Marine Pollution Bulletin, Vol. 13, No. 4, pp. 123–125, 1982.
Printed in Great Britain.

0025-326X/82/040123-03 $03.00/0

Differentiation of the Sensitivity to Copper and Cadmium in Different Life Stages of a Copepod

G. VERRIOPOULOS and M. MORAÏTOU-APOSTOLOPOULOU
Zoological Laboratory, University of Athens, Greece

Copper is more toxic to all life stages of the copepod *Tisbe* than cadmium. The most sensitive life stage of *Tisbe* to both copper and cadmium is the one-day-old nauplius. The resistance of larval stages of *Tisbe* increases with age (one-day-old nauplii 48h LC_{50} = 0.3142 mg Cu l^{-1}. and 0.5384 mg Cd l^{-1}, 0.3415 mg Cu l^{-1}. and 0.645 mg Cd l^{-1}. for five-days-old nauplii and 0.5289 mg Cu l^{-1}. and 0.9061 mg Cd l^{-1}. for ten-days-old nauplii. The two reproductive stages of *Tisbe* tested (females with ovigerous bands and females bearing the first ovigerous sac) demonstrated an increased sensitivity to metals and proved more sensitive than the ten-days-old copepodids (only females with ovigerous bands had a similar sensitivity to copper with the ten-days-old copepodids).

Recently the living resources of estuarine, coastal and even oceanic ecosystems are being harmed by discharging of waste material and runoff of highly polluted waters. In creating standards and guidelines to follow in marine environmental management, there is urgent need for information on the effects of pollutants upon marine resources. Both field and laboratory tests have proved the impact of pollutants on aquatic life.

Heavy metals are considered to be among the most harmful aquatic pollutants. The toxicity of metals to marine organisms has been found to vary not only between, but also within, species. This has been attributed to various abiotic factors: temperature, salinity (Vernberg *et al.*, 1974; Moraïtou-Apostolopoulou, in press) or biotic factors: population density (Verriopoulos & Moraïtou-Apostolopoulou, 1981) or life stage (Calabrese *et al.*, 1973, 1977; Calabrese & Nelson, 1974). These authors have emphasized that the different embryo or larval stages of invertebrates present different sensitivities from the adults.

If the impact of various heavy metals is to be accurately appraised, more information of this kind is required. In this paper we have studied the acute toxicity of copper and cadmium, two of the most toxic heavy metals, to various life stages of the benthic marine copepod *Tisbe holothuriae* Humes.

Materials and Methods

Tisbe holothuriae, Humes, a benthic harpacticoid copepod, is a common organism in the littoral environment of Mediterranean coasts. The test animals were taken from laboratory cultures. Ovigerous females of *Tisbe* were isolated individually in 100 ml glass containers. Immediately after egg hatching the parental females were removed. Consequently in each container all *Tisbe* had the same (and known) age.

Five different ontogenetic stages of *Tisbe* were exposed separately (a) to copper and (b) to cadmium, as follows:

(1) One-day-old nauplii;
(2) Five-days-old nauplii;
(3) Ten-days-old copepodids;
(4) Adult females with ovigerous bands (soon after mating and about 15 days old);
(5) Adult females with ovigerous sacs (first ovigerous sac).

The 48 h LC_{50} (concentration of a toxicant lethal to 50% of the test animals after 48 h exposure) values were determined for each of the five life stages of *Tisbe* (a) for copper and (b) for cadmium, according to the Bliss (1938) method. For the determination of each 48 h LC_{50} the appropriate life stage of *Tisbe* was exposed to five to seven concentrations of each metal. The range of metal concentration tested was established by preliminary tests. Twenty *Tisbe*, in groups of five, were exposed at each metal concentration (100–140 *Tisbe* were used for the determination of each 48 h LC_{50}). All experiments were run in constant temperature rooms at 18 ± 0.5°C. The sea water used was previously filtered with a millipore filter (0.8 μm) and autoclaved. No food was added during the 48 h of experiments.

Fresh stock solutions containing 20 mg Cu l^{-1} and 20 mg Cd l^{-1} were prepared immediately prior to each test by dissolving $CuSO_4.5H_2O$ and $CdCl_2.2H_2O$ in sea water. These solutions were diluted appropriately to make the test metal ion concentrations.

Results

Copper was more toxic (about two times) to *Tisbe* than cadmium. The 48 h LC_{50} values calculated according to the method of Bliss (1938) are given in Table 1.

