HELGOLÄNDER MEERESUNTERSUCHUNGEN
Helgoländer Meeresunters. 33, 59–67 (1980)

Cardiac and ventilatory responses of *Crangon crangon* to cadmium, copper and zinc

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ABSTRACT: The acute (30 min) responses of heart and scaphognathite activities of *Crangon crangon* on exposure to concentrations of 1–20 mg Cd, Cu or Zn l^{-1} are increased beat frequencies. The relative magnitude of response *(△f)* is linearly related to immediate pretreatment frequency *(f)* and standardised responses *(△fs)* are given for *f* values of 70 and 100 beats min^{-1} for hearts and scaphognathites, respectively. △*fs* values for each organ are also linearly related to test concentration for each metal. Qualitative changes to organ activities described include an increased incidence of scaphognathite reversals in concentrations of 5.0 mg Cu l^{-1} and in 20.0 mg Zn l^{-1}. Chronic (13 days) exposure to incipient lethal levels of the test metals produced increases in scaphognathite rates of the Cd-treated animals and in heart and scaphognathite rates of the copper-treated animals. The general applicability of these methods to studies of pollution stress in decapods is discussed.

INTRODUCTION

Death is an unequivocal endpoint, but a crude index of stress, in pollution studies. Because many metals are known to exert their toxic effects by acting as non-specific enzyme inhibitors (see Dixon & Webb, 1964) it is reasonable to assume that measurement of altered metabolic status would be a more sensitive index of pollutant stress on an animal. Commonly, oxygen-consumption changes have been used to this end. Brown & Newell (1972) and Scott & Major (1972) found that copper depressed oxygen consumption in *Mytilus edulis* but Collier et al. (1973) found no change in oxygen consumption of *Eurypanopeus depressus* on exposure to high levels of cadmium. Similar results of the effects of cadmium on *Palaemonetes pugio* led Vernberg et al. (1977) to conclude that respiration rates (as O_2 consumption) were not "predictable and reliable" indices of cadmium pollution.

Because of their intimate role in the satisfaction of oxygen demands in decapod crustaceans, cardiac and ventilatory activities have received recent attention as potential indicators of metabolic rate variability. The possibility also exists that such organ activities may show variations from "normal" patterns when the animals are exposed to pollutants.

Impedance techniques have been used successfully to record heart and scaphognathite activities in *Carcinus maenas* (Cumberlidge, 1977; Cumberlidge & Uglow, 1977a, b, 1978) and *Crangon crangon* (Dyer & Uglow, 1977, 1978a, b). An advantage of these techniques is that they allow organ activities to be monitored using non-invasive techniques and with minimal restriction of the normal repertoire of whole animal movements.

 0017-9957/80/0033/0059/$ 02.00

The aim of these studies was to carry out preliminary investigations of the effects of cadmium, copper and zinc on the cardiac and ventilatory behaviour of *Crangon crangon* (L.) and to assess the potential use of such activities as indicators of pollution stress.

MATERIALS AND METHODS

Animals in moult stages C_b, D_0 and D_1 and within the size range 55–60 mm body length (tip of rostrum to tip of telson) were selected for these studies. The larger shrimps are easier, technically, to wire up with electrodes and have a relatively longer intermoult period than smaller animals. The left scaphognathite only was monitored as Dyer (unpublished) demonstrated an almost constant bilateral synchrony of the scaphognathites in *Crangon crangon*. Details of electrodes and their attachment have been described elsewhere (Dyer & Uglow, 1977). Animals were left for four complete days to recover from electrode affixation, before recordings were made.

Impedance techniques were used to record organ activities; the transduced outputs were fed to an automatic timer/counter and recorded as a digital printout of elapsed beats within a preselected time period. Outputs were, when necessary, produced also as a pen trace.

