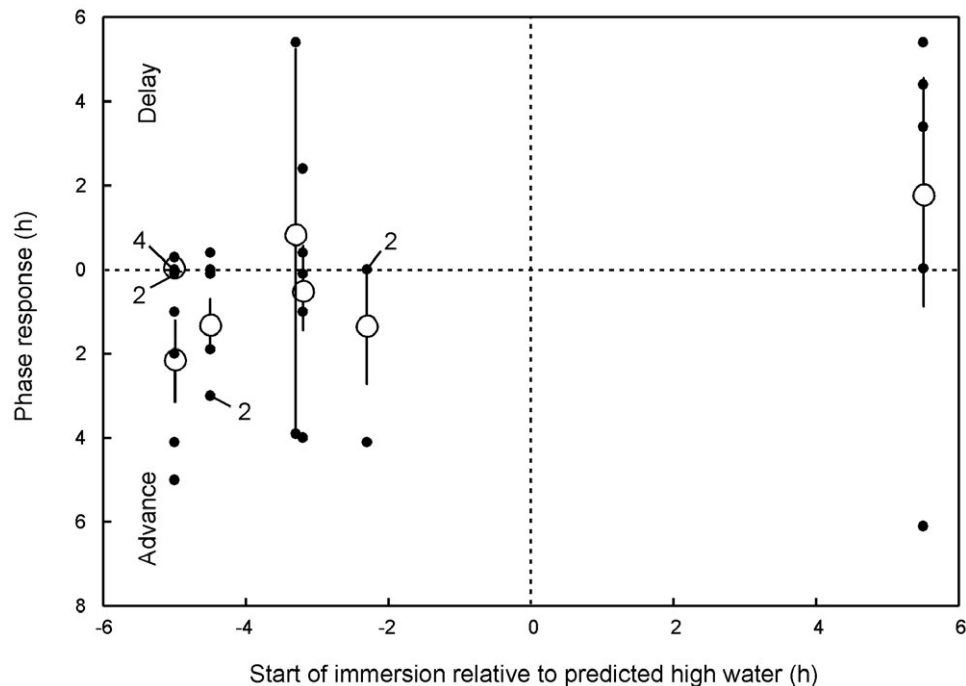


Figure 6. Phase response of individual snails (dots) that were immersed at different times relative to the predicted high water. Numbers adjacent to points indicate the number of overlapping observations. The average phase response of batches of snails that were immersed at the same time is also shown (circles with standard error bars). Only individuals that displayed a significant cross-correlation between crawling activity and tidal height are shown.

and evolutionary significance of inter-individual variability of tidal rhythms are poorly known, however, because tidal behaviour is usually studied in ensembles of individuals, in an attempt to reduce the inherent noise (Palmer, 1995). The overt circatidal rhythmicity detected in the present study does not match the results obtained previously in constant conditions (Barnes, 1986). The methodologies of the two studies were different in the sense that the latter study was based on ensembles of individuals that had to resort to human and light interference for detection of activity, which may have affected the expression of the rhythm.

Delays in the start of immersion relative to the expected tidal cycle could not be consistently tested in the present study, although the phase response curve obtained still presents useful information. Inundation earlier than expected resulted in an advance of the active phase, whereas late inundation delayed crawling. This entraining effect of immersion has been demonstrated in intertidal animals (Palmer, 1995) and is one of the mechanisms by which their internal clocks are timed to the tide cycle. This type of response may constitute an especially useful adaptation for *H. ulvae* because individuals of the species may be flushed up and down estuaries by tides (Armonies & Hartke, 1995; Meireles & Queiroga,

2004) and be exposed to different timings of the tide after resettlement. However, many of the snails did not respond to delays or advances in the start of immersion and sometimes showed opposite phase responses when subjected to the same delays in immersion, suggesting that one-time immersion is a relatively weak *zeitgeber* (Naylor & Williams, 1984). Other *zeitgebers* associated with the tidal cycle, but not examined in the present study, may be involved in the entrainment of the rhythm, such as temperature (Williams & Naylor, 1969; Williams, Palmer & Hutchinson, 1993) and salinity (Reid & Naylor, 1989) shocks, as well as hydrostatic pressure (Gibson, 1971; Abelló, Reid & Naylor, 1991).

