TOXICITY BIOASSAYS FOR THE PITI POWER PLANT, GUAM, ON THE EFFECTS OF TOTAL RESIDUAL CHLORINE ON THE EARLY LIFE STAGES OF SELECTED TROPICAL MARINE SPECIES

by

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ABSTRACT

Static 96-hr, controlled-temperature bioassay systems were developed, with NaOCl as chlorine source, to investigate the effects of single doses of chlorine-induced oxidants (CIO) on the phytoplankters Chaetoceros gracilis and Dunaliella tertiolecta, plutei of the sea urchin Echinometra mathaei, veligers of the opisthobranch Stylocheilus longicauda, 4-month-old juveniles of the topshell Trochus niloticus, and two reef-flat fish species, the mullet Chelon engeli and the cardinal-fish Apogon lateralis. LC50's were interpolated from a simplified log-probit regression analysis. Results indicate that the phytoplankters were affected (LC50's) at concentrations as low as 0.1 ppm CIO with a general range of 0.16-0.29 ppm. The mid-range LC50's for the fish were 0.2-0.3 ppm, followed by the urchin plutei at 0.4 to 0.8 ppm and juvenile and larval molluscs (>1.95 ppm CIO).

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The biologically influenced physical characteristics measured for samples of experimental and control seawater were as follows: salinity ranged from 31.4°/₀₀ to 34.2°/₀₀; NH_4^+ was consistently less than 0.2 µg-at ℓ^{-1} but did go to 3.0 µg-at ℓ^{-1} during the fish runs; dissolved oxygen ranged from 4.95 to 5.60 ppm; and the pH varied from 7.93 to 8.06.

Plots of chlorine decay rates in saltwater are displayed in Figure 2. The presence of test organisms in the water greatly increased the rate of decay. The addition of 2.4×10^5 cells of Dunaliella per mℓ to chlorinated, filtered (0.45µ) seawater reduced the CIO level from approximately 2.7 to 0.5 ppm after 1 hour. Similar results were obtained when fish were present. An initial CIO concentration of 0.47 ppm in a 40-ℓ aquarium with 12 apogonids dropped below 0.1 ppm within 1 hr and reached 0.00 ppm within 6 hr. Although these results indicate that one-time exposure to moderate chlorine concentrations was relatively short-termed, one should keep in mind that the chlorine probe measures the available chlorine and chlorine-induced oxidants but does not measure chlorine or its by-products that have been absorbed or bound by the organisms. On the basis of the observed decay rates, it was deemed safe to aerate aquaria 1 hr after chlorination when necessary to maintain the test organisms, without concern for driving off measurable amounts of chlorine.

The results of each bioassay are plotted as percent mortality versus ppm CIO (Figures 3-8). Although data collected at 48 hr are plotted, curves (broken lines) are drawn only for 96-hr data. A curve indicating the general response appears as solid line, with the symbol "L" indicating the LC50 value determined by log-probit analysis of the combined 48- and 96-hr data.

Dunaliella tertiolecta cultures were assayed at temperatures of 28.0°C, 30.0°C, 33.0°C, and 34.4°C over a range of 0.06 to 1.43 ppm CIO. The results are illustrated in Figures 3 and 4. The LC50 values for all Dunaliella bioassays ranged from 0.16 to 0.29 ppm CIO (Table 2). It should be noted that the results with phytoplankters like Dunaliella probably reflect the effects of chlorine on the growth rate, rather than actual mortality. This is because acellular organisms undergo a considerable amount of binary fission during the 96-hr test period. The percent mortality is computed by comparing the density of organisms in the chlorinated water to the density of controls in unchlorinated seawater. The number of cells per mℓ in the control increases over 96 hr (Table 1). The density of organisms in the test beakers can increase if the rate of division exceeds the mortality. The result is reported as mortality, but in fact represents a combination of lethal effects and sublethal inhibition of cell division. This should be kept in mind when

one interprets the results of the phytoplankton bioassays, which are reported here as percent mortality and concentration of chlorine causing 50% mortality (LC50).

The average LC50 for Chaetoceros was 0.18 ppm CIO (Figure 5). Since correlations between the transformed mortality and concentration data were high (r = 0.85-0.92) and no consistent differences between temperatures were noted, the 48-hr and 96-hr data from both temperatures were combined for this LC50 interpolation. The LC50 at 29°C was approximately 0.3 ppm and the LC50 at 33°C was approximately 0.15 ppm. The Chaetoceros controls grew faster at 33°C than at 29°C (Table 1).

Bioassay results with Echinometra are shown in Figure 6. Ciliate contamination was marked after 96 hr in cultures that received low doses of chlorine and especially in the controls. To correct for this problem, counts of control cultures at 48 hr were used as a reference concentration to compute both 48-hr and 96-hr mortality rates. The LC50 determined by log-probit analysis at 28°C was 0.84 ppm CIO, and at 33°C it was 0.46 ppm CIO. The 96-hr LC50 values ranged from approximately 0.1 to 1.0 ppm for two runs at four temperatures (Figure 6).

Juvenile Trochus mortalities never exceeded 50% over a CIO range of 0.057 to 3.35 ppm (Table 2). Hence, no calculated LC50 can be reported for this organism, and the reasons for its apparent resistance to chlorine are discussed below.

Bioassay results with the other mollusc, Stylocheilus, are presented in Table 2. As with Trochus, no 100% mortalities were obtained; therefore, no mortality curves were plotted. The apparent anomaly in the percent mortality at 0.4 ppm between the 48-hr and 96-hr counts will be discussed below. The LC50 was estimated to be greater than 1.95 ppm CIO. Fifty-percent mortality was never reached at 29°C.

The LC50 for the cardinalfish Apogon at 30.1°C was 0.21 ppm CIO (Figure 7). In two of the six runs the mortalities never exceeded 20% and hence were not used in the calculation of this LC50 value. Possible explanations for the variation in tolerance to chlorine are discussed below. The effect of any given chlorine concentration was usually all-or-none. Partial kills were observed in only 38% of the bioassay tanks. During the bioassays it was observed that any fish exhibiting abnormal swimming behavior immediately after chlorination would invariably die within three hours.

The mullet Chelon showed even more striking all-or-none responses to the chlorine (Figure 8). The LC50 determined by log-probit analysis was 0.20 ppm CIO at 30.1°C. However, the average LC50 for the four runs was 0.30 ppm and was probably a more accurate representation of their response.

INTRODUCTION

Late in 1979 the U.S. Navy, through its Fena Laboratory, contracted the University of Guam Marine Laboratory to investigate the effects of chlorine on selected tropical marine organisms. This report presents the results of these studies.

Historically speaking, most chlorine toxicity work has focused on freshwater organisms; work on marine organisms is more recent. Many studies in both areas were reviewed in Brooks and Seegert (1978), Davis and Middaugh (1977), and Morgan and Carpenter (1978). Mattice and Zittel (1976) presented a compilation showing test organisms, experimental conditions, and effects of chlorine for saltwater studies available to them. We have expanded and modified their compilation in the Appendix, which includes our data and the unpublished results of the only other study which we know of that deals with tropical organisms (Davis 1971).

The chemistry of chlorine in seawater is complex and still poorly understood. When chlorine is introduced into seawater it reacts rapidly with bromide and with organic compounds, and some of the chlorine is "lost" or at least unaccounted for by ordinary analytical tests (Goldman et al. 1979). Chloramine, a slow-decaying product of the seawater-clorine reaction, has been found to be a more potent biocide to temperate lobster larva than chlorine itself (Capuzzo 1977); and the nonoxidative "loss" could also be a potential biocide (Goldman 1979). Jolley (1977) found over 50 chloro-organic constituents in municipal sewage effluent. He estimated that more than 5,000 tons of these compounds are released into aquatic ecosystems each year. The potential damage to the marine environment is enormous. Bio-magnification, slow degradation, and other known effects of such clorinated organics as DDT and PCB account for the concern of marine ecologists. Only recently have advances in analytical methods for the quantitative and qualitative assessment of chlorine-induced oxidants, or CIO, enabled the biologist to examine the acute effects of chlorine on aquatic organisms. There is still much discussion about the relative merits of these analytical methods (Bender 1978, White 1972).

The deleterious effects of chlorine on marine organisms depend on many factors, but residual concentrations as low as 0.05 ppm have been shown to be potent fertilization inhibitors (Muchmore and Epel 1973) and to reduce primary production by 75% in entrained phytoplankton (Carpenter et al. 1972). A concurrent rise in temperature and chlorine concentration, such as might be found in power plant effluent channels, was shown to have an adverse synergistic effect on juvenile salmon (Stober and Hanson 1974), on trout and yellow perch (Brooks and Seegert 1977), and on many other fish and fish-food organisms (Thatcher et al. 1976).

Interesting patterns emerge from studies of mortality versus concentration for some selected marine organisms. The work by Capuzzo et al. (1977) at Woods Hole showed no mortality of juvenile fish until chlorine concentrations reached approximately 1 mg/ℓ, after which, with only a slight rise in clorine concentration, mortality was 100%. Conversely, invertebrate larvae and zooplankters showed gradual increases in mortality with increased chlorine concentrations. The responses of marine invertebrate sperm and some phytoplankters to chlorine seem to be more pronounced (lower LC50) than those of larval and juvenile fish, which in turn are generally more sensitive to chlorine than are adult invertebrates.

These are only general patterns; and, as in most toxicological studies, it must be stressed that the responses of aquatic organisms to chlorine seem to be species-dependent. Broad conclusions concerning the responses of major taxonomic groups to chlorine-induced oxidants should be avoided until specific data, generated under similar experimental conditions, are available for major families and genera. In relation to the sensitivity hierarchy, Goldman et al. (1978) pointed out that ecological impact does not always mirror laboratory results. Although both phytoplankton and invertebrate larvae are affected at very low levels (0.01 mg/ℓ), complete populations of invertebrate species that spawn intermittently could be seriously threatened by chlorination while any entrained phytoplankton exposed to chlorine represents only a small fraction of the total standing crop.

Ferguson-Wood and Johannes (1975) compared some tropical and temperate parameters and discussed the possible effects of chemical disinfectants in the tropical marine environment. They noted that the potency of these toxins increases with increasing temperature but that faster degradation is predicted in the tropics. Furthermore, any physiological or toxicological study with tropical reef organisms must consider the possibility that if tropical species (especially fish) are living at the lower limits of their oxygen demand tolerance, they would be more susceptible to a respiratory tissue oxidizer such as chlorine than would their temperate cognates.

As previously noted, many research groups have evaluated the toxic effect of CIO on selected temperate biota with either continuous or intermittent exposure methods. However, only limited data are available on the effects of single-dose exposures of CIO on tropical marine organisms. On Guam, the Navy's Piti Power Plant is the only known source of chlorine contamination other than illegal fishing; chlorine in the form of Cl_2 gas dissolved in water is added to the condenser cooling water twice a day to control the growth of fouling organisms. Hence, the objective of this study was to quantify the acute effects of chlorine on selected tropical marine biota by utilizing a single-dose LC50 method. The relative sensitivities of key species under similar experimental conditions can then be compared.

MATERIALS AND METHODS

Test organisms were selected on the basis of local availability, ability to withstand laboratory manipulation, good survivorship for 96 hours in a static test system, and common occurrence in the outfall of the Piti Power Plant. A variety of phyla and trophic levels was included. Whenever possible, larval or juvenile forms were chosen for the bioassays because they were expected to be more sensitive to toxic compounds.

The test organisms in this investigation included:

1) Dunaliella tertiolecta Butcher, a cultured tropical phytoplankter;
2) Chaetoceros gracilis Shutt, a cultured tropical phytoplankter;
3) Echinometra mathaei (de Blainville), a local sea urchin, at the larval pluteus stage;
4) Stylocheilus longicauda (Quoy and Gaimard), a local opisthobranch, at the larval veliger stage;
5) Trochus niloticus L., an introduced gastropod, at a post-settling (juvenile) stage;
6) Chelon engeli (Bleeker), a local mullet, at a juvenile stage;
7) Apogon lateralis Valenciennes, a local cardinalfish, at an adult stage.

Both phytoplankton cultures were maintained at the Marine Laboratory. Adult Echinometra were collected from the Piti intake canal and gametes were obtained for fertilization by peristomial injection of 0.5 M isotonic KCl. After 24 hours the pluteus larvae were then put into the experimental system, where they had no additional opportunity for feeding during the 96-hour test period. Stylocheilus veligers were collected by incubating naturally occurring gelatin strings which were deposited by adults on the walls of flow-through tanks at the lab. Trochus juveniles were reared from natural spawnings of adults held in laboratory tanks. The fish were collected by cast net from the reef flats of Agana and Tumon Bays.

Single-dose, 96-hour static bioassay systems were employed throughout this investigation. All the test runs were carried out with natural seawater in a temperature-controlled water bath regulated by a Braun contact thermoregulator which controlled a PSG normal-closed relay and two 1000-watt immersion heaters. Appropriate modifications of the actual set-up were used when necessary to accommodate the diverse group of test organisms.

Generally, the bioassay system was identical for Dunaliella, Chaetoceros, Echinometra plutei, Stylocheilus veligers and Trochus juveniles. This system consisted of two complete sets of acid-washed non-

aerated Pyrex beakers, one set at each of two controlled temperatures, with 100 ml of solution consisting of test species and filtered (45μ membrane filters) fresh seawater. Each set had two experimental beakers plus a control of each of six CIO concentrations. For monitoring chlorine concentration, reagents were added to an additional 100 ml beaker, without organisms, at each concentration. This method allowed the initial chlorine demand of the filtered seawater to be satisfied, so the recorded CIO was the concentration available to the organisms. Except where noted, counts were made at least at 48- and 96-hr intervals.

Dunaliella and Chaetoceros samples were scored on a hemacytometer under a compound microscope. Echinometra and Stylocheilus larvae were scored by placing an aliquot in a small, ruled petri dish and counting under a dissecting microscope. These scoring techniques are particularly time-consuming, since there were at least three beakers at each of six concentrations (2 experimental and one control), a complete set of two temperatures, and, in the case of Stylocheilus and Echinometra, three samples were aliquoted from each of the 36 beakers by three workers for each interval. For the phytoplankton counts, four individual aliquots (two for each of two workers) from each culture were scored at each interval. Only a hand lens was necessary to score the macroscopic Trochus juveniles. Lack of movement or degeneration were used as the criteria for death.

The two species of fish, juveniles of the common mullet Chelon engeli and adults of the cardinalfish Apogon lateralis, were subjected to CIO bioassays at one controlled temperature (30.1°C). The fish system required 40-ℓ aquaria containing 1 -filtered seawater. Only four concentrations were used per replicate because of limited space in the tempreature-controlled water table. Therefore, at least four replicate 96-hr runs were made for each species. Replicate tanks of exactly the same volume of filtered seawater were set up for each CIO measurement. Twelve fish were placed in each static aerated tank one hour prior to NaOCl addition. This sequence allowed some time for the fish to acclimate before the toxin was added but minimized the time that excreted ammonia could accumulate. A constant, low-level overhead fluorescent light regime was used throughout the fish runs because preliminary work indicated that they react violently to sudden light-dark changes. Scoring the fish bioassays was much simpler and more precise. The number of living or dead fish in each tank was noted by near-continual monitoring for the first 3-4 hours then once every 12-24 hours during the 96-hr experimental period.

Single-dose 96-hr LC50's, the concentrations at which 50% of the test organisms die when compared to the controls, were calculated in the following manner: since the toxicity response curve for each organism was sigmoidal, a log-probit transformation was performed to enable us to do a common linear regression and thus calculate a 50% mortality concentration. Test concentrations were log-transformed and percent mortality data were transformed to unweighted simple probits, which are normal deviates plus five (Fisher and Yates 1962). Using this method, we considered only mortalities between 5 and 95% (except where noted in the Results section) because this enabled us to do a simplified probit analysis without weighting for the extremes, i.e., a 0 and 100%

mortality, which when graphed on probability or probit paper, are undefined and infinity, respectively. Regression statistics include: r, the regression coefficient; m, slope of the line; intcp, the Y-intercept; and N, the sample size, which in these cases are the number of concentrations at each, or both, temperature. We define percent mortality as:

$$\% \text{ Mortality} = \left(1 - \frac{N_e}{N_c}\right) \times 100$$

where N_e is the number of organisms, or concentration of organisms, in the experimental samples; and N_c is the number of organisms, or concentration of organisms, in the control samples. ClO concentrations versus percent mortalities were graphed, with the interpolated LC50's, on semi-log paper for each species.

