

Mortality and Settlement Success of *Pocillopora damicornis* Planula Larvae during Recovery from Low Levels of Nickel¹

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ABSTRACT: Effects on mortality and settlement of *Pocillopora damicornis* planula larvae during recovery from low levels of Ni⁺⁺ were investigated. Results indicated that a nickel concentration of 9 ppm over 12 hr was sufficient to cause 50% mortality in larvae 39.6 hr after removal of the toxicant. Settlement in larvae was more sensitive, showing significantly reduced settlement rates from 9 days into recovery, after exposure to 1 ppm Ni⁺⁺ at durations of 12–96 hr. It is recommended that coral planula larvae be utilized more extensively in pollution studies.

MOST STUDIES ON THE TOXIC effects of heavy metals on marine organisms to date have concentrated on the more potentially toxic metals like mercury, copper, cadmium, and zinc (e.g., Barnes and Stanbury 1948, Corner and Sparrow 1956, Raymont and Shields 1962, Wisely and Blick 1966, Steemann and Wium-Anderson 1970, Eisler and Gardner 1973, Ahsanullah 1976, Ahsanullah and Arnott 1978, Mirkes et al. 1978, Lang et al. 1981). Fewer studies have been carried out on iron, lead, and nickel, which are known to be associated with the crude oil industry and exploration (Hodgins et al. 1977). Nickel, in particular, is important in the manufacture of steel and alloys (Moore and Ramamoorthy 1984) and is mined in the Americas, the Caribbean Islands, and Oceania, with some mining and smelting activities located very close to coral reefs (Carey 1981). Investigating the toxic effects of these metals is especially important in tropical developing countries with growing industries, where proper management stan-

dards for the treatment of industrial effluents are often overlooked.

Early investigations into the effects of heavy metals have used the death of organisms as an indicator of acute toxic stress (e.g., Wisely and Blick 1966, Connor 1972, Eisler and Gardner 1973, Ahsanullah 1976, Ahsanullah and Arnott 1978). More recently, there has been an increased interest in the use of ecotoxicological tests in which sublethal effects of metals are studied (Stebbing and Brown 1984, Brown and Howard 1985a).

Indicators of sublethal stress include growth rates (Brown and Ahsanullah 1971, Bryan 1971), reproductive success and embryo viability (Calabrese et al. 1973, Engel and Sunda 1979, Heyward 1988), physiological effects (Collier et al. 1973, Brown and Howard 1985b, Harland and Brown 1989), and metabolism (Howard et al. 1983).

Coral planula larvae (planulae) have been proposed as potentially useful organisms in ecotoxicological work (Stebbing and Brown 1984), as well as organisms for monitoring stress on coral reefs (Brown and Howard 1985a, Brown 1988). Apart from the work by Rinkevich and Loya (1977, 1979) on the effects of oil pollution on *Stylophora pistillata* planulae and a short-term copper bioassay on *Pocillopora damicornis* planulae by Esquivel (1983), little else has been done to explore this potential in pollution studies.

This short study examined how nickel exposure affected planulae from the coral *P.*

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damicornis in the laboratory. In the environment, this can be illustrated in a situation where coral reefs are subjected to occasional, but not chronic metal pollution. It investigated both acute and sublethal effects on planulae during recovery, after they were subjected to low levels of the metal. Mortality and settlement success were used as indicators of acute toxicity and sublethal toxicity, respectively.

MATERIALS AND METHODS

Collection

Pocillopora damicornis from Kaneohe Bay, Hawaii, has been documented to planulate monthly with a lunar periodicity (Richmond and Jokiel 1984). A single large type "B" (see Richmond and Jokiel 1984) *P. damicornis* head ca. 15 cm in diameter was removed from reef no. 10 in Kaneohe Bay on 18 June 1989. Experimental planulae were collected from the *P. damicornis* head on 23 June 1989 using the method described by Richmond and Jokiel (1984). The planulae were maintained in 0.2- μm -filtered seawater and held in 250-ml glass fleaker bottles at a density of 80 planulae per bottle. The fleaker bottles were placed in flowing seawater at ambient temperature and shaded from direct sunlight. To prevent the larvae from settling, the seawater was changed daily (Richmond 1983) and glassware washed regularly with dishwashing soap and distilled water following the suggestion of F. T. Te (pers. comm.). The planulae were kept from settling for 21 days before the start of the bioassay.

