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Assessment of factors affecting heart rate of the limpet *Patella vulgata* on the natural shore

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Abstract Heart rate variations of a population of the limpet *Patella vulgata* were monitored in the natural environment (Lough Hyne, southern Ireland) by non-invasive, optoelectronic recording. The heart rates of 145 limpets of different sizes, living on vertical and horizontal substrata, were measured both in air and water at different environmental temperatures, while the animals were inactive on their home scars. The heart rates of emersed, inactive limpets were positively related to air temperature and negatively related to limpet size. These relationships were similar for limpets on vertical and horizontal substrates. In contrast, no significant relationship between heart rate and temperature was found in submerged limpets, probably due to the narrow thermal range of the water during the study period. During submersion, a significant negative relationship between heart rate and size was evident for limpets on vertical surfaces but not for limpets on horizontal surfaces. In general, submerged limpets had a higher heart rate, 1.16 times that of limpets exposed to air. Moreover, the heart rates of nine animals were recorded while they were moving and while inactive on their home scars. Active limpets had a faster heart rate, 1.6 times that of limpets resting on their home scars. The dependence of heart rate on environmental temperature, size, respiratory medium and activity, as observed in limpets on the shore, agrees well with laboratory data and with previous findings of the correlation of oxygen consumption with the same factors. Such in situ measurements may, therefore, prove useful in attempts to determine natural levels of energy expenditure in models on the behaviour of foraging molluscs.

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Introduction

The effect of a variety of factors on the metabolic rate of limpets has been investigated thoroughly (see Newell 1979; Branch 1981; Branch et al. 1988 for reviews). Most of the studies were based on the measurement of oxygen consumption (e.g. Davies 1966; Branch and Newell 1978; Dye 1987), but, in some cases, metabolic rate was indirectly assessed by the monitoring of cardiac activity (e.g. Jones 1968; Marshall and McQuaid 1992, 1994), which has been shown to be linearly related to oxygen consumption rates in several limpet species (see Marshall and McQuaid 1992; Santini et al. 1999).

Investigations based on respirometry have usually been conducted under laboratory conditions, with limpets removed from their natural environment. Such manipulations have been shown to influence metabolic rate (Houlihan and Newton 1978; Santini et al. 1999) and thus to bias the assessment of the dependence of metabolic rate on various environmental and internal factors. The assessment of metabolic rate by the recording of cardiac activity has traditionally employed invasive techniques, such as visual inspection of heart activity through a hole drilled in the shell (e.g. Segal 1956; Markel 1974) or impedance methods using implanted electrodes (e.g. Jones 1968; Marshall and McQuaid 1992). These techniques, however, can also introduce artefacts in the evaluation of variations in heart rate (Aagard et al. 1991).

The recent development of non-invasive techniques to monitor heart rate in shelled invertebrates (Depledge and Andersen 1990) has allowed more reliable recordings of cardiac activity directly on the shore and has greatly reduced manipulative stress (Chelazzi et al. 1999; Santini et al. 1999). This method has been employed in the present study to obtain heart rate recordings from an Irish population (Lough Hyne) of the limpet *Patella vulgata*; these measurements were taken directly in the natural environment. *P. vulgata* is a homing limpet which returns to a fixed location (the home scar) after each foraging excursion (Orton 1929; Hartnoll and Wright 1977; Chelazzi et al. 1998). Timing of activity depends on both tidal and day/night cycles (Hawkins and Hartnoll 1982; Little 1989; Williams and Morritt 1991; Della Santina et al. 1994). The activity patterns of this species can easily be divided into discrete resting, moving and foraging phases, and attempts have been made to model the energy costs of foraging in *P. vulgata* (Evans and Williams 1991; Santini and Chelazzi 1996). Behavioural observations of *P. vulgata* at Lough Hyne (Williams et al. 1999) have shown that limpets living on horizontal or vertical rocks adopt different patterns of foraging activity. The difference in energy expenditure by the limpets inhabiting these substrates has been suggested as one of the possible causes of this variation in the activity pattern.