Chlorine is a difficult toxin to study because its concentration changes rapidly for many reasons once it is in solution. This means that no stock solution or dilution series can be trusted to have a specific concentration. It is necessary to measure the initial chlorine concentration at the beginning of each 96-hr run. An Orion selective-ion electrode (97-70) was used to measure chlorine-induced oxidants for the bioassay systems. The basic principle of this electrode involves the oxidation of an iodide reagent in the presence of a buffered acidic solution (pH 4). The probe is then used to measure the concentration of the free molecular iodine (or other halogen compounds) released. The probe was tested against nine other methods in a matrix of concentrations and water sources by Bender (1978). Regrettably, seawater was not included in the matrix, but precision was good; consistently less than 2% relative standard deviation within the expected experimental concentrations (0.2 to 1.0 ppm) and less than 5% in all cases. The relative accuracy, when compared to the iodometric (PAE) forward titration method, for all water types except very polluted waters (raw sewage), was within the same limits. In addition, Bender titrated the Orion-supplied standard and found it to be correct. We tested the accuracy of the probe against the HACH DPD method. The results of this comparison are shown in Figure 1. The two methods are in agreement over a range of 0.1 to 2.0 ppm (mg/ℓ) total residual chlorine. Because of its precision and ease of use, the probe was used throughout the project.

Since maintaining consistent or safe levels of important water quality parameters is a major concern in static bioassay systems (Alabaster and Abram 1965), ammonia, dissolved oxygen, pH, and salinity were periodically checked throughout the study. NH_4^+ and dissolved oxygen were checked with an Orion ammonia probe (95-10) and an Orion O_2 probe (97-08) attached to a Beckman expanded-scale pH meter. A description of the function of the ammonia probe can be found in the appendix of Nelson et al. (1980). Salinity was monitored with a refractometer, and pH was measured with a Corning triple-purpose probe attached to the Corning 135 meter.

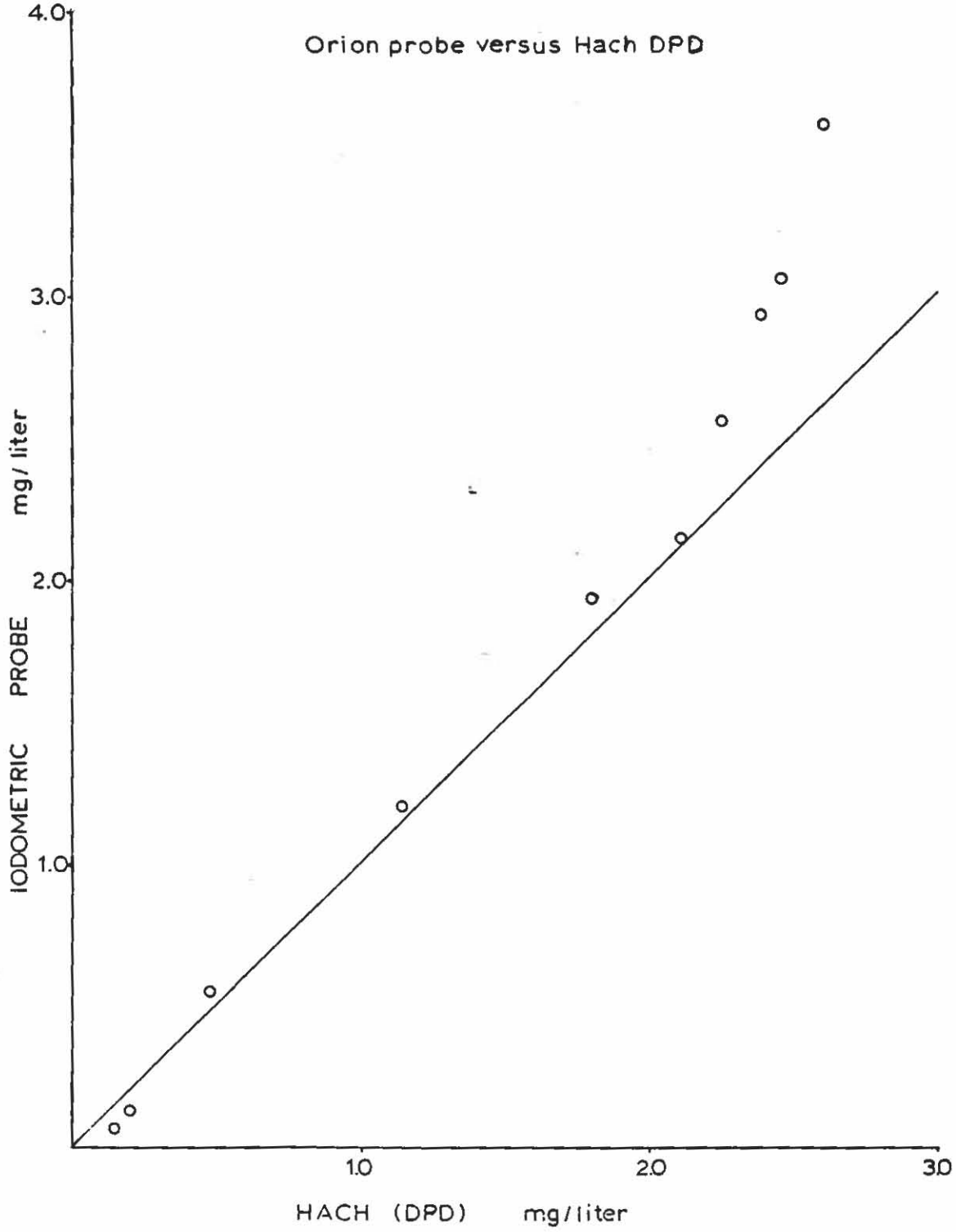

Figure 1. Comparison of analytical results with the chlorine probe and the Hach DPD method.

RESULTS

The biologically influenced physical characteristics measured for samples of experimental and control seawater were as follows: salinity ranged from 31.4°/₀₀ to 34.2°/₀₀; NH_4^+ was consistently less than 0.2 µg-at ℓ^{-1} but did go to 3.0 µg-at ℓ^{-1} during the fish runs; dissolved oxygen ranged from 4.95 to 5.60 ppm; and the pH varied from 7.93 to 8.06.

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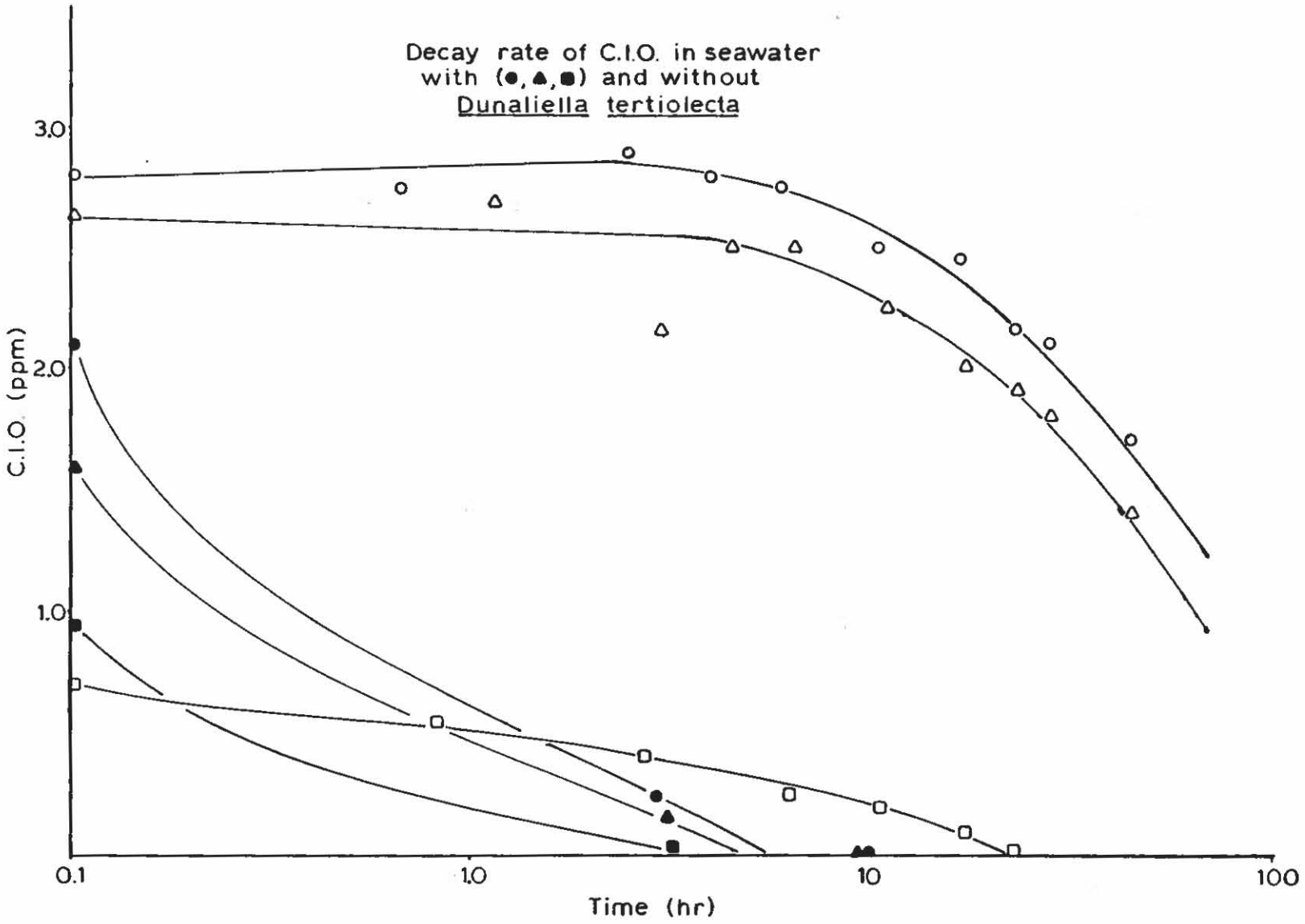

Figure 2. Decay rates of chlorine-induced oxidants in seawater with phytoplankton cells and with no organisms.

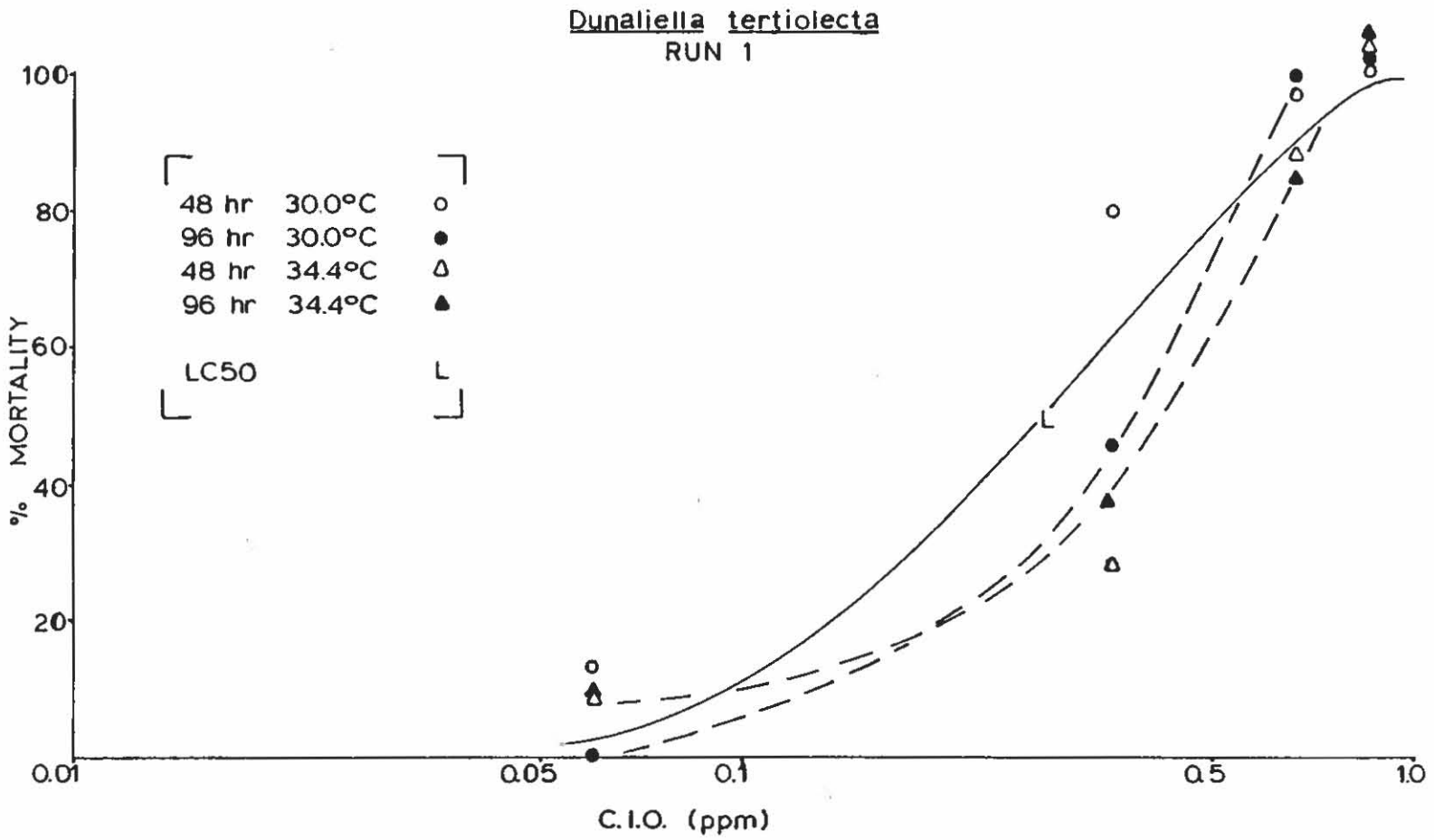

Figure 3. Mortality in Dunaliella tertiolecta, Run 1.

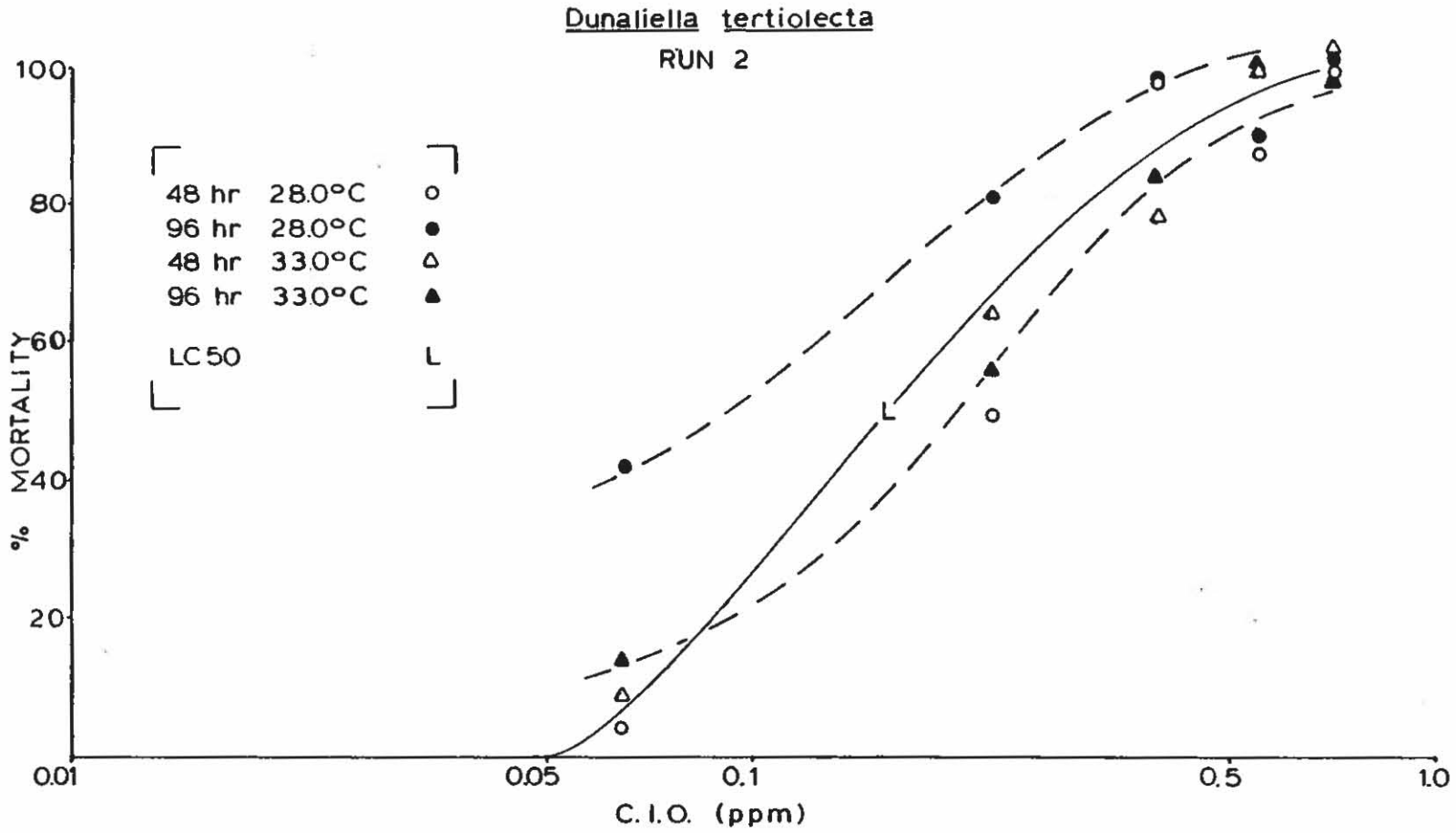

Figure 4. Mortality in Dunaliella tertiolecta, Run 2.