Benthic microalgae are one of the main sources of food for *Hydrobia* (Fenchel *et al.*, 1975) and are known to perform vertical migrations along the top few millimeters of the sediment in response to internal clocks and external stimuli from the environment (Consalvey *et al.*, 2004; Serôdio *et al.*, 2006). These vertical movements result in periodic increases of biomass at the surface of the sediment during diurnal low tides when the sediment surface is freely exposed to sunlight. At other times, biomass concentration increases below the surface, with maximum values occurring at depths of 0.5–2 mm (De Brouwer & Stal, 2001; Herlory *et al.*, 2004; Van Leeuwe *et al.*, 2008).

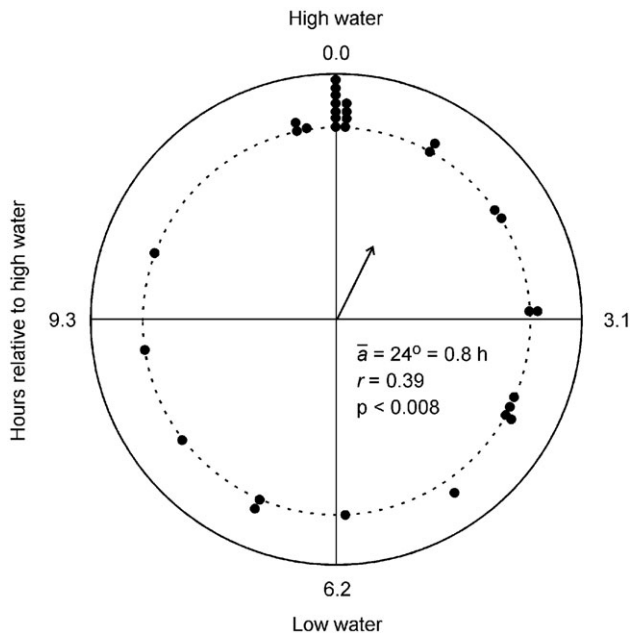


Figure 7. Phase relationship between peak activity under constant dark conditions and tidal cycle at the collecting sites. Each point represents the time lag between maximum crawling activity and the expected high water, as determined by cross-correlation analyses. The dotted circle represents a correlation (r) of 1; the vector is aligned to the mean angle (\bar{a}) and has a length of 0.39; P represents the significance value of the Rayleigh test.

The phasing of the endogenous rhythm of *H. ulvae* detected in the present study, with crawling activity peaking during expected high water, indicates that feeding on large concentrations of benthic microalgae at the very surface of the sediment does not appear to be a major selective force shaping adaptive behaviours in the species, at least in the Ria de Aveiro. It is possible that: (1) the snails can feed to depths of a few millimeters, thus becoming independent of fluctuations of microalgae concentration at the surface (Lopez & Cheng, 1983) or (ii) other types of food, namely bacteria (Fenchel *et al.*, 1975; Blanchard *et al.*, 2000), are of equal or greater importance for *H. ulvae* from the Ria de Aveiro.

Intertidal gastropods form the mid and upper littoral of rocky, sandy, and mangrove habitats (Zann, 1973; Petpiroon & Morgan, 1983; Kitching, Hughes & Chapman, 1987; Vannini *et al.*, 2008) time their active phase to coincide with the period of flooding, whereas those living in the lower littoral tend to move during expected low tide. These patterns have been interpreted in terms of a balance between the needs to forage and to avoid predators (Vadas, Burrows & Hughes, 1994; Rochette & Dill, 2000) and desiccation (Chapman & Underwood, 1996). Mid and upper inter-