The aim of the present study was to provide information on heart rate of *Patella vulgata* under natural conditions, trying to assess whether the dependence of heart rate on different factors observed in the natural environment is consistent with patterns of oxygen consumption previously obtained from *P. vulgata* and other limpet species in the laboratory (e.g. Davies 1966, 1969; Branch 1981; Dye 1987). This comparison is important to test the conclusion drawn from laboratory studies (Marshall and McQuaid 1992; Santini et al. 1999) that non-invasive heart rate monitoring could be used as an alternative to respirometric techniques in the assessment of energy costs of limpets in their natural habitat. Moreover, the heart rates obtained from limpets living on vertical and horizontal surfaces were compared, in order to evaluate possible differences in metabolic rate which could explain the observed differences in their patterns of foraging activity.

Materials and methods

Study site

The heart rate of *Patella vulgata* was measured in populations inhabiting the shore at Lough Hyne, Co. Cork, Ireland (51°31′N; 9°10′W) during September 1998. Lough Hyne is a semi-enclosed sea lough which experiences an irregular tidal regime (4 h flood tide, 8.5 h ebb tide, see Kitching 1987) of low amplitude (~2 m during the study period). The low tidal amplitude and reduced wave action make this site ideal for in situ recordings of heart rate. Details of the study site and limpet populations can be found in Kitching (1987), Little et al. (1991), Williams and Morritt (1991) and Williams et al. (1999).

In situ recording of cardiac activity

Heart rate was monitored using an optoelectronic method derived from the technique of Depledge and Andersen (1990), as described in Santini et al. (1999). The sensor (CNY70) consisted of an infrared light emitting diode axially coupled with a phototransistor. Each sensor was either glued (Attack, Loctite) or fixed temporarily (using Blue-Tac, Bostick) to the shell in a position corresponding to the heart. The limpets were not removed from the substrate but, prior to sensor attachment, the shell of each limpet was gently cleaned. Cleaning and positioning of the sensor took < 1 min. After attachment, each limpet was left undisturbed for at least 5 min before the start of recording, to allow recovery from this procedure. Preliminary observations showed that this time period was sufficient for the heart rate to return to normal levels. Signals from the phototransistor were filtered, amplified and relayed to a Fluke 105B portable oscilloscope. For each specimen, heart rate was measured during three consecutive 20 s periods (30 s in between), and an individual average heart rate was computed (HR, beats s^{-1}). Marking of individuals ensured that each animal was monitored only once, except for the activity/inactivity comparisons, where repeated measures from the same limpet were used (see below).

The shell length (SL, mm) of individual limpets was measured with Vernier callipers (± 0.1 mm). The slope of the substrate was assessed by clinometer (± 1°). Limpets were scored as "vertical" when the slope of their home scar exceeded 60° and as "horizontal" when the slope was < 20° (see Williams et al. 1999). Air or water temperature was measured (± 0.1 °C) with a thermocouple (Omega K-type) placed 5 cm above the limpet's shell and connected to a Fluke 52 temperature meter. During the study period, air temperature ranged from 8.4 to 15.6 °C, while water temperature ranged from 14 to 15.9 °C.

We monitored 145 inactive limpets to test the effect on cardiac activity of temperature and body size in various conditions of exposure to air/water and vertical/horizontal slope of the substrate. Due to the variety of combinations of these factors under natural conditions, it was impossible to perform a balanced, full factorial analysis of variance on changes in heart rates under different situations (Underwood 1997). Instead, multiple regression was employed to relate heart rate to limpet size and environmental temperature under the different combinations of tidal exposure and substrate inclination. Comparisons between the obtained regressions were performed according to Zar (1996), and analyses were carried out with the JMP statistical package (SAS Institute Inc.).

Two samples of limpets, emerged and submerged ($n = 24$ for each), of similar size and experiencing the same range of temperatures were considered to assess the effect of respiratory medium (air vs water) on heart rate.

Recordings from the same individuals ($n = 9$), obtained when limpets were at rest (shell margin fitting the home scar) and when moving (rotated on or moved away from the home scar), were compared using repeated measures *t*-test (see Winer 1971 for details). The effective sample size was limited by the necessity to compare active/inactive measurements of the same limpet under the same respiratory medium and temperature (± 0.5 °C).