Set-up

A static bioassay in which test solutions were not renewed throughout the experiment was carried out using covered plastic petri dishes 8.5 cm in diameter at the base. The petri dishes were soaked overnight in 10% nitric acid and rinsed with filtered seawater over several hours before the start of the experiment. Twelve randomly picked planulae were placed in each of the petri dishes, which were first filled up to two-thirds with test solution. Four replicate experiments at each test con-

centration, including controls in which only 0.2- μm -filtered seawater was used, and at each exposure regime were carried out simultaneously. The petri dishes were placed under continuous fluorescent light at ambient room temperature (25–28°C) throughout the experiment.

Test Concentrations

All test concentrations were made using $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ and 0.2- μm -filtered seawater. A brief, range-finding preliminary experiment indicated that acute lethal effects were observable at between 1 ppm and 100 ppm Ni^{++} . Based on these preliminary data, test solutions for this experiment were made to concentrations of 0.5 ppm, 1 ppm, 10 ppm, and 25 ppm Ni^{++} , respectively. Water samples from the bioassays were taken at intervals during the experiment and analyzed using the Atomic Absorption Furnace and Inductive Couple Plasma to determine actual Ni^{++} concentrations to which the planulae were exposed, as well as to determine background levels of the metal in the seawater used.

Exposure Regime

The planulae were exposed to low concentrations of Ni^{++} under four time regimes, namely, 12 hr, 24 hr, 48 hr, and 96 hr. At the end of the exposures the Ni^{++} -spiked seawater in each petri dish was replaced with natural filtered seawater and the planulae observed for mortality and settlement.

Mortality Studies

Planulae mortality during recovery from Ni^{++} treatment was monitored using a binocular microscope at regular 12-hr intervals over 3 days. Death was determined by immobility, tissue disintegration, and ciliate infestation. Dead planulae were removed from the petri dishes and observed again for possible signs of revival.

Settlement Studies

One glass slide primed for 2 days in seawater was placed in each petri dish at the end

of the treatment regimes. The planulae were observed at 24-hr intervals for settlement on the glass slides or at the sides of the petri dishes. Settlement success was determined by the formation of a primary calcified corallite and extension of tentacles. The planulae were also observed for de-settlement, in which settled larvae bail out of their primary skeletons (Richmond 1985a).

Data Analyses

Planula mortality under stress from low levels of Ni^{++} was plotted graphically to determine the time at which 50% mortality was observed during recovery.

Percentage settlement success of planulae was analyzed using two-way analysis of variance (ANOVA) to determine significant differences in settlement ability between the controls and the various treatment regimes, in terms of concentrations used and time of exposure. The tests were carried out using a package program, MINITAB, performed on a mainframe computer terminal of the National University of Singapore.

Significant treatment effects were further analyzed using Duncan's new multiple range test, an a posteriori test (Duncan 1955) to identify the particular treatment regimes that were significantly detrimental to the settlement ability of the planulae.

RESULTS

Analyses of water samples from the bioassays using the Atomic Absorption Furnace and Inductive Couple Plasma indicated that the actual Ni^{++} concentrations the planulae were exposed to were 0.2 ppm, 1 ppm, 9 ppm, and 23 ppm, respectively. Background levels of Ni^{++} in the controls were low (<0.005 ppm) (Table 1).

Effects on Mortality

Because the effect observed during recovery from nickel stress was investigated, the presentation of the results does not follow the usual convention of the determination of LC_{50} and EC_{50} values.

TABLE 1
ESTIMATED AND ACTUAL [Ni] IN BIOASSAYS

ESTIMATE (mg/l)	MEASURED [Ni] (mg/l)	
	MEAN	SD
Control	< 0.002	
0.2	0.18	0.01
1.0	1.00	0.05
10.0	9.08	0.32
25.0	23.03	0.22

Planula mortality observed over 96 hr following the end of exposure to Ni^{++} is presented in Table 2. Experimental planulae in control petri dishes and 0.2-ppm Ni^{++} treatments at all exposure regimes, and 1-ppm treatments at 12-hr and 24-hr exposure durations did not show significant mortalities. In these treatments, mortalities during recovery over the 96-hr observation period was less than 5%.