The most sensitive life stage of *Tisbe* for both metals was the one-day-old nauplius and the lowest 48 h LC_{50} values were found for this life stage (0.3142 ± 0.0052 mg Cu l^{-1} and 0.5384 ± 0.0062 mg Cd l^{-1}). The resistance of *Tisbe* to copper and cadmium progressively increased with larval age and the most resistant larval stage was the ten-days-old copepodids (0.9061 mg Cd l^{-1} and 0.5289 mg Cu l^{-1}). However, the increase of *Tisbe* resistance to the two metals was not extended to the two adult stages tested. The *Tisbe* with ovigerous bands were

TABLE 1

48 h LC_{50} in mg ions $l.^{-1}$ of Cu in the form of $CuSO_4\,5H_2O$ and Cd in the form of $CdCl_2.2H_2O$ to various life stages of *Tisbe holothuriae*.

Life stage	Metal	
	Cu	Cd
One-day-old nauplii	0.3142 ± 0.0052	0.5384 ± 0.0062
Five-days-old nauplii	0.3415 ± 0.0004	0.6450 ± 0.0092
Ten-days-old copepodids	0.5289 ± 0.0011	0.9061 ± 0.0066
Females with ovigerous bands	0.4473 ± 0.0021	0.9166 ± 0.0056
Females with ovigerous sacs	0.4281 ± 0.0027	0.8727 ± 0.0166

more resistant to metals than those bearing ovigerous sacs. In the case of copper, females with ovigerous bands were more sensitive than the ten-days-old copepodids while they demonstrated a slightly higher resistance to cadmium.

Females with ovigerous sacs showed an increased sensitivity and for both metals were more sensitive than the ten-days-old copepodids. The more resistant life stage of *Tisbe* was the ten-days-old copepodids for copper, while in the case of cadmium the ten-day copepodites and females with ovigerous bands showed the higher resistance to cadmium (0.906 for the ten-days-old copepodids and 0.916 for the females bearing ovigerous sacs).

Discussion

This paper concerns the acute toxicity of copper in the form of $CuSO_4.5H_2O$ and cadmium in the form of $CdCl_2.2H_2O$ to various life stages of *Tisbe holothuriae* at 18°C and 38‰S. The form of the metal salt (Moraïtou-Apostolopoulou & Verriopoulos, in press, a), temperature and salinity (as has been previously referred to) are among the factors that influence the toxicity of metals to marine animals. The effects of metals are further complicated by biological variables such as sex, life stage and prior adaptation to pollution conditions (Bryan & Hummerstone, 1971; Vernberg *et al.*, 1974; Brown, 1976; Moraïtou-Apostolopoulou, 1978).

Copper and cadmium are listed among the metals most highly toxic to aquatic organisms (Doudoroff & Katz, 1973). Copper proved more toxic to *Tisbe* than cadmium. This is in accordance with the literature data that copper is more toxic to marine animals than cadmium (Moraïtou-Apostolopoulou *et al.*, 1979; Moraïtou-Apostolopoulou & Verriopoulos, in press, b). *Tisbe* was more resistant to both copper and cadmium than other (planktonic) copepods (Gibson & Grice, 1977; Moraïtou-Apostolopoulou, 1978; Moraïtou-Apostolopoulou *et al.*, 1979; Ahsanullah & Arnott, 1979) or benthic animals (Eisler, 1971).

The sensitivity of *Tisbe* to both metals presented differences according to the life stage. The most sensitive ontogenetic stage of *Tisbe* was one-day-old nauplii. The resistance of larval stages of *Tisbe* to copper and cadmium increased with age. Five-days-old nauplii were more resistant than the one-day-old, while the most resistant larval stage was the ten-days-old copepodids. In crustaceans there is evidence that metals concentrate in the body covering and their entry into the body is thus reduced. Bertine & Goldberg (1972) reported higher levels of mercury in the proteinaceous moults than in the tissues of the shrimp *Crangon crangon*. The sensitivity of young larval forms may be associated with the thinness of their body covering. The completion of body covering diminishes the entry of metal into the body, thereby increasing the resistance of older forms. According to Bryan & Hummerstone (1971) the regulation of metal ions is less effective to young than adults. It seems that in young forms the mechanisms of detoxification observed in adults (Bernard & Lane, 1961; Bryan, 1974) have not yet developed. Laboratory studies have demonstrated that larval stages of marine invertebrates are more sensitive to metals than are adults (Vernberg *et al.*, 1973; Calabrese *et al.*, 1973; Wier & Walter, 1976).

Surprisingly enough, the important increase of *Tisbe* resistance to the two metals with age was not continued to the adult stages. An abrupt decrease of resistance to copper was observed to the adult females bearing ovigerous bands while in the case of cadmium these females showed a very slight increase in the resistance to cadmium. Furthermore, the females with ovigerous sacs demonstrated a pronounced sensitivity to both metals and were more sensitive than the females bearing ovigerous bands and the ten-days-old copepodids.

