International Council for
the Exploration of the Sea

The use of a continuous flow apparatus in the study of longer-term toxicity of heavy metals

by

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SUMMARY

A continuous flow apparatus has been built at the Fisheries Laboratory at Burnham-on-Crouch that is capable of administering different, yet constant, concentrations of toxin to twenty experimental tanks. Using this apparatus, the toxicity of mercury (as mercuric chloride) and cadmium (as cadmium chloride) to the brown shrimp (Crangon crangon) has been studied over several weeks. After 1500 hours' exposure these metals were toxic at 1/1000 and 1/100 respectively of their 48 hr LC_{50}. No lethal threshold concentration was established. Although the toxins did not affect the rates at which the animals moulted, at the higher concentrations newly-moulted animals were much more vulnerable. This was shown as a decrease in survival time after moulting. A correlation between the size of the shrimp and concentration was established, with smaller shrimps being more sensitive.

INTRODUCTION

The techniques of acute toxicity testing in sea water have followed those developed by the freshwater toxicologists, where the determination of the concentration of a toxin required to kill 50 per cent of the test organisms within 48 or 96 hours (48 or 96 hour LC_{50}) is a well established practice. However, it is becoming increasingly recognized that these figures give little or no indication of the pattern of intoxication, and the 'safe' concentration of two chemicals with the same 48 hour LC_{50} can differ markedly (Alabaster 1970). A more important parameter is the maximum concentration at which acute toxicity is not apparent. This concentration, also known as the incipient LC_{50} or lethal threshold concentration, is not necessarily the safe concentration at which a fishery would survive, for it takes no account of sub-lethal effects but is, as Sprague (1969) points out, " .... a convenient and reproducible reference point: that concentration which would kill the average fish on long exposure". He went on to point out that in most cases this threshold of acute toxicity is reached within

4 days; this was not the case for metals. Corner and Rigler (1958) demonstrated that, in the static conditions of their test, mercury was lost from a solution of mercuric chloride in sea water by evaporation, by adsorption to the glass walls of the aquarium, and by absorption by bacteria in the water. Boetius (1960) demonstrated the uptake of the same metal from solution by his test fish. These losses lead to a decrease in the desired concentration which becomes significant at low concentrations and results in an underestimation of the toxicity of the solution.

The present paper describes an apparatus designed and built to deliver continuously low concentrations of toxin at such a rate that losses to the tank and animals had negligible effects on the external concentration. The apparatus is simple and reliable and enables tests to be carried out for long periods with a minimum amount of maintenance. Some preliminary results achieved with the apparatus, using salts of cadmium and mercury, are described.

METHODS

The continuous flow apparatus will be described in detail elsewhere (Connor and Wilson, in preparation) so that only a brief description is given here. The apparatus was built to supply each of twenty 10-litre 'Perspex' treatment aquaria with its own flow of sea water containing toxin at a constant concentration. Settled sea water from the laboratory supply is filtered and enters the reservoir tank where it is heated (or cooled) to $15^{\circ}C$ (see Figure 1). Water in the tank is pumped continuously to the header tank at a rate which exceeds the demand of the aquaria, the excess sea water overflowing back into the reservoir tank. The header tank feeds the treatment aquaria individually, each separate flow passing through its own adjustable and calibrated flowmeter into the mixing chamber. Toxin held in the storage bottle is metered into the mixing chamber at a constant rate by a peristaltic pump. Here it mixes with the flowing sea water, and the solution thus formed leaves the chamber and flows into the treatment aquarium before running to waste. All flows are adjustable, but in the present experiments sea water was supplied to each aquarium at 10 l/hour and toxin at 6.67 ml/hour; the desired concentrations in the aquaria were achieved by making up stock solutions of toxin at concentrations 1500 times greater than that desired. Stock solutions were made up every 48 hours in distilled water using 'Analar' cadmium chloride ($CaCl_2$ $2\frac{1}{2}H_2O$) and mercuric chloride ($HgCl_2$). Fifteen adult brown shrimps (Crangon crangon) were held in each aquarium but they were individually retained in small, extruded

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plastic ('Netlon') cages. The animals were fed to satiation every third day on chopped mussel. The aquaria were inspected twice daily and all dead animals and moulted cuticles were removed. The carapace length of all animals was measured with callipers at death. The experiments ran for two months.

