Bull. Environ. Contam. Toxicol. 31, 344-351 (1983)

Comparative Study of the Effects of Metals on the Settlement of *Crassostrea gigas*

H. R. Watling

Zoology Department, University of Port Elizabeth, P.O. Box 1600, Port Elizabeth 6000, South Africa

The effects of zinc and cadmium on the embryonic development, larval growth and settlement of the oyster Crassostrea gigas have been studied individually in some detail (BRERETON et al. 1973; BOYDEN et al. 1975; WATLING 1978). BOYDEN et al. (1975) reported that settlement of C. gigas larvae was both reduced and delayed in the presence of zinc but that those larvae which settled were as viable as controls when on-grown in clean water. The results reported by WATLING (1978) indicated the extreme toxicity of cadmium to C. gigas larvae and spat but contrasted with those results obtained from the two studies on the effects of zinc (BRERETON et al. 1973; BOYDEN et al. 1975). Cadmium appeared to be more toxic than zinc to both larvae and spat; the effect of cadmium was longer lasting than that of zinc; and cadmium appeared to induce early settlement and the numbers settling and their viability were markedly reduced.

Of course the results obtained in these three studies are not strictly comparable because under normal laboratory conditions the development of oyster embryos and larvae in repetitive tests is variable, some cultures being more hardy and faster growing. In the experiments described here, the effects of up to eight elements on C. gigas larval settlement and spat growth have been investigated using a single larval culture for each comparative test. The metal concentrations were chosen in the range to cause sub-lethal rather than lethal effects. Lethal concentrations (LC_{50}; causing 50% mortality in a specified period) were not determined except where they occurred in the selected concentration range. Experimental observations included growth and behaviour in addition to mortality.

MATERIALS AND METHODS

The experimental conditions were the same as those used by WATLING (1982) with the following modifications. The stabilities of sea water solutions containing low metal concentrations were investigated for the conditions of this experiment. Losses of up to 25% for lead were recorded at the end of the 24-h period but it was also found that the greatest losses occurred during the final 6 h. No losses were detected for the other elements. The experimental solutions were prepared to contain a combination of the flagellates Monochrysis lutherii, Isochrysis galbana, Chaetoceros calcitrans and Tetraselmis chui to give a final combined concentration of 150 cells/μl. The algal mix was supplied daily from the hatchery.

C. gigas larvae and spat were obtained from hatchery stock cultures. Hatchery procedure included the sieving of larvae and spat through nylon screens of selected mesh sizes. Thus the range of individual sizes was restricted at the beginning of each experiment. Samples from each treatment were collected at selected intervals and behaviour and structure examined. Measurements made included width across the valve for larvae and length for spat. The ages of larvae and spat always refer to the date of initiation of the culture.

Larvae were encouraged to settle on discs of black PVC placed in the bottom of each beaker. These discs were slightly arched so that both upper and lower surfaces were available for settlement. The number of larvae settling on the black discs in each beaker were counted but spat were not scraped off. Those which settled in the presence of metals were grown for an additional period in clean water.

Small cultchless-spat were suspended on a 400 μm nylon mesh; older spat were suspended on screens of larger mesh size. Samples were examined daily and measured at intervals during both the treatment and the succeeding period in clean sea water. Gaping individuals were removed. Spat in the size range tested may have been dead for up to two days before the shells gaped (A. GENADE personal communication).

RESULTS

The effects of eight elements on 19-day-old C. gigas larvae were compared. The treatments, 10 and 20 μg/l of each element prepared in triplicate, were continued throughout the 20-day experiment. Settlement was plotted on a daily basis and the settlement patterns for zinc, cadmium and copper (Fig. 1) illustrate three possible effects;

a) maximum settlement delayed with respect to that in the control (zinc, also lead);
b) maximum settlement coincident with that in the control (cadmium, also manganese, nickel and chromium);
c) maximum settlement occurring earlier than that in the control (20 μg/l copper, also 20 μg/l cobalt).