In a previous paper (Moraïtou-Apostolopoulou & Verriopoulos, in press, b), working also with ovigerous *Tisbe* females, the 48 h LC_{50} of Cd were found higher than those observed in this paper (0.97 ± 0.04 mg Cd l^{-1}). We cannot say if the observed differences are due to the difference in the age between the ovigerous females (the females of the previous paper were not young ones bearing their first ovigerous sac) or to experimental conditions (mainly differences in the organic substances content of the two waters resulting in different complexation of the metal). Furthermore, copper has been found to affect the reproduction of marine zooplankton (Reeve *et al.*, 1977; Moraïtou-Apostolopoulou & Verriopoulos, 1979). It is possible that in some phase of the reproductive mechanism a substance, e.g. an enzyme, eventually important for survival, becomes sensitive to metals. It is also possible that the reproductive effort contributes to the observed sensitivity.

Although research with pollutants using adult animals is useful for demonstrating the effect of a pollutant to marine organisms, it does not seem to provide enough information for the animals' response to a toxic substance. Young stages are generally more sensitive than adults and may suffer harmful effects at concentrations considered safe for the adults. From our results it seems also that even adult animals, when in the reproductive phase, may present increased sensitivity. It became evident from this study that research which includes various life stages of an animal provides a much more realistic idea of what the responses of an animal to a pollutant might be in nature.

The toxicity results concern the nominal added metal concentrations. As discussed by Portmann (1970), losses due to uptake by the organisms and absorption onto the walls of the test containers as well as the existence of inorganic ligands or complexation of metals in natural sea water may occur. However, according to the same author, in test lasting up to 48 h it is unlikely that a significant amount of metal ion is removed.

This paper is part of the Med IV research program of the UNEP. All experiments were conducted at the Zoological Laboratory of the University of Athens. We thank the Director of the Laboratory, Prof. V. Kiortsis, for laboratory facilities and kind advice.

Ahsanullah, M. & Arnott, G. H. (1979). Acute toxicity of copper, cadmium and zinc to three species of marine copepods. *Aust. J. mar. Freshwat. Res.*, **30**, 63–71.

Bernard, F. S. & Lane, C. E. (1961). Absorption and excretion of copper ions during settlement and metamorphosis of the barnacle *Balanus amphitrite*. *Biol. Bull.*, **121**, 438–448.

Bertine, K. K. & Goldberg, E. D. (1972). Trace elements in clams, mussel and shrimp. *Limnol. Oceanogr.*, **17**, 877–884.

Bliss, G. J. (1938). The determination of the dosage mortality curve from small numbers. *Q. J. Pharm.*, **2**, 192–216.

Brown, B. (1976). Observations on the tolerance of the isopod *Asellus meridianus*. Rac. to copper and lead. *Wat. Res.*, **10**, 555–559.

Bryan, G. W. (1974). Adaptation of an estuarine polychaete to sediments containing high concentrations of heavy metals. I. General observations and adaptation to copper. *J. mar. Biol. Ass. U.K.*, **51**, 845–863.

Bryan, G. W. & Hummerstone, L. G. (1971). Adaptation of polychaete *Nereis diversicolor* to estuarine sediments containing high concentrations of heavy metals. I. General observations and adaptation to copper. *J. mar. Biol. Ass. U.K.*, **51**, 845–863.

Calabrese, A., Collier, R. S., Nelson, D. A. & MacInnes, S. R. (1973). The toxicity of heavy metals to embryos of the American oyster *Crassostrea virginica*. *Mar. Biol.*, **18**, 162–166.

Calabrese, A., MacInnes, S. R., Nelson, D. A. & Miller, S. E. (1977). Survival and growth of bivalve larvae under heavy metals stress. *Mar. Biol.*, **41**, 179–184.

Calabrese, A. & Nelson, D. A. (1974). Inhibition of embryonic development of the red clam *Mercenaria mercenaria* by heavy metals. *Bull. envir. contam. Toxic.*, **2**, 92–97.

Doudoroff, P. & Katz, M. (1953). Critical review of literature on the toxicity of industrial wastes and their components to fish–II. The metals as salts. *Sewage ind. Wastes*, **25**, 802–839.

Eisler, R. (1971). Cadmium poisoning in *Fundulus heteroclitus* (Pisces. Cyprinodontidae) and other marine organisms. *J. Fish. Res. Bd Can.*, **28**, 1225–1234.

Gibson, V. R. & Grice, G. D. (1977). Response of macro-zooplankton populations to copper: controlled ecosystem pollution experiment. *Bull. mar. Sci.*, **27**, 85–91.