RESULTS

The distribution of the survival times of the shrimps at each concentration was found to be log normal (typical results for cadmium are given in Figure 2); the results were analysed graphically to determine the mean survival times (ET_{50}) and 95 per cent confidence limits (Litchfield 1949). The resulting survival-concentration curves for mercury and cadmium and the results of 48 hour LC_{50} determinations (Portmann 1970) are shown in Figure 3. In both cases there is a similar curvilinear relationship between survival time and concentration, but the incipient threshold concentration, indicated by the curve becoming parallel with the time axis, was not reached after 1500 hours' (62 days') exposure. Mortality occurred even after continuing exposure at low concentrations. This is indicative of the accumulative nature of the poisons, and it seems likely that the threshold concentration would only become apparent when the effects of uptake of the toxin are balanced by excretion or other methods of detoxification.

By retaining the shrimps in separate cages, it has been possible to study some of the biological factors affecting their susceptibility to poisoning. Newly-moulted animals were found to be more vulnerable; for example, of the 15 animals that had moulted after 297 hours' exposure to 0.1 ppm cadmium, 11 had died, whereas only one of the 15 unmoulted shrimps had died. The difference was significant ($X^2 = 8.32$; P = 0.01-0.001). The distribution of the times of moult was found to be log normal (Figure 4), and the significance tests of Litchfield (1949) showed them to be the same for the controls and for shrimps exposed to all concentrations of toxins. However, post-moult survival was inversely related to the concentration of metal (Figure 5). This increased susceptibility may be due to increased permeability to the toxin following moult, to the increased physiological stresses associated with moulting, or to a combination of these factors.

The effect of carapace length and therefore body size on the susceptibility of shrimps to the toxins is shown in Figure 6. From the survival distribution, the population was divided into 20 per cent intervals (see inset to Figure 6), and the average carapace length of the animals dying

in each interval was calculated. To enable comparison of lengths and survival times from different concentrations the average length of each 20 per cent interval was expressed as a percentage of the average carapace length of the total distribution at that concentration. The results shown in Figure 6 are in agreement with those of Portmann (1968), with smaller shrimps being much more sensitive.

CONCLUSIONS

The continuous flow apparatus has proved reliable and accurate during preliminary tests lasting more than two months, and from these longer-term studies it has been possible to recognize toxins that probably exert their influence by becoming slowly accumulated. Analyses of the tissues of dead animals for the metals are planned shortly to confirm this possibility. These and other tests suggest that considerable caution is needed in predicting the effects of long-term exposure to toxins on the basis of their 48 hour LC_{50} values, unless a clear lethal threshold concentration has been demonstrated. In the tests described, concentrations of mercury and cadmium at 1/1000 and 1/100 of the 48 hour LC_{50} values respectively have been shown to be lethal in the longer term.

Sources of variation in toxicity tests are as often derived from heterogeneity between test populations as from differences in test procedures. The present paper describes the difference of body size and moulting, but other biological variables - such as age, condition and sex - are likely to be significant. The importance of these factors is not always evident in short-term toxicity tests.