The acute responses to cadmium, copper and zinc were determined under conditions of static seawater (17 °C; 34 ‰ S). The experimental tanks (39 × 17 × 19.5 cm) were subdivided by perforated "Perspex" partitions and contained a 2-cm layer of sand and, initially, 2 l of aerated seawater. Test metals were added by syphoning 2 l of test solution into the experimental tank so that required concentrations were achieved in a total of 4 l of seawater. Metals (as sulphates) and sodium sulphate for controls were added giving final concentrations of 0.1, 1.0, 5.0 and 20 mg metal l^{-1}. Simultaneous recordings of heart and scaphognathite activity of each test animal were taken as 6 × 5 min of recording prior to, and from 5 min after, metal addition. Subsequently, activities were recorded again (10 min for each organ) 48 h after addition of the metals. Animals were not fed during these experiments.

The effects of chronic exposure to the test metals were determined on batches ($n = 6$) of shrimps in seawater contaminated with 0.005 mg Cd l^{-1} or 0.75 mg Cu l^{-1} or 5.5 mg Zn l^{-1}. These concentrations had been found previously to correspond to the incipient lethal levels of these metals to *Crangon* (Price & Uglow, unpublished results).

After the contaminated seawater had been added, the water in the tanks was syphoned off and replaced with more containing the same concentration of the test metal. This procedure was repeated 3–4 times to prevent dilution with uncontaminated water in the sand of the tank. During the 13 days of the experiment, the experimental and control solutions were changed in the same manner immediately after organ activities had been recorded on Days 4 and 9. Organ activities were recorded as 10-min traces/printouts at the same time of day at each recording session. All test concentrations were monitored daily, using a Perkin Elmer 103 atomic absorption spectrophotometer (AAS) coupled to a Perkin Elmer 56 chart recorder. The metal-contaminated seawater samples were diluted or concentrated so that final concentrations fell within the linear working range of the AAS. Standards and blanks were made up in artifical seawater (Analar grade reagents) and operating parameters and instrument settings used were as recommended in the Perkin Elmer handbook.

Daily values of each metal were found not to vary by more than 5 % over the experimental period.

RESULTS

The considerable individual variation of beat frequency of hearts and scaphognathites (ca. 20 fold in each case) of untreated *Crangon crangon* minimises the value of absolute rate data in comparative studies of acute responses to heavy metals. However, relative values *($\triangle f$)*, taken as the percentage change from pretreatment values *(f)* after a selected time, are more useful. Figure 1 illustrates a plot of $\triangle f$ (30 min) against f for each organ type at a particular concentration of each of the 3 metals. The linearity of these plots allows a standardised response *($\triangle fs$)* to be calculated for a chosen frequency of any organ. We have selected 70 and 100 beats min^{-1} for hearts and scaphognathites respectively – approximately mean values for a reasonably large sample of animals ($n > 50$) under these temperature/salinity conditions.

When $\triangle fs$ values for different concentrations are plotted against test concentrations, they produce response curves as shown in Figure 2. In each case there is a direct, linear increase of response within the concentration range 1–20 mg metal l^{-1}. We have not found that a sufficiently large or consistent response occurs within 30 min at concentrations of 0.1 mg l^{-1} for any of these metals so, once the threshold of response is reached, the acute response is a substantial change in organ frequencies.

At these concentrations, of course, mortalities reduce the number of animals available for analysis of $\triangle f_{48\text{-h}}$ responses. However, it is possible to comment on the trends observed. Table 1 gives the mean $\triangle f_{48\text{-h}}$ value for each organ in terms of the change from

Table 1. Forty-eight-h $\triangle f$ values of heart and scaphognathite rates calculated from the initial and the 30-min post-treatment frequencies for the various concentrations used. Values are presented as means ± standard errors