Death in experimental planulae was characterized by immobility and the failure to respond to gentle prodding with blunt forceps, and then eventual tissue disintegration following infestation of the bodies of the planulae by ciliates. Mortality curves under the various Ni^{++} treatments and exposure regimes are given in Figure 1.

The most lethal treatment regimes were the 23-ppm and 9-ppm Ni^{++} treatments under the 96-hr exposure duration. All planulae in these two bioassay regimes eventually died before the end of the exposure durations. This was followed by the 48-hr, 23-ppm treatment where a 97.8% mortality was already observed at the end of the exposure regime and 100% mortality only 12 hr into recovery (Figure 1).

Graphical analysis of the data indicated that 50% mortality was observed after 14.7 hr into recovery at the 24-hr, 23-ppm regime; 17.1 hr at the 48-hr, 9-ppm regime; 22.5 hr at the 24-hr, 9-ppm regime; 28.5 hr at the 12-hr, 23-ppm regime; and 39.6 hr at the 12-hr, 9-ppm regime (Figure 1). At the end of the experiment, mortalities in the 1-ppm treatment at both the 48-hr and 96-hr exposure regimes were still below 50%.

TABLE 2
MORTALITY (%) OF PLANULAE DURING RECOVERY FROM NICKEL TREATMENT

EXPOSURE TIME (hr)		12		24		48			96		
TREATMENT CONC. (ppm)		9	23	9	23	1.0	9	23	1.0	9	23
DURATION AFTER TREATMENT (hr)		MORTALITY (%)									
0	Mean	0	0	0	0	0	14.6	97.9	0	100.0	100.0
	SE	0	0	0	0	0	12.0	2.1	0	0	0
12	Mean	0	0	0	38.1	2.1	25.0	100.0	0		
	SE	0	0	0	9.7	2.1	19.5	0			
24	Mean	10.8	31.4	56.5	95.8	2.1	85.4		2.3		
	SE	6.4	4.9	17.3	4.2	2.1	5.2		2.3		
36	Mean	38.5	82.9	85.1	100.0	6.3	95.8		2.3		
	SE	7.5	7.5	4.1	0	4.0	4.2		2.3		
48	Mean	80.9	95.8	97.9		25.0	100.0		6.8		
	SE	5.2	4.2	2.1		6.8	0		4.4		
60	Mean	100.0	100.0	100.0		27.1			6.8		
	SE	0	0	0		7.1			4.4		
72	Mean					39.6			11.2		
	SE					13.8			5.8		
84	Mean					39.6			11.2		
	SE					13.8			5.8		
96	Mean					45.9			13.5		
	SE					14.6			7.9		