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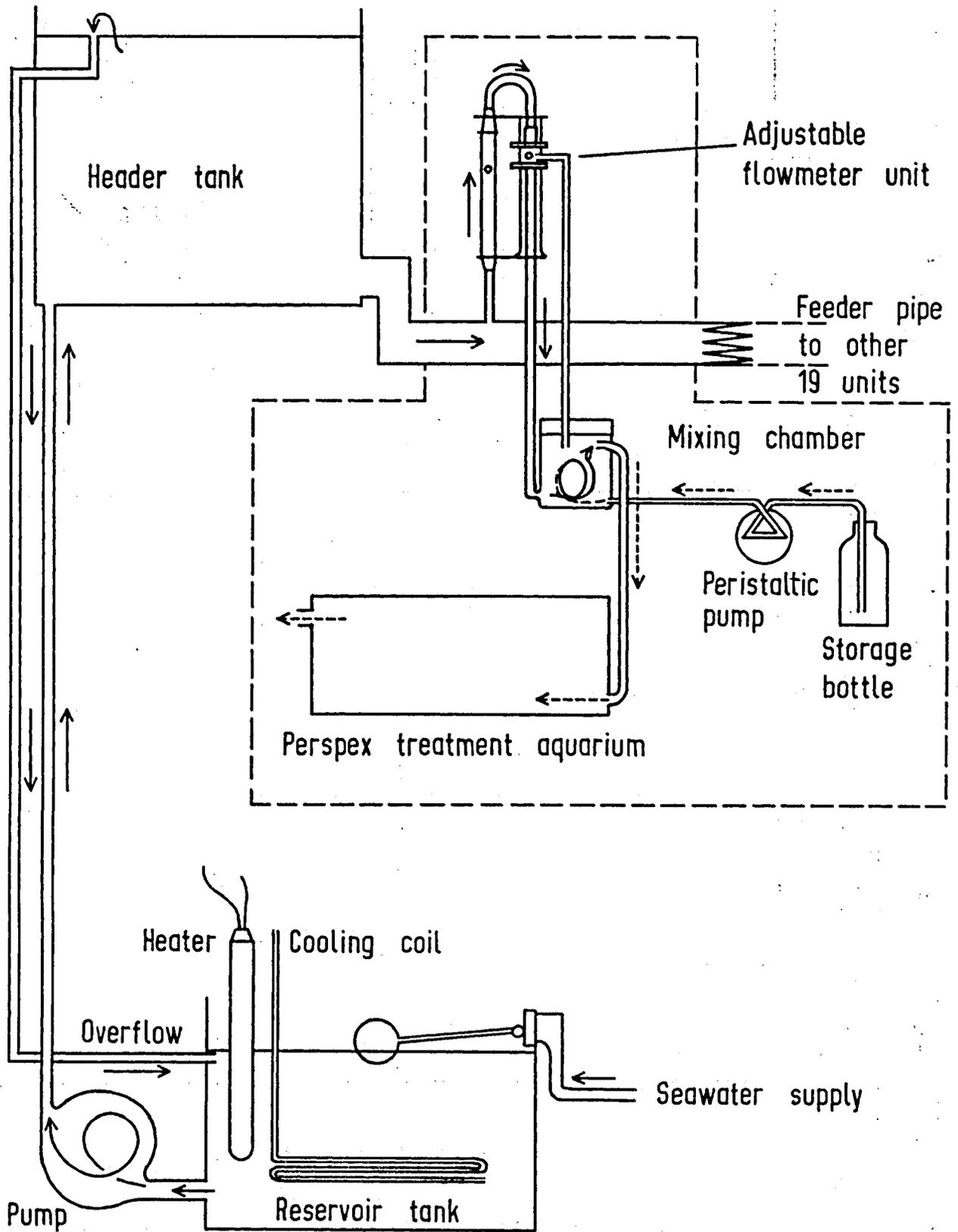
Figure 1   Diagram of the continuous flow apparatus. Only one treatment unit is shown.