Metal	n	$\triangle f$ 48 h (beats min^{-1} ± S.E.) Heart		Scaphognathite	
		Initial	30-min post-treatment	Initial	30-min post-treatment
Cadmium (mg l^{-1})					
0.1	9	+ 27 ± 8	+ 28 ± 8	+ 58 ± 23	+ 43 ± 17
1.0	6	+ 60 ± 9	+ 40 ± 5	+ 124 ± 28	+ 61 ± 10
5.0	4	+ 127 ± 18	+ 76 ± 18	+ 246 ± 50	+ 107 ± 33
20.0	2	+ 161 ± 20	+ 56 ± 3	+ 277 ± 44	+ 37 ± 10
Copper (mg l^{-1})					
0.1	10	+ 4 ± 4	− 2 ± 5	+ 25 ± 13	+ 20 ± 14
1.0	10	+ 8 ± 4	− 32 ± 3	+ 8 ± 6	− 40 ± 3
5.0	7	+ 14 ± 4	− 26 ± 5	+ 8 ± 6	− 50 ± 3
20.0	3	+ 279 ± 44	+ 87 ± 3	+ 144 ± 10	+ 58 ± 18
Zinc (mg l^{-1})					
0.1	4	+ 2 ± 7	+ 3 ± 5	0 ± 8	− 2 ± 2
1.0	11	0 ± 3	− 22 ± 3	+ 9 ± 6	− 43 ± 3
5.0	10	+ 5 ± 5	− 27 ± 4	+ 5 ± 7	− 44 ± 5
20.0	7	+ 60 ± 12	+ 1 ± 6	+ 80 ± 23	− 20 ± 5