Moraïtou-Apostolopoulou, M. (1978). Acute toxicity of copper to a copepod. *Mar. Pollut. Bull.* **9**, 278–280.

Moraïtou-Apostolopoulou, M. & Verriopoulos G. (1979). Some effects of sub-lethal concentration of copper on a marine copepod. *Mar. Pollut. Bull.*, **10**, 88–92.

Moraïtou-Apostolopoulou, M., Verriopoulos, G. & Palla, P. (1979). Temperature and adaptation as factors influencing the acute toxicity of Cd to the planktonic copepod *Acartia clausi*. *Tethys*, **9**, 97–101.

Moraïtou-Apostolopoulou, M. & Verriopoulos, G. (in press, a). Toxicity of chromium to the marine planktonic copepod *Acartia clausi*. *Hydrobiologia*.

Moraïtou-Apostolopoulou, M. & Verriopoulos, G. (in press, b). Individual and combined toxicities of three heavy metals, Cu, Cd, and Cr for the marine copepod *Tisbe holothuriae*. *Hydrobiologia*.

Portman, J. E. (1976). A discussion of results of acute toxicity tests with marine organisms, using a standard method. F.A.O. Technical conference on Marine Pollution and its Effects on Living Resources and Fishing FIR MP/70/E-31, pp. 1–13.

Reeve, M. R., Walter, M. A., Darcy, K. & Ikeda, T. (1977). Evaluation of potential indicators of sublethal toxic stress on marine zooplankton (feeding, fecundity, respiration and excretion): Controlled ecosystem pollution experiment. *Bull. mar. Sci.*, **27**, 105–113.

Vernberg, W. B., De Coursey, P. J. & Padgett, W. J. (1973). Synergetic effects of environmental variables on larvae of *Uca pugilator*. *Mar. Biol.*, **22**, 307–312.

Vernberg, W. B., De Coursey, P. J. & O'Hara, J. (1974). Multiple environmental factors effects on physiology and behaviour of the fiddler crab *Uca pugilator*. In *Pollution and Physiology of the Marine Organism* (J. W. Vernberg, ed.). Academic Press, New York.

Verriopoulos, G. (1980). La toxicité du Cr sur le copépode harpacticoïde *Tisbe holothuriae* en relation avec la temperature. *Ves Journées Etudes Pollutions*. 797–802. Cagliari. C.I.E.S.M.

Verriopoulos, G. & Moraïtou-Apostolopoulou, M. (1981). Effects of some environmental factors on the toxicity of cadmium to the copepod *Tisbe holothuriae*. *Archs. Hydrobiol.*, **91**, 287–293.

Wier, C. F. & Walter, W. M. (1976). Toxicity of cadmium to the freshwater snail, *Physa gyrina* Say. *J. Envir. Quality*, **5**, 359–362.

Marine Pollution Bulletin, Vol. 13, No. 4, pp. 125–127, 1982.
Printed in Great Britain.

0025-326X/82/040125-03 $03.00/0

The Acute Toxicity of Motor Fuels to Brackish Water Organisms

MARIA TARKPEA and OLOF SVANBERG

The National Environment Protection Board, Brackish Water Toxicology Laboratory, Studsvik, S-611 82 Nyköping, Sweden

The addition of synthetic additives, such as methanol and methyl tertiary butyl ether (MTBE) to the base fuel, with the aim of raising the octane content of motor fuel, is currently of special interest in Sweden. A question, which has to be solved, is whether it is preferable to carry the additives by ship in separate tanks, because of the probable enhanced toxicity of the motor fuels by the addition of solvents, or not. The acute toxicity of these blend fuels has been compared with the acute toxicity of base fuel, gasoline and low leaded gasoline. As test organisms, two representative species of the Baltic Sea have been used, the harpacticoid *Nitocra spinipes* and the bleak, *Alburnus alburnus*. The results of the tests show that the acute toxicity of base fuel to aquatic organisms is not increased by the addition of 15% methanol or 5% MTBE.

A large amount of the motor fuel destined for Sweden is transported by ship in the Baltic Sea. New synthetic additives are continually being introduced on the market. To be able to forecast and reduce the effects of possible spills, in the event of accidents, it is essential to know the sensitivity of the aquatic organisms to these products, whether isolated or in combination with petroleum, as in blend fuels.

It could be expected that the toxicity of the gasoline is enhanced by the addition of solvents with the ability to dissolve more of the non-polar components into the water phase. Because of this, the question was raised as to whether it would be advisable to carry the additives in separate tanks in order to reduce the negative effects on biota in the event of shipwreck.

The aim of this study was to see whether the acute toxicities to representative species from the Baltic Sea of the