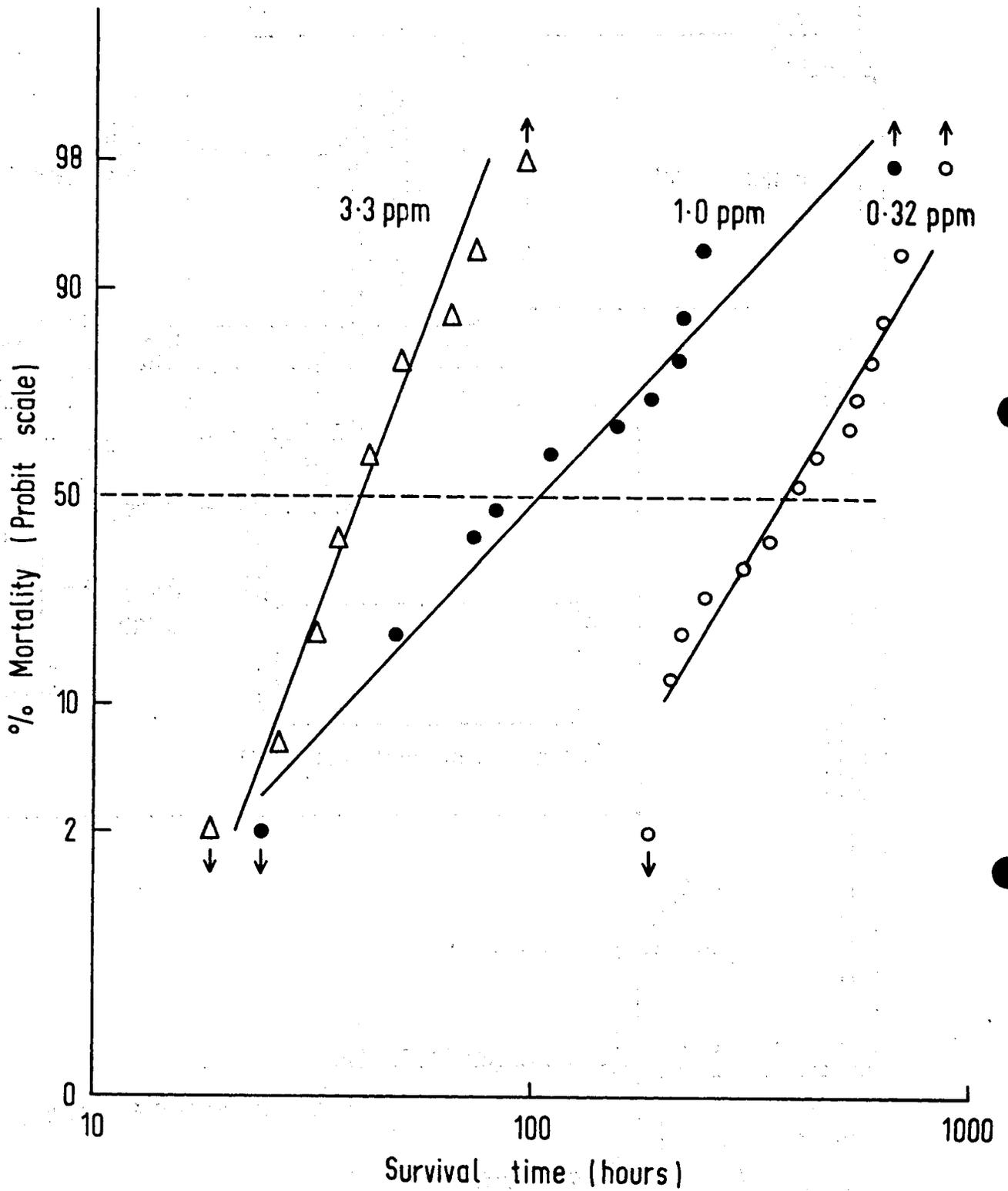
Figure 2   The accumulative percentage mortality curves for the brown shrimp in different concentrations of cadmium as cadmium chloride. The ET_{50} is the time that each line intersects 50 per cent mortality.