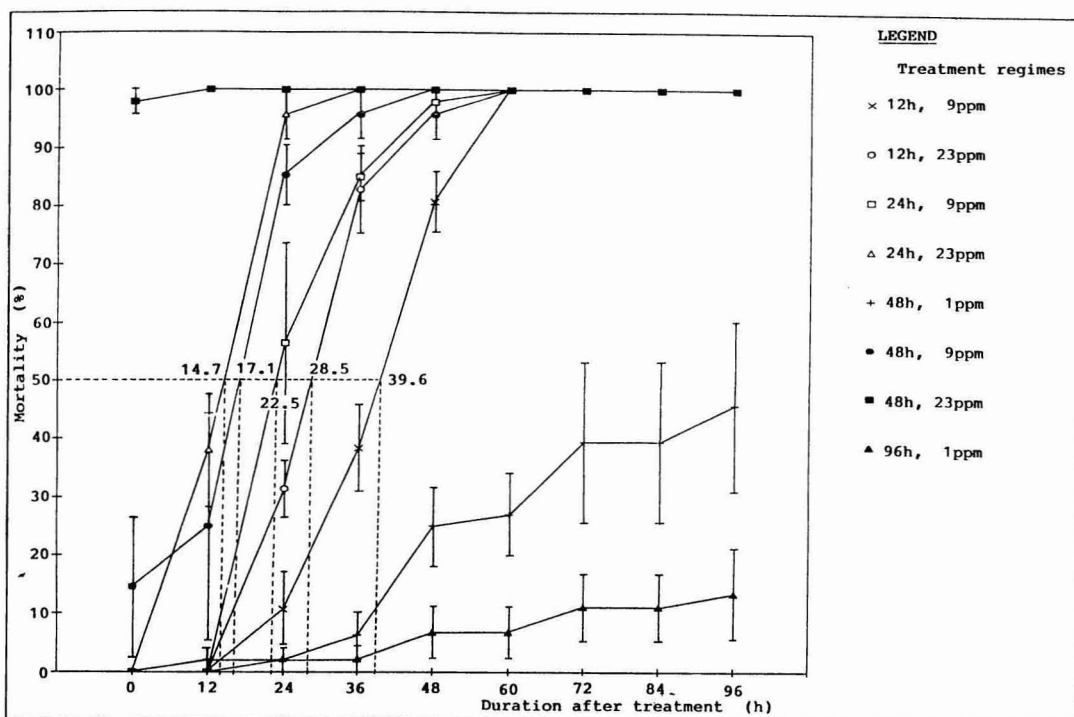


FIGURE 1. Percentage mortality (\pm SE) of planulae during recovery from Ni^{++} treatment.

Effects on Settlement

Glass slides primed in seawater used in the experiment did not serve as effective settlement surfaces. Less than 10% of the settlements recorded actually took place on the slides. Planulae had a greater tendency to settle on the edges, or at the bases of the petri dishes under the glass slides, where light intensity was reduced.

Planula settlement observed during recovery is recorded in Table 3. In general, settlement rates were low (< 50%), even in control

experiments. Percentage settlement success of planulae under the four time regimes and two treatment concentrations and controls are plotted (Figures 2-5).

Percentage settlement fluctuated in the first 10 days after treatment because of the settlement/de-settlement behavior of the planulae. This was observed in the treatment as well as control experiments. In the few examples of this behavior, settled planulae that bailed out of their corallites were observed to move around without any impairment to their swimming abilities. It could not be deter-

TABLE 3
SETTLEMENT SUCCESS (%) OF PLANULAE DURING RECOVERY FROM NICKEL TREATMENT

EXPOSURE TIME (hr)		12			24			48			96		
		0	0.2	1.0	0	0.2	1.0	0	0.2	1.0	0	0.2	1.0
TREATMENT CONC. (ppm)		CONTROL			CONTROL			CONTROL			CONTROL		
DURATION AFTER TREATMENT (days)		SETTLEMENT SUCCESS (%)											
1	Mean	4.2	2.1	0	0	0	0	2.1	2.1	4.2	6.3	6.3	0
	SE	2.4	2.1	0	0	0	0	2.1	2.1	2.4	6.3	4.0	0
2	Mean	4.2	2.1	0	2.1	2.1	0	4.2	4.2	0	6.3	10.4	0
	SE	2.4	2.1	0	2.1	2.1	0	2.4	2.4	0	6.3	5.3	0
3	Mean	4.2	4.2	2.1	4.2	2.1	0	4.2	4.2	0	6.3	10.4	0
	SE	2.4	2.4	2.1	2.4	2.1	0	2.4	2.4	0	4.0	5.3	0
4	Mean	6.3	4.2	0	4.2	4.2	2.1	6.3	4.2	0	8.4	10.4	0
	SE	4.0	2.4	0	2.4	2.4	2.1	4.0	2.4	0	4.8	5.3	0
5	Mean	2.1	4.2	4.2	4.2	8.3	2.1	6.3	2.1	2.1	10.4	10.4	2.1
	SE	2.1	2.4	2.4	2.4	3.4	2.1	4.0	2.1	2.1	4.0	5.3	2.1
6	Mean	2.1	2.1	4.2	4.2	8.3	2.1	6.3	2.1	2.1	12.5	10.4	2.1
	SE	2.1	2.1	2.4	2.4	3.4	2.1	4.0	2.1	2.1	2.4	5.3	2.1
7	Mean	2.1	2.1	4.2	4.2	6.3	2.1	6.3	2.1	2.1	14.6	10.4	2.1
	SE	2.1	2.1	2.4	2.4	4.0	2.1	4.0	2.1	2.1	4.0	5.3	2.1
8	Mean	6.3	2.1	4.2	4.2	6.3	4.2	6.3	4.2	2.1	18.8	10.4	2.1
	SE	4.0	2.1	2.4	2.4	4.0	2.4	4.0	4.2	2.1	2.1	6.3	2.1
9	Mean	4.2	4.2	4.2	4.2	6.3	4.2	6.3	4.2	0	20.9	10.4	0
	SE	2.4	4.2	2.4	2.4	4.0	2.4	4.0	4.2	0	2.4	6.3	0
10	Mean	6.2	4.2	4.2	6.3	6.3	4.2	8.3	4.2	0	22.9	10.4	0
	SE	2.1	4.2	2.4	4.0	4.0	2.4	5.9	4.2	0	4.0	6.3	0
11	Mean	8.3	6.3	4.2	6.3	6.3	4.2	12.5	6.3	0	20.8	14.6	0
	SE	3.4	4.0	2.4	4.0	4.0	2.4	9.9	4.0	0	5.4	8.6	0
12	Mean	8.3	4.2	4.2	6.3	10.4	4.2	14.6	6.3	0	20.8	14.6	0
	SE	3.4	2.4	2.4	4.0	6.3	2.4	12.0	4.0	0	5.4	8.6	0
13	Mean	10.4	4.2	4.2	6.3	10.4	4.2	14.6	8.3	0	20.8	14.6	0
	SE	4.0	2.4	2.4	4.0	6.3	2.4	12.0	5.9	0	5.4	8.6	0
14	Mean	10.4	4.2	4.2	6.3	10.4	4.2	14.6	8.3	0	33.3	27.1	6.3
	SE	4.0	2.4	2.4	4.0	6.3	2.4	12.0	5.9	0	3.4	5.3	4.0
16	Mean	10.4	4.2	4.2	6.3	10.4	4.2	16.7	10.4	2.1			
	SE	4.0	2.4	2.4	4.0	6.3	2.4	14.0	6.3	2.1			
17	Mean	12.5	4.2	4.2	6.3	10.4	4.2						
	SE	2.4	2.4	2.4	4.0	6.3	2.4						