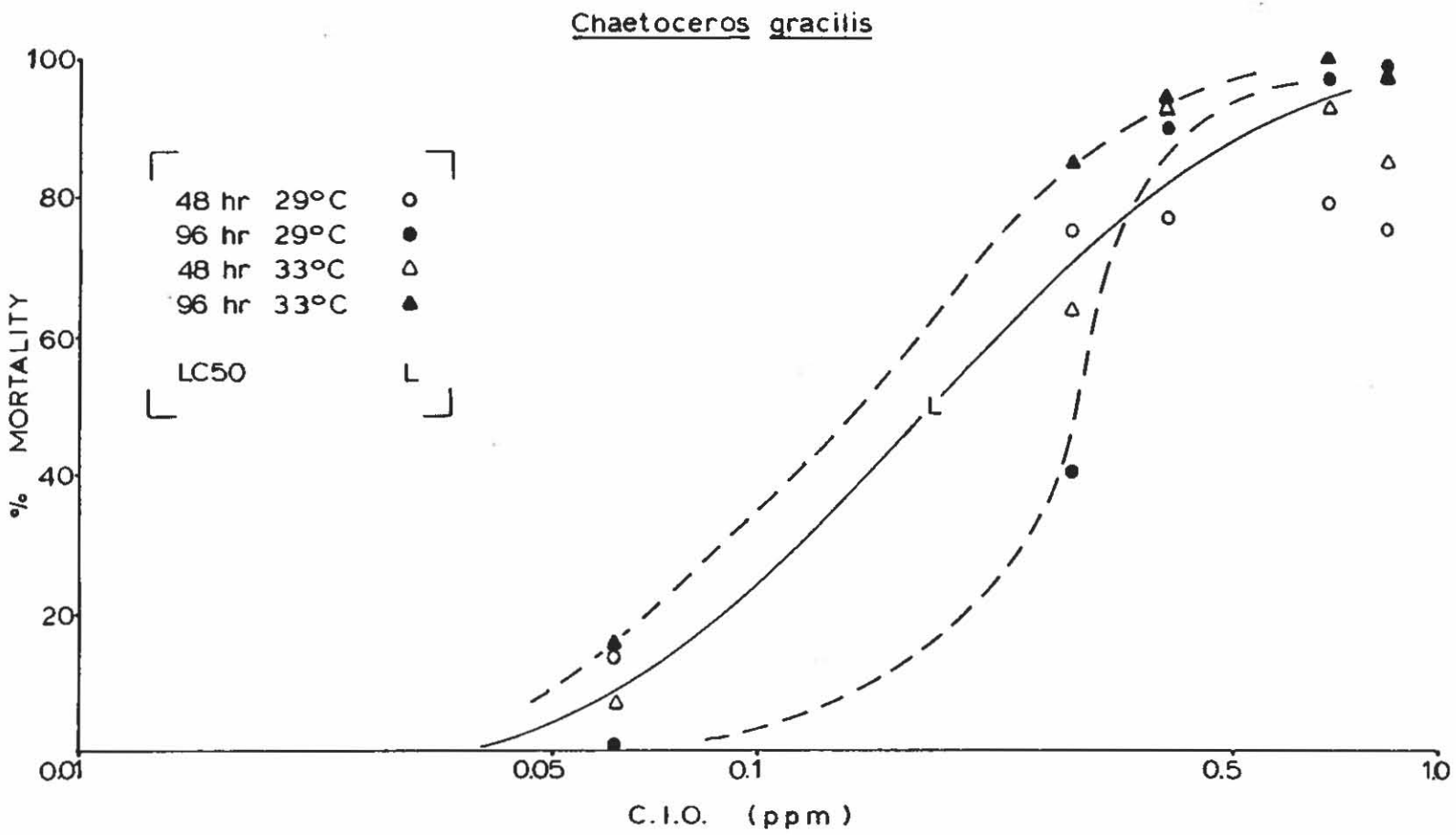

Figure 5. Mortality in Chaetoceros gracilis.

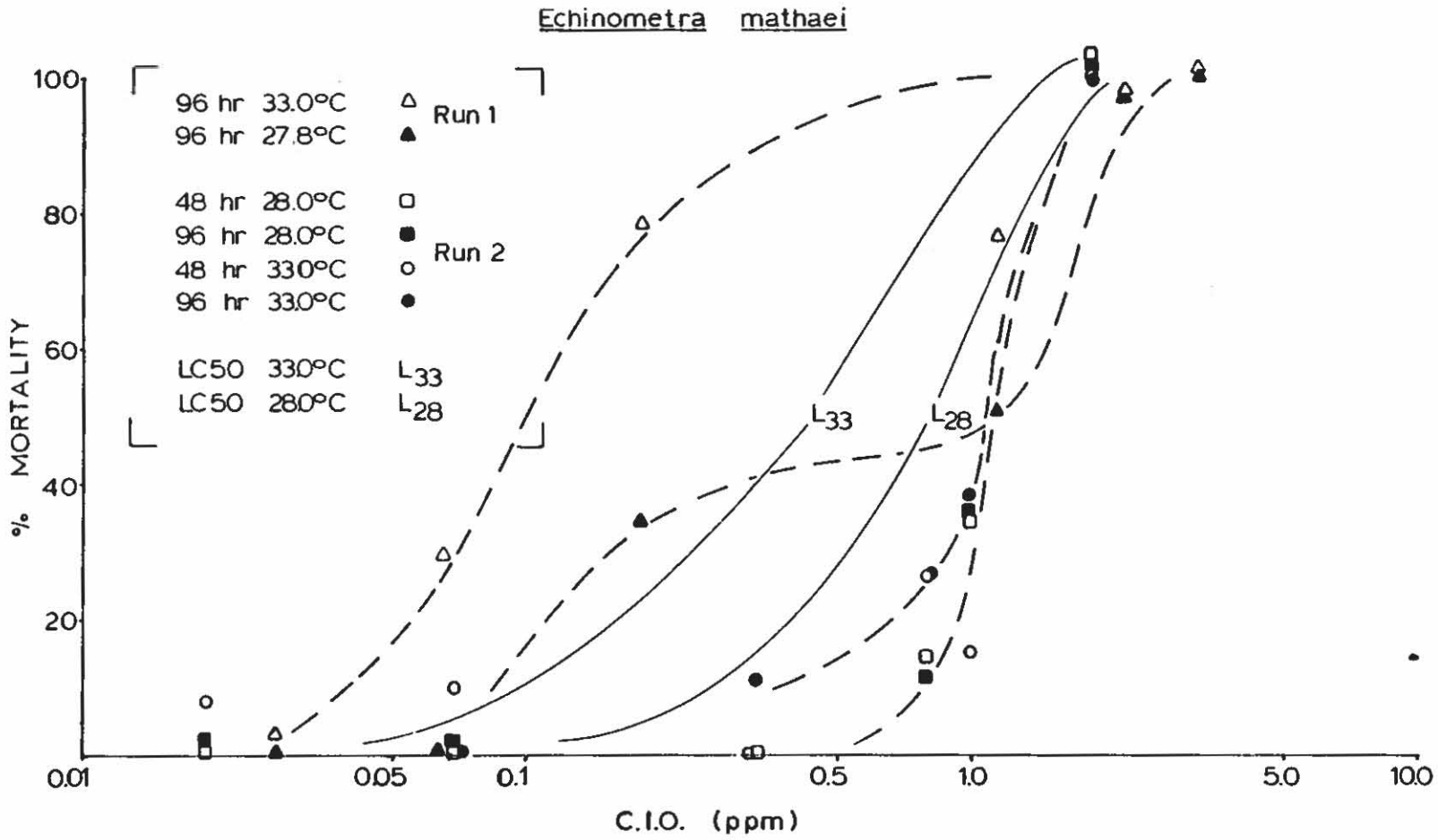

Figure 6. Mortality in Echinometra mathaei.

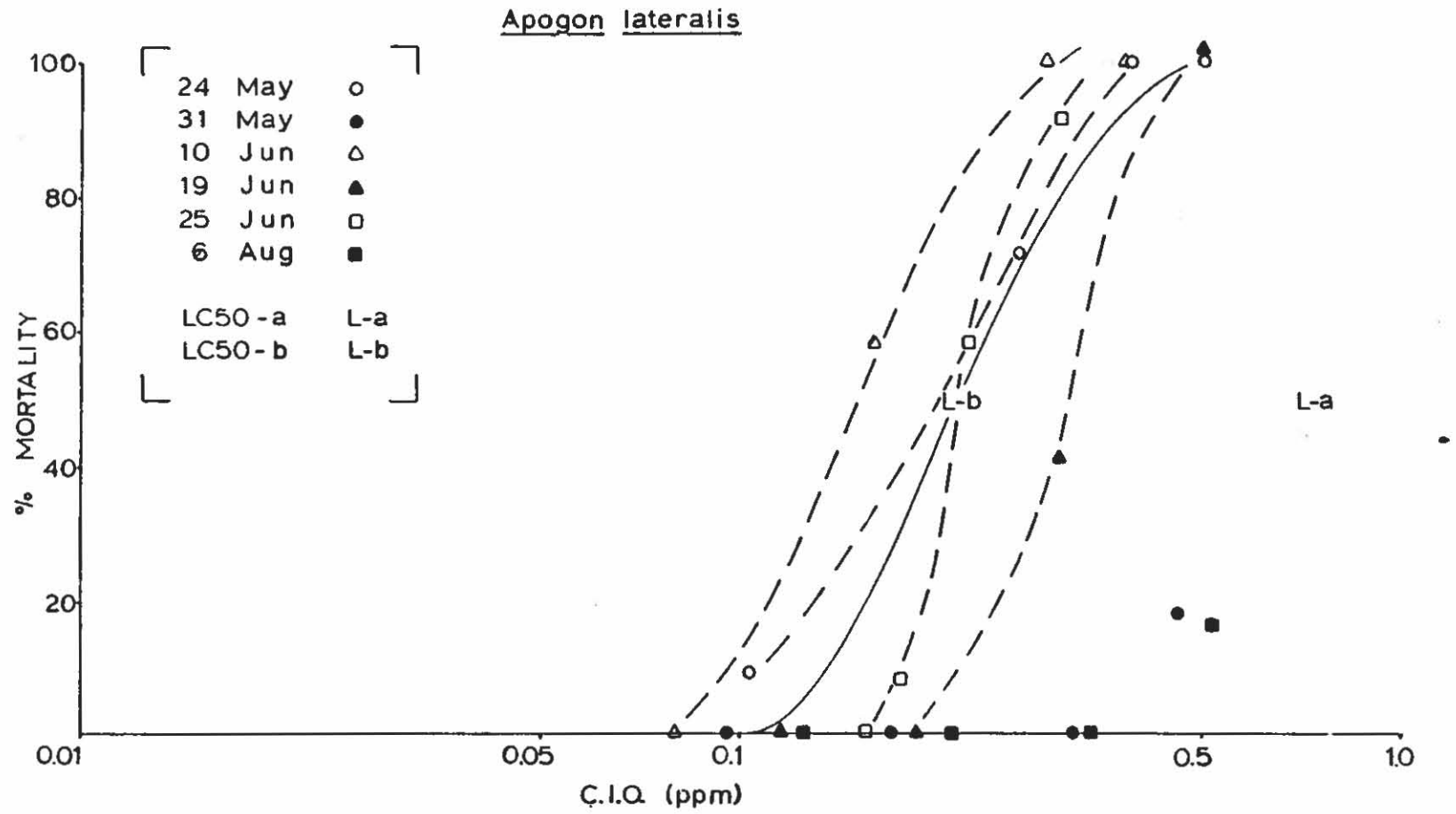

Figure 7. Mortality in Apogon lateralis.

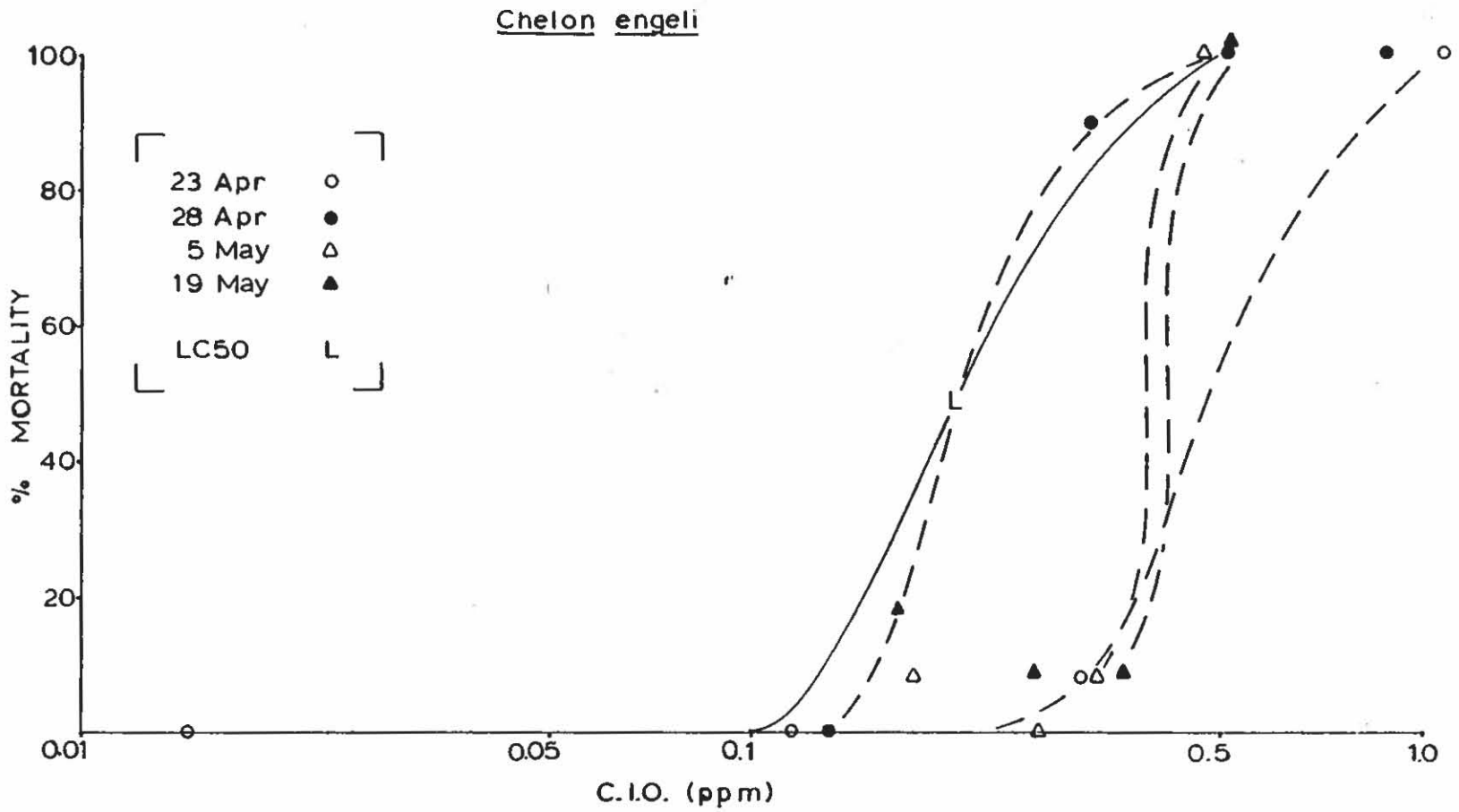

Figure 8. Mortality in Chelon engeli.

Table 1. Data for bioassay control runs. Means ($\bar{X}$), standard deviations (S.D.), and sample size (N), which is the number of control beakers per temperature.

$\bar{X}$ (cells x 10^4/mℓ)	S.D.	N	Temperature (°C) (± 0.1)	Time (hr)
		Chaetoceros gracilis		
		(starting concentration ca. 10 x 10^4 cells/mℓ)		
14.14	1.53	6	29.0	48
17.50	1.89	6	33.0	48
12.07	2.36	6	29.0	96
23.51	3.39	6	33.0	96
		Dunaliella tertiolecta -- Run 1		
		(starting concentration ca. 6.12 x 10^4 cells/mℓ)		
7.88	1.48	6	30.0	43
6.25	0.80	6	34.4	43
6.09	1.34	6	30.0 (+8)*	96
6.24	1.22	6	34.4	96
		Dunaliella tertiolecta -- Run 2		
		(starting concentration ca. 7 x 10^4 cells/mℓ)		
19.15	11.58	6	28.0	48
15.73	1.27	6	33.0	48
44.0	6.65	6	28.0	96
17.0	1.21	6	33.0	96

*An aberrant temperature spike was realized during this run.