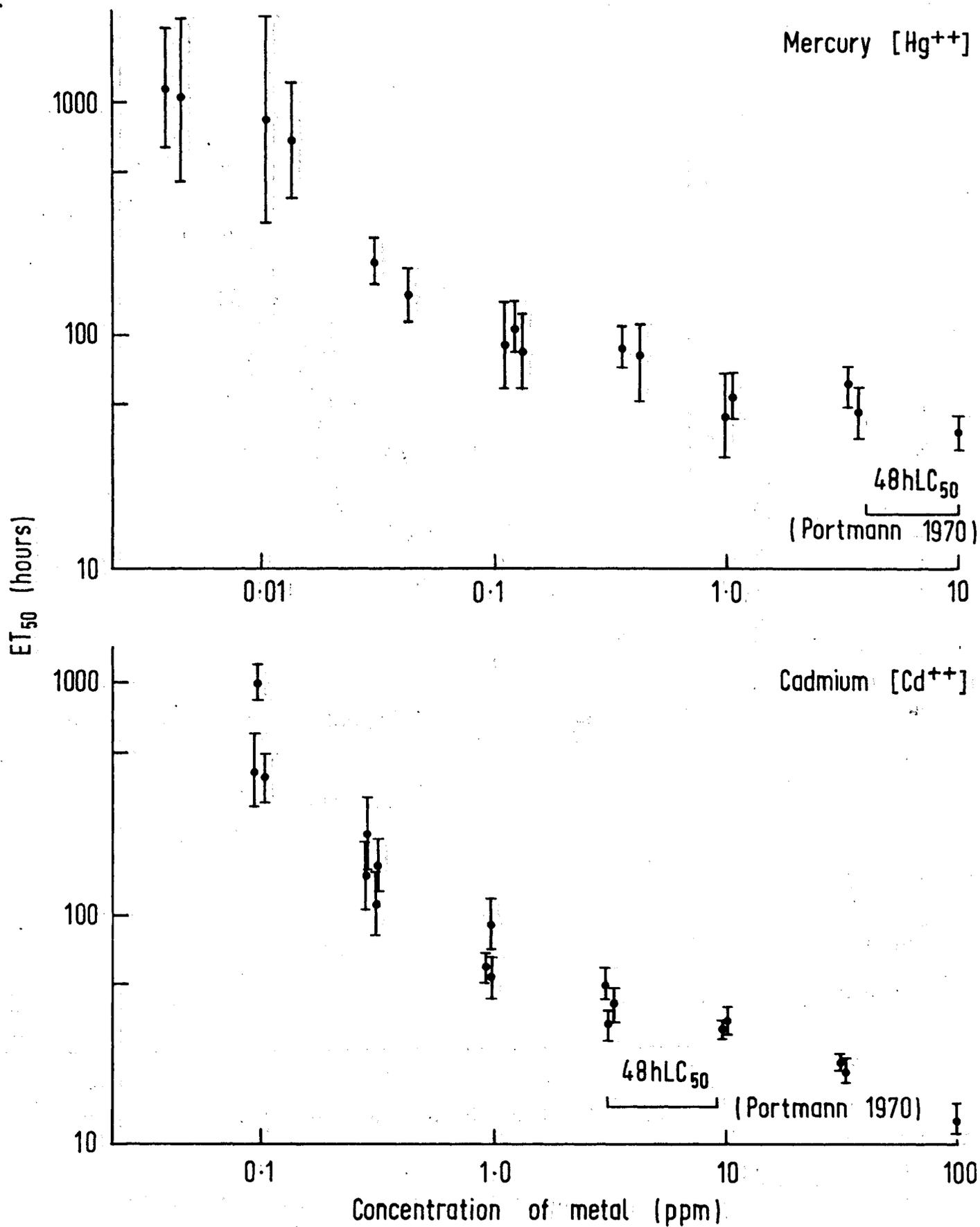
Figure 3   Survival-concentration curves for <u>Crangon</u> in mercuric chloride and cadmium chloride solutions.   