In terms of the numbers settling in each treatment, the only element which appeared to promote metamorphosis and settlement was copper, for which 80 and 95% of the larvae settled in the 10 and 20 μg/l treatments respectively, compared with 72% in the controls and 68% in the presence of manganese. All other elements tested caused a significant decrease in the total numbers of larvae settling (between 35-45%). On the basis of numbers settling, the order of element toxicity is Cr>Ni>Pb>Co>Zn>Cd>Mn>Control>Cu.

In view of the apparent positive response to the presence of copper, two further experiments were carried out. Twenty-nine-day old fast-growing larvae (those retained by a 200 μm screen at age 16 days) and slow-growing larvae (those which passed through a

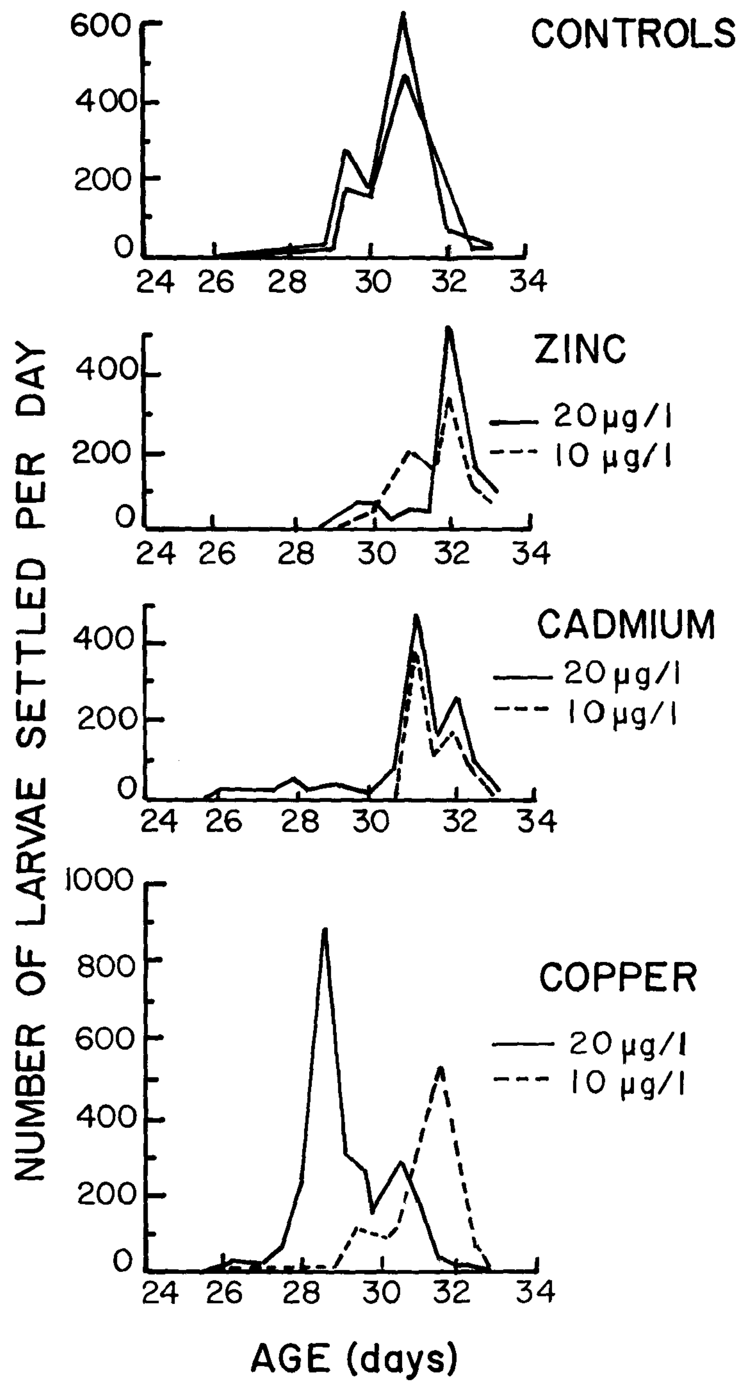

Fig. 1 Patterns of settlement for <u>C. gigas</u> larvae exposed to zinc, cadmium and copper.