Results

Heart rate of limpets exposed to air was consistently affected both by body size and environmental temperature, as shown by multiple regression analysis (Table 1). For both limpets on horizontal and vertical surfaces, heart rate increased with temperature and decreased with size. There was no significant difference between the regressions for limpets on horizontal or vertical surfaces ($F_{3,104} = 1.54$, $P > 0.05$); when the samples were combined, a significant effect of both temperature and size was evident (Table 1; Fig. 1).

Recordings from submerged limpets ($n = 35$) revealed no significant effect of temperature on cardiac activity, for either limpets resting on vertical or horizontal surfaces. The temperature variation of the sea

Table 1 *Patella vulgata*. Multiple linear regressions of heart rate on shell length (*SL*, mm) and environmental temperature (*T*, °C) for emersed and submerged limpets on horizontal and vertical rock surfaces. Sample size (*n*), partial regression coefficient (*β*), standard error (*SE*), *t*-test (*t*) and probability level (*P*) are also shown

Sample	*n*	Variable	*β*	SE	*t*	*P*
Emerged						
Horizontal	59	SL	−0.007	0.002	−4.21	<0.0001
		T	0.034	0.006	5.89	<0.0001
Vertical	51	SL	−0.009	0.120	−6.08	<0.0001
		T	0.021	0.008	2.80	0.0070
Overall	110	SL	−0.008	0.001	−7.21	<0.0001
		T	0.029	0.005	6.32	<0.0001
Submerged						
Horizontal	16	SL	−0.004	0.003	−1.67	>0.1
		T	−0.014	0.077	−0.19	>0.8
Vertical	19	SL	−0.009	0.003	−3.00	<0.01
		T	0.031	0.024	1.31	>0.2

water was slight under these conditions (~2 °C). There was a significant reduction of heart rate with increasing size in vertical limpets (Table 1; Fig. 2), while this relationship was not statistically significant in horizontal individuals.

The effect of respiratory medium (air vs water) on the heart rate of *Patella vulgata* was assessed by two sets of recordings ($n = 24$ each) from animals of the same size (*t*-test: $t = 1.53$, $df = 46$, $P > 0.1$), under similar temperature conditions (14 to 15.9 °C, *t*-test: $t = 1.52$, $df = 46$, $P > 0.1$). Heart rates measured during emersion (mean HR = 0.50 ± 0.02 SE) were slightly, but significantly, lower than those measured during immersion (mean HR = 0.58 ± 0.02, *t*-test: $t = 2.55$, $df = 46$, $P < 0.05$).

Heart rate during activity was, on average, 1.6 times greater (±0.11 SE, $n = 9$) than during inactivity (paired *t*-test: $t = -5.66$, $df = 8$, $P < 0.001$). The increase in heart rate during activity was, however, rather variable, ranging from 1.3 to 2.2 times the rate when inactive (Fig. 3). The available data are not sufficient to clarify if this variability was due to the inclination of the substrate or to other factors (temperature, size).

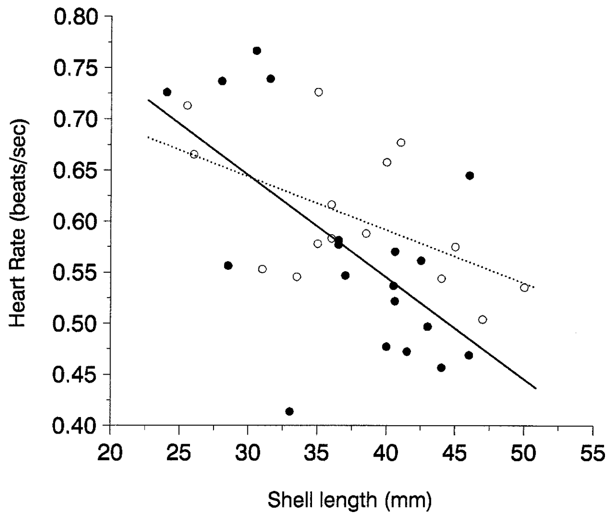

Fig. 2 *Patella vulgata*. Relationship between heart rate and individual's shell length during submersion in limpets on horizontal (*open symbols*, *dotted line*) and vertical (*closed symbols*, *continuous line*) rock surfaces

Discussion and conclusions

The heart rate of *Patella vulgata* inhabiting the shore is clearly affected by environmental temperature and body size. Moreover, these effects interact with exposure to air (low tide) and to submergence (high tide) and vary between limpets resting on vertical and on horizontal substrates.