Mean and 95 per cent confidence limits are shown for each determination.
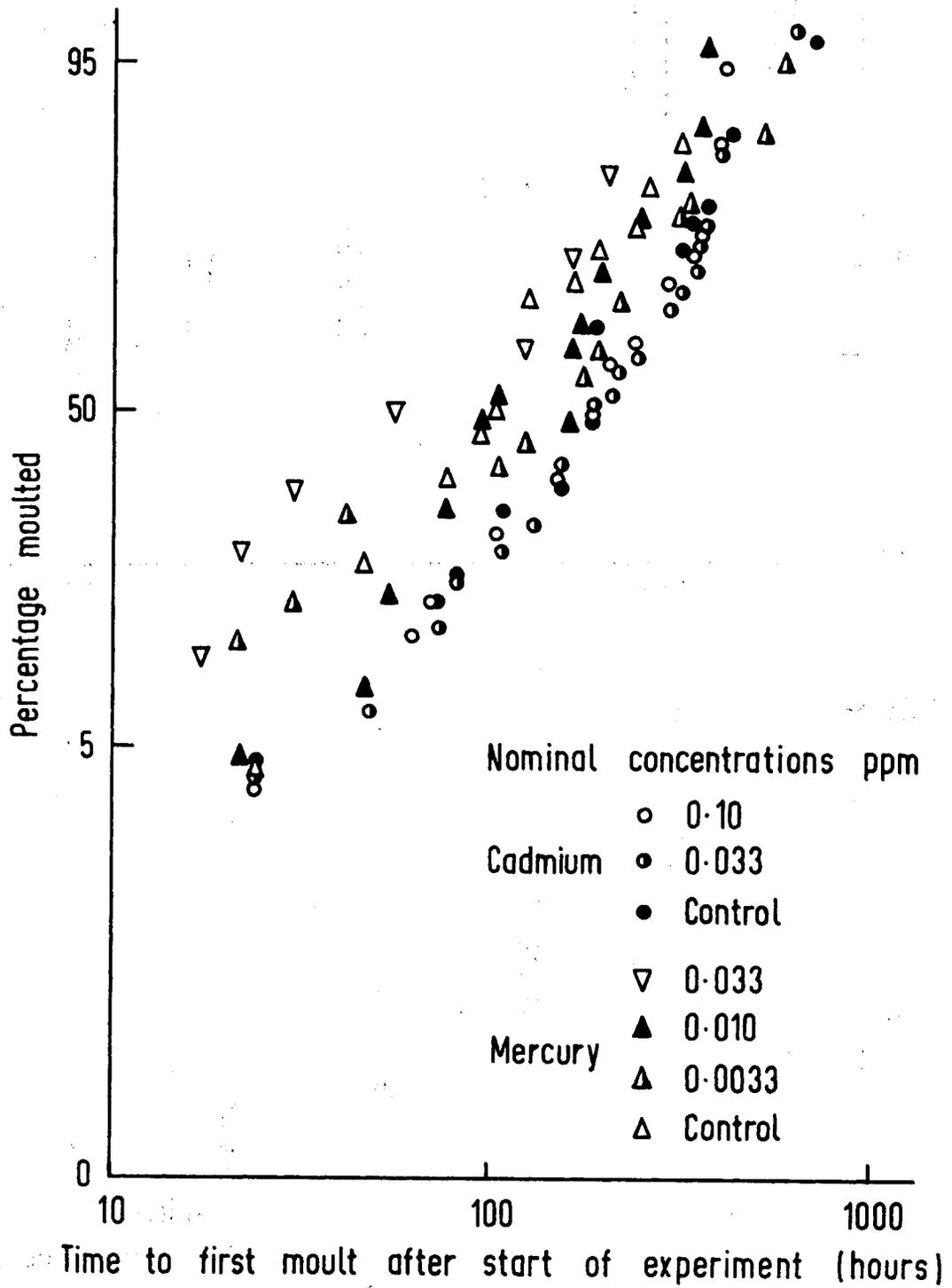
Figure 4   The time distributions of first moults after the start of the experimental period.