200 μm screen at age 16 days) were placed in duplicate sets of beakers containing control, 20, 40 and 60 μg/l copper. These treatments were continued for 20 days.

In the case of the fast-growing larvae, copper was added to the solution for 3 days before substantial settlement occurred; low copper concentrations (<60 μg/l) appeared to promote settlement (Fig. 2.1). The slower growing larvae were exposed to copper for 8 days before substantial settlement occurred; fewer larvae settled in the presence of copper, the effect increasing with increased copper concentration (Fig. 2.2).

A third experiment on 16-day-old larvae was carried out. Larvae were exposed to zinc, cadmium and copper in the range 0-100 μg/l (treatments prepared in triplicate) for 6 days, after which the larvae were resuspended in clean sea water. Substantial settlement occurred 3 days later (age 25 days). Without exception increased metal concentration resulted in fewer individuals settling. Copper was toxic under these conditions.

The effects of these three metals can be compared directly, not on the basis of total settlement but rather in terms of the metal concentrations which prevent 50% of the larvae from settling, as compared with settlement in the control. Percentage settlement was plotted against concentration for each element and the concentration which caused a 50% reduction estimated. The results (30-35 μg/l zinc, 20-25 μg/l cadmium and 35-45 μg/l copper) indicate that metamorphosis/settlement is a very sensitive stage in the life of the oyster.

The effects of eight elements on 51-day-old cultchless *C. gigas* spat were compared (Table 1). All the metals which were tested caused a reduction in growth during the 14-day treatment period. However, recovery was such that individuals in all treatments were approximately the same size as those in the controls after a further 14 days in clean sea water.

The effects of zinc, cadmium and copper in the range 0-50 μg/l were tested on 35-day-old cultchless *C. gigas* spat (Table 2). Growth was reduced in the presence of cadmium and copper to a greater extent than in the presence of zinc but a general decrease in growth rate with increase in metal concentration was observed in all cases. On the basis of recovery during the subsequent period in control solutions, zinc is the least toxic element.

Mortality after the 11-day treatment was a maximum of 75% in the 50 μg/l copper solution, but this increased during the subsequent period in clean sea water. Twenty-three day LC_{50} values have been estimated to be 75 μg/l zinc, 50 μg/l cadmium and 60 μg/l copper.