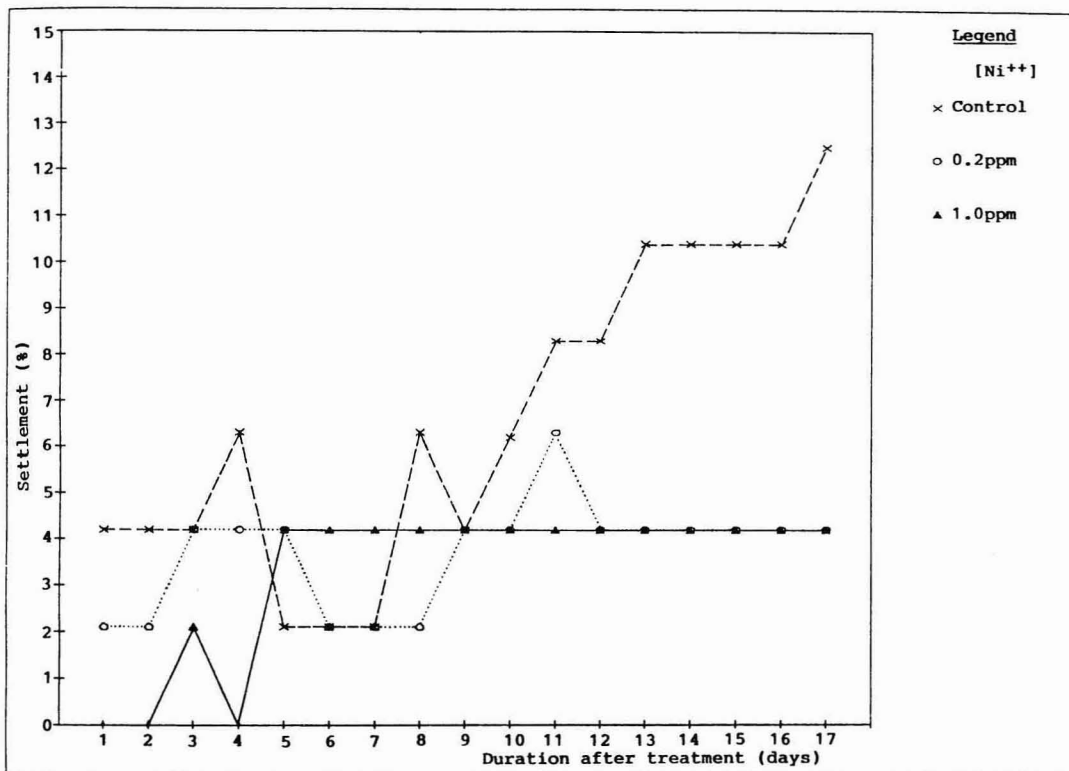


FIGURE 2. Percentage settlement of planulae during recovery from Ni⁺⁺ (12-hr treatment).