$\bar{X}$ (#larvae/3 mℓ)	S.D.	N	Temperature (°C) (± 0.1)	Time (hr)
		Echinometra mathaei -- Run 1		
		1-day-old larvae		
168.4**	36.8**	5	27.8	96
185.9**	65.7**	5	33.0	96

**Note: These data were combined for the mortality calculations: $\bar{X}$ = 177.14, S.D. = 50.7, N = 10.

Table 1. Continued.

$\bar{X}$ (#larvae/2 mℓ)	S.D.	N	Temperature (°C) (± 0.1)	Time (hr)
		Echinometra mathaei -- Run 2		
		1-day-old larvae		
52.0	13.2	5	28.0	48
50.6	23.7	5	33.0	48
51.3	18.1	10	28.0, 33.0	96

$\bar{X}$ (#larvae/ 5 mℓ)	S.D.	N.	Temperature (°C) (± 0.1)	Time (hr)
		Stylocheilus longicauda		
		1-day-old veligers		
52.0	17.6	8	29, 33	48
34.6	9.4	10	29, 33	96

$\bar{X}$ (#juveniles/100 mℓ)	S.D.	N	Temperature (°C) (± 0.1)	Time (hr)
		Trochus niloticus		
		4-month-old-juveniles		
13.8	1.9	6	32	48
14.0	1.1	6	28	48
13.3	1.2	6	32	96
12.8	1.9	6	28	96

$\bar{X}$ (#/40 ℓ)	S.D.	N	Temperature (°C) (± 0.1)	Time (hr)
		Apogon lateralis		
		adults		
11.6	0.5	6	30.1	48 & 96
		Chelon engeli		
		juveniles		
11.0	0.8	4	30.1	48 & 96

Table 2. Percent mortalities for various concentrations of chlorine-induced oxidants (CIO) and LC50's calculated by simplified log-probit analysis. Column (A) equals percent mortality when compared to controls at each temperature and time interval, or, in the case of the phytoplankton bioassays, percent decrease in growth when compared to the controls.

(A) % Mortality	(B) Probit Transformation of (A)	(C) Initial CIO (ppm)	(D) Log Transformation of (C)
	Chaetoceros gracilis		
13.1 @ 29°C @ 48 hr	3.88	0.062	-1.208
7.1 @ 33°C @ 48 hr	3.53	0.062	-1.208
0 @ 29°C @ 96 hr	--	0.062	-1.208
15.6 @ 33°C @ 96 hr	3.99	0.062	-1.208
75.2 @ 29°C @ 48 hr	5.68	0.29	-0.538
63.6 @ 33°C @ 48 hr	5.35	0.29	-0.538
40.5 @ 29°C @ 96 hr	4.76	0.29	-0.538
84.4 @ 33°C @ 96 hr	6.01	0.29	-0.538
76.1 @ 29°C @ 48 hr	5.71	0.40	-0.398
92.9 @ 33°C @ 48 hr	6.47	0.40	-0.398
90.2 @ 29°C @ 96 hr	6.29	0.40	-0.398
94.2 @ 33°C @ 96 hr	6.57	0.40	-0.398
78.7 @ 29°C @ 48 hr	5.80	0.69	-0.161
92.1 @ 33°C @ 48 hr	6.41	0.69	-0.161
96.6 @ 29°C @ 96 hr	6.83	0.69	-0.161
99.6 @ 33°C @ 96 hr	7.65	0.69	-0.161
75.2 @ 29°C @ 48 hr	5.68	0.84	-0.076
85.0 @ 33°C @ 48 hr	6.04	0.84	-0.076
98.5 @ 29°C @ 96 hr	7.17	0.84	-0.076
98.3 @ 33°C @ 96 hr	7.12	0.84	-0.076
92.0 @ 29°C @ 48 hr	6.41	1.90	0.279
93.6 @ 33°C @ 48 hr	6.52	1.90	0.279
97.9 @ 29°C @ 96 hr	7.03	1.90	0.279
99.2 @ 33°C @ 96 hr	7.41	1.90	0.279
Results: LC50 = 0.18 ppm; N = 17; r = 0.85; m = 1.85 ; intcp. = 6.38 .			
	Dunaliella tertiolecta -- Run 1		
8.0 @ 34.4°C @ 43 hr	3.59	0.06	-1.222
12.7 @ 30.0°C @ 43 hr	3.86	0.06	-1.222
9.0 @ 34.4°C @ 96 hr	3.66	0.06	-1.222
0 @ 30.0°C @ 96 hr	--	0.06	-1.222
28.0 @ 34.4°C @ 43 hr	4.42	0.35	-0.450
79.4 @ 30.0°C @ 43 hr	5.82	0.35	-0.450
37.2 @ 34.4°C @ 96 hr	4.67	0.35	-0.450

Table 2. Continued

(A) % Mortality	(B) Probit Transformation of (A)	(C) Initial CIO (ppm)	(D) Log Transformation of (C)
45.7 @ 30.0°C @ 96 hr	4.89	0.35	-0.450
88.0 @ 34.4°C @ 43 hr	6.18	0.66	-0.180
96.8 @ 30.0°C @ 43 hr	6.85	0.66	-0.180
84.7 @ 34.4°C @ 96 hr	6.02	0.66	-0.180
100 @ 30.0°C @ 96 hr	∞	0.66	-0.180
100 @ 34.4°C @ 43 hr	∞	0.84	-0.076
100 @ 30.0°C @ 43 hr	∞	0.84	-0.076
100 @ 34.4°C @ 96 hr	∞	0.84	-0.076
100 @ 30.0°C @ 96 hr	∞	0.84	-0.076
Results: LC50 = 0.286 ppm; N = 9; r = 0.89; m = 2.04; intcp. = 6.11.			
	Dunaliella tertiolecta -- Run 2		
4.6 @ 28.0°C @ 48 hr	3.34	0.064	-1.193
9.0 @ 33.0°C @ 48 hr	3.66	0.064	-1.193
42.1 @ 28.0°C @ 96 hr	4.80	0.064	-1.193
14.1 @ 33.0°C @ 96 hr	3.92	0.064	-1.193
49.6 @ 28.0°C @ 48 hr	4.99	0.225	-0.648
64.4 @ 33.0°C @ 48 hr	5.37	0.225	-0.648
81.2 @ 28.0°C @ 96 hr	5.89	0.225	-0.648
56.2 @ 33.0°C @ 96 hr	5.16	0.225	-0.648
98.0 @ 28.0°C @ 48 hr	7.05	0.390	-0.409
78.6 @ 33.0°C @ 48 hr	5.79	0.390	-0.409
98.4 @ 28.0°C @ 96 hr	7.14	0.390	-0.409
84.2 @ 33.0°C @ 96 hr	6.00	0.390	-0.409
87.8 @ 28.0°C @ 48 hr	6.16	0.55	-0.260
99.8 @ 33.0°C @ 48 hr	7.88	0.55	-0.260
90.2 @ 28.0°C @ 96 hr	6.29	0.55	-0.260
100 @ 33.0°C @ 96 hr	∞	0.55	-0.260
99.5 @ 28.0°C @ 48 hr	7.58	0.71	-0.149
99.8 @ 33.0°C @ 48 hr	7.88	0.71	-0.149
99.9 @ 28.0°C @ 96 hr	8.09	0.71	-0.149
98.3 @ 33.0°C @ 96 hr	7.12	0.71	-0.149
100 @ 28.0°C @ 48 hr	∞	1.43	0.155
100 @ 33.0°C @ 48 hr	∞	1.43	0.155
100 @ 28.0°C @ 96 hr	∞	1.43	0.155
100 @ 33.0°C @ 96 hr	∞	1.43	0.155
Results: LC50 = 0.16 ppm; N = 11; r = 0.92; m = 2.26 ; intcp. = 6.82.			
	Echinometra mathaei -- Run 1		
0 @ 27.8°C @ 96 hr	--	0.027	-1.57
3.15 @ 33.0°C @ 96 hr	3.14	0.027	-1.57

Table 2. Continued

(A) % Mortality	(B) Probit Transformation of (A)	(C) Initial CIO (ppm)	(D) Log Transformation of (C)
0.25 @ 27.8°C @ 96 hr	2.13	0.063	-1.20
29.8 @ 33.0°C @ 96 hr	4.47	0.063	-1.20
35.0 @ 27.8°C @ 96 hr	4.61	0.174	-0.76
78.4 @ 33.0°C @ 96 hr	5.79	0.174	-0.76
51.45 @ 27.8°C @ 96 hr	5.04	1.14	0.057
75.9 @ 33.0°C @ 96 hr	5.70	1.14	0.057
97.45 @ 27.8°C @ 96 hr	6.95	2.20	0.34
98.4 @ 33.0°C @ 96 hr	7.14	2.20	0.34
99.8 @ 27.8°C @ 96 hr	7.88	3.70	0.57
100 @ 33.0°C @ 96 hr	∞	3.70	0.57
	Echinometra mathaei -- Run 2		
0 @ 28°C @ 48 hr	--	0.019	-1.72
8.35 @ 33°C @ 48 hr	3.62	0.019	-1.72
0 @ 28°C @ 96 hr	--	0.019	-1.72
* @ 33°C @ 96 hr	--	0.019	-1.72
0 @ 28°C @ 48 hr	--	0.069	-1.16
0 @ 33°C @ 48 hr	--	0.069	-1.16
0 @ 28°C @ 96 hr	--	0.069	-1.16
0 @ 33°C @ 96 hr	--	0.069	-1.16
0 @ 28°C @ 48 hr	--	0.33	-0.48
0 @ 33°C @ 48 hr	--	0.33	-0.48
* @ 28°C @ 96 hr	--	0.33	-0.48
11.3 @ 33°C @ 96 hr	3.79	0.33	-0.48
0 @ 28°C @ 48 hr	--	0.79	-0.10
26.75 @ 33°C @ 48 hr	4.38	0.79	-0.10
8.9 @ 28°C @ 96 hr	3.65	0.79	-0.10
26.9 @ 33°C @ 96 hr	4.38	0.79	-0.10
34.7 @ 28°C @ 48 hr	4.61	0.99	-0.01
15.2 @ 33°C @ 48 hr	3.97	0.99	-0.01
36.1 @ 28°C @ 96 hr	4.64	0.99	-0.01
38.6 @ 33°C @ 96 hr	4.71	0.99	-0.01
100 @ 28°C @ 48 hr	∞	1.85	0.27
100 @ 33°C @ 48 hr	∞	1.85	0.27
100 @ 28°C @ 96 hr	∞	1.85	0.27
99.5 @ 33°C @ 96 hr	7.58	1.85	0.27

Combined Results for 33°C, Runs 1 & 2, 48 & 96 hr: LC50 = 0.45 ppm;
N = 12; r = 0.64 ; m = 1.25 ; intcp. = 5.44.

Combined Results for 28° and 27.8°C, Runs 1 & 2, 48 & 96 hr: LC50 = 0.77 ppm;
N = 8; r = 0.85; m = 2.64; intcp. = 5.31.

Table 2. Continued

(A) % Mortality	(B) Probit Transformation of (A)	(C) Initial CIO (ppm)	(D) Log Transformation of (C)
	Stylocheilus longicauda larvae		
0 @ 29°C @ 96 hr	--	0.055	-1.26
0 @ 33°C @ 96 hr	--	0.055	-1.26
0 @ 29°C @ 48 hr	--	0.10	-1.00
0 @ 33°C @ 48 hr	--	0.10	-1.00
0 @ 29°C @ 96 hr	--	0.10	-1.00
0 @ 33°C @ 96 hr	--	0.10	-1.00
60.6 @ 29°C @ 48 hr	5.27	0.36	-0.44
57.7 @ 33°C @ 48 hr	5.19	0.36	-0.44
0 @ 29°C @ 96 hr	--	0.36	-0.44
0 @ 33°C @ 96 hr	--	0.36	-0.44
46.2 @ 29°C @ 48 hr	4.90	0.84	-0.076
32.5 @ 33°C @ 48 hr	4.55	0.84	-0.076
8.0 @ 29°C @ 96 hr	3.59	0.84	-0.076
21.0 @ 33°C @ 96 hr	4.19	0.84	-0.076
0 @ 29°C @ 48 hr	--	1.38	0.14
42.3 @ 33°C @ 48 hr	4.81	1.38	0.14
25.3 @ 29°C @ 96 hr	4.33	1.38	0.14
92.8 @ 33°C @ 96 hr	6.46	1.38	0.14
0 @ 29°C @ 96 hr	--	1.95	0.29
84.1 @ 33°C @ 96 hr	6.00	1.95	0.29
Results: LC50 estimated to be > 1.95 ppm CIO.			
	Trochus niloticus juveniles		
7.15 @ 28°C @ 48 hr	3.54	0.057	-1.24
6.60 @ 32°C @ 48 hr	3.46	0.057	-1.24
3.12 @ 28°C @ 96 hr	3.14	0.057	-1.24
29.85 @ 32°C @ 96 hr	4.47	0.057	-1.24
0 @ 28°C @ 48 hr	--	0.21	-0.68
0 @ 32°C @ 48 hr	--	0.21	-0.68
3.12 @ 28°C @ 96 hr	3.14	0.21	-0.68
2.25 @ 32°C @ 96 hr	2.99	0.21	-0.68
3.57 @ 28°C @ 48 hr	3.19	0.47	-0.33
0 @ 32°C @ 48 hr	--	0.47	-0.33
3.12 @ 28°C @ 96 hr	3.14	0.47	-0.33
4.89 @ 32°C @ 96 hr	3.34	0.47	-0.33
7.15 @ 28°C @ 48 hr	3.54	1.45	0.16
16.85 @ 32°C @ 48 hr	4.04	1.45	0.16
7.05 @ 28°C @ 96 hr	3.53	1.45	0.16
13.53 @ 32°C @ 96 hr	3.90	1.45	0.16
0 @ 28°C @ 48 hr	--	2.20	0.34
13.85 @ 32°C @ 48 hr	3.91	2.20	0.34

Table 2. Continued

(A) % Mortality	(B) Probit Transformation of (A)	(C) Initial CIO (ppm)	(D) Log Transformation of (C)
0 @ 28°C @ 96 hr	--	2.20	0.34
51.14 @ 32°C @ 96 hr	5.08	2.20	0.34
3.57 @ 28°C @ 48 hr	3.19	3.35	0.52
0 @ 32°C @ 48 hr	--	3.35	0.52
3.12 @ 28°C @ 96 hr	3.14	3.35	0.52
0 @ 32°C @ 96 hr	--	3.35	0.52

Results: LC50 > 3.35 ppm CIO.

*Ciliate contamination, not calculated.

Table 3. LC50's and percent mortalities for selected fish at various concentrations of chlorine-induced oxidants (CIO). LC50's were calculated by a simplified log-probit analysis discussed in the text. Temperature for all runs was 30.1°C; all bioassays were performed in 1980.

(A) Date of run	(B) % Mortality (96 hr)	(C) Probit Transformation of (B)	(D) Initial CIO (ppm)	(E) Log_{10} of (D)	(F) Mean Standard Length. N=12 [$\bar{X}$mm (S.D.)]
		Apogon lateralis			
24 May	81.8	5.91	0.26	-0.58	31.3 (2.1)
24 May	100	∞	0.39	-0.41	31.0 (2.9)
24 May	9.1	3.66	0.104	-0.98	32.3 (2.0)
24 May	100	∞	0.50	-0.30	30.6 (3.6)
24 May	control	--	0	--	33.6 (1.9)
31 May	0	--	0.17	-0.93	33.6 (1.8)
31 May	0	--	0.32	-0.49	34.7 (2.2)
31 May	0	--	0.096	-1.02	34.3 (2.5)
31 May	18.2	4.09	0.48	-0.32	34.2 (4.1)
31 May	control	--	0	--	33.1 (2.7)
10 Jun	100	∞	0.29	-0.54	25.8 (1.2)
10 Jun	0	--	0.08	-1.10	26.4 (0.8)
10 Jun	100	∞	0.38	-0.42	24.9 (1.7)
10 Jun	58.3	5.21	0.16	-0.80	26.0 (1.7)
10 Jun	control	--	0	--	27.3 (2.7)
19 Jun	41.7	4.79	0.30	-0.52	32.5 (2.7)
19 Jun	0	--	0.186	-0.73	33.1 (2.3)
19 Jun	0	--	0.116	-0.94	33.7 (2.7)
19 Jun	100	∞	0.50	-0.30	32.6 (2.8)
19 Jun	control	--	0	--	33.5 (1.2)
25 Jun	58.3	5.21	0.22	-0.66	35.5 (2.4)
25 Jun	0	--	0.156	-0.81	37.0 (2.8)
25 Jun	8.33	3.62	0.176	-0.75	36.9 (3.7)
25 Jun	91.66	6.38	0.30	-0.52	34.9 (3.4)
25 Jun	control	--	0	--	36.4 (4.0)
6 Aug	0	--	0.34	-0.47	43.1 (3.2)
6 Aug	16.7	4.03	0.52	-0.28	40.6 (2.8)
6 Aug	0	--	0.13	-0.89	40.9 (3.7)
6 Aug	0	--	0.21	-0.68	41.9 (4.5)
6 Aug	control	--	0	--	39.9 (5.0)

Results of all data: LC50 = 0.72 ppm; N = 9; r = 0.12; m = 0.51; intcp. = 5.1.