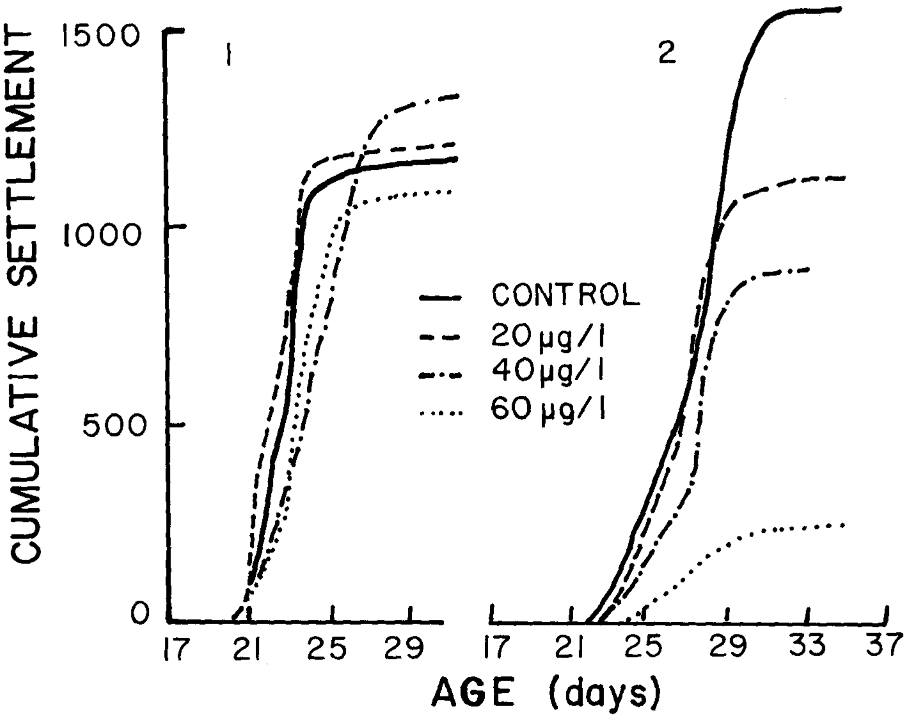

Fig. 2 Cumulative settlement during 20-day exposure to copper. 1. Fast growing larvae; 2. Slow-growing larvae.

TABLE 1

Growth and recovery of 51-day-old cultchless spat (mean length 3.7 mm) exposed to eight elements (results as mean spat length (mm))

Treatment	After 14 days treatment	After further 14 days clean water	Treatment	After 14 days treatment	After further 14 days clean water
Control	6.3	8.2	Mn 10µg/l	5.2	7.9
Control	6.2	8.2	Mn 20µg/l	5.4	7.6
Zn 10µg/l	5.3	8.1	Ni 10µg/l	5.1	7.8
Zn 20µg/l	4.9	7.9	Ni 20µg/l	5.5	7.9
Cd 10µg/l	4.9	7.6	Co 10µg/l	5.5	7.5
Cd 20µg/l	5.2	7.7	Co 20µg/l	5.7	7.4
Cu 10µg/l	5.7	8.0	Cr 10µg/l	5.5	7.7
Cu 20µg/l	5.2	7.8	Cr 20µg/l	5.3	7.5
Pb 10µg/l	5.0	8.0			
Pb 20µg/l	5.3	7.8			

TABLE 2

Growth and recovery of 35-day-old cultchless spat (mean length 2.6 mm) exposed to zinc, cadmium and copper (results as mean spat length (mm))

Treatment	After 11 days treatment	After further 12 days clean water
Control	4.2	6.2
Zn 10µg/l	4.1	6.5
20µg/l	4.2	6.3
50µg/l	3.6	5.1
Cd 10µg/l	3.7	5.7
20µg/l	4.0	5.2
50µg/l	3.8	4.7
Cu 10µg/l	3.9	5.1
20µg/l	3.8	5.0
50µg/l	3.6	4.8

DISCUSSION

Of the eight elements tested, only copper and manganese were not detrimental to C. gigas settlement success. Larval behaviour (as evidenced by foot extension and crawling movements) was not noticeably different for the various treatments in this experiment. Such differences were reported previously for greater zinc and cadmium concentrations (BOYDEN et al. 1975; WATLING 1978). Element effects on settlement patterns can be divided into three groups: induced early settlement, settlement at the same time as that in the controls and delayed settlement. However, the effects were not consistent for all the experiments. For example, prolonged exposure to low zinc concentrations resulted in delayed settlement whereas short-term exposure to slightly higher zinc concentrations induced early settlement. Copper induced early settlement in those cases where its presence was apparently beneficial but caused delayed settlement in those experiments where fewer larvae ultimately settled. Apparently induced changes in settlement patterns do not only depend upon the metal which is present and further research is required to elucidate the mechanisms by which metals affect the processes of metamorphosis and settlement.

The effect of copper has been accorded particular attention because PRYTHERCH (1934) reported that, of all the elements he tested, this was the only one which stimulated settlement, and that within a concentration range of 50-600 µg/l the number of individuals responding to copper stimulation was directly proportional to the amount present. Higher copper concentrations (>800 µg/l) were extremely toxic to C. virginica larvae. These concentrations are much higher than the ones tested in the present study, but the larvae were exposed to copper for much shorter periods during which time they were observed continuously.