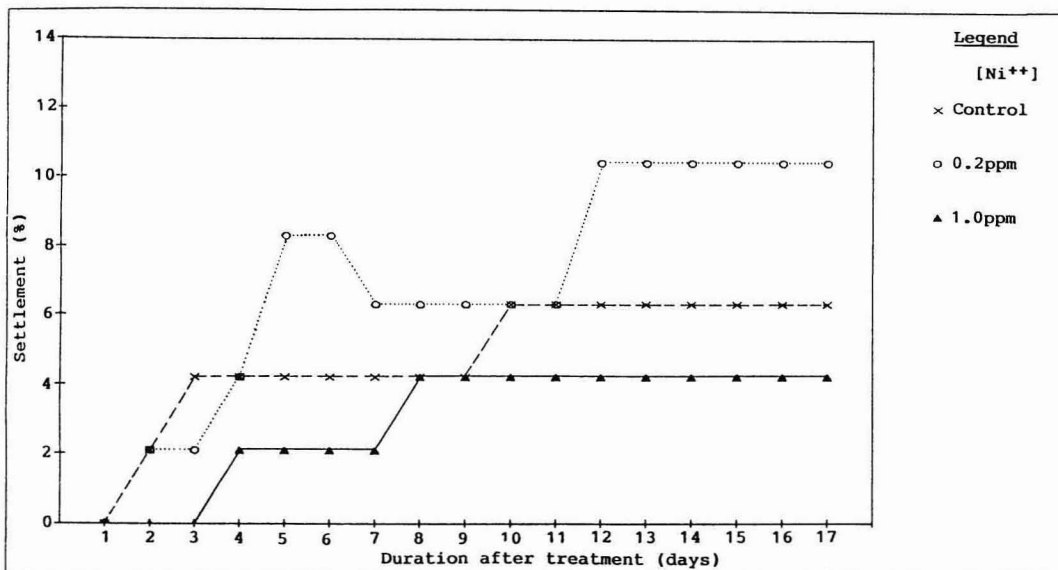


FIGURE 3. Percentage settlement of planulae during recovery from Ni⁺⁺ (24-hr treatment).

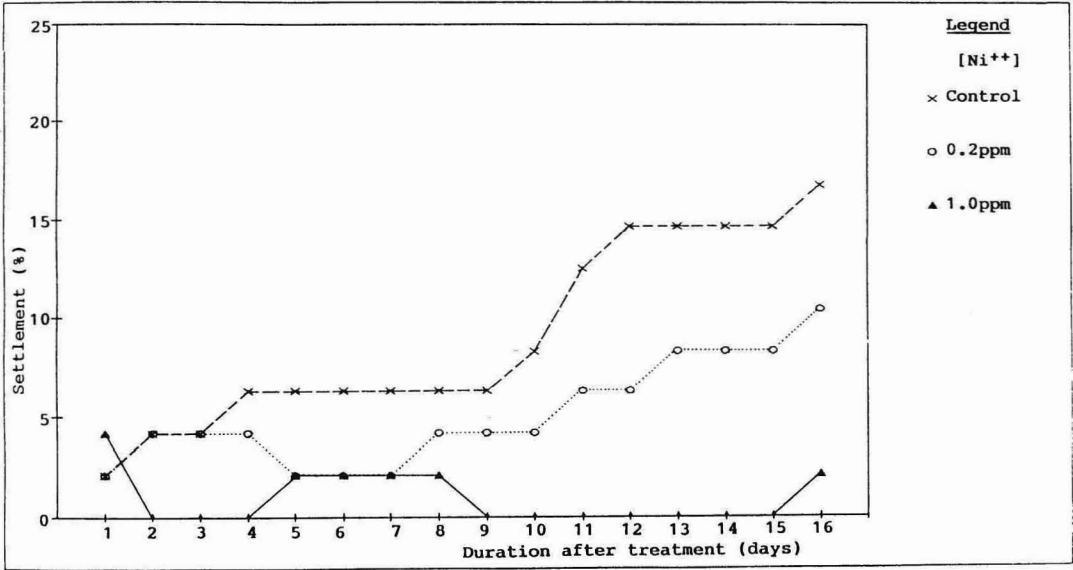


FIGURE 4. Percentage settlement of planulae during recovery from Ni⁺⁺ (48-hr treatment).