tidal species time their active phase to the moment when the shore is flooded, allowing the snails to seek refuge in crevices and retract into the shell during low tide, avoiding water loss and predation by birds. Exposure to predatory fish during flood may be relatively low in the splash zone. Low intertidal species forage during low water, being protected from desiccation in the comparatively wetter habitat and reducing their exposure to predatory fish when the tide is high. It is tentative to interpret that the crawling behaviour of *H. ulvae*, which lives in the upper and middle zones of sheltered shores, has evolved as an adaptation to feed during high water to avoid predation by birds as well as to remain inactive and protected inside the shell when the tide is low to prevent water loss. However, the role of predation should be carefully considered because it is uncertain how predation pressure on *Hydrobia* is distributed along the tidal cycle. Studies indicate that *Hydrobia* is likely to be preyed upon by waders and other birds during low tide (Goss Custard *et al.*, 1977; Moreira, 1995), although fish (Aarnio & Mattila, 2000) and invertebrates (Mearthar, 1998) prey on them at other phases of the tide. Moreover, ingestion rates along the tidal cycle have not been measured, and the true effects of ingestion by vertebrates on the mortality of *Hydrobia* require better quantification given that sometimes up to 90% of individuals may pass the digestive tract of flat fish alive (Aarnio & Bonsdorff, 1997).

Feeding in marine gastropods, as in most non-sessile animals, is linked to locomotion (Underwood, 1979; Lopez-Figueroa & Niell, 1987). Given this link, the results obtained in the present study suggest that feeding of *H. ulvae* in the natural environment should not change at semi-lunar time scales related to the spring/neap tidal cycle, because the levels of crawling are not influenced by the previous conditions of the tidal range to which the snails were subjected in nature. Moreover, feeding should take place mostly during high water and be independent of periodic fluctuations of microphytobenthos biomass at the surface of the sediment. A formal test of these hypotheses would require an investigation of the behaviour and feeding of *H. ulvae* in the presence of controlled changes of light, water, and food levels, which is a challenging task. An alternative approach would be the development of techniques to estimate *in situ* feeding rates through different phases of the natural environmental cycles.

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APPENDIX

Table A1. Sampling site, shell height, and period of the crawling rhythm of individual snails under constant dark conditions

Site	Tidal range	Original series length (h)	Shell height (mm)	Main period (h)	Secondary period (h)
VA	Neap	120	2.16	13.2	NS
VA	Neap	120	2.00	29.8	11.9
VA	Neap	120	2.08	9.9	NS
VA	Neap	50	2.04	11.7	NS
VA	Neap	50	1.96	9.4	NS
VA	Neap	120	1.61	14.9	NS
VA	Spring	120	1.61	13.0	NS
VA	Spring	120	1.84	13.0	NS
VA	Spring	120	1.65	13.0	NS
VA	Spring	120	1.95	13.0	NS
VA	Spring	120	1.95	13.0	NS
VA	Spring	120	1.76	6.8	10.6
ER	Neap	60	2.47	NS	NS
ER	Neap	240	2.55	14.8	27.3
ER	Neap	180	2.82	16.1	NS
ER	Neap	60	2.51	8.1	NS
ER	Neap	60	2.78	14.2	NS
ER	Neap	240	2.23	14.8	8.8
ER	Neap	240	2.39	13.9	19.7
ER	Neap	240	2.16	14.8	NS
ER	Neap	180	2.20	14.7	NS
ER	Neap	120	2.39	13.0	NS
ER	Neap	240	2.04	14.8	NS
ER	Neap	240	1.96	13.9	NS
GE	Spring	180	9.80	14.7	11.1
GE	Spring	140	10.08	15.2	NS
GE	Spring	180	9.66	12.6	NS
GE	Spring	140	6.44	9.1	NS
GE	Spring	160	8.40	14.3	NS
GE	Spring	160	7.14	14.3	NS
GE	Neap	75	8.96	14.4	9.0
GE	Neap	60	10.08	14.2	NS
GE	Neap	145	9.80	14.2	20.3
GE	Neap	120	9.24	14.6	NS
GE	Neap	0	8.68	Did not move	
GE	Neap	90	7.98	14.5	NS
GE	Spring	0	1.76	Did not move	
GE	Spring	0	1.72	Did not move	
GE	Spring	60	2.00	14.2	NS
GE	Spring	0	1.80	Did not move	
GE	Spring	170	2.16	18.6	12.8
GE	Spring	180	1.96	19.7	12.6
GE	Neap	240	2.47	12.5	9.5
GE	Neap	150	2.23	21.5	24.5
GE	Neap	240	1.80	13.2	6.6
GE	Neap	240	2.74	13.1	NS
GE	Neap	180	2.35	8.4	12.6
GE	Neap	240	2.90	12.5	NS

VA, Vista Alegre; E, Ermida; GE, Gafanha da Encarnação; NS, significant period was not detected.