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The use of cardiac monitoring in the assessment of mercury toxicity in the subtropical pebble crab *Gaetice depressus* (Brachyura: Grapsidae: Varuninae)*

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SUMMARY: Heart rates were monitored in the pebble crab, *Gaetice depressus* using a non-invasive, computer aided cardiac monitoring system. A high degree of intra- and interindividual variability was observed, as was the presence of endogenous circatidal and circadian rhythms. Both acute and sublethal toxicities of mercury were determined. LC_{50}'s (96) for mercury were between 0.16-0.20 mg l^{-1}. Exposure to $HgCl_2$ above LC_{50} (96) (0.3 mg Hg l^{-1}) resulted in rapid and statictically significant increases in heart rate whereas exposure to a concentration lower than LC_{50} (96) (0.1 mg Hg l^{-1}) resulted in progressive reduction in heart rate similar to that of control crabs.

Key words: cardiac activity, mercury, pebble crab, *Gaetice depressus*, endogenous rhythms.

INTRODUCTION

The pebble crab, *Gaetice depressus* (De Haan), is a small crab (max. carapace width: 25 mm) found in subtropical regions. It lives along shores, in the intertidal zone (Kikuchi *et al.*, 1981), and is known to express a circatidal rhythm of behaviour (Depledge, 1989). Earlier studies on marine and freshwater decapods have demonstrated that variations in locomotive activity are related to variations in physiology e. i. variations in oxygen consumption and heart rates (Fingerman and Lago, 1957; Aagaard *et al.*, 1995; Styrishave *et al.*, 1999). It is therefore possible that the expression of circadian and circatidal behaviour observed for *G. depressus* is coupled to circadian and circatidal variations in heart rate and oxygen consumption.

Several heavy metals are known to influence the expression of rhythmic physiology and behaviour in decapod crustaceans (Depledge, 1984; Styrishave and Depledge, 1996). In general, these metals work by stimulating heart rates, oxygen consumption and locomotive behaviour. So far, however, primarily temperate species have been investigated with respect to their response to pollutants. There is, therefore, a lack of information concerning the ecotoxicology of subtropical and tropical species. It is commonly believed that subtropical species are more sensitive to pollutants due to higher tempera-

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tures which presumably results in higher metabolic rates and higher metal uptake rates. However, this assumption needs further investigation, especially in developing countries in subtropical and tropical regions, which face rapid expansion of industrial manufacturing output at a time when lack of resources, expertise and infrastructure limit their capability to assess pollution threats.

In the present study, heart rates were monitored in the pebble crab *Gaetice depressus* to investigate the general levels of cardiac activity and the physiological expression of endogenously modulated circatidal and circadian rhythms. Also, heart rates were measured during sublethal exposure to mercury ($HgCl_2$) to examine any metal induced changes in cardiac activity. In addition, the toxicity of mercury has been determined to permit comparison with data for temperate decapod species.

MATERIALS AND METHODS

Gaetice depressus were collected by hand in the intertidal zone of the beach adjacent to the Swire Institute of Marine Sciences, Hong Kong. The animals were held in the laboratory in aerated seawater (34‰ salinity; 27±1°C), prior to initiating the experiments. Unless otherwise stated, the crabs were maintained in a natural light regime (15h light: 9h dark).

Estimation of LC_{50}'s (96) for mercury

Five glass tanks (10 litres capacity) were filled with aerated seawater (34‰ salinity; 27±1°C). 20 crabs were then placed in each tank (17 males and 3 females - mean carapace widths = 17±3 mm; total number of crabs = 100). Four concentrations of $HgCl_2$ (0.1, 0.3, 0.8, and 1.8 mg Hg l^{-1}) were made up in the tanks. The fifth tank was used to hold control animals in clean seawater. Deaths occuring during the experiment were recorded daily and bodies removed. LC_{50} (96) was the concentration at which 50% of all crabs died within 96 hours.

