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Field and laboratory measurement of heart rate in a tropical limpet, *Cellana grata*

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Heart rate of the tropical limpet *Cellana grata* was monitored on the shore (Cape d'Aguilar, Hong Kong) and in the laboratory using a non-invasive technique. Individual field measurements performed on inactive limpets, in a variety of thermal conditions during a diurnal low tide, showed a general increase in heart rate with increasing body temperature. This relationship was not always evident when monitoring individual responses over a diurnal low tide period, since under some circumstances, heart rate of individuals decreased with increasing the temperature of the substrate and foot. A factorial laboratory experiment showed that heart rate was faster at higher temperatures but slower in larger animals. The combined evaluation of field and laboratory data suggests that limpets in some habitats may be able to regulate their metabolic rate when resting on hot rock substrates.

Cellana grata Gould, a non-homer limpet abundant in the mid–high intertidal on exposed rocky shores in Hong Kong (Williams, 1993), forages whilst awash, and remains inactive during low tide. Williams & Morritt (1995) compared body and environmental temperatures, and haemolymph osmolality, of inactive limpets of this species from a variety of sites, showing that selection of certain habitats can reduce thermal and desiccation stress. On the contrary, resting in suboptimal refuges can result in limpet mortality due to thermal stress and dehydration (see also Williams & McMahon, 1998). The observation that metabolic rate of limpets increases with increasing temperatures (e.g. Branch et al., 1988; Marshall & McQuaid, 1994), suggests that exposure to sublethal high temperatures may modify metabolic rates of *C. grata*, therefore affecting energetic costs. Since heart rate of gastropods has been demonstrated to be almost linearly correlated to oxygen consumption, and hence metabolic rate (Marshall & McQuaid, 1992), heart rate and thermal variation was investigated in limpets inactive in different sites on the shore during a hot emersion period, and in limpets of different size exposed to different ambient temperatures in the laboratory. Investigations were performed at Cape d'Aguilar, Hong Kong, in October 1997.

Heart rate was monitored in the field and in the laboratory using a method similar to that introduced by Depledge & Andersen (1990). The sensor consisted of an infrared (IR) light emitting diode axially coupled with a phototransistor, fixed onto the limpet shell using a removable adhesive (Blue-Tac, Bostick Ltd), in a position directly over the heart. The IR light emitted by the diode is reflected by the internal parts of the limpet and generates a current on the phototransistor. Variations in the light-dependent current produced by heart beat were amplified and filtered, and recorded using a portable oscilloscope (Fluke 105B). The whole system was battery powered. Body temperature was monitored using Omega K-type Teflon coated thermocouples (diameter 0.25 mm) connected to a portable digital thermometer (Fluke 52). The thermocouple was inserted through the gaps between the shell margin and the substrate, without damaging the animal, so as to touch the foot of the limpet just above the substrate (see Williams & Morritt, 1995).

Fieldwork was conducted on west facing, moderately exposed shores within the Cape d'Aguilar Marine Reserve. Heart rates of 27 limpets of different size (shell length 23.7–38.9 mm) and the temperature of the foot were monitored on the shore under sunny conditions during the diurnal low tide (0900–1300 hours), when limpets were completely exposed to air. Some of the limpets were resting in shaded habitats (N=13) whilst others were exposed to direct sunlight (N=14). About 5 min after placing the sensor, heart rate of each limpet was recorded for a period of ~3 min. Limpets resting in the shade had an average body (foot) temperature lower (29.9 ± 1.0°C) than those exposed to direct sunlight (35.0 ± 2.1°C). Heart rate (HR, Hz) showed a significant positive relationship with body temperature (T_b) (N=27, r=0.713, $P<0.001$; regression line: HR$=-1.581 + 0.098 \times T_b$; Figure 1). When considering animals in the shade

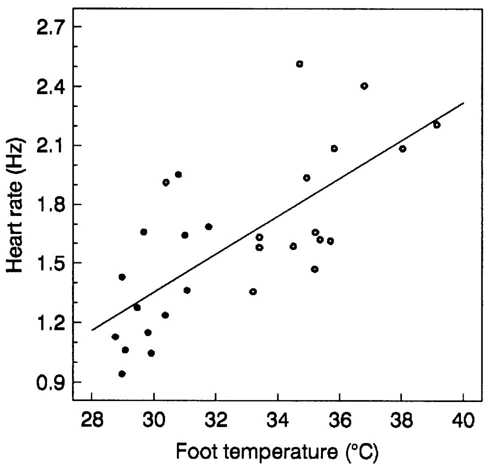

Figure 1. Relationship between heart rate (Hz) and foot temperature in *Cellana grata* on the shore at Cape d'Aguilar, Hong Kong. (●), limpets inactive in the shade; (○), limpets exposed to direct sunlight.

and direct sunlight separately, however, a significant correlation between HR and T_b exists only for limpets in the shade (N=13, r=0.638, $P<0.02$), but not for limpets in the sun (N=14, r=0.425, $P>0.1$). No effect of size on heart rate in the field was detected from the entire field data set (N=27, r=0.004, $P>0.98$) or for limpets from the two separate habitats. Moreover, two limpets from a west facing vertical rock (30.6 and 35 mm shell length) and two from a south facing rock (32.9 and 33.7 mm shell length) were continuously monitored during a diurnal low tide (Figure 2). Foot temperature of animals on the west wall was higher during the afternoon (1330–1600), when directly exposed to the sun, than in the morning (1030–1300), and their heart rate increased accordingly during the afternoon. Foot temperature of south facing limpets was high during the morning when they were exposed to direct radiation from the sun, and continued to increase during the afternoon despite animals being shaded. Heart rate in these animals was, however, higher during the morning, and decreased as direct sun radiation ceased.

