AN ABSTRACT OF THE THESIS OF Bruce R. Best for the Master of Science in Biology presented February 27, 1981.

Title: Effects of Chlorine-Induced Oxidants on Selected Inshore Organisms around Guam.

Approved: Lucius G. Eldredge

Lucius G. Eldredge, Chairman, Thesis Committee

Static 96-hr controlled temperature bioassay systems were developed, with NaOCl as a chlorine source, to investigate the effects of single doses of chlorine-induced oxidants (ClO) on the phytoplankters Chaetoceros gracilis and Dunaliella tertiolecta, plutei of the urchin Echinometra mathaei, the hermit crab Clibanarius humilis, and two species of fish—the mullet Chelon engeli and the cardinalfish Apogon lateralis.

LC50- and LT50-values were interpolated by log-probit analysis. Results indicate that the phytoplankters were affected at concentrations as low as 0.09 ppm ClO (28°C 96-hr LC50), the fish at around 0.2-0.3 ppm (30.1°C 96-hr LC50) followed by the plutei at 0.2-1.0 ppm (for 48- and 96-hr LC50s at temperatures from 28 to 33°C). The LC50s for the hermit crabs were above 2.4 ppm for temperatures of 27.8 and 31.6°C. LT50 data for fish indicate a fast response (<3 hr) to the toxic effect of introduced chlorine compared to the general response by other nonvertebrate organisms of increased mortality with increased exposure time.

TO THE GRADUATE SCHOOL AND RESEARCH

The members of the Committee approve the thesis of Bruce R. Best presented February 27, 1981.

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EFFECTS OF CHLORINE-INDUCED OXIDANTS ON SELECTED INSHORE ORGANISMS AROUND GUAM

by

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INTRODUCTION

In recent years the introduction of chlorine to marine ecosystems has prompted concern from various sectors. In the near-shore communities of Guam the use of chlorine for illegal fishing purposes has continued to be a chronic problem, and legislation prohibiting this activity has been virtually unenforceable. Chlorine also is used as a disinfectant in island water and waste systems and as a biocide in power-generating plants. Therefore, there is a need for basic studies on the toxicity of chlorine on tropical marine organisms.

The chemistry of chlorine in seawater is exceedingly complex and poorly understood. When chlorine is introduced into seawater, it rapidly reacts with bromide and organics and some of the chlorine becomes "lost" or at least unaccounted for by ordinary analytical tests (Goldman et al. 1979). This nonoxidative loss could be a potential biocide (Goldman 1979). Chloramine, a slowly decaying product of the seawater-chlorine reaction, has been found to be a more potent biocide to temperate lobster larva than chlorine (Capuzzo 1977). Jolley (1977) found over 50 chloro-organic constituents in municipal sewage effluent. He estimated that more than 5000 tons of these compounds are released into the North American aquatic ecosystem each year. The potential damage to the marine environment is enormous. Biomagnification, slow degradation, and other known effects of such chlorinated organics as DDT and PCB account for the grave concern of marine ecologists. Only recently have advances

in analytical methods for the quantitative and qualitative assessment of chlorine-induced oxidants (ClO) 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).

Historically, the majority of chlorine toxicity work has focused on freshwater. More recently, studies have been carried out on marine organisms. Many works in both areas are reviewed in Brooks and Seegert (1978), Davis and Middaugh (1978), and Morgan and Carpenter (1978). Best et al. (1981) have compiled, in tabular form, many major marine works including unpublished tropical Pacific bioassay results. Most of the previous studies were carried out in quest of the biocidal properties of chlorinated power plant or sewage effluents.

The deleterious effects of chlorine on marine organisms depend on many factors. Residual chlorine concentrations as low as 0.05 ppm have been shown to be a potent fertilization inhibitor (Muchmore and Epel 1973) and to cut primary production by 76% in entrained phytoplankton (Carpenter et al. 1972). Metabolic activity of larval zooplankton was seriously affected at ClO levels below 0.01 mg/ℓ (Capuzzo et al. 1977). A concurrent rise in temperature and chlorine concentration, such as might be found in power plant effluent areas or in isolated reef-flat moat areas, which are favored by illegal fisherman, 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 fishfood organisms (Thatcher et al. 1976).

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

These are only general patterns and, as in most toxicological studies, it must be stressed that since response of aquatic organisms to chlorine seems to be species-dependent, broad conclusions concerning the responses of major taxonomic groups to chlorine-induced oxidants should be avoided until specific data, generated from similar experimental conditions, exist for major families and genera of major taxa. Also, in relation to the sensitivity hierarchy, Goldman et al. (1978) pointed out that the ecological impact does not always mirror the results of laboratory studies. For instance, although both phytoplankton and invertebrate larvae are affected at very low levels, complete populations of invertebrate species which spawn intermittently could be seriously threatened by chlorination, while any entrained phytoplankton exposed to chlorine represents only a small fraction of the standing crop.