The relationship between heart rate and temperature observed during emersion, both on vertical and horizontal substrates, agrees with the temperature dependence of the metabolic rate of *Patella vulgata* assessed by measurement of the oxygen consumption of limpets

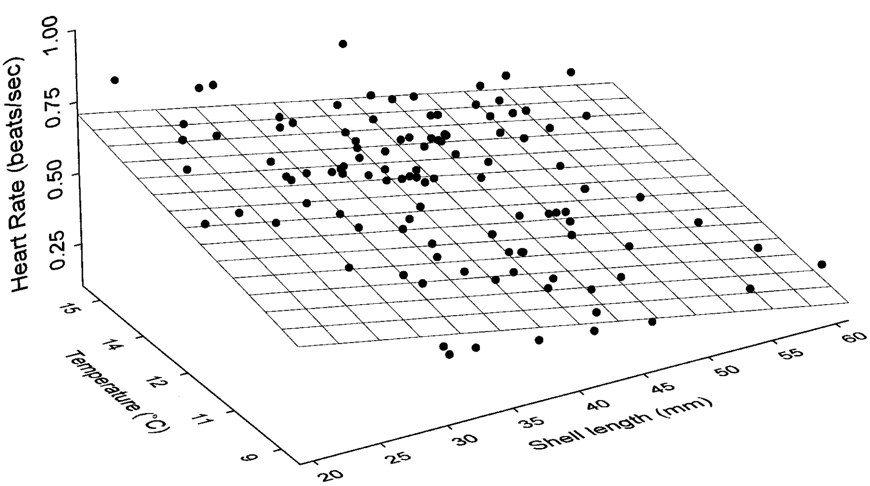

Fig. 1 *Patella vulgata*. Effect of temperature and body size on the heart rate of limpets exposed to air. Observed values (*dots*), predictions from multiple regression (*grid*)

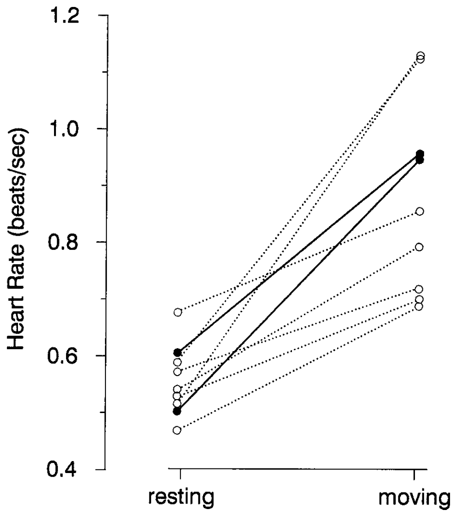

Fig. 3 *Patella vulgata*. Heart rate measured during inactive periods and during spontaneous activity (n = 9 limpets). Limpets on horizontal (*open circles, dotted line*) and vertical (*closed circles, continuous line*) rock surfaces

exposed to air in the laboratory (e.g. Davies 1966). On the other hand, the lack of a significant correlation of heart rate with temperature in submerged limpets from both vertical and horizontal substrates is not surprising given the narrow thermal range of the sea water during the tests.

The negative relationship between cardiac activity and body size in limpets exposed to air (independent of the inclination of the substrate) and in submerged limpets from vertical substrates agrees with the size dependence of heart rate and oxygen consumption observed in other intertidal invertebrates (Newell 1979), including limpets (Davies 1966, 1969; Branch 1981; Dye 1987). Such a dependence is expected on the basis of Hemmingsen's (1960) equation for the size dependence of metabolic rate.