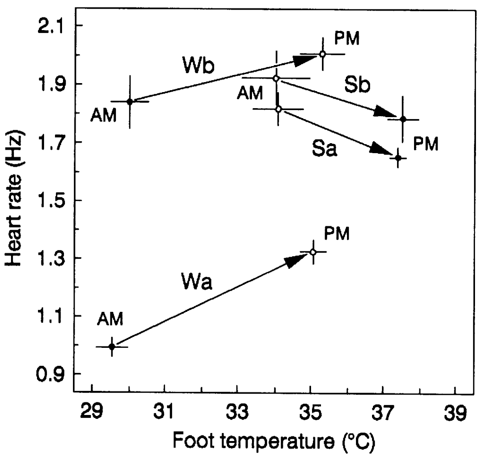

Figure 2. Variation in the heart rate and foot temperature of individual *Cellana grata* during a diurnal low tide at Cape d'Aguilar, Hong Kong. Wa &Wb, two limpets from a west facing rock; Sa & Sb, two limpets from a south facing rock. (●) and (○), average individual heart rate (ordinate) and foot temperature (abscissa) recorded when limpets were in the shade and when exposed to direct sunlight, respectively. Vertical and horizontal segments indicate standard error. AM, 1030–1300 hours; PM, 1330–1600 hours.

Laboratory experiments were performed in the Swire Institute of Marine Science, close to the field study site. Limpets were collected from a south facing, moderately exposed shore. On two different days 40 limpets, 20 small (shell length 18.7–24.5 mm) and 20 large (29.5–43.7 mm) were collected whilst active and awash by the tide, placed on plastic trays and immediately transferred to the laboratory. Limpets of each size class were randomly assigned to two groups (cold and warm) which were maintained at 21.3 ±1.5°C and 27.8 ±1.5°C, respectively, for 90–180 min prior to and during testing. The warmer temperature was close to the average daytime air temperature (shade) experienced by the limpets in the field during this period. The average individual heart rate was computed as above. Small limpets showed a faster heart rate than larger animals in both cool and warm conditions, and in both small and large limpets exposure to the lower temperature produced a decrease in heart rate as compared to animals maintained at the higher temperature (Table 1). Two-way ANOVA (Table 2) showed a significant effect of temperature and size on heart rate, but no combined effect of the two factors.

In synthesis, laboratory results for *C. grata* agree with the general finding that, in limpets, heart rate is related to size and temperature (see references above). Field recordings, however, showed that this relationship, significant for limpets resting in shaded habitats, was less clear for individuals exposed to direct sunlight, and that there was no relationship between heart rate and size for limpets on the shore. Moreover, under some circumstances, heart rate of individuals decreased with increasing the temperature of the substrate and foot. The relative independence between heart rate and foot temperature sometimes observed in the field suggests that in *C. grata* this relationship may not be linear along the whole range of possible temperatures experienced in the natural environment, as observed in *Patella caerulea* (Bannister, 1974) and in different South African *Patella* spp. (Branch & Newell, 1978). Occasionally bradycardia independent

Table 1. *The effect of size and temperature on the heart rate of* Cellana grata *in the laboratory (cold ~21°C; warm ~28°C; small average shell length ~22 mm; large ~34 mm). Mean rate, standard deviation, minimum and maximum values are in Hz.*

	Small		Large	
	Cold	Warm	Cold	Warm
N	10	10	10	10
Mean rate ±SD	1.111 ±0.162	1.663 ±0.221	0.883 ±0.101	1.299 ±0.155
Min	0.882	1.303	0.761	0.997
Max	1.341	1.974	1.117	1.503

Table 2. *Two-way ANOVA to compare heart rates of* Cellana grata *(large and small) at different temperatures (warm and cold). Variances were homogeneous (Cochran's C-test=0.449,* P*=0.140).*

Factors	Sum of squares	d.f.	Mean square	F-ratio	Significance level
Size	0.878	1	0.878	32.142	$P<0.001$
Temperature	2.341	1	2.341	85.692	$P<0.001$
Size × Temperature	0.047	1	0.047	1.708	$P>0.1$
Residual	0.983	36	0.027		

of temperature have been reported in the pulmonate limpet *Siphonaria oculus*, which normally shows a positive correlation of heart rate with ambient temperature (Marshall & McQuaid, 1994).

A second explanation for the apparent occasional independence of heart rate from body temperature might be that, if direct solar radiation is not too strong, the cardiac region of *C. grata* can be maintained at a lower temperature than the foot, which could be achieved by cooling the haemolymph before it enters the heart, possibly by evaporating water at the gills. The finding that temperatures of the foot and the visceral mass in the very dorsal region of *C. grata* are similar (Williams & Morritt, 1995) does not contrast with this hypothesis as evaporative cooling at the gills could just affect the cardiac region, while the dorsal part of the visceral mass could maintain higher temperatures due to direct solar radiation. Evaporative cooling may be achieved by *C. grata* lifting the shell above the substrate ('mushrooming') and exposing the foot and pallial tentacles to air when stressed by high temperatures (Williams & Morritt, 1995; Williams & McMahon, 1998). In conclusion, these preliminary data suggest that although metabolism of *C. grata* is strongly dependent on temperature, as in other limpets, this species may have developed physiological mechanisms allowing a partial control of the increase in metabolism when resting on hot substrates, which would presumably be extremely adaptive for animals living high on tropical shores.

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