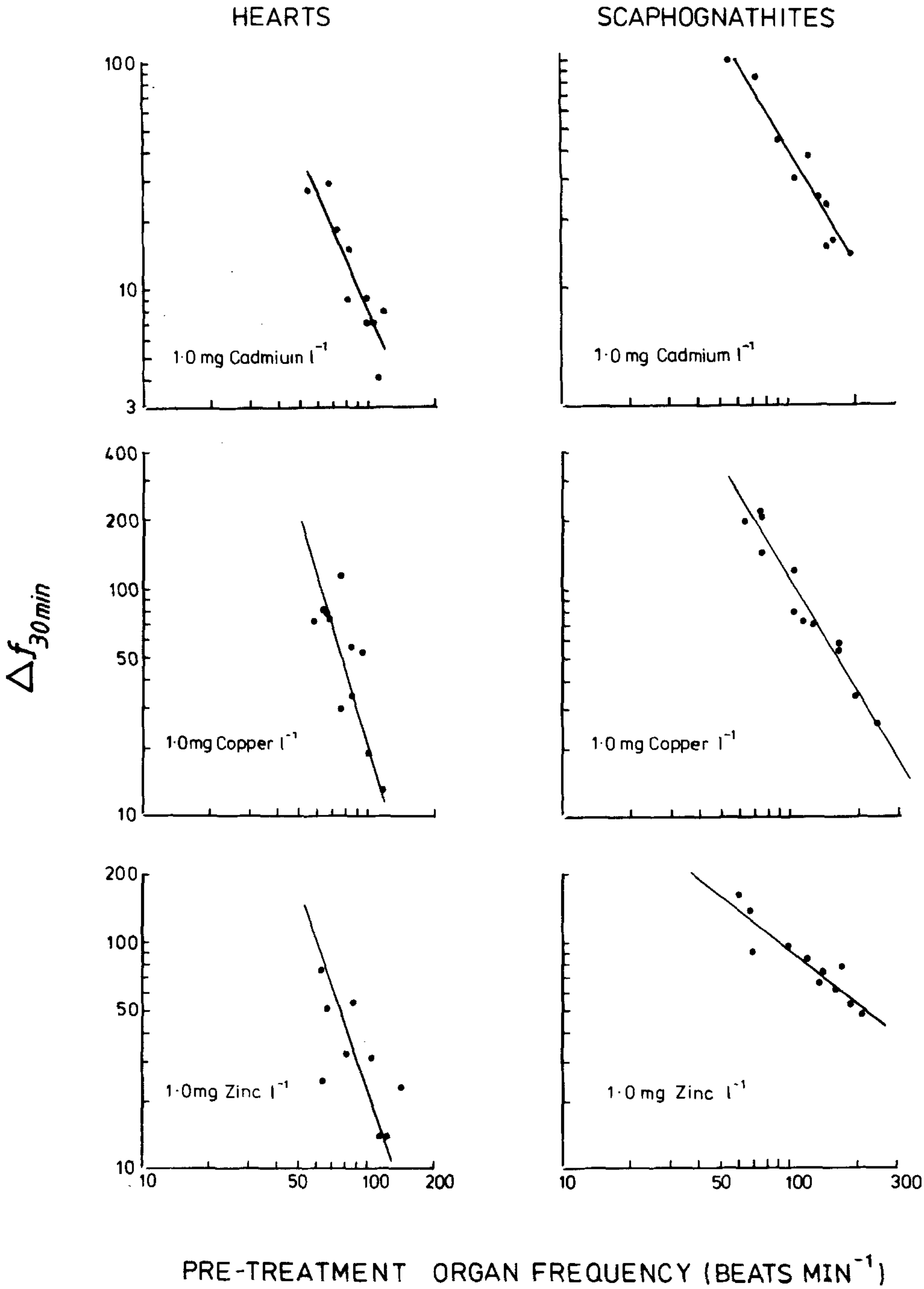

Fig. 1. *Crangon crangon* heart and scaphognathite activities: the relationship between organ responses ($f_{30\ min}$) and immediate pre-treatment frequencies *(f)* after exposure to concentrations of 1.0 mg Cd, Cu or Zn l^{-1}

(a) initial *f* values and (b) the 30-min post-treatment *f* values. In the 20 mg Cu l^{-1} solution and in all the cadmium solutions, the rates of both organs had continued to increase during the 48–h period but, in 1.0 or 5.0 mg Zn or Cu l^{-1}, rates had nearly returned to original values. After 48 h in 0.1 mg Cu l^{-1}, rates were substantially higher than the 30-min post-treatment values whilst in 20 mg Zn l^{-1} the heart rates were much the same as the 30-min post-treatment values but the scaphognathite rates were about 20 % less than the 30-min post-treatment values.

Figure 3a gives typical traces of heart and scaphognathite activity from untreated animals and shows that this species has a fairly regular heart beat and scaphognathite activity which includes frequent apnoeas (ca. 5–6 min^{-1}). On exposure to 0.1–5.0 mg l^{-1} of the metals or of Na_2SO_4, scaphognathite activity either ceases completely for 20–25 sec and then resumes at an increased rate or it displays alternate periods of apnoea and bursts (5–15 sec duration) of higher (cf. pre-exposure) frequency beating (Fig. 3b). These responses last just a few minutes usually and we interpret them as being evoked by the mechanical stimulus of adding the test solutions.

When the high concentrations (20 mg l^{-1}) of the solutions are added, the majority of animals emerge from the sand and spend 3–4 min swimming vigorously. Arrhythmic events become abolished and beat rates increase to high values (200–450 beats min^{-1}) for hearts and scaphognathites respectively – these values often persisting for 10 min (Fig. 3d.). After this initial period has passed, the copper- and zinc-treated animals showed scaphognathite activity which included bursts of high frequency beating reversals (Dyer & Uglow, 1978b). These occurred at ca. 8–10 min^{-1} (cf. < 1min^{-1} in normal, undisturbed animals), and persisted until the animals died. Regular reversals occurred also after exposure to 1.0 or 5.0 mg Cu l^{-1} (Fig. 3e). Animals treated with cadmium solutions all