Cardiac monitoring

Preliminary investigations of cardiac activity were conducted with adult male *Gaetice depressus* (ca. 20 mm carapace width). The crabs were held in constant darkness in individual 200 ml perforated containers and submerged in a continuous flow of clean seawater (34‰ salinity; 27±1°C). A layer of coarse gravel (ca. 2 cm deep) was added to each container as contact with sediment is known to influence the resting behaviour and cardiac activity of crabs (Florey and Kreibel, 1974). Heart rates were recorded using an infra-red technique developed by Depledge and Andersen (1990), and later refined by Aagaard *et al*. (1991). The technique involved affixing an infra-red emitter/detector to the dorsal carapace in the cardiac region. Data was recorded automatically for up to 72 hours and stored on diskette for later analysis. Heart rate time series for each individual were analysed for the presence of free running rhythms using periodogram analysis (Williams and Naylor, 1978).

Short-term mercury exposure

Cardiac responses to mercury exposure were examined in individual *G. depressus*. Using the technique described above, heart rates were recorded during a 24h control period and then for a further 24h during exposure to mercury. The crabs were held individually in 200 ml perforated containers (without sediment) in constant darkness and submerged in a tank containing 10 l aerated seawater (34‰ salinity; 27±1°C). Four animals were simultaneously used as controls whilst being maintained in clean seawater. Two groups of six crabs were exposed to 0.1 and 0.3 mg l^{-1} $HgCl_2$ respectively. Since recorded heart rates were not normally distributed even after standard transformation, nonparametric Mann-Whitney U-test was employed to detect significant changes (Zar, 1984).

RESULTS

Heart rates (beats min^{-1}) recorded for 72 hours from 8 *Gaetice depressus* under constant conditions were not normally distributed, even after standard transformation. Therefore, time series data are presented as medians and ranges (Table 1). Median heart rates for individuals were between 75-190 beats min^{-1}. However, most values were in the range of 80 to 100 beats min^{-1} (Table 1). Variability within each time series was high with heart rates ranging from 0 to 395 beats min^{-1}. The time series data were reduced by conversion to average heart rates (beats min^{-1} recorded over successive 20 minute periods). This was undertaken to facilitate periodogram analysis. Both circatidal and circadian endogenous

TABLE 1. – Heart rates as beats min^{-1} in adult male *Gaetice depressus* under constant conditions.

Median	Min	Max
75	23	297
84	16	286
88	16	340
92	48	366
189	80	395
102	0	405
89	6	368
100	53	358

rhythms were evident in three of the eight time series recorded, as well as combinations of the two (Fig. 1). Heart rates were found to be higher at expected high tide than at expected low tide. Similarly, heart rates were higher during night than during day.

LC_{50}'s (96) were between 0.16-0.20 mg l^{-1} for mercury. Time series of heart rates for the 24 h periods prior to and following the addition of the two different concentrations of mercury are shown in

TABLE 2. – Median and range of heart rate (beats min^{-1}) in adult male *Gaetice depressus* before and during exposure to mercury. Positive Z-values indicate an increase in heart rate after metal exposure. (P): significance level.

[Hg]	Before exposure		After exposure		Z	P
mg l^{-1}	Median	Range	Median	Range		
0.3	59	4-177	64	0-228	9.8	< 0.001
	92	70-146	114	53-338	17.4	< 0.001
	143	72-430	254	11-428	13.2	< 0.001
	91	75-291	114	26-291	12.5	< 0.001
	98	36-327	108	38-355	5.4	< 0.001
0.1	201	67-335	237	135-340	11.5	< 0.001
	98	58-255	74	57-217	-25.4	< 0.001
	98	60-247	70	41-168	-26.7	< 0.001
	218	85-433	195	104-353	-20.5	< 0.001
	118	49-317	100	61-321	-9.9	< 0.001
	164	97-342	128	108-317	-19.5	< 0.001
Control	107	0-286	69	0-267	-24.1	< 0.001
	89	62-256	74	56-214	-17.4	< 0.001
	83	0-239	83	38-251	2.95	= 0.003
	91	63-261	82	59-264	-15.1	< 0.001

Fig. 2. The non-parametric Mann-Whitney U-test was used to compare heart rate data obtained during periods in clean seawater with that from periods