Results of data without 6 Aug and 31 May runs: LC50 = 0.21; N = 7; r = 0.71; m = 4.42; intcp. = 8.01.

Table 3. Continued.

(A) Date of run	(B) % Mortality (96 hr)	(C) Probit Transformation of (B)	(D) Initial ClO (ppm)	(E) Log_{10} of (D)	(F) Mean Standard Length. N=12 [$\bar{X}$mm (S.D.)]
		Chelon engeli			
23 Apr	100	∞	1.08	0.033	46.4 (4.6)
23 Apr	8.3	3.61	0.31	-0.51	48.0 (9.2)
23 Apr	0	--	0.015	-1.82	46.8 (6.8)
23 Apr	0	--	0.115	-0.94	48.5 (5.9)
23 Apr	control	--	0	--	46.1 (7.0)
28 Apr	90	6.28	0.32	-0.49	42.7 (5.4)
28 Apr	100	∞	0.51	-0.29	44.4 (7.8)
28 Apr	100	∞	0.88	-0.056	43.1 (6.0)
28 Apr	0	--	0.131	-0.88	--
28 Apr	control	--	0	--	--
5 May	100	∞	0.47	-0.33	49.6 (5.4)
5 May	0	--	0.27	-0.57	45.2 (11.2)
5 May	8.3	3.61	0.174	-0.76	52.3 (5.9)
5 May	8.3	3.61	0.34	-0.47	52.7 (6.3)
5 May	control	--	0	--	46.7 (9.0)
19 May	100	∞	0.52	-0.28	38.2 (4.8)
19 May	9.1	3.67	0.265	-0.58	39.1 (6.8)
19 May	18.2	4.09	0.166	-0.78	37.8 (4.7)
19 May	9.1	3.67	0.36	-0.44	38.1 (6.2)
19 May	control	--	0	--	39.8 (4.4)

Results: LC50 = 0.20 ppm; N = 7; r = 0.17; m = 1.19; intcp. = 4.76.

DISCUSSION

Although the bioassay results reported in the previous section are straightforward, there are additional matters relating to the bioassays, individually and collectively, that deserve mention.

Dunaliella was assayed at four temperatures, ranging from 29.0°C to 34.4°C. At 28.0°C the LC50 was less than 0.1 ppm, while at 34.4°C the the LC50 was approximately 0.4 ppm. This would seem to indicate that the deleterious effects of chlorine are greater at lower temperatures. The opposite result might be expected. A possible explanation is the difference in growth rates at different temperatures; controls grew faster at 28°C than at higher temperatures (Table 1). As discussed previously, the LC50's computed for phytoplankton cultures reflect growth rate as well as mortality since growth rate in experimental cultures is calculated as a percentage of that in controls. A difference between the growth rates of controls at different temperatures would produce different LC50's, even if the initial mortalities were the same. Therefore, from our data it is impossible to determine how much of the difference between LC50's obtained from the Dunaliella bioassays reflects the effects of temperature on sensitivity to chlorine.

As a phytoplankter, Chaetoceros is subject to the same difficulties in determining lethal effects of chlorine as in Dunaliella. In this case, however, the Chaetoceros seems to display greater sensitivity at the higher temperatures (33°C vs. 29°C). The difference in temperature effects between the two species is probably due to the fact that Chaetoceros controls grew faster at the higher temperature, while Dunaliella controls grew faster at the lower (Table 1).

Our chlorine LC50's for both Dunaliella (0.16-0.29 ppm CIO) and Chaetoceros (0.18 ppm) fall within the range of 0.11 ppm to 0.60 ppm reported in other studies (Gentile et al. 1974, Videau et al. 1979).

Bioassays with Echinometra larvae indicate a marked increase in toxic effects when the temperature is elevated. It should be noted, however, that the LC50's at 28°C and 33°C are based on an average of control counts made at 48 hr in one run. This was necessary because of the ciliate contamination which occurred in the control cultures between 48 and 96 hr. After 96 hr, the mortality was higher in control cultures because of ciliate contamination than in cultures with moderate amounts of chlorine, which inhibits ciliate growth. For this reason, data taken at 48 hours, before ciliate contamination became acute, are more reliable.

The only other studies of chlorine toxicity to echinoderms report effects on fertilizaton rather than mortality (Appendix, Table A-3). They indicate that fertilization is quite sensitive to chlorine, since

0.125 ppm was sufficient to reduce fertilizaton success in Strongylocentrotus purpuratus to less than 6% of that in controls (Muchmore and Epel 1973). This means that even sublethal doses of chlorine could prevent reproduction of organisms within an outfall area.

Juvenile Trochus are apparently quite tolerant to chlorine, since concentrations up to 3.35 ppm failed to cause more than 50% mortality (Table 2). The explanation of this result involves both the structure and behavior of gastropods. Trochus has a thick, impervious shell and an operculum with which it can effectively shut itself off from a hostile environment. Since the chlorine concentration in seawater drops very rapidly (Figure 2), the Trochus are able to remain sealed off until it has decreased to a sublethal level. For these organisms, continuous-flow bioassays with sustained levels of chlorine might reveal a much greater sensitivity than a single-dose static system.

Stylocheilus veligers are also capable of avoidance behavior and are therefore more resistant to chlorine than are Echinometra and other invertebrates. There was an apparent temperature effect on toxicity at most concentrations. The 96-hr mortality at 29°C never exceed 25%, even at CIO concentrations as high as 1.95 ppm, whereas it reached 92.8% at 33°C (Table 2). For 48-hr intervals, the maximum mortality was 60.6% at 29°C and 57.7% at 33°C for an initial CIO concentration of 0.36 ppm, but mortalities at the other concentrations were always higher at 33°C. The sensitivity of Stylocheilus veligers is apparently comparable to that of temperate molluscs like Mytilus edulis and Ostrea edulis (Appendix, Table A-3). The former had 100% mortality at 2.5 ppm chlorine, and larvae of the latter stopped all swimming activity at 0.5 ppm.

In general, the 96-hr LC50 values for the fish, Apogon and Chelon, were in the same range as those for the phytoplankton and invertebrates. With both fish, the results varied considerably between runs. Some of the variability in the chlorine toxicity to Apogon was due to size. Fish collected later in the year (August) averaged larger than those caught in the spring (May). When taking fish length into consideration, it was found that there was a 5-8% size effect at intermediate chlorine concentrations; larger fish were slightly more resistant to chlorine. The LC50's for Apogon and Chelon reported here are consistent with LC50's reported for many temperate fishes (Appendix, Table A-3).

This investigation indicates that phytoplankton would be acutely affected by chlorine concentrations as low as 0.1 ppm, fish at approximately 0.2 ppm, followed by sea urchin larvae between 0.4 and 0.8 ppm and larval and juvenile gastropods at levels above 1.95 ppm. These results suggest that the most serious temperate biofouling organisms, the molluscs, are exceptionally tolerant to chlorine, while other, nonfouling species may be quite sensitive. Many hatcheries culturing bivalve molluscs routinely chlorinate their larval cultures to inhibit bacteria and protozoa.

Although the data reported here indicate lethal effects of chlorine-induced oxidants on selected marine organisms, responses such

as behavioral, physiological and other changes induced by exposure to chlorine were not measured. The need for information on the sublethal effects of chlorine is obvious and should be considered when chlorine-effluent standards are established.

The mechanisms of the toxic action of chlorine-induced oxidants in marine organisms are unclear. Many studies (reviewed in Brooks and Seegert 1978) suggest the gills as the target tissue in fish. Our results are compatible with this hypothesis. Fish reacted to chlorine in a consistent pattern: erratic movements followed by a gaping, gasping behavior, terminating with death. This reaction was complete within a period of 15 to 45 minutes after chlorine was added to the aquaria. As previously described, juvenile Trochus and the shelled Stylocheilus veligers immediately closed up upon exposure to chlorine. When the ClO concentration had decayed, they re-emerged.

A survey of Appendix Tables A-2 and A-3 reveals how few chlorine toxicity studies have been done in the tropics. These data included in this report are among the first from tropical marine bioassays. In general, our results are in keeping with the results of similar temperate-region studies. More work is needed in the tropics if proper comparisons of tolerances of tropical and temperate species are to be made. There is no evidence at present, however, that tropical organisms, including larval forms, are more sensitive to chlorine than related temperate species.

Much variability is obvious in our results. Such variability is characteristic of this kind of study, as can be seen by a survey of the literature summarized in the Appendix and the Annotated Bibliography. An examination of Tables A-2 and A-3 further reveals the great variety of experimental conditions, chlorine sources, analytical procedures, sublethal effects, organisms and life stages in other studies. There is, so far, no generally utilized "standard procedure"; and this may not even be desirable in studies of this nature. In particular, a 96-hr bioassay may not be the most desirable procedure for chlorine toxicity studies. We do, however, feel that the single-dose approach is the most appropriate protocol for simulating potential conditons of chlorine exposure for organisms in the outfall area of the Piti Power Plant; and we feel generally confident about our results.

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Alderson, R. 1974. Seawater chlorination and the surival and growth of the early developmental stages of plaice, Pleuronectes platessa L., and dover sole, Solea solea (L.). Aquaculture 4:41-53.

Eggs found to be more tolerant than larvae for both species. LC50's of 0.64 to 0.025 recorded for eggs and larvae, respectively.

American Public Health Association. 1971. Standard methods for the examination of water, sewage, and industrial wastes, 13th ed. Washington, APHA. 834 p.

Anderson, B. G. 1950. The apparent thresholds of toxicity to Daphnia magna for chlorides of various metals when added to Lake Erie water. Trans. Am. Fish. Soc. 78:96-113.

Basch, R. E., and J. G. Truchan. 1976. Toxicity of chlorinated power plant cooling waters to fish. U. S. Environ. Prot. Agency, Ecol. Res. Ser. R-800700, Duluth, Minn. 105 p.

Beauchamp, R. S. A. 1966. Low level chlorination for the control of marine fouling. Cent. Electr. Res. Lab., Lab. Memo RD/L/M 147. 16 p.

Beauchamp, R. S. A. 1969. The use of chlorine in the cooling water system of coastal power stations. Chesapeake Sci. 10:280.

Explains the British system for mussel control; the continuous injection of 0.5 ppm chlorine.

Bender, D. F. 1978. Comparison of methods for the determination of total available residual chlorine in various sample matrices. U. S. Environ. Prot. Agency. EPA-600/4-78-019, 30 p.

The Orion selective-ion electrode was one of the methods used in this sample matrix. Accuracy and precision good in unpolluted fresh water.

Betts, J. L., T. W. Beak, and G. G. Wilson. 1967. A procedure for small-scale laboratory bioassays. J. Wat. Pollut. Control Fed. 39(1):89-96.

Methods for a small-scale, continuous flow, bioassay system for fish are presented.

Bick, H. 1963. A review of central European methods for the biological estimation of water pollution levels. Bull. Wld. Hlth Org. 29:401-413.

Bliss, C. I. 1937. The calculation of the time-mortality curve. Ann. Appl. Biol. 24:815-852.

Block, R. M., G. R. Helz, and W. P. Davis. 1977. The fate and effects of chlorine in coastal waters: summary and recommendations. Chesapeake Sci. 18(1):97-101.

Summarizes the need for information on the chemistry of chlorine in seawater and the need for sublethal response studies. Suggests bioassay systems and important parameters that should be reported.

Bradley, B. P. 1977. Comparison of residual biotoxicity of chlorine and bromine chloride to copepods. Univ. of Maryland Water Resources Research Center. Tech. Rep. 47. 15 p.

Acartia tonsa had a 24-hr LC50 of 0.40 ppm for Cl_2 and 0.36 ppm for BrCl. Eurytemora affinis tested for reproduction capacity.

Brereton, A., H. Lord, I. Thorton, and J. S. Webb. 1973. Effects of zinc on growth and development of larvae of the Pacific oyster Crassostrea gigas. Mar. Biol. 19:96-101.

Amounts over 50 µg/ℓ were deleterious; larvae were stripped; water treated with U.V. and penicillin for bacterial control.

Brook, A. J., and A. L. Baker. 1972. Chlorination at power plants: impact on phytoplankton productivity. Science 176:1414-1415.

Brooks, A. S., and G. L. Seegert. 1976. Toxicity of chlorine to fresh water organisms under varying environmental conditions. p. 277-298. In: R. L. Jolley (Ed.), Proceedings of the conference on environmental impact of water chlorination, Oct. 22-24. Oak Ridge Nat. Lab., Conf. 751096, Oak Ridge, Tenn. Oct. 1975.

Brooks, A. S., and G. L. Seegert. 1977a. The effects of intermittent chlorination on rainbow trout and yellow perch. Trans. Am. Fish. Soc. 106:278-296.

Juvenile freshwater fish subjected to single 30-min. and triple 5-minute doses of residual chlorine. Results indicated marked effect of temp. (LC50 0.7 mg/ℓ at 30°C, LC50 8.0 mg/ℓ at 10°C). Analyzed both amperometrically and with DPD.

Brooks, A. S., and G. L. Seegert. 1977b. The effect of intermittent chlorination on the biota of Lake Michigan. Univ. Wisconsin-Milwaukee Center for Great Lakes Studies, Spec. Rep. 31. 167 p.

Brooks, A. S., and G. L. Seegert. 1978. The toxicity of chlorine to freshwater organisms under varying environmental conditions. p. 261-282. In R. L. Jolley (Ed.), Water chlorination: environmental impact and health effects. Vol. I. Ann Arbor Sci. 439 p.

A review of the literature which presented sufficient data to permit comparisons. Factors considered include species composition, size, avoidance, exposure time, and temperature.

Brungs, W. A. 1973. Effects of residual chlorine on aquatic life. J. Wat. Pollut. Control Fed. 45(10):2180-2193.

Recommends test procedures and reviews some literature. Summarizes results and conclusions. Good reference list included.

Brungs, W. A. 1976. Effects of wastewater and cooling water chlorination on aquatic life. Environ. Prot. Agency Ecol. Res. Ser. EPA-600/3-76-098.

Burdick, G. E. 1967. Use of bioassays in determining levels of toxic wastes to aquatic organisms. Trans. Am. Fish. Soc. 96(1) Suppl., Spec. Publ 4:7-12.

Burton, D. T. 1977. General test conditions and procedures for chlorine toxicity tests with estuarine and marine macro-invertebrates and fish. Chesapeake Sci. 18(1):130-136.

A review with terms, recommended set-up (continuous-flow systems) and test procedures.

Burton, D. T., L. W. Hall, Jr., S. L. Margrey, and R. D. Small. 1979. Interactions of chlorine, temperature change (ΔT), and exposure time on survival of striped bass (Morone saxatilis) eggs and prolarvae. J. Fish. Res. Board Can. 36:1108-1113.

Prolarvae were more sensitive than eggs. TRC, exposure time and ΔT were important in causing mortality in prolarvae.

Cairns, J., Jr., and K. L. Dickson, (Eds.). 1973. Biological methods for the assessment of water quality. ASTM STP 528, American Society for Testing and Materials.

Capuzzo, J. M. 1977. The effects of free chlorine and chloramine on growth and respiration rates of larval lobsters (Homarus americanus). Wat. Res. 11:1021-1024.

Sublethal effects monitored for 19 days following 60-min exposure @ 25°C to 1.0 mg/ℓ of chlorine and chloramine. Chloramine had more drastic effect. Estimated 85% of initial chlorine taken up by seawater.

Capuzzo, J. M., J. C. Goldman, J. A. Davidson, and S. A. Lawrence. 1977. Chlorinated cooling waters in the marine environment: development of effluent guidelines. Mar. Pollut. 8(7):161-163.

Stresses sublethal effects from chlorine and chloramine. Results from killfish and lobster larvae included somewhat cryptically.

Carpenter, J. H., and D. L. Macalady. 1978. Chemistry of halogens in sea water. In R. L. Jolley (Ed.), Water chlorination: environmental impact and health effects. Vol. 1. Ann Arbor Sci. 439 p.

Furthers knowledge of the complexity of chlorine in seawater. Suggests that brominated or brominated-chlorinated organic compounds are formed; present methods fail to satisfactorily measure these compounds.

Carpenter, E. J., B. Peck, and S. Anderson. 1972. Cooling water chlorination and productivity of entrained phytoplankton. Mar. Biol. 16:37-40.

Disputes Hirayama & Hirano (1970). Found primary prod. down 76% with less than 0.05 ppm Cl (Enslo chlorimeter) on entrained phytoplankton. Recommends mechanical methods for removal of bio-fouling organisms.

Clendenning, K. A., and W. J. North. 1959. Effects of wastes on the giant kelp (Macrocystis pyrifera). Proc. 1st Intl. Conf. on waste disposal in the marine environ., Berkeley, Calif.

Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. U. S. Environ. Prot. Agency. Ecol. Res. Ser. EPA-660-3-75-009. 61 p.

Cope, O. B. 1961. Standards for reporting fish toxicity tests. Progress. Fish Cult. 23:187-189.

Davies, R. G. 1971. Computer programming in quantitative biology. Academic Press, New York. 492 p.

Davis, K. K. 1971. The levels of residual chlorine in Kaneohe Bay, Oahu, Hawaii, and the effects of residual chlorine on coral planulae. Univ. of Hawaii, M. S. Thesis. 62 p.

Coral planulae (3 spp.) were immobile after short exposures to 0.49 mg/ℓ chlorine but recovered from all concentrations up to 40 mg/ℓ. These are the most resistant animals reported to date.

Davis, W. P., and D. P. Middaugh. 1977. A revised review of the impact of chlorination processes upon marine ecosystems: update 1977. p. 283-310. In R. L. Jolley (Ed.), Water chlorination: environmental impact and health effects. Vol. 1. Ann Arbor Sci.

A review of bioassay literature and presents a degradation model of chlorine added to marine waters.

De Filippis, L. F., and C. K. Pallaghy. 1976. The effect of sublethal concentrations of mercury and zinc on Chlorella. II. Photosynthesis and pigment composition. Pflanzenphysiol. 78. 5:314-322.

Delistraty, D. A., J. M. Carlberg, J. C. Van Olst, and R. F. Ford. 1976. Ammonia toxicity in cultured larvae of the American lobster (Homarus americanus). San Diego State Univ. Center for Marine Studies. Contribution 25.

Looks at NH_3/NH_4, pH, Temp, °/oo (pH up, then ammonia toxicity down; T up, then toxicity increased; salinity, no effect). Interesting ideas in methods.

Dickson, K. L., J. Cairns, Jr., B. C. Gregg, D. I. Messenger, J. L. Plafkin, and W. H. Van Der Schalie. 1977. Effects of intermittent chlorination on aquatic organisms and communities. J. Wat. Pollut. Control Fed. 49:35-44.

Using different exposure times and frequencies, the group tested goldfish (Carrasius auratus L.) and a protozoan community.

Dove, R. A. 1970. Reactions of small dosages of chlorine in seawater. Central Electr. Generating Board, Res. Rep. 42/70, F. Le No. 03070/ID.

Dowden, B. F., and H. J. Bennett. 1965. Toxicity of selected chemicals to certain animals. J. Wat. Pollut. Control Fed. 37:1308-1316.

Dressel, D. M. 1971. The effects of thermal shock and chlorine on the estuarine copepod (Acartia tonsa). Univ. of Virginia, M. S. Thesis.

Eaton, J. G. 1973. Recent developments in the use of laboratory bioassays to determine "safe" levels of toxicants for fish. p. 107-115. In G. E. Glass (Ed.), Bioassay techniques and environmental chemistry. Ann Arbor Sci. 499 p.

Developes the "safe" level concept using chronic biossay tests (must include reproduction cycle and exposure of eggs and young) and acute biossays.

Engstrom, D. G., and J. B. Kirkwood. 1974. Median tolerance limits of selected marine fish and lobster larvae to temperature and to chlorinity. Supplement to pre-operational report on pilgrim nuclear studies for Division of Marine Fisheries, Commonwealth of Massachusetts.

Eppley, R. W., E. H. Renger, and P. M. Williams. 1976. Chlorine reactions with seawater constituents and the inhibition of photosynthesis of natural marine phytoplankton. Estuar. Coast. Mar. Sci. 4:147-161.

Decay rates in filtered, UV-treated and nonfiltered seawater were reported. Some natural (field) and lab assays were preformed.

Erickson, S. J., and A. E. Freeman. 1978. Toxicity screening of fifteen chlorinated and brominated compounds using four species of marine phytoplankton. p. 307-310. In R. L. Jolley (Ed.), Water chlorination: environmental impact and health effects. Vol. 2. Ann Arbor Sci.

Esvelt, I. A., et al. 1972. Toxicity removal from municipal wastewaters. State Water Resources Control Board Publ. 44, Vol. 4, Sanitary Eng. Res. Lab. Rep. 71-7.

Esvelt, L. A., W. J. Kaufmann, and R. E. Selleck. 1973. Toxicity assessment of treated municipal waste waters. J. Wat Pollut. Control Fed. 45:1558-1572.

Fandrei, G. L. 1977. Total residual chlorine: its effect on the emerald shiner Notropis atherinoides (Rafinesque). Univ. of Minnesota, M. S. Thesis. 53 p.

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Finney, D. J. 1952. Probit analysis. Cambridge Univ. Press, London. 318 p.

Fisher, R. A., and F. Yates. 1963. Statistical tables for biological, agricultural and medical research, 6th ed. Oliver and Boyd, London. 124 p.

Galtsoff, P. S. 1946. Reaction of oysters to chlorination. U. S. Dept. of Int., Fish and Wildl. Serv. Res. Rep. 11.

Gaufin, A. R. 1973. Use of aquatic invertebrates in the assessment of water quality. p. 96-116. In Biological methods for the assessment of water quality. ASTM STP 528, American Society for Testing and Materials.

Argues for bioassay versus chemical analysis since pollutants' primary effect is on living organisms.

Gentile, J. H. 1972. Unpublished data, EPA, Natl. Mar. Wat. Qual. Lab., West Kingston, R. I.

Gentile, J. H., et al. 1974. Toxicity to marine organisms of free chlorine and chlorinated compounds in seawater. EPA Res. Dev. Rep.

Glass, G. E. (Ed.). 1973. Bioassay techniques and environmental chemistry. Ann Arbor Sci. 499 p.

Goldman, J. C. 1979. Chlorine in the marine environment. Oceanus 22(2):36-43.

Popular review including impact, effects, source, results of Capuzzo's bioassays (sudden death of fish), future prospects.

Goldman, J. C., J. M. Capuzzo, and G. T. F. Wong. 1978. Biological and chemical effect of chlorination at coastal power plants. p. 291-303. In R. L. Jolley (Ed.), Water chlorination: environmental impact and health effects. Vol. II. Ann Arbor Sci.

Decay rates and bioassays; Cl chemistry is complex, phytoplankton less affected than invertebrate larvae by TRC.

*Ferguson Wood, E. J., and R. E. Johannes. 1975. See Wood.

Goldman, J. C., and J. A. Davidson. 1977. Physical model of marine plants. Environ. Sci. Technol. 11:908-913.

Model incorporated temp. rise with NaOCl injection, 45 min. exposure time then temp. dropped and sodium thiosulfate added to take up any chlorine. Amperometric titration and culture absorbance techniques. Varying phosphorus conc. were used to keep a steady conc. of plankters.

Goldman, J. C., and H.L. Quinby. 1979. Phytoplankton recovery after power plant entrainment. J. Wat. Pollut. Control Fed. (7):1816-1823.

Using natural populations, they found essentially complete recovery of entrained phytoplankton populations.

Goldman, J. C., H. L. Quinby, and J. M. Capuzzo. 1979. Chlorine disappearance in sea-water. Wat. Res. 13:315-323.

A rapid loss followed by a continuous, but slow, loss was found. Main loss attributed to Br^- but continuous slow loss was/is unexplained. These are suspects for potential biocides.

Hall, L. W., Jr., D. T. Burton, and S. L. Margrey. 1979. The influence of acclimation temperature on the interactions of chlorine, elevated temperature, and exposure duration for grass shrimp, Palaemonetes pugio. Trans. Am. Fish. Soc. 108:626-631.

Matrix with increased temperatures of 2, 6 and 10°C. Models introduced. Essentially, TRC more important than temp. change in inducing mortality.

Hamilton, D. H., A. W. Flemer, C. Keefe, and J. A. Mihursky. 1970. Power plants: effects on estuarine primary production. Science 169:197-198.

Heath, A. G. 1977. Toxicity of intermittent chlorination to freshwater fish: influence of temperature and chlorine form. Hydrobiologia 56(1):39-47.

Fingerling rainbow trout, coho salmon, carp, golden shiner, channel catfish were exposed to pulses of free chlorine or monochloramine. Temperature had little effect on toxicity and free chlorine was more toxic than monochloramine. LC- and LT50's reported.

Heinle, D. R., and M. S. Beaven. 1977. Effects of chlorine on the copepod Acartia tonsa. Chesapeake Sci. 18(1):140.

LC50's of 0.028 to 0.175 were reported for exposures of over 10,000 minutes. No materials or methods.

Henderson, C., and C. M. Torzwell. 1957. Bioassays for control of industrial effluents. Sewage Ind. Wastes 29:1002-1017.

Heslinga, G. A. 1976. Effects of copper on the coral-reef echinoid Echinometra mathaei. Mar. Bio. 35:155-160.

Uses early life stages of E. mathaei. We followed much the same technique for our spawnings.

Hiatt, R. W., et al. 1953. Effects of chemicals on a schooling fish (Kuhlia sandvicensis). Biol. Bull. 104:28.

Hiatt, R. W., et al. 1953. Relation of chemical structure to irritant responses in marine fish. Nature 172:904.

Hirayama, K., and R. Hirano. 1970. Influences of high temperature and residual chlorine on marine phytoplankton. Mar. Biol. 7:205-213.

Chlamydomonas can tolerate 20 ppm chlorine. Skeletonema growth inhibited at 35°C, but 1.5 ppm chlorine will damage phytoplankton. Conclusions disputed in literature.

Holland, E. A., et al. 1960. Toxic effects of organic and inorganic pollutants on young salmon and trout. Washington Dept. Fish. Res. Bull. 5:198-214.

Holmes, N. 1970. Marine fouling in power plants. Mar. Pollut. Bull. 1(7):105-106.

British power plants use up to 5 mg/ℓ chlorine gas to sustain a 0.5 residual.

Hoss, D. E., L. C. Coston, J. P. Babtist, and D. W. Engle. 1974. Effects of temperature, copper and chlorine on fish during simulated entrainment in power plant condensor cooling systems. p. 519-527. Vienna: Int. At. Energy Agency (Ref. I AEA-SM-189/19).

Jensen, D., (Ed.). 1977. Bio-fouling control procedures: technology and ecological effects. Marcel Dekker, Inc., New York. 113 p.

Johnson, J. D. 1978. Measurement and persistence of chlorine residuals in natural waters. In R. L. Jolley (Ed.), Water chlorination: environmental impact and health effects. Vol. 1. Ann Arbor Sci. 439 p.

Paper breaks apart the products — some are better disinfectants (i.e. HOCl, chloramines) — of the chlorine-in-water reaction. Must selectively measure for these chemical species.

Johnson, A. G., T. D. Williams, and C. R. Arnold. 1977. Chlorine-induced mortality of eggs and larvae of spotted seatrout (Cynoscion nebulosus). Trans. Am. Fish Soc. 106:466-469.

48-hr TLm's for NaOCl, chloramine, 4-chlorouracil on 2-h-old, 10-h eggs, and 1-h posthatch larval trout are reported. Eggs were more resistant than larvae.

Jolley, R. L., (Ed.). 1976. Proceedings of the conference on environmental impact of water chlorination. (Oct. 22-24) Oak Ridge Nat. Lab., Conf.-751096, Oak Ridge, Tenn. Oct. 1975.

Jolley, R. L. 1977. Identification of organic halogen products. Chesapeake Sci. 18(1):122-126.

Over 50 chloro-organic constituents were identified and quantified from waste-water effluents. The author anticipates many more bromo-organics will be found.

Jolley, R. L., (Ed.). 1978. Water chlorination: environmental impact and health effects. Vol. I. Ann Arbor Sci. 439 p.

Jolley, R. L., H. Gorchev, and D. H. Hamilton, (Eds.). 1978. Water chlorination: environmental impact and health effects. Vol. II. Ann Arbor Sci.

Kobayashi, N. 1971. Fertilized sea urchin eggs as an indicating material for marine pollution bioassays, preliminary experiments. Publ. Seto Mar. Biol. Lab. 18(6):379-406.

Eggs and embryos were used as indicators of polluted waters. Use is suggested for simplicity, speed and sensitivity. Available year around.

Kott, Y., and J. Edlis. 1969. Effect of halogens on algae — I. Chlorella sorokiniana. Wat. Res. 3:251-256.

Bromine alone or in conjunction with chlorine had superior algacidal properties. Chlorine toxicity increased in the dark.

Kott, Y., G. Hershkovitz, A. Shemtob, and J. B. Sless. 1966. Algicidal effect of bromine and chlorine on Chlorella pyrenoidosa. Appl. Microbiol. 14:8-11.

Krock, H., and D. T. Mason. 1971. Effects of chlorinated domestic effluent on plankton. In Bioassays of lower trophic levels. Calif. State Wat. Resources Control Board, Publ. 44, Vol. VI.

Latimer, D. L., A. S. Brooks, and A. M. Beeton. 1975. Toxicity of 30-minute exposures of residual chlorine to the copepods Limnocalamus macrurus and Cyclops bicuspidatus thomasi. J. Fish. Res. Board. Can. 32:2495-2501.

Freshwater work: the higher the temperature the lower the LC50. Also found increased pH had no effect on mortality.

Lee, F. G. 1979. Persistence of chlorine in cooling water from electric generating station. J. Environ. Eng. Div. Proc. Am. Soc. Civil Eng. 105:757-773.

Delaware freshwater station. Conclusion: "no adverse effects on aquatic life present." No bioassays. Noted that TRC of 0.2-0.5 mg/ℓ may persist for 4 hr per day.

Lewis, B. G. 1966. Chlorination and mussel control. I: The chemistry of chlorinated seawater, a review of the literature. Leatherhead, Surrey, England Central Electr. Res. Lab. (Lab. note Cent. Elect. Res. Lab. RD/LN106/66, Job 10266.10 p.)

Litchfield, J. T. 1949. A method for rapid graphic solution of time-percent curves. J. Pharmacol. Exp. Ther. 97:399-408.

The well used method for mortality-dose bioassay analysis.

Litchfield, J. T., and F. Wilcoxon. 1949. A simplified method of evaluating dose effect experiments. J. Pharmacol. Exp. Ther. 96:99-113.

A well followed method for deriving LC50's, slopes, and confidence limits.

Marsh, J. A., Jr., M. I. Chernin, and J. E. Doty. 1977. Power plants and the marine environment in Piti Bay and Piti Channel, Guam: 1976-1977 observations and general summary. Univ. of Guam Mar. Lab., Tech. Rep. 38. 93 p.

Compiles data on physical parameters. Includes some biological observations in the Piti area.

Mattice, J. S., and M. E. Zittel. 1976. Site-specific evaluation of power plant chlorination. J. Wat. Pollut. Control Fed. 48:2284-2308.

A good review of freshwater and marine organisms respose to chlorine toxicity. 123 references.

Matulova, D. 1967. The application of Chalamydomonas cultures as bio-assay test organisms. Hydrobiologia 30:494-502.

McLean, R. I. 1971. Chlorine and temperature stress on selected estuarine invertebrates. Am. Soc. Zool., 1:649.

McLean, R. I. 1972. Chlorine tolerance of the colonial hydroid Bimeria franciscana. Chesapeake Sci. 13:229.

McLean, R. I. 1973. Chlorine and temperature stress in estuarine invertebrates. J. Wat. Pollut. Control Fed. 45(5):837-841.

Although heat had little effect on the selected invertebrates, chlorine had a major effect and synergistic effects were predicted. Copepods more sensitive than barnacle or grass-shrimp larvae.

Meldrim, J. W., et al. 1974. The effect of temperature and chemical pollutants on the behavior of several estuarine organisms. Ichthyological Associates, Inc., Bull. 11.

Middaugh, D. P., J. A. Couch, and A. M. Crane. 1977. Responses of early life history stages of the stripped bass, Morone saxatilis, to chlorine. Chesapeake Sci. 18:141-153.

TRC effects on embryos and juveniles. Aspects looked at include histopathology, avoidance responses and LC50's. Freshwater. Concentrations down to 0.01 ppm caused adverse effects.

Middaugh, D. P., A. M. Crane, and J. A. Couch. 1977. Toxicity of chlorination to juvenile spot, Leiostomus xanthurus. Wat. Res. 11:1089-1096.

LC50 values were 0.12 mg/ℓ TRC at @ 10°C and 0.06 mg/ℓ @ 15°C. Avoidance and histological studies were carried out.

Middaugh, D. P., J. M. Dean, R. G. Domey, and G. Floyd. 1978. Effect of thermal stress and total residual chlorination on early life stages of the mummichog Fundulus heteroclitus. Mar. Biol. 46:1-8.

Short-term tests (7.5 to 60 min.), checked 24 hr later for survival rates. Synergistic effects possible. Temp. is more important in embryonic stages.

Morgan, R. P., II, and R. G. Stross. 1969. Destruction of phytoplankton in the cooling water supply of a stream electric station. Chesapeake Sci. 10:165-171.