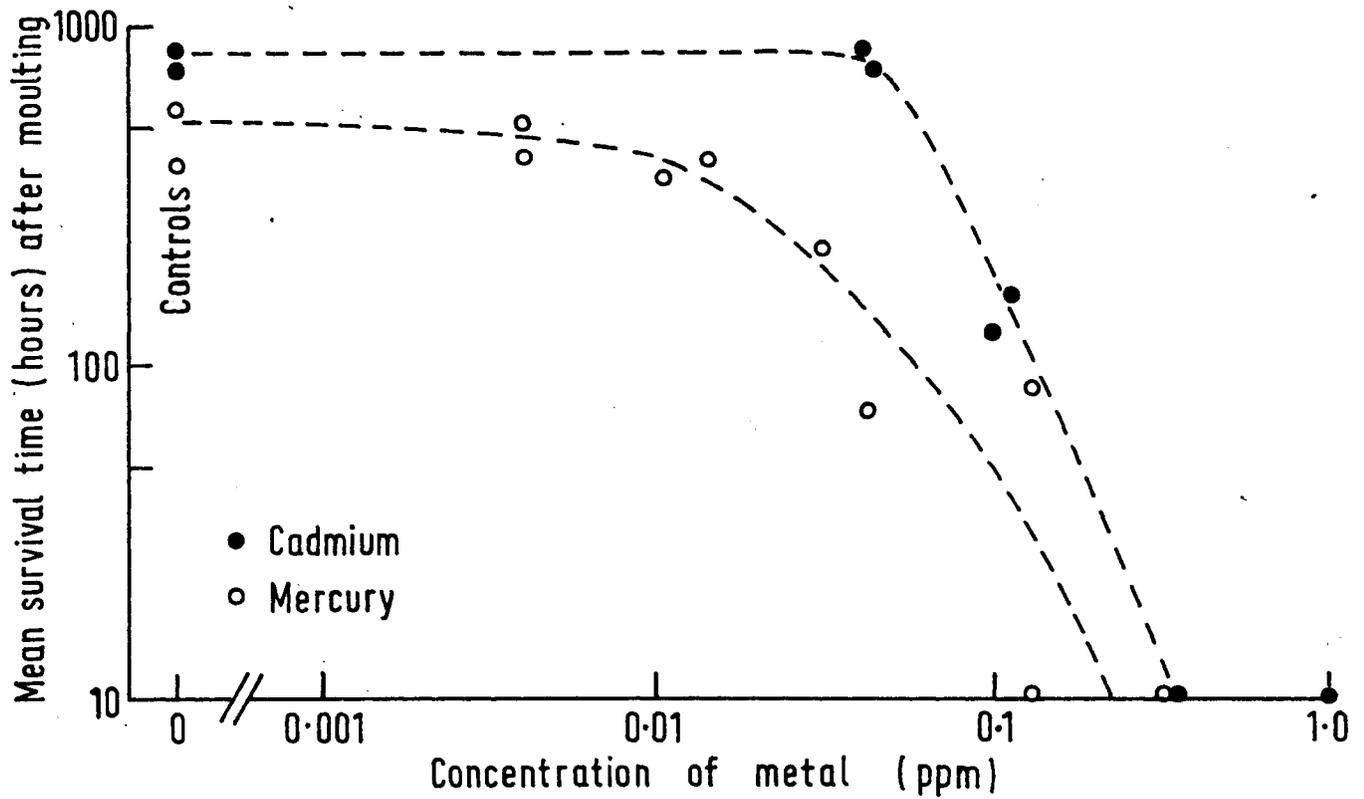
Figure 5   Effect of concentration on time of survival after moulting.
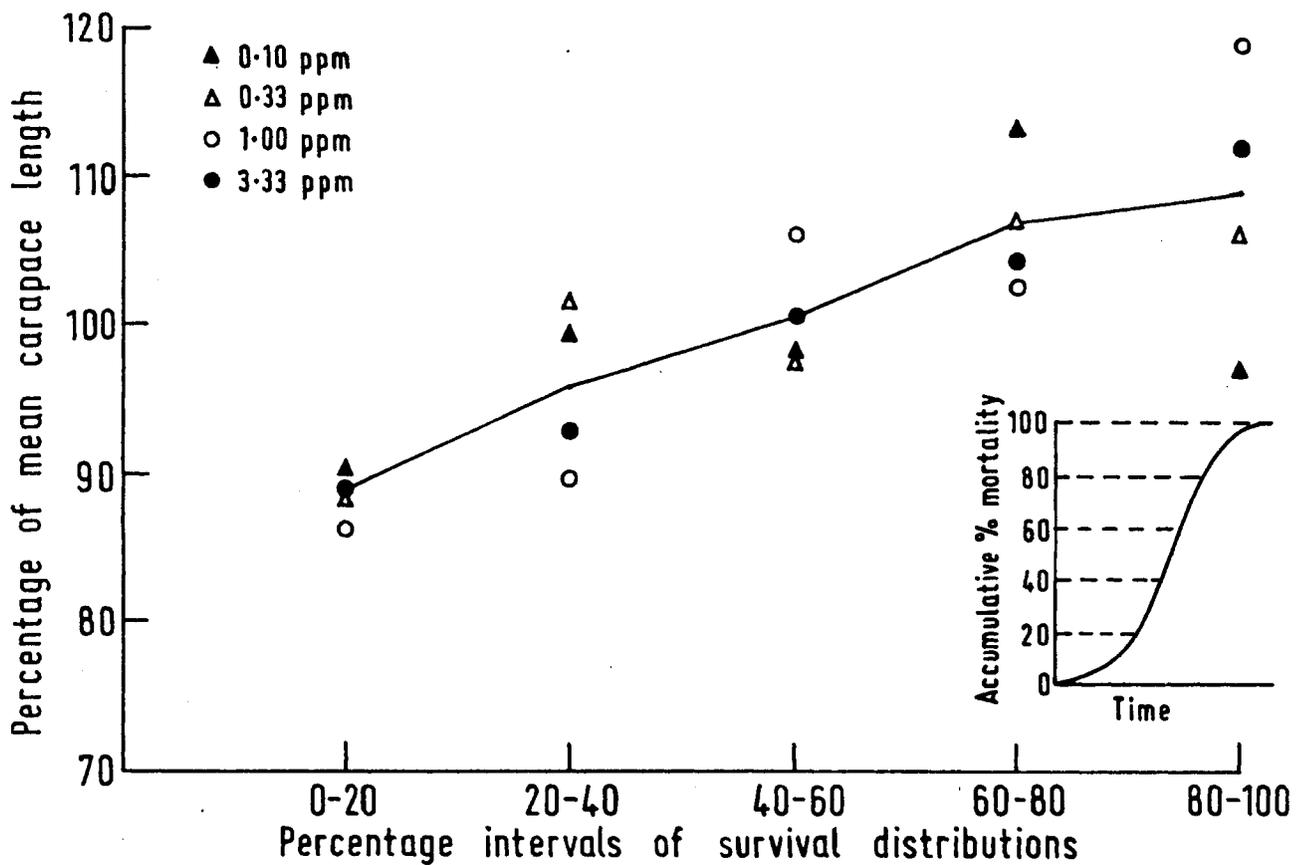
Figure 6   Influence of carapace length on the susceptibility of <u>Crangon</u> to mercury
poisoning.   ● 3.33; O 1.0;   △ 0.33; ▲ 0.10 ppm of mercury.
Inset shows a typical survival distribution for one concentration with the
20 per cent intervals indicated.