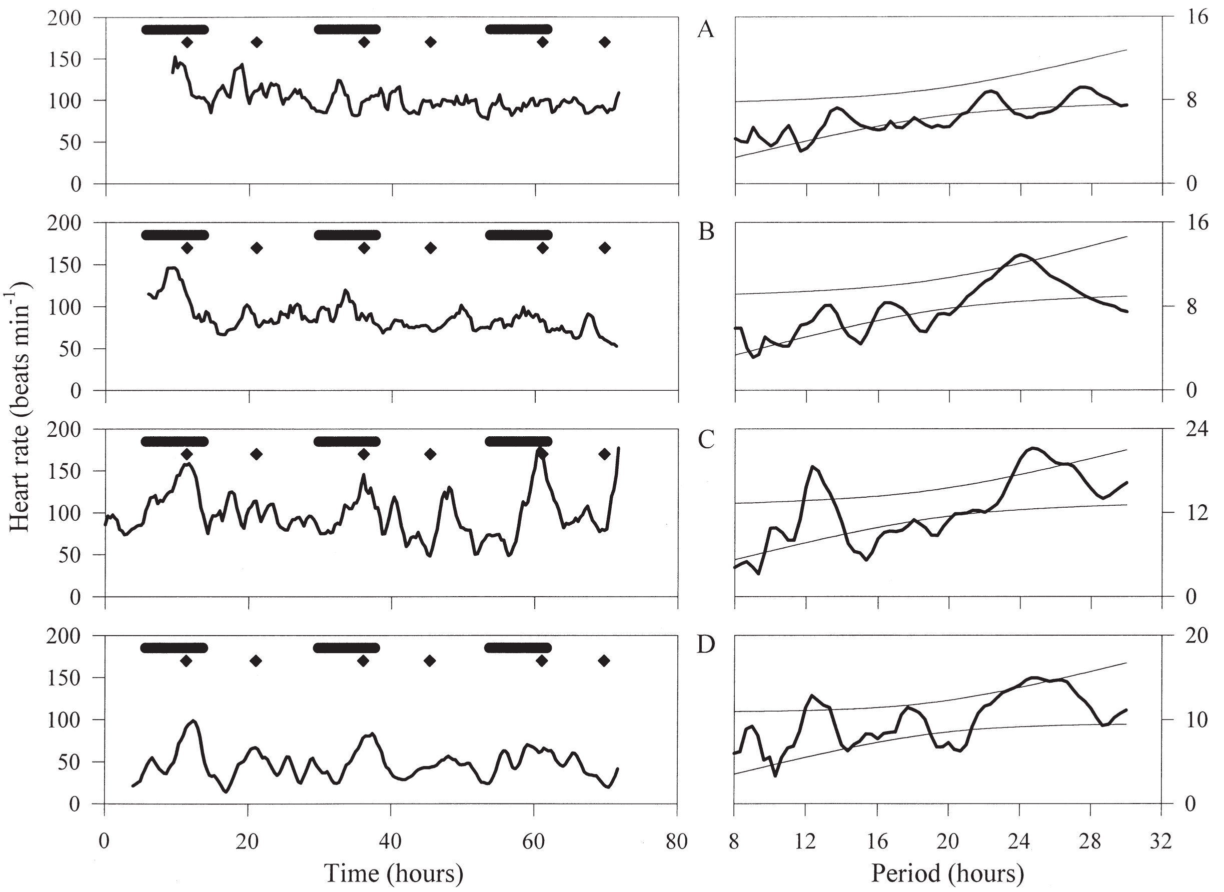

FIG. 1. – Time series and periodogram analysis of cardiac activity in male *Gaetice depressus* under constant conditions. Examples of a crab without endogenous rhythmicity (A), with circadian rhythmicity (B), with circatidal rhythmicity (C) and with circadian modulation of a circatidal rhythmicity in heart rate (D). (u): expected high tide; (nnn): expected night time. Thin lines in periodograms denote ±95% confidence intervals (see Williams and Naylor, 1978 for details).