Ferguson Wood and Johannes (1975) compared some tropical and temperate environmental characteristics and discussed the possible

effects of chemical disinfectants in the tropical marine environment. They noted that while the potency of these toxins increased with increasing temperature that faster toxin degradation is predicted in the tropics. Furthermore, any physiological or toxicological study with tropical reef organisms must consider the assumption that if tropical species—especially fish—are living at the lower limits of their oxygen demand tolerance, then they would be more susceptible to a respiratory tissue oxidizer such as chlorine than would their temperate cognates.

Although virtually no chlorine bioassay studies on tropical organisms have been published, some casual observations emphasize the need for such studies. Birkeland (pers. comm.) recently noted the detrimental effects of shallow-water open-coast "cloroxing" on the coral community in Samoa. His concern was that in Acanthaster-devastated areas the only refuge for corals is in the shallow surf zone—the main target areas for illegal chlorination. Johannes (Ferguson Wood and Johannes 1975) recalled a once richly encrusted cave off Hawaii that had not recolonized a year after it was illegally bleached.

Many research groups have extensively evaluated the toxic effects of ClO on selected temperate biota by either continuous or intermittant exposure methods. However, only very limited data are available on the effects of single-dose exposures of ClO on tropical marine organisms. Because of the widespread use of chlorine as an illegal inshore fishing method, Guam's reef inhabitants are often exposed to such a single-dose regime. Therefore, it was

the objective of this study to quantify the acute effects of sodium hypochlorite on selected tropical reef-flat biota by single-dose LC50 and LT50 methods (the concentration or time at which 50% of the test organisms die, respectively) and to determine the relative sensitivity of selected species.

Results from these studies will be valuable to fishery officers for predicting the impact of an illegal fishing method, and to planners responsible for shoreline sewage treatment or power plant development. The managers of Guam's power plants are presently reevaluating the need for chlorine as a biocide in cooling pipes and, indeed, some are considering complete stoppage of chlorination.

MATERIALS AND METHODS

Species selected for the bioassays represent a variety of taxonomic groups characteristic of the reef flats of Guam. They are species which are able to withstand laboratory manipulation and which are locally available in sufficient numbers to carry out the many runs necessary. Two species of tropical-cultured phytoplankton Dunaliella tertiolecta Butcher and Chaetoceros gracilis Schutt, plutei of the urchin Echinometra mathaei (de Blainville), the aggregating reef-flat hermit crab Clibanarius humilis Dana, and two species of reef-flat schooling fish—the mullet Chelon engeli (Bleeker) and the cardinalfish Apogon lateralis Valenciennes—were selected.

Dunaliella and Chaetoceros cultures were maintained at the University of Guam Marine Laboratory. Adult Echinometra were collected by hand from the Piti area. Gametes were obtained for fertilization by peristomial injection of 0.5M isotonic KCl. After fertilization, larvae were maintained for 24 hr at 27°C before they were introduced into the bioassay system. Specimens of Clibanarius were collected from the Pago Bay reef flat and held 8-24 hr at 30°C prior to the tests. The fish were collected by cast-net from the reef flats of Agana and Tumon Bays and maintained in the lab for 18-24 hr at 30°C prior to the bioassays. Organisms were not fed during the bioassays.

A single-dose static system was used with commercial sodium hypochlorite ("Clorox") as a chlorine source (constituents of "Clorox" are in Appendix 1). An Orion selective-ion electrode (97-70) attached to a Corning 135 meter was used to measure chlorine-induced oxidants (ClO). 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 measures the concentration of released iodine. In another study, this probe was extensively tested against nine other methods in a matrix of concentrations and water sources (Bender 1978). Regrettably, seawater was not included in the matrix, but the precision of the probe was good, i.e. consistently less than 2% relative standard deviation within the expected experimental concentration (0.2 to 1.0 ppm) and less than 5% in all cases. The relative accuracy of the probe when compared to an iodometric (PAO) forward titration method, for all water type except very polluted waters, such as raw sewage, was within the same limits. In addition, Bender (1978) titrated the Orion-supplied standard and found it to be correct. Additional comparisons and chlorine-in-seawater decay rate curves are included in Best et al. (1981).

The bioassay regime for Dunaliella, Chaetoceros, and the one-day-old Echinometra larvae bioassays consisted of two complete sets of acid-washed unaerated pyrex beakers, one at each of two controlled temperatures, with each beaker containing 100 mℓ of 0.45μ (membrane) fresh-filtered seawater. Each set had two experimental beakers, plus a control without added chlorine, at each of six ClO concentrations. The initial culture concentrations were 10×10^4 cells/mℓ for Chaetoceros and 7×10^4 cells/mℓ for Dunaliella.

Approximately 59 Echinometra larvae/mℓ were assayed for Run I and ca. 25 larvae/mℓ were assayed for Run 2. Reagents were added to an additional 100 