Morgan, R. P., II, and E. J. Carpenter. 1978. Biocides. p. 95-133. In J. R. Schubel, and B. C. Marcey, Jr., (Eds.), Power plant entrainment: biological assessment. Academic Press, Inc. New York. 271 p.

A good review of the temperate chlorine work to 1978. Major papers mentioned on phytoplankton, invertebrate and fish bioassays.

Morgan, R. P., II, and R. D. Prince. 1977. Chlorine toxicity to eggs and larvae of five Chesapeake Bay fishes. Trans. Am. Fish. Soc. 106:380-385.

Stripped eggs and fish larvae in continuous flow system. Amperometric titration method used. Egg LC50's between 0.20 and 0.40 ppm. Larvae: 0.2 to 0.32 ppm LC50's.

Morgan, R. P., II, and R. D. Prince. 1978. Chlorine effects on larval development of stripped bass (Morone saxatilis), white perch (M. americana) and blueback herring (Alosa aestivalis) Trans. Am. Fish. Soc. 107(4):636-641.

Sublethal effects of TRC studied; used amperometric titrations and calcium hypochlorite. Temp. 16.7°C. Results: TRC up, then larval length down; TRC up, then development down; TRC up, then production of larvae down. Ecological impact discussed.

Morse, D. E., H. Duncan, N. Hooker, and A. Morse. 1977. Hydrogen peroxide induces spawning in mollusks, with activation of prostaglandin endoperoxide synthetase. Science 196:298-300.

H_2O_2 method for induced spawning. We have enlarged the list of known mollusks to respond to this method.

Morse, D. E., M. Hooker, and A. Morse. 1979. Chemical control of reproduction in bivalve and gastropod molluscs, III: an inexpensive technique for mariculture of many species. Proc. of World Mariculture Soc. Ninth Annu. Meeting. Atlanta. Jan. 1979.

H_2O_2 method for spawning molluscs. We experimented with this method.

Muchmore, D., and D. Epel. 1973. The effects of chlorination of wastewater on fertilization in some marine invertebrates. Mar. Biol. 19:93-95.

Residual as low as 0.05 ppm was a potent spermicide. Animals: Strongylocentrotus purpuratus (urchin), Urechis caupo (echiuroid), Phragomatopoma califranica (Polychaete). Calif. study.

Reviews theory of ecological change brought about by heated effluents. Modes and effects are addressed.

Nelson, S. G., R. N. Tsutsui, and B. R. Best. 1980. Evaluation of seaweed mariculture potential on Guam: I. Ammonium uptake by, and growth of two species of Gracilaria (Rhodophyta). Univ. of Guam Mar. Lab., Tech. Rep. 61. 20 p.

Appendix contains the theory and the application of the Orion NH_3 probe in saltwater.

Okubo, R., and T. Okubo. 1962. Study on the bio-assay method for the evaluation of water pollution. II. Use of fertilized eggs of sea urchins and bivalves. Bull. Tokai Reg. Fish. Res. Lab. 32:131-140.

Palin, A. J. 1974. Analytical control of water disinfection with special references to differential DPD methods for chlorine, chlorine dioxide, bromine, iodure and ozone. J. Inst. Wat. Engrs. 20:139-154.

For best overall accuracy and precision the DPD titrimetric method is advised over the orthotolidine, amperometric and iodometric methods.

Patrick, R., J. Cairns, and A. Schneier. 1968. The relative sensitivity of diatoms, snails and fish to 20 common constituents of industrial wastes. Progve. Fish Cult. 30:137-140.

Patrick, R., and R. Mclean. 1970. Chlorine and thermal bioassay studies of some marine organisms for the Potomac Electric Power Company. Progress Rep. for Potomac Electr. Power Company.

Patrick, R., and R. Mclean. 1971. Entrainment simulation studies on some estuarine organisms for the Potomac Electric Power Company. Acad. Nat. Sci. Phil., Dept. of Limnology.

Pearse, J. S. 1968. Patterns of reproductive periodicities in four species of Indo-Pacific echinoderms. Proc. Indian Acad. Sci. 68:247-279.

Echinometra mathaei near the equator are continual breeders (also discusses Holothuria atra, Linckia laevigata, Diadema setosum).

Roberts, M. H., Jr. 1977. Bioassay procedures for marine phytoplankton with special reference to chlorine. Chesapeake Sci. 18(1):137-139.

Suggests advantages of dialysis (membrane). Continuous culture methods have advantages over static systems.

Roberts, M. H., Jr. 1978. Effect of chlorinated sea water on decapod crustaceans. p. 329-339. In R. L. Jolley (Ed.), water chlorination: environmental impact and health effects. Vol. II. Ann Arbor Sci.

Had problems with constant dosage and bacterial/fungal infections. Eggs and larvae: eggs more tolerant than larvae; 96-hr LC50's 0.06 to 0.12 for larvae.

Rupp, J. H. 1973. Effects of temperature on fertilization and early cleavage of some tropical echinoderms, with emphasis on Echinometra mathaei. Mar. Biol. 23:183-189.

High temperature inhibits fertilization and early cleavage. E. mathaei most resistant.

Schubel, J. R., and B. C. Marcey, Jr. (Eds.). 1978. Power plant entrainment: a biological assessment. Academic Press, New York. 271 p.

Introductions explains and defines entrainment concept.

Scott, G. I., and D. P. Middaugh. 1978. Seasonal chronic toxicity of chlorination to the American oyster, Crassostrea virginica (G.). p. 311-328. In R. L. Jolley (Ed.), Water chlorination: environmental impact and health effects. Vol. II. Ann Arbor Sci.

Used CPO (chlorine-produced oxidant); 45- 60- and 75-day exposures. Survival, growth, gonads, and fecal production were monitored. Severe sublethal effects and mortality but many variables (seasons, temp., etc.).

Seegert, G. L., and A. S. Brooks. 1978. The effects of intermittent chlorination on colo salmon, alewife, spottail shiner, and rainbow smelt. Trans. Am. Fish. Soc. 107(2):346-353.

Uses 30-min exposures at different temps. LC50's were specie- and temp.-dependent. T. up, then LC50 down. LC50's from 0.3 to 2.4 mg/ℓ. Mortalities usually occurred within 24 hr. S. F. (safe factor) = Conc. below which no mortality occurs/ LC50.

Sprague, J. B. 1969. Measurement of pollutant toxicity to fish. I. Biosassay methods for acute toxicity. Wat. Res. 3:793-821.

First of a series of reviews encompassing the field of bioassays on aquatic organisms. Introduces LC50 techniques, reviews other toxicity tests and discusses replacement rates in flow-thru systems.

Sprague, J. B. 1970. Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. Wat. Res. 4:3-32.

A standard in the field. Methods are given for predicting joint toxicity, multivariable analysis, and reporting results, etc. Directed to fish but includes other organisms. Good references.

Sprague, J. B. 1971. Measurement of pollutant toxicity to fish. III. Sublethal effects and "safe" concentrations. Wat. Res. 5:245-266.

The third in the classic three-part review. Changes in behavior, feeding, avoidance reactions, and reproduction success must be tested when feasible.

Stephan, C. E., and D. I. Mount. 1973. Use of toxicity tests with fish in pollution control. p. 164-177. In Biological methods for assessment of water quality. ASTM STP 528, American Society for Testing and Materials.

Argues for chronic tests which study effects of toxic agents on survival, growth, and reproduction. Fish toxicologists should watch for avoidance, flavor impairment, and the accumulation of residues.

Stober, Q. J., and C. H. Hanson. 1974. Toxicity of chlorine and heat to pink (Oncorhynchus gorbuscha) and chinook salmon (O. tshawytscha). Trans. Am. Fish. Soc. 103:569-576.

Tested a matrix of temp. and exp. time to Cl. Found decreased tolerance with increasing temp. LC50's, 0.045 mg/ℓ to 0.5 mg/ℓ Orthotolidine method; examined decay rates.

Texas Instruments Inc. 1974. Acute and chronic effects of evaporative cooling tower blow down and power plant chemical discharges on white perch (Morone americana) and stripped bass (M. saxatilis).

Thatcher, T. O. 1978. The relative sensitivity of Pacific Northwest fishes and invertebrates to chlorinated seawater. p. 341-349. In R. L. Jolley (Ed.), Water chlorination: environmental impact and health effects. Vol. II. Ann Arbor Sci.

States composition of clorox. Sensitivity to chlorine by 15 fish & crustacean, is recorded. Sensitivity (LC50): fish greater than crustaceans. A good comparative study although critical decimals are misplaced in results.

Thatcher, T. O., M. J. Schneider, and E. G. Wolf. 1976. Bioassays on the combined effects of chlorine, heavy metals, and temperature on fishes and fish food organisms. Bull. Environ. Contam. Toxicol. 15:40-48.

At 20°C adverse synergistic effects of temp. and clorine were evident. Amperometric method; freshwater.

Thomas, N. A. 1973. Assessment of fish flesh tainting substances. p. 178-193. In Biological methods for assessment of water quality. ASTM STP 528, American Society for Testing and Materials.

Fish from discharge areas were tested by a panel and tainting of fish flesh was noted.

Turner, H. J., et al. 1948. Chlorine and sodium pentachlorophenate as fouling preventives in sea-water conduits. Ind. and Eng. Chem. 40:450.

Van Olst, S. C., R. F. Ford, J. M. Carlberg, and W. R. Dorband. 1975. Use of thermal effluent in culturing the American lobster. p. 71-97. In Power plant waste heat ultilization in Aquaculture — Workshop I. Nov. 6-7, 1975. PSE&G Co. Newark, N. J.

Includes static bioassay work with chlorine. LC50's of 0.31 to 0.21 ppm.

Vernberg, W. B. 1979. Seasonal effects of chlorine-produced oxidants on the growth, survival, and physiology of the American oyster, Crassostrea virginica (Gmenlin). In Marine pollution; functional responses. Academic Press, New York.

Videau, C. M., M. Khalanski, and M. Penot. 1979. Preliminary results concerning effects of chlorine on monospecific marine phytoplankton. J. Exp. Mar. Biol. Ecol. 36:111-123.

Static tests. LD50's using concentrations from 0.4 to 4.0 ppm on 3 species were reported. Results showed some dependency on cellular density (chlorine toxicity increases with decreasing cellular concentration). Influence of light also noted. Decay rates in seawater graphed.

Waugh, G. D. 1964. Observations on the effects of chlorine on the larvae of oysters (Ostrea edulis (L.)) and barnacles (Elminius modestus (Darwin)). Ann. Appl. Biol. 54:423-440.

Barnacle larva were much more sensitive than oyster larvae to chlorine. Some oysters lived at levels to 200 ppm chlorine.

White, G. C. 1972. Handbook of chlorination. Van Nostrand Reinhold, New York. 744 p.

A fair history of fresh water chlorination: includes present use in industry and domestic systems, and chemistry. Poor on biology.

White, W. R. 1966. The effect of low-level chlorination on mussels at Poole Power Station. Cent. Electr. Generating Board Rep. RD/L/N 7/66.

Wisely, B., and R. A. P. Blick. 1967. Mortality of marine invertebrate larva in mercury, copper, and zinc solutions. Aust. J. Mar. Freshwat. Res. 18:63-72.

Wong, G. T. F., and J. A. Davidson. 1977. The fate of chlorine in sea water. Wat. Res. 371-798.

Chlorine demand in seawater has no limit. Residual sodium hypochlorite decreased rapidly within the first hour then slowed. Hypobromite is the major species measured. Conc. used were up to 30 ppm.

Wood, E. J. F., and R. E. Johannes (Eds.). 1975. Tropical marine pollution. Elsevier Scientific, New York. 192 p.

A good reference for any tropical pollution work. Discusses different patterns found in tropical versus temperate pollution problems. Chapters on coral reef, mangrove, coral, and seagrass systems. Good references.

Zeitoun, I. H. 1976. The effects of intermittent chlorination on survival of fish at the James H. Campbell Plant. Consumers Power Co., Jackson, Mich. 127 p.

APPENDIX

Table A-1. Constituents of the chlorine source used in this investigation.

Chlorine source:	Commercial Clorox	
	90.42%	water
	5.25%	sodium hypochlorite
	4.12%	sodium chloride
	0.20%	sodium carbonate
	0.01%	sodium hydroxide

Information from the Clorox Co., Oakland, Calif. and USEPA Registrations Office in Seattle, Washington.

Table A-2. Selected Data from Investigations on the Toxicity of Chlorine to Marine Organisms. [Expanded and modified from Mattice and Zittel 1976.]

Reference	Method	Concentration (mg/l)	Duration (min)	Type of Chlorine Measured	Type of Test*	Chlorine Source
1. Alderson (1970)	Palins DPD orthotolidine	0.015-0.7	4320-11520	Total	CF	Electrolysis
2. Alderson (1974)	Palin DPD	0.025-0.64	2880-5760	Total	CF	Direct electrolysis
3. Block et al. (1977)	Amperometric titration	0.8	60-480	Total residual	CF	CaOCl
4. Bradley (1977)	Palin DPD	0.1 -0.3	1440	Chlorine	S	NaOCl
5. Capuzzo et al. (1977)	Amperometric titration	0.01 -10	30-60	Total residual	S	NaOCl
6. Capuzzo (1977)	Amperometric titration	0.15	60	Total residual	CF	NaOCl
7. Carpenter et al. (1972)	Orthotolidine	0.1 -1.2	N.G.‡	Free	S	Gas
8. Clendenning and North (1959)	N.G.	5-10	5760	Chlorine	S	
9. Davis (1971)	Iodometric	0.018-40	10-60	Residual	S	
10. Dressel (1971)	Palins DPD	0.75 -1.2	2	Free and combined	S	
11. Engstrom and Kirkwood (1974)	Titration	0.55 -1.2	30-300	Chlorine	CF	
12. Esvelt et al. (1972)**	Amperometric titration	0.03 -1.3	5760	Total	FO	
13. Galtsoff (1946)	Colorimetric	0.01 -1.8	< 1	Free	CF	
14. Gentile (1972)	Iodometric	1 -10	6-360	Chlorine	FO & S	
15. Gentile et al. (1974)	Iodometric	0.1 -10.0	10-1440	Residual	FO & S	
16. Goldman and Davidson (1977)	Amperometric titration	0.5 -1.86	60	Total residual	S	NaOCl
17. Heinle and Beaven (1977)	N.G.	0.062-0.175	>10000	Total residual	NG	NG
18. Hirayama and Hirano (1970)	Iodometric	0.1 -1.8	5-10	Total residual	S	NG
19. Holland et al. (1960)	Orthotolidine and iodometric	0.05 -2.0	1155-33000	Total residual	CF	

Table A-2. Continued

Reference	Method	Concentration (mg/l)	Duration (min)	Type of Chlorine Measured	Type of Test*	Chlorine Source
20. Hoss et al. (1974)	Amperometric titration	0.3 -0.5	0-10	Free residual	CF	
21. Johnson et al. (1977)	DPD Colorimetric	0.01 -100	2880	Total	S	NaOCl
22. Krock and Mason (1971)	Amperometric titration	0.009-1.1	N.G.	Total	S	
23. McLean (1971)	N.G.	2.5	180	Total residual	CF	Gas
24. McLean (1972)	Amperometric titration	1-4.4	60-180	Total	FO & CF	Gas
25. McLean (1973)	Amperometric titration	2.5	5-600	Total	CF	Gas
26. Meldrim et al. (1974)	Amperometric titration	0.02 -0.6	10	Total	CF	
27. Middaugh et al. (1977a)	Amperometric titration	0.02 -1.42	2880+	Total residual	CF	NaOCl
28. Middaugh et al. (1977b)	Amperometric titration	0.02 -2.48	11520	Total residual	CF	NaOCl
29. Middaugh et al. (1978)	Amperometric titration	0.07 -0.99	75-60	Total residual	CF	NaOCl
30. Morgan II and Prince (1977)	Amperometric titration	0.048-2.15	1440-2880+	Total residual	CF	CaOCl
31. Morgan II and Prince (1978)	Amperometric titration	0.046-0.84	N.G.	Total residual	CF	CaOCl
32. Muchmore and Epel (1973)	Iodometric	0.2 -1.0	5	Total available	S	NaOCl
33. Patrick and McLean (1970)	Amperometric titration	0.1 -8.0	5760	Total	CF	
34. Patrick and McLean (1971)	Amperometric titration	2.5	5-180	Total	CF	
35. Roberts Jr. (1978)	Amperometric titration	0.05 -3.9	1440-7200	Total residual	CF	
36. Scott and Middaugh (1978)	Amperometric titration	0.12 -2.98	45-75 days	Chlorine-produced oxidant	CF?	NaOCl

Table A-2. Continued

	Reference	Method	Concentration (mg/l)	Duration (min)	Type of Chlorine Measured	Type of Test*	Chlorline Source
36.	Stober and Hanson (1974)	Orthotolidine	0.05 -1.0	0-60	Residual	S	NaOCl
37.	Texas Instruments (1974)	Dilution	N.G.	1800-40320	Chlorine	CF	
38.	Thatcher (1978)	Amperometric titration	0.026-1.530	5760	Total residual	CF	NaOCl
39.	Turner et al. (1948)	N.G.	1-10	1440-21600	Residual	CF	
40.	Videau et al. (1979)	Colorimetric DPD	0.1 -6.0	1440-4320	Total residual	S	NaOCl
41.	Waugh (1964)	Iodometric	0.5 -5	N.G.	Free	S	NaOCl
42.	White (1966)	Orthotolidine	0.2	Continuous	Residual	CF	
43.	This report (1980)	Chlorine Electrode (Orion)	0.015-3.70	5760	Total residual	S	NaOCl

*S --- static; CF --- continuous flow; FO --- field observation.