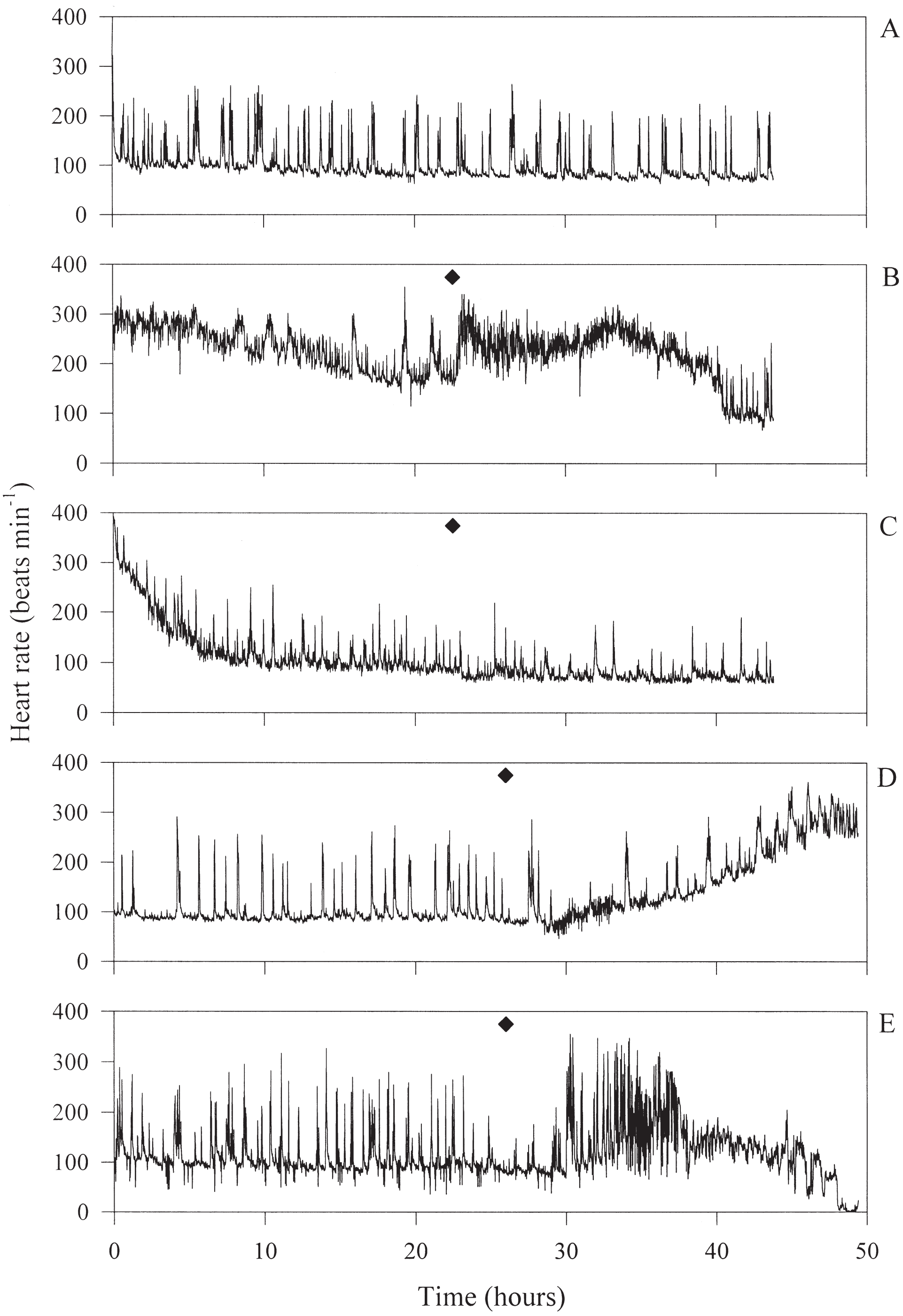

FIG. 2. – Cardiac activity over 48 hours in male *Gaetice depressus* before and during exposure to mercury. Control crab (A), crabs exposed to 0.1 mg Hg l^{-1} (B+C) and crabs exposed to 0.3 mg Hg l^{-1} (D+E). (u): start of mercury exposure.

with mercury exposure. Medians and ranges prior to, and following exposure are shown in Table 2. Positive Z-values denote heart rate increases. All five crabs exposed to 0.3 mg Hg l^{-1} responded with significant increases in heart rate while only one of the individuals exposed to 0.1 mg Hg l^{-1} exhibited such a response. In the remaining crabs exposed to 0.1 mg Hg l^{-1}, heart rate decreased significantly following mercury exposure. Similar decreasing trends were observed in animals in the control group. The wide range of heart rates shown in Table 2, both before and after mercury addition reflect the high degree of temporal variation in individual heart rates.