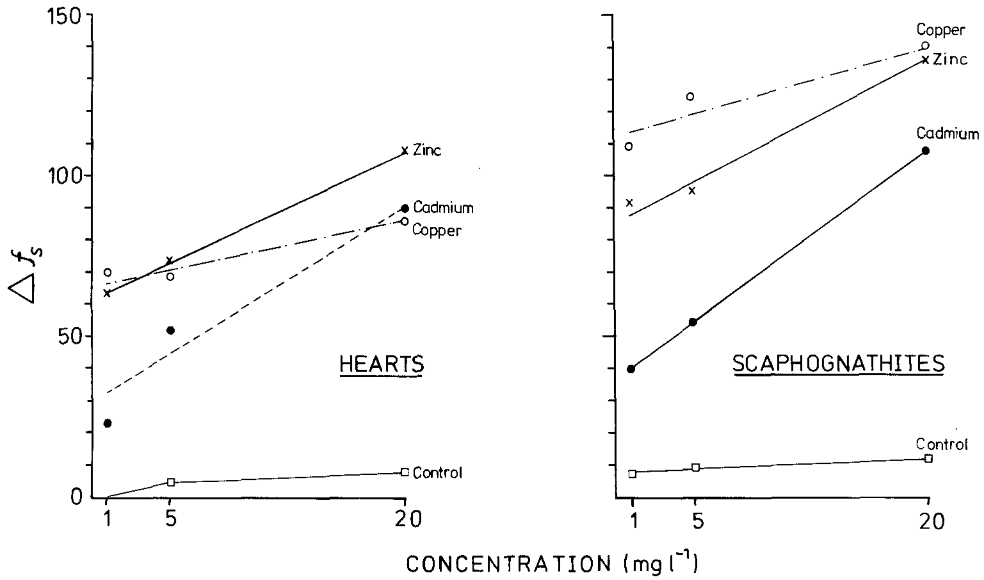

Fig. 2. Magnitude-of-response curves of hearts and scaphognathites of *Crangon crangon* after 30 min exposure to various concentrations of cadmium, copper or zinc