‡N.G --- not given.

** --- Wastewater chlorination.

Table A-3. Summary of Available Data on Toxicity of Chlorine to Marine Organisms. See Table for numbered reference. All animals are adults unless otherwise noted. [Modified and expanded from Mattice and Zittel 1976.]

		Concentration (mg/l)*	Duration (min)	Effect	Reference
Plants:					
N.G.†	Phytoplankton	<0.1	240	71% decrease in productivity	7
N.G.	Phytoplankton	0.03	N.G.	50% decrease in photosynthesis‡	22
Chlamydomonas sp.	Phytoplankton	1.5	5-10	Decreased growth	18
Dunaliella primolecta	Phytoplankton	0.6	1440	Decrease in growth ≈LC50	40
Dunaliella tertiolecta	Phytoplankton	0.11	1440	50% decrease in growth	15
Dunaliella tertiolecta	Phytoplankton	0.16	5760	50% decrease in growth	43
Dunaliella tertiolecta	Phytoplankton	0.29	5760	50% decrease in growth	43
Phaeodactylum tricornutum	Phytoplankton	0.6-0.8	1440	Decreased growth	40
Phaeodactylum tricornutum	Phytoplankton	0.5-1.86	60	Washout but recovered	16
Pavlova lutheri	Phytoplankton	4.0	1440	Decrease in growth ≈LC50	40
Asterionella japonica	Phytoplankton	0.4	0.27	50% decrease in growth	14
Asterionella japonica	Phytoplankton	0.2	2	50% decrease in growth	14
Chaetoceros decipiens	Phytoplankton	0.14	1440	50% decrease in growth	15
Chaetoceros didymum	Phytoplankton	0.125	1440	50% decrease in growth	15
Chaetoceros gracilis	Phytoplankton	0.18	5760	50% decrease in growth	43
Detonula confervacea	Phytoplankton	0.8	0.6	50% decrease in growth	14
Skeletonema costatum	Phytoplankton	0.095	1440	50% decrease in growth	15
Skeletonema costatum	Phytoplankton	0.6	1.7	50% decrease in growth	14
Thalassiosira nordensholkii	Phytoplankton	0.195	1440	50% decrease in growth	15
Thalassiosira pseudonana	Phytoplankton	0.075	1440	50% decrease in growth	15
Thalassiosira pseudonana	Phytoplankton	0.2	6.8	50% decrease in growth	15
Thalassiosira pseudonana	Phytoplankton	0.5	0.3	50% decrease in growth	14
Thalassiosira rotula	Phytoplankton	0.33	1440	50% decrease in growth	15
Monochrysis lutheri	Phytoplankton	0.2	1440	50% decrease in growth	15
Rhodomonas baltica	Phytoplankton	0.11	1440	50% decrease in growth	15

Table A-3. Continued

	Descriptive Name	Concentration (mg/l)*	Duration (min)	Effect	Reference
Phaeophyta					
Macrocystis pyrifera	Giant kelp	5-10	5760	50% decrease in photosynthesis	8
Invertebrate animals:					
Cnidaria					
N.G.	Sea anemone	1.0	21600	No effect	39
Bimeria franciscana	Hydroid	2.5	180	Slight decrease in growth	24
+*Cyphastrea ocellina*	Coral planulae	0.49	10-60	Immobile	9
+*Pocillopora damicornis*	Coral planulae	0.49	10-60	Immobile	9
+*Tubastrea aurea*	Coral planulae	0.49	10-60	Immobile	9
Annelida					
Phragmatopoma californica	Polychaete worm	0.2	5	17% decrease in sperm motility‡	32
Phragmatopoma californica	Polychaete worm	0.4	5	70% decrease in sperm motility‡	32
Mollusca					
Crassostrea virginica	Oyster	0.2	N.G.	~46% decrease in cilliary beat rate	13
Crassostrea virginica	Oyster	1.0	20-90	Pumping threshold	13
Crassostrea virginica	Oyster	0.18	4320	50% decrease in time open	33
Crassostrea virginica	Oyster	0.65-1.23	45-70 days	22 to 88% mortality depending on season	36
Ostrea edulis	Oyster larvae	0.5	2	Swimming stopped	41
Mytilus edulis	Mussel	1.0	21600	100% mortality	39
Mytilus edulis	Mussel	2.5	7200	100% mortality	39
Crepidula and *Littorina*	Gastropods	0.2	N.G.	Stop growth	42
Stylocheilus longicauda	Sea hare veligers	>1.95	5760	50% mortality	43
Trochus niloticus	Topshell juveniles	>3.35	5760	50% mortality	43

Table A-3. Continued

	Descriptive Name	Concentration (mg/l)*	Duration (min)	Effect	Reference
Arthropoda					
Acartia tonsa	Copepod	0.75	2	30% mortality @ 20°C after 96 hr	10
Acartia tonsa	Copepod	0.75	2	70% mortality @ 25°C after 96 hr	10
Acartia tonsa	Copepod	1	120	50% mortality	15
Acartia tonsa	Copepod	10.0	0.7	50% mortality	15
Acartia tonsa	Copepod	2.5	5	90% mortality-after 3 hr	25
Acartia tonsa	Copepod	0.40	1440	50% mortality	4
Acartia tonsa	Copepod	0.028-0.175	>10000	50% mortality @ 15°C	17
Eurytemora affinis	Copepod	1	360	50% mortality	15
Eurytemora affinis	Copepod	10.0	2	50% mortality	15
Pseudodiaptomus coronatus	Copepod	2.5	45	50% mortality	15
Pseudodiaptomus coronatus	Copepod	10	5	50% mortality	15
Neomysis sp.	Adult mysid	0.162	5760	50% mortality	38
Balanus improvisus	Barnacle larvae	2.5	5	80% mortality-after 3 hr	25
N.G.	Barnacles	1.0	21600	Most dead	9
Elminius modestus	Barnacle nauplii	0.5	10	Threshold mortality	41
Elminius modestus	Barnacle nauplii	1	10	Heavy losses-no growth	41
Anonyx sp.	Adult amphipod	0.145	5760	50% mortality	38
Corophium sp.	Tube dwelling amphipod	10	410	No mortality after 24 hr	14
Gammarus tigrinus	Amphipod	2.5	180	25% mortality after 96 hr	25
Melita nitida	Amphipod	2.5	120	50% mortality	25
Melita nitida	Amphipod	2.5	5	Some mortality	25
Pontogenia sp.	Juvenile amphipod	0.687	5760	50% mortality	38
Callinectes sapidus	Blue crab	10	1140	50% mortality	33
Callinectes sapidus	Blue crab	0.1	5760	50% mortality	33
Hemigrapsus nudus	Juvenile and adult shore crab	1.418	5760	50% mortality	38
Hemigrapsus oregonensis	Juvenile and adult shore crab	1.418	5760	50% mortality	38
Panopeus herbstii	Crab larvae	0.055-0.41	2880	50% mortality	35
Pagurus longicarpus	Crab larvae	0.062-0.102	5760	50% mortality	35

Table A-3. Continued

	Descriptive Name	Concentration (mg/l)*	Duration (min)	Effect	Reference
Crangon nigricauda	Adult shrimp	0.134	5760	50% mortality	38
Crangon septemspinosus	Sand shrimp	0.15	900	50% mortality	33
Crangon septemspinosus	Sand shrimp larvae	5	10	42% mortality	15
Crangon septemspinosus	Sand shrimp larvae	10	5	55% mortality	15
Palaemonetes pugio	Grass shrimp	2.5	180	98% mortality-after 96 hr	25
Pandalus danae	Juvenile and adult coon stripe shrimp	0.178	5760	50% mortality	38
Pandalus goniurus	Shrimp	0.090	5760	50% mortality	38
Homarus americanus	Stage I lobster larvae	0.3	30 or 60	50% mortality after 48 hr (25°C)	5
Homarus americanus	Stage I lobster larvae	0.01	60	Significant respiratory stress (25°C)	5
Homarus americanus	Stage I lobster larvae	0.15	60	15% mortality after 48 hr (25°C)	6
Ectoprocta					
Bugula sp.		2.5	2880	100% mortality	39
Bugula sp.		10	1440	100% mortality	39
Echinodermata					
Echinometra mathaei	Sea urchin plutei	0.46	5760	50% mortality @ 33°C	43
Echinometra mathaei	Sea urchin plutei	0.84	5760	50% mortality @ 28°C	43
Strongylocentrotus purpuratus	Sea urchin	0.125	5	1-6% fertilization success‡	32
Urechis caupo	Echiuroid	0.2	5	78% fertilization success‡	32
Urechis caupo	Echiuroid	0.4	5	0% fertilization success‡	32
Vertebrate animals					
Chordata					
Botryllus sp.		10	1440	100% mortality	39
Molgula sp.		1	4320	100% mortality	39

Table A-3. Continued

	Descriptive Name	Concentration (mg/l)*	Duration (min)	Effect	Reference
Pleuronectidae					
Limanda ferruginea	Yellowtail flounder	0.1	1440	50% mortality	15
Pleuronectes platessa	Plaice larvae	0.028	5760	50% mortality	1
Pleuronectes platessa	Plaice larvae	0.05	460	50% mortality	1
Pleuronectes platessa	Plaice larvae	0.075	75	50% mortality	1
Pleuronectes platessa	Plaice larvae	0.25	4320	Mortality threshold	1
Pleuronectes platessa	4-day-old eggs of plaice	0.64	4320	50% mortality (5.6°C)	2
Pleuronectes platessa	Larvae of plaice	0.025	5760	50% mortality @ 7.5°C	2
Pleuronectes platessa	Metamorphosed plaice	0.095	5760	50% mortality (14.5°C)	2
Parophrys vetulus	Juvenile English sole	0.073	5760	50% mortality	38
Pseudopleuronectes americanus	Winter flounder	2.5	15	50% mortality	15
Pseudopleuronectes americanus	Winter flounder	10.0	0.3	50% mortality	15
Pseudopleuronectes americanus	Winter flounder eggs	10.0	20	No mortality	15
Salmonidae					
Oncorhynchus gorbuscha	Pink salmon	0.05	5760	50% mortality	19
Oncorhynchus gorbuscha	Pink salmon	0.5	7.5	50% mortality (13.6°C)	36
Oncorhynchus gorbuscha	Pink salmon	0.25	15	50% mortality (13.6°C)	36
Oncorhynchus gorbuscha	Juvenile pink salmon	0.023-0.052	5760	50% mortality	38
Oncorhynchus kisutch	Juvenile coho salmon	0.032	5760	50% mortality	38
Oncorhynchus kisutch	Coho salmon	0.08	< 7200	50% mortality	19
Oncorhynchus tshawytscha	Chinook salmon	0.1	60	Distressed-no mortality	19
Oncorhynchus tshawytscha	Chinook salmon	0.25	130	Mortality threshold	19
Oncorhynchus tshawytscha	Chinook salmon	1.0	23	Mortality threshold	19
Oncorhynchus tshawytscha	Chinook salmon	0.5	7.5	50% mortality (11.7°C)	36
Oncorhynchus tshawytscha	Chinook salmon	0.25	30	50% mortality (11.7°C)	36
Oncorhynchus tshawytscha	Juvenile chinook salmon	0.038-0.065	5760	50% mortality	38
N.C.	Young salmon	0.05	33123	Threshold mortality	19

Table A-3. Continued

	Descriptive Name	Concentration (mg/l)*	Duration (min)	Effect	Reference
Atherinidae					
Menidia beryllina	Eggs of tidewater silversides	0.21-0.32	1440-2880	50% mortality	30
Menidia menidia	Eggs of Atlantic silversides	0.30-0.38	1440-2880	50% mortality	30
Menidia menidia	Atlantic silverside	0.58	90	50% mortality	11
Menidia menidia	Atlantic silverside	1.2	30	50% mortality	11
Clupeidae					
Alosa aestivalis	Blueback herring	0.67	60	50% mortality	11
Alosa aestivalis	Blueback herring	1.2	15	50% mortality	11
Alosa aestivalis	Blueback herring eggs	0.33	4800	50% mortality	30
Alosa aestivalis	Blueback herring eggs	0.38	N.G.	**47.0% developed to prolarvae	31
Alosa aestivalis	Blueback herring larvae	0.25-0.32	1440-2880	50% mortality	30
Brevoortia tyrranus	Atlantic menhaden	0.21	300	50% mortality	11
Brevoortia tyrranus	Atlantic menhaden	1.2	30	50% mortality	11
Brevoortia tyrranus	Atlantic menhaden larvae	0.5	3	0 mortality	20
Clupea harengus	Juvenile Pacific herring	0.065	5760	50% mortality	38
Gasterosteidae					
Gasterosteus aculeatus‡	Threespine stickleback	0.09-0.13	5760	50% mortality	12
Gasterosteus aculeatus‡	Juvenile and adult threespine stickleback	0.167	5760	50% mortality	38

Table A-3. Continued

	Descriptive Name	Concentration (mg/l)*	Duration (min)	Effect	Reference
Ameiuridae					
Ictalurus catus	White catfish	0.1	2880	50% mortality	12
Cyprinidae					
Notemigonus chrysoleucas‡	Golden shiner	0.03-0.23	5760	50% mortality	12
Bothidae					
Paralichthys sp.	Flounder	0.3	5	Threshold mortality	20
Mugilidae					
Chelon engeli	Mullet juveniles	0.20	5760	50% mortality @ 30.1°C	43
Mugil cephalus	Striped mullet juveniles	0.3	5	Threshold mortality	20
Percichthyidae					
Morone americana	White perch eggs	0.27	4560	50% mortality	30
Morone americana	White perch eggs	0.16	N.G.	***53.8% developed to prolarvae	31
Morone americana	White perch larvae	0.31	1440	50% mortality	30
Morone americana	White perch	0.8	60-480	Breakdown of physiological processes	3
Morone saxatilis	Striped bass eggs	0.19	N.G.	50.6% developed to prolarvae	31
Morone saxatilis	Striped bass eggs	0.20-0.22	2880	50% mortality	30
Morone saxatilis	2-day-old prolarvae of striped bass	0.04	2880	50% mortality	27
Morone saxatilis	Striped bass larvae	0.20	1440	50% mortality	30
Morone saxatilis	12-day-old larvae	0.07	2880	50% mortality	27
Morone saxatilis	30-day-old juveniles	0.04	2880	50% mortality	27

Table A-3. Continued

	Descriptive Name	Concentration (mg/l)*	Duration (min)	Effect	Reference
Cyprinodontidae					
Fundulus heteroclitus	Mummichog eggs and larvae	0.07-0.99	7.5-60	Ill effects for embryos dependent on temperature: larvae effects dependent on TRC	29
Fundulus heteroclitus	Juvenile killfish	0.3	30	Significant respiratory stress (25°C)	5
Fundulus heteroclitus	Juvenile killfish	0.8	30	50% mortality after 48 hr (25°C)	5
Sciaenidae					
Cynoscion nebulosus	Spotted sea trout 2-hr-old eggs	0.21	2880	50% mortality	21
Cynoscion nebulosus	10-hr eggs	0.21	2880	50% mortality	21
Cynoscion nebulosus	1-hr posthatch larvae	0.17	2880	50% mortality	21
Soleidae					
Solea solea	Dover sole larvae	0.028	2880	50% mortality @ 17°C	2
Solea solea	Metamorphosed dover sole	0.07	5760	50% mortality @ 15°C	2
Ammodytidae					
Ammodytes hexapterus	Juvenile and adult Pacific sand lance	0.082	5760	50% mortality	38
Apogonidae					
Apogon lateralis	Cardinal fish	0.21-0.72	5760	50% mortality @ 30.1°C	43

Table A-3. Continued

	Descriptive Name	Concentration (mg/l)*	Duration (min)	Effect	Reference
Embiotocidae					
Cymatogaster aggregata	Juvenile and adult shiner perch	0.071	5760	50% mortality	38
Leiostomus xanthurus	Juvenile spot	0.12	11520	50% mortality @ 10°C	28
Leiostomus xanthurus	Juvenile spot	0.06	11520	50% mortality @ 15°C	28

*Mg/l and ppm were treated as equivalent units.

†Not given.

‡Wastewater chlorination.

+Coral planulae "recovered" after exposures up to 40 ppm (Davis 1971)

**89.7% of control group developed to prolarvae stage.

***Only 70.1% of control embryos developed to prolarvae stage.