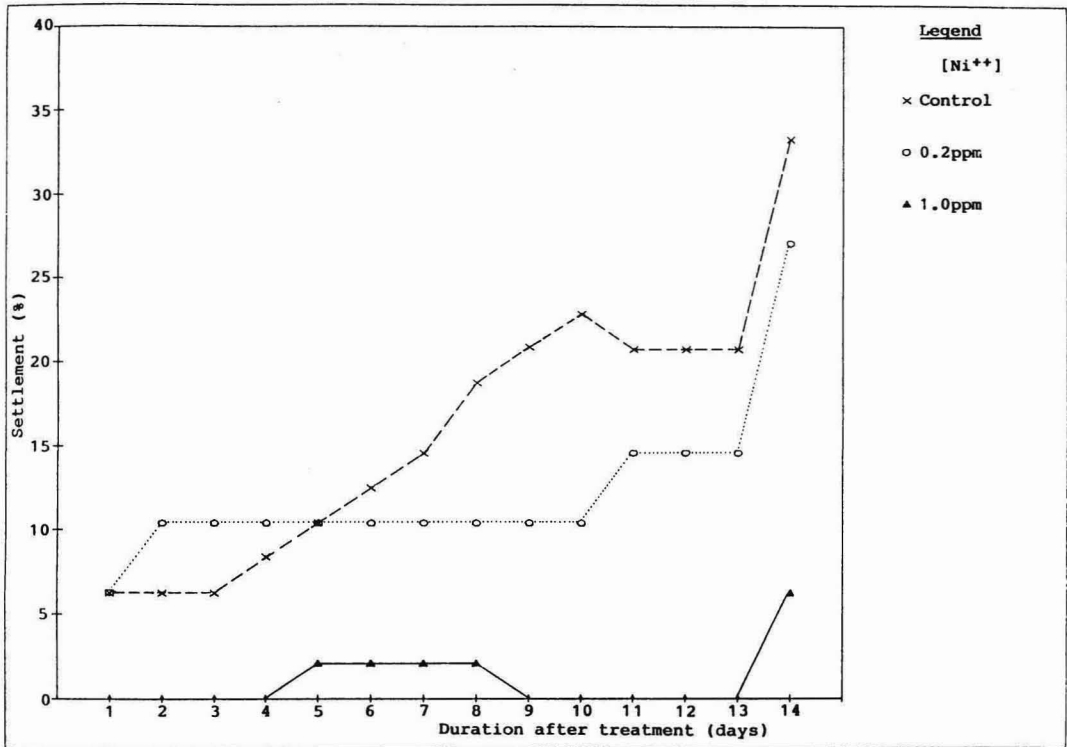


FIGURE 5. Percentage settlement of planulae during recovery from Ni⁺⁺ (96-hr treatment).

mined if the larvae that bailed out eventually settled successfully again.

Two-way ANOVA on the data revealed that significant treatment effects due to the concentration of Ni^{++} used were apparent from the ninth day of recovery ($F = 4.16$; $df = 2, 36$; $P < .025$), regardless of the duration of the treatment. Duncan's new multiple range test comparing mean settlement in treatments and controls indicated that a concentration of 1 ppm Ni^{++} was sufficient to cause significant effects on the settlement ability of planulae from day 9 of recovery ($P < .05$).

The effects of treatment durations on planula settlement were significant only at 14 days into recovery (two-way ANOVA: $F = 6.59$; $df = 3, 36$; $P < .01$). Duncan's multiple range test on the data revealed that mean settlement of planulae subjected to treatment durations of 96 hr differed significantly from the 12-hr, 24-hr, and 48-hr time regimes ($P < .05$).

Two-way ANOVA also indicated no significant interaction effects between the concentration of Ni^{++} used and the duration of treatment ($P > .05$).