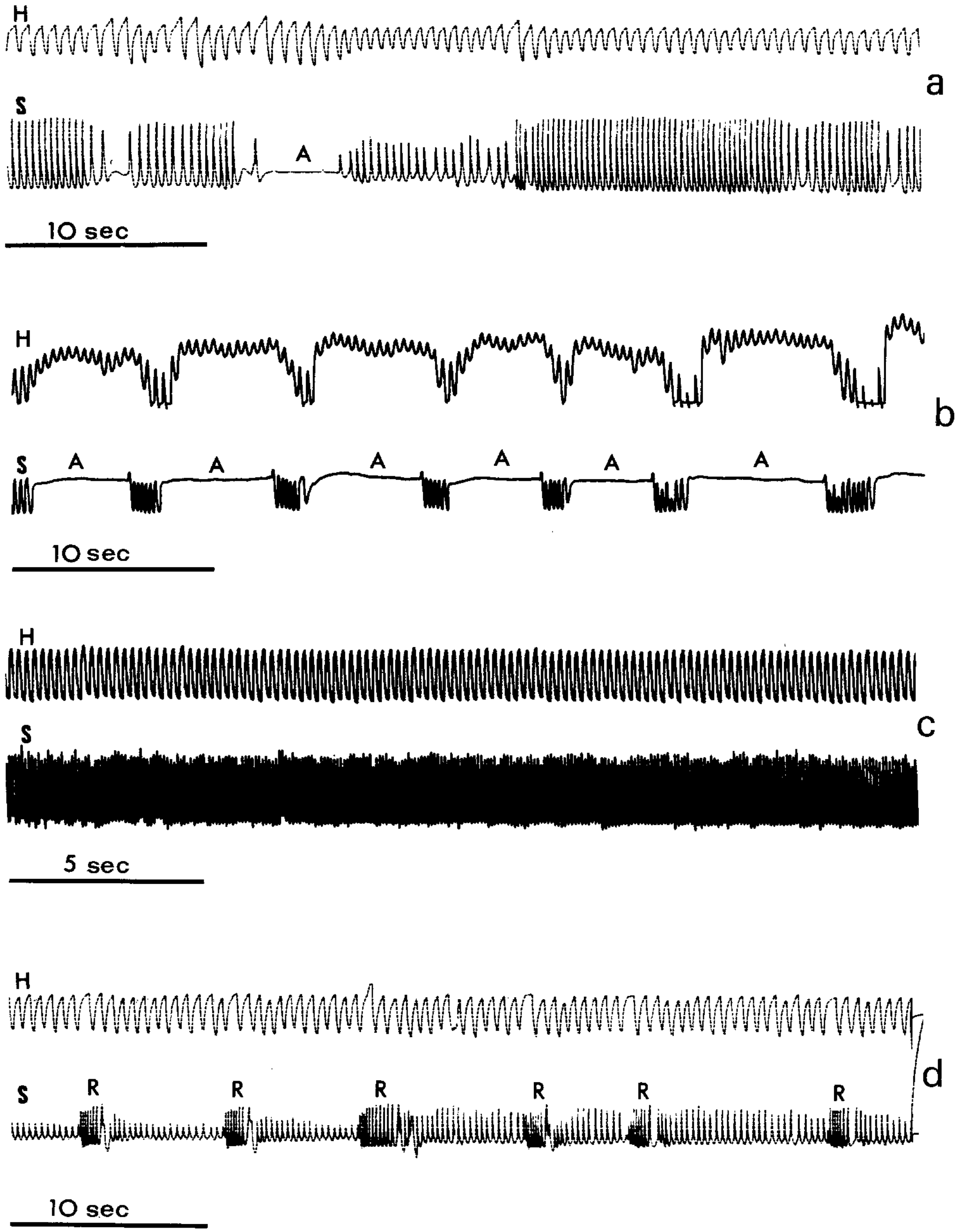

Fig. 3. Typical impedance traces of heart (H) and scaphognathite (S) beating of *Crangon crangon*: (a) undisturbed, control animal, (b) the transient (ca. 3 min.) response following the addition of low (5.0 mg l^{-1}) concentrations of test metals or seawater sham, (c) the response of addition of high (20 mg l^{-1}) concentrations of test metals, (d) in high concentrations of copper or zinc or after chronic exposure to incipient lethal levels of these metals. (A = apnoea, R = reversal)

developed very arrhythmic patterns of heart and scaphognathite beating. Interestingly, only the animals subjected to 0.1 mg metal l^{-1} (1.0 mg l^{-1} with zinc) were ever observed to rebury in the sand.

The concentrations used in the acute response experiments are relatively high and the effects of lower concentrations (at the incipient lethal levels, ILL) were observed in groups (n = 8 each) of animals during a 13-day period. Figure 4 illustrates the data obtained. The progressive drop in the mean rates of control animals was highly significant ($P < 0.001$) in the case of heart rates only but we have observed such declines in several other experiments; possibly this drop, represents acclimatisation of the animals to the holding conditions. At the end of the experiment the heart rates of the cadmium-treated group were not significantly ($P > 0.05$) different from original values but scaphognathite rates had increased significantly ($P > 0.001$). No major qualitative changes to organ-beat behaviour were observed (cf. the effects of higher concentrations, above). In the copper-treated group, organ rates increased progressively so that final values were significantly ($P < 0.05$) greater than original frequencies. After Day 7, scaphognathite apnoeas were abolished and reversal occurrence rose to 5–10 min^{-1} – similar to animals exposed to higher concentrations.

Thirteen days of exposure to zinc resulted in organ rates which were not significantly ($P > 0.05$) different from original values, although the numbers of animals in this group were reduced because of moulting and electrode detachment.