The lack of a statistically significant correlation between heart rate and size in limpets resting on horizontal substrates during submersion could be due to an increase in the variability of the readings, as a consequence of limpets becoming active when covered by the flooding tide. Visual inspection showed that some of these individuals lifted their shell from the substrate and started ventilation movements (see also Daniel 1980; Williams and Morritt 1991) a few minutes after being covered by the incoming tide. On the contrary, such behaviour was not observed in limpets resting on vertical substrates, at least with the same immediacy after submergence. This finding also agrees with behavioural observations; Williams et al. (1999) found that limpets inhabiting horizontal surfaces were mainly active during diurnal high tide, while limpets from vertical substrates were mostly active during nocturnal low tides (see also Hawkins and Hartnoll 1982). However, we must point out that even though the regression line relating heart rate to body size in specimens resting on horizontal surfaces was not significant, the line had a negative slope and the observed lack of fit could be due to the small sample size (n = 16).

No extensive reports on heart rate variations in *Patella vulgata* are available for comparison, the only existing being those of Jones (1968), which only include a limited number of observations. More direct comparisons between the temperature or size dependence of heart rate and the same sort of relationships observed for oxygen consumption are difficult to infer. In this species, in fact, dependence of respiration rates on temperature changes or body size has been shown to be influenced by other factors such as acclimation temperature and food availability (e.g. Davies 1966, 1969; Wright and Hartnoll 1981), rendering direct comparison difficult.

Differences in oxygen consumption between emersed and submerged animals have been described for several limpet species (see Hughes 1986; McMahon 1988 for reviews). Contrasting evidence has been found for the limpet *Patella vulgata*. Higher oxygen consumption rates during exposure to air were described by Houlihan and Newton (1978), whereas significantly higher oxygen consumption rates during submersion were reported by Gompel (1938, quoted in Houlihan and Newton 1978) and by Wright and Hartnoll (1981). Moreover, "a slight, but significant, increase [in heart rate] when the tide was in" was reported by Jones (1968). The present study supports the latter findings, since at similar temperatures, inactive submerged limpets showed a slightly, but significantly, faster heart rate than emerged animals.

Finally, this study revealed a marked effect of activity. During movements on or away from their home scar, limpets' heart rates increased by ~1.6 times their resting levels. This increase agrees with field data on the cardiac activity of the Mediterranean limpet *Patella caerulea*, in which a 1.5- to 1.7-fold increase in heart rate was observed during locomotion (Santini et al. 1999). These values are similar to those found in other gastropods (e.g. trochids and nassarids: Crisp 1979; Houlihan and Innes 1982), but are lower than those reported for littorinids (Newell and Roy 1973).

In general, our in situ observations on the dependence of the heart rate of *Patella vulgata* on temperature, size, respiratory medium and activity agree with previous findings on the effects of the same factors on oxygen consumption of limpets. This lends further support to the conclusions drawn from laboratory studies on other *Patella* species, which showed that heart rate can reliably be used to assess metabolic activity (Marshall and McQuaid 1992; Santini et al. 1999). Despite this, no direct evidence is available for *P. vulgata* and all the above inferences should be considered with caution.

Our results did not provide conclusive evidence to support the hypothesis that differences in energy costs between limpets from horizontal and vertical substrates contribute to the differences in foraging patterns

(Williams et al. 1999), since the recordings were mostly from resting limpets. In fact, the observed difference in heart rates between vertical and horizontal inactive limpets during submergence seems to be a consequence of the difference in foraging patterns shown by the two groups, rather than its cause. Moreover, the number of recordings from active animals was not sufficient to permit comparisons among media and substrates.

The current method allows a large amount of data to be collected directly in the natural habitat, with minimal disturbance to the experimental animals. Once it has been established that heart rate variations can be used to infer metabolic rate, this technique could be employed to derive realistic, natural assessments of metabolic costs. Such data could be used with greater confidence than laboratory-derived estimates and would help to refine current foraging models (e.g. Burrows et al. 2000) and the assessment of individual and population energy budgets (e.g. Wright and Hartnoll 1981).

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