The results of the four copper tests indicate that low copper concentrations (20-40 μg/l), added to the sea water immediately prior to larval settlement and for only two to three days, may stimulate larval settlement. However, the same copper concentrations, added too early or for longer periods, had the usual toxic effect of causing fewer larvae to settle. The effects of copper on larval development in oysters should be investigated more thoroughly as it may be possible to improve settlement success by the addition of small amounts of copper at a particular stage in larval development. This could be of significant value to the shellfish cultivation industry.

Zinc, cadmium, copper, lead, manganese, nickel, cobalt and chromium in the 10-20 μg/l range all caused a reduction in spat growth during metal exposure. However, recovery is such that individuals in all treatments grew to approximately the same size as those in the controls during the subsequent 14 days in clean sea water. The results of a second experiment indicated that zinc is less toxic to *C. gigas* than either cadmium or copper.

The results which have been obtained apply only to the experimental conditions which have been used, these being controlled water quality, food quality, temperature and aeration. Low metal concentrations which have had a measurable effect under these conditions might be expected to have a more serious effect under normal conditions where the parameters listed are variable. CALABRESE *et al.* (1973) have remarked that retardation of growth serves to prolong the pelagic life of larvae, thus increasing their chance of loss through predation, disease and dispersion. On the other hand, under normal conditions, pollutant concentrations are also very often variable, allowing periods of clean water conditions to occur during which times recovery and growth of both larvae and spat can take place as has been demonstrated. However, it is possible that recovery immediately prior to or during the process of settlement may not be as successful.

The most important and critical stage in the life history of the oyster is reported to be that of metamorphosis and settlement. STAFFORD (1913) states: "Spatting is the all important event. The value of the oyster harvest does not depend upon the number of eggs spawned, nor upon the number of larvae in the water, but upon the number of successful spat". Sufficient data are available to compare the sublethal effects of zinc, cadmium and copper on *C. gigas* embryos, larvae and spat (Table 3). The results of these experiments indicate that the process of settlement is the most sensitive period with respect to the effects of these three metals.

ACKNOWLEDGEMENT

The author thanks Mr A. Genade, Fisheries Development Corporation, Knysna, for his invaluable assistance and encouragement during these experiments. The research was carried out as part

TABLE 3

Summary of zinc, cadmium and copper toxicities to *C. gigas* embryos, larvae and spat

Effect	Element (µg/l) Zn	Cd	Cu
50% fewer straight-hinge larvae (WATLING 1981)	>>100	>>100	180
50% reduction in growth WATLING 1982) 6-day-old larvae	80	75	50
50% reduction in numbers settling 15-day-old larvae	30-35	20-25	35-45
50% reduction in growth of 35-day-old larvae	>50	>50	>50

of the National Programme for Environmental Sciences (Marine Pollution Section) and was financed by the Department of Environment Affairs.

REFERENCES

BOYDEN, C.R., H. WATLING and I. THORNTON: Mar. Biol. 31, 227-234, (1975).

BRERETON, A., H. LORD, I. THORNTON and J.S. WEBB: Mar. Biol. 19, 96-101 (1973).

CALABRESE, A., R.S. COLLIER, D.A. NELSON and J.R. MACINNES: Mar. Biol. 18, 162-166 (1973).

CALABRESE, A. and H.C. DAVIS: Helgoländer. wiss. Meeresunters, 20, 553-564 (1970).

PRYTHERCH, H.F.: Ecol. Monogr. 4, 49-107 (1934).

STAFFORD, J.: The Canadian Oyster. Its development, environment and culture. Report to the Canadian Commission of Conservation. Committee on Fisheries, Game and Fur-bearing Animals. Ottawa, The Mortimer Company. 159p. (1913).

WATLING, H.R.: Trans. roy. Soc. S. Afr. 43, 125-134 (1978).

WATLING, H.R.: S. Afr. J. Sci. 77, 134-135 (1981).

WATLING, H.R.: Bull. Environ. Contam. Toxicol. 28, 195-201 (1982).

Accepted May 19, 1983