Although the settlement success of planulae subjected to 0.2 ppm Ni^{++} under the 24-hr treatment regime was higher than that of controls (Figure 3), this was not significant in the ANOVA test where all data were pooled.

DISCUSSION

Esquivel's (1983) study of the short-term effects of copper on *P. damicornis* planulae reported LC_{50} 's of 120 ppb, 115 ppb, 90 ppb, and 63 ppb Cu^{++} at 12-hr, 24-hr, 48-hr, and 96-hr exposure regimes, respectively. In the present study, median lethal effect at the 12-hr exposure and 9-ppm nickel regime was observed only 39.6 hr after the removal of the toxicant. It seems that nickel is far less toxic than copper, as deleterious levels to planulae lie only in the ppm range.

Calabrese et al. (1973) classified nickel as a metal "relatively toxic" to *Crassostrea virginica* embryos. They reported a 48-hr LC_{50} of 1.18 ppm Ni^{++} in their toxicity tests, a level that lies closer to the concentrations used in the present study. Nickel concentrations of

0.03–0.05 ppm were also reported to reduce fecundity, reproduction, and respiration in *Daphnia magna* (Biesinger and Christensen 1972) and sea urchin embryos (Timourian and Watchmaker 1972). Planula larvae are more developed than embryos and, understandably, are less susceptible to nickel toxicity. However, considering the fact that many coral species rely on external fertilization and embryological development, the actual concentration of nickel at which the embryos of coral spawners will be affected may be much lower than the threshold levels encountered in this study.

The settlement rates of control planulae observed in this study were low compared to those of *Stylophora pistillata* planulae as documented by Rinkevich and Loya (1977). In their laboratory experiments with crude oil, control planulae had percentage settlements of 55%. In the present study, planulae settlement in controls only reached a maximum of 33.3% in the 96-hr experiments. Richmond (1987) reported that the settlement viability of *P. damicornis* planulae he kept for 103 days was 3%. Although it is usually assumed that high settlement rates of *P. damicornis* planulae occur in areas with large populations of this species of coral (Richmond 1985b), there are little quantitative data on actual settlement rates in the laboratory to make direct comparisons. The percentage settlement of control planulae recorded in this study may well be the norm for *P. damicornis*. However, it must be noted that the settlement rates observed here may also have been affected by the length of time the planulae were kept from settling. Possible effects of delaying settlement in planulae and their development and eventual settlement abilities must be investigated further.

Reversible metamorphosis in *Pocillopora damicornis* planulae was documented by Richmond (1985a). His observations indicated that settled planulae could bail out of their calcareous skeletons and revert to a planktonic form when stressed. The small percentage of the settlement/de-settlement behavior or polyp bail out observed in the present study could be attributed to stress caused by the experimental set-up. It cannot,

however, be determined if the stress was also due to the low Ni^{++} concentrations present, as this behavior was observed in both treatment and control dishes.

It should be noted that survival and settlement of planulae in these experiments were significantly affected by levels of nickel as low as 1 ppm, even after the source of pollutant was removed. This has ecological significance, as a reduction in the planula's viability and ability to settle successfully directly affects the percentage of viable recruits into the coral reef.

The threshold concentration of nickel above which the planulae were adversely affected in this study was between 1 ppm and 9 ppm. This concentration may not be encountered in the natural environment, as published data for nickel levels in uncontaminated coastal waters are merely 1.8 ppb (Snodgrass 1980), and waters affected by anthropogenic inputs are estimated to contain 2.5–15 ppb (Moore and Ramamoorthy 1984). However, contaminated marine sediments have been reported to contain an average of 3.5–36 ppm of nickel (Helz 1976, Trefry and Presley 1976, Knauer 1977, Salomons and Mook 1977, Pilotte et al. 1978, Carey 1981). The results obtained here are therefore valid.

The results of this short study indicate that the use of *P. damicornis* planulae as a tool in pollution monitoring has great potential. The ease of collection, maintenance, and manipulation of the larvae make them attractive organisms with which to work. However, the methods used in the bioassays need to be refined further for better sensitivity and viability. Some immediate proposals are for more detailed studies into the effects of nickel concentrations between 1 ppm and 9 ppm on planulae for a longer term, and determining if the sensitivity of planulae to stress is affected when settlement is delayed.

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