DISCUSSION

The LC_{50}'s (96) recorded values are similar to those reported for *Carcinus maenas* from temperate regions, but are slightly higher than those reported for many other decapod crustaceans (Mance, 1987). These data suggest that the sensitivity of subtropical *G. depressus* exposed to trace metal toxicity in ambient conditions is similar to that of temperate decapods. This might be interpreted as preliminary evidence supporting the view that toxicity test data obtained using temperate organisms exposed in test conditions appropriate to the temperate environment, may retain validity when making predictions regarding the toxicity of chemicals to subtropical and tropical organisms. However, until a great deal more evidence has been collected for diverse species with a wide range of chemicals, it would be unwise to base managerial strategies on such findings.

Heart rates recorded during the present study are consistent with earlier reports for *G. depressus* and for similar sized individuals (Depledge, 1986). In *G. depressus* Depledge (1986) reported a mean heart rate of 214±7 beats min^{-1} at 25°C. In the present study, a mean heart rate of 75-189 beats min^{-1} was observed at 27°C, with minimum and maximum heart rate ranging from 0-405 beats min^{-1}. The high degree of intra- and inter-individual variability in heart rates observed for *G. depressus* in the present study is common in marine decapods but appears to be less common in freshwater decapods. This is probably due to periods of cardiac arrest (Depledge, 1984) a phenomenon not yet observed in freshwater decapods.

Continuous recordings of heart rate of *G. depressus* in constant conditions revealed the presence of endogenous heart rate rhythms. Previously, Depledge (1989) presented preliminary evidence of tidal and diurnal feeding activity rhythms in this species. Monitoring heart rate may therefore be a useful method to investigate general rhythmic behaviour. The expression of rhythms is an important consideration in relation to the execution of both lethal and sublethal toxicity tests. If *G. depressus* with rhythms intact were to be used in testing programmes it is likely that individuals at different stages in the rhythm cycle would respond differently. Recent work with the shore crab *C. maenas* indicates that crabs are less susceptible to toxic agents when exposed in the low activity phase of the rhythmic cycle compared with exposure during the high activity phase (Depledge, unpubl.). Disturbance of biological rhythms in cardiac activity apparently occurs at similar mercury concentrations in *C. maenas*, *Potamon potamios* and *Astacus astacus* (Depledge, 1984; Styrishave and Depledge, 1996).

This study is the first occasion on which the infra-red cardiac monitoring technique has been applied to *G. depressus*. Heart rate data was readly obtained with minimal disturbance of test animals, despite the fact that the individual crabs used were much smaller than representatives of other species used in earlier studies. The fact that only three out of eight individuals expressed endogenous rhythms may indicate that the pebble crabs were restrained or otherwise disturbed during the experiments. However, studies on other decapod crustaceans demonstrates that not all individuals in a population express endogenous biological rhythms at any given time (Aagaard *et al.*, 1995; Styrishave *et al.*, 1999), which testifies to the reliability and versatility of the technique.

Cardiac activity altered during exposure to 0.3 mg Hg l^{-1}, with heart rate increasing in each individual at times when it would normally have been expected to decline due to acclimatization and starvation (Ansell, 1973; Depledge, 1985). At lower exposure concentrations (0.1 mg Hg l^{-1}) and in control conditions heart rate declined as expected. It is likely, however, that a longer exposure period (several days) to 0.1 mg Hg l^{-1} or lower concentrations would result in a stimulation of heart rates as has been observed for other decapods (Depledge, 1984; Styrishave and Depledge, 1996). It is interesting to note that exposure to 0.1 mg Hg l^{-1} being lower than the observed LC_{50} (96) has no apparent influence on the heart rate within 24 hours, whereas exposure to 0.3 mg Hg l^{-1} which is slightly above the LC_{50} (96) observed for *G. depressus* results in an increase in heart rate within the same period of time.

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