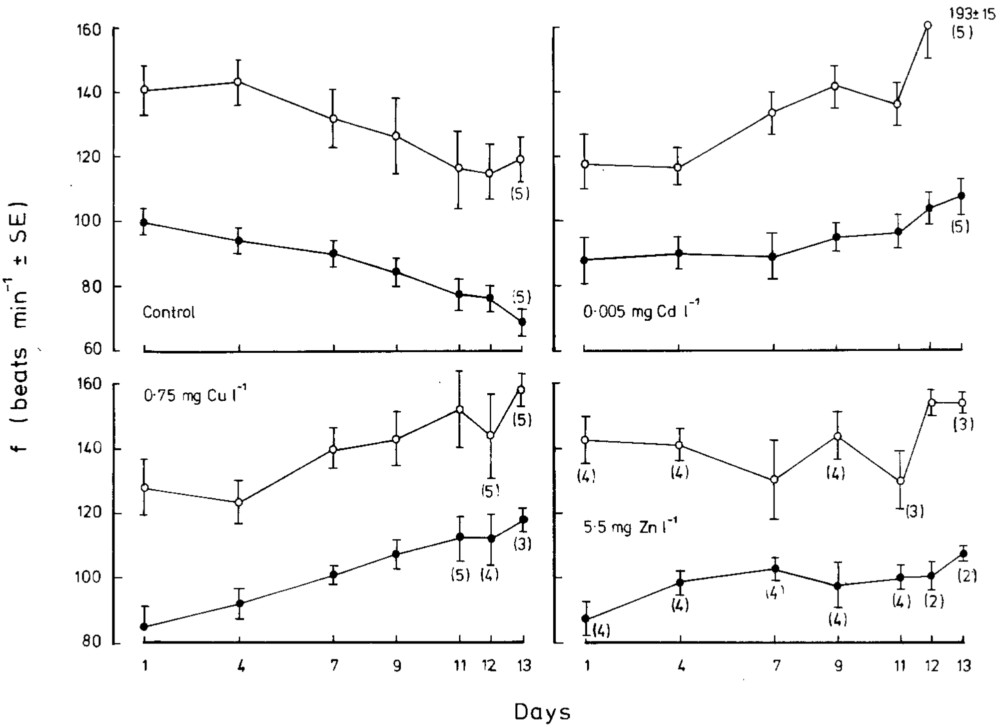

Fig. 4. Heart and scaphognathite frequencies (beats min^{-1}) of *Crangon crangon* obtained at intervals during 13 days of exposure to clean seawater, 0.005 mg Cd l^{-1}, 0.75 mg Cu l^{-1}, 5.5 mg Zn l^{-1}. Values are given as means ± standard errors

Interestingly, none of the treated groups showed the typical acclimatisation reduction of rates shown by the control groups.

DISCUSSION

The results described suggest that there is a threshold concentration of these heavy metals below which *Crangon crangon* does not display an overt response to their presence. Perhaps suprisingly, this threshold appears to lie between 0.1 and 1.0 mg l^{-1} for all three metals. All metals at 1.0 mg l^{-1} induced a marked increase in heart and scaphognathite frequency but this was not accompanied by any apparent change to locomotor activity – the animals mostly remaining buried in the sand. At these concentrations and for the short time span of the experiment, all 3 metals would be completely in solution which suggests the responses are to chemical rather than mechanical stimuli. At the highest concentration of each metal used, however, the animals did show increased walking/swimming activity and this would contribute to the extent of the frequency changes observed.

The hierarchy of magnitude for the 30-min response values was Cu > Zn > Cd but this altered subsequently to Cd > Cu > Zn after 48 h – thus corresponding to the hierarchy of toxicity of these metals (unpublished data).

Scaphognathite reversal frequency increased in copper- and zinc-treated animals. Where high concentrations were used, it is possible that they were evoked by particulate metal-complex precipitates on the gills. This reasoning would explain also why increased reversals were not found in the cadmium-treated groups, as cadmium would still be in stable solution at the concentrations used.

Whatever the functional significance of heart and scaphognathite behaviour may be in the context of satisfaction of oxygen or ionic demands, the present findings indicate that they offer supportive evidence of changes induced by these heavy metals. Although the responses were not specific to these metals, the technique does show promise of being a useful and sensitive means of measuring the magnitude of pollution stress, by particular toxicants, to decapod crustaceans.

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