# The Influence of EDTA on the Mortality and Burrowing Activity of the Clam (*Venerupis decussata*) Exposed to Sub Lethal Concentrations of Copper

by R. R. STEPHENSON* and D. TAYLOR
*ICI Brixham Laboratory*
*Freshwater Quarry*
*Brixham, Devon, England*

## 1. Introduction

The harmful effects of certain heavy metal ions on marine life is giving cause for concern. Until recently investigations were focussed on the acute toxic effects of metals, but now attention is being given to their chronic and long term effects.

Most experimental techniques involve the exposure of test animals, for different periods of time, to a range of concentrations of the particular metal. The majority of workers have however, ignored the physico chemical state of the metal that is found in the natural environment and have simply used solutions of metal salts for their experimental work in the laboratory.

This paper presents some preliminary results of investigations with a small bivalve mollusc (*Venerupis decussata*) which clearly indicate the importance of taking into consideration the species of ion to be found in the natural environment.

Copper has been investigated because it is commonly found in industrial effluents, its acute toxicity has been extensively studied (VAN DER PLAS 1972) and the physico chemical state of copper in natural waters has recently been studied by several workers (STIFF 1972a,b; MONTGOMERY et al 1971; VAN DUIN 1972; ZITKO et al 1972).

## Methods

The experimental animals were obtained from the White Fish Authority Hatchery at Conway. They ranged in diameter from 94 to 118 mm and a weight (without shell) of 85 to 160 mg.

During the experiments the animals were left in glass dishes (diam.150 mm, depth 70 mm)three-quarters filled with coarse sand, taken from an unpolluted beach in Tor Bay. Each dish was supplied with coarsely filtered sea water (150 ml/min), which contained sufficient fine particulate matter to provide a food source for the animals. All the experimental dishes were placed in a water bath and the temperature of both the bath and the water flowing over the animals was maintained at 15°C ± 1.

*Present address: Tecoyatitla 365, Colonia Agricola San Angel, Mexico DFZP20



Bulletin of Environmental Contamination & Toxicology,
Vol. 14, No. 3 

The clams were placed in the experimental dishes 2 weeks prior to the start of experiments to allow them to acclimatise to the conditions.

During the experiments the burrowing activity of the clam was measured at frequent intervals. Animals were removed by sieving and their ability to burrow into the substrate was measured. The percentage of each population burrowed 30 mins after having been placed on the sand surface was used as the index of activity. A clam was considered to have burrowed when more than 50% of its shell was below the sand surface.

Daily, throughout the experiment, the concentration of soluble copper in the water in each experimental dish was monitored using the method of RILEY and TAYLOR (1968).

## Results

Four populations of *Venerupis d.* were exposed to copper for a period of 75 days. The surviving animals were then maintained under similar conditions in clean sea water for a further 35 days.

Two of the populations were exposed to concentrations of 0.1 and 0.01 ppm of copper by introducing copper sulphate solution of an appropriate concentration into their sea water supply. Two populations were also exposed to 0.1 and 0.01 ppm of copper but in the presence of an excess (1.0 ppm) of EDTA. In addition two control populations, exposed respectively to sea water and sea water + 1.0 ppm EDTA were maintained throughout the experiment.

The results are presented in two sections:

### (1) Mortality

The two populations exposed to copper in the presence of EDTA and the two control populations had total mortalities throughout the experiment of >2%. Figure 1 summarises the mortality in the two populations exposed to 0.1 and 0.01 ppm of copper in the absence of EDTA.

It is clear from Figure 1 that high mortalities occurred when the *Venerupis* were exposed to 0.1 ppm of copper, all the animals died within 50 days exposure. Of the population exposed to 0.01 ppm of copper none of the experimental animals died until the 30th day of exposure. In this experiment, the exposure to copper was terminated at the 75 th day but the mortality rate in the population was in excess of 2%/day from day 69 until day 91 after which there were no further deaths.

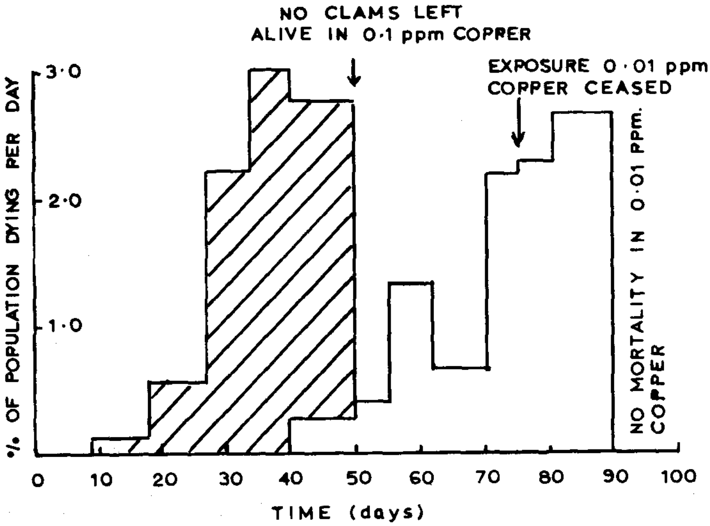


Figure 1 : The Mortality of Clams Exposed to 0.1 and 0.01 ppm of Copper

(2) Burrowing Activity

The results of these observations are shown in Figure 2.

It can be seen in Figure 2 that both of the contol populations and the two populations exposed to copper in the presence of EDTA showed no inhibition of burrowing during the 75 days of exposure, but the two populations exposed to copper in the absence of EDTA showed a significant decline.

As a result of the high mortalities amongst the animals exposed to 0.1 ppm of copper it was not possible to follow the recovery of burrowing activity after cessation of exposure, but the population exposed to 0.01 ppm of copper began to recover its burrowing ability within a few days of the exposure being terminated and within 30 days burrowing activity was similar to that of the control population.

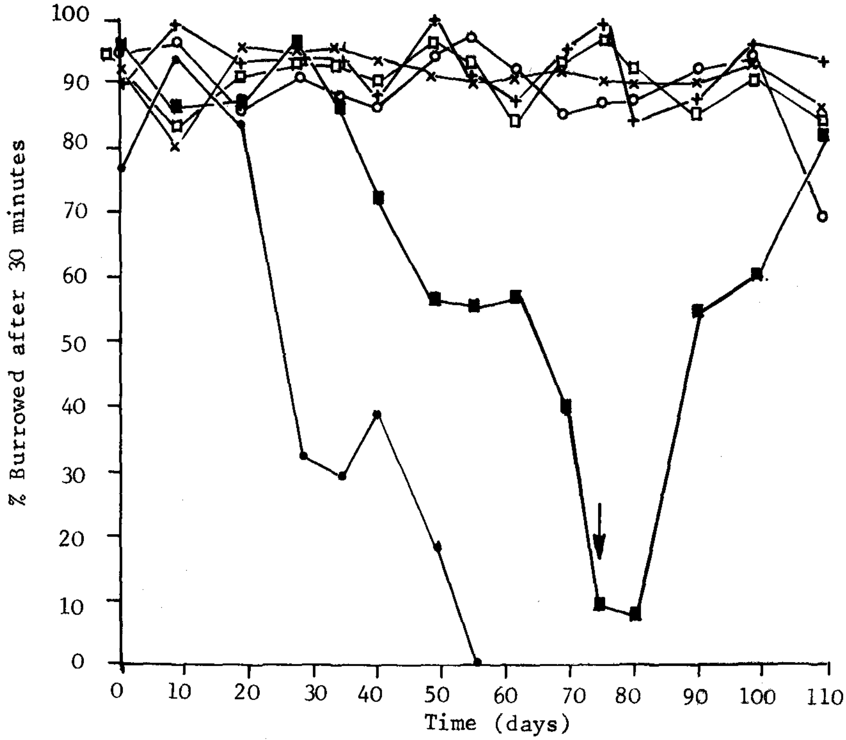


Figure 2 : Effect of Exposure to Copper on Burrowing Activity (Key : X = Control, 0 = 0.1 ppm Cu + EDTA, □ = 0.01 ppm Cu + EDTA ● = 0.1 ppm Cu, ■ = 0.01 ppm Cu).

Discussion

It is clear from the results of these experiments that there is a very substantial difference in the long term effects of copper on *Venerupis d.* caused by the presence of EDTA.

There have been a number of reports in the literature that complex formation, ie the binding of the metal ion to a large organic molecule, can modify the acute toxicity of copper to fish. SPRAGUE (1968) found that the acute toxicity of copper was reduced by the presence of the chelating agent nitriloracetic acid, and more recently ZITKO et al (1973) showed that humic acids reduced the toxicity of copper to Atlantic salmon.

In our experiments, in the tests where EDTA was present, all the copper would have been in the form of an EDTA complex. The results show that, under these conditions, even at relatively high concentrations of copper ie 0.1 ppm no significant differences from the controls were noted.

The data from the experiments without EDTA show that at 0.01 ppm of copper, toxic effects were caused to the *Venerupis d.* that would have led to the death of the total population within a relatively short time.

The actual situation in the sea is probably represented by neither of these extremes. ZIRINO and YAMAMOTO (1972) predict, from their mathematical model, that the free ionic copper present in sea water represents less than 1% of the total and that the predominant species present are 90% $Cu(OH)_2$ and 8% $CuCO_3$.

Thus the assessment of the long term effects of copper by using a solution of a copper salt bears little relation to the problems to be found in the marine environment. Much more attention needs to be paid to the physico-chemical state of the heavy metals in sea water before adequate toxicity data can be established for predicting long term effects in the environment.

Acknowledgements

We thank Mr R.C. Barr for technical assistance. Miss Stutchbury for typing the manuscript, Mr E.E. Ritson for preparing the diagrams and Dr L. Carter for his critical appraisal of the manuscript.

References

MONTGOMERY H.A.C and STIFF M.J: in Identification and Measurement of Environmental Pollutants. Westley B Editor pp375 National Res. Council of Canada, Ottawa (1971)
RILEY J.P. and TAYLOR D: Anal. Chim. Acta (1968)
SPRAGUE J.B: Nature 220 1345 (1968)
STIFF M.J: Wat. Res. 5 171 (1971 a,b)
VAN DER PLAS E.H.H: TNO News 460 (1972)
VAN DUIN P.J: TNO News 457 (1972)
ZIRINO A. and YAMAMOTO S: Limnol. Oceanog. 17 661 (1972)
ZITKO P., CARSON W.V. and CARSON W.G: Bull. Environ. Contam. Toxicol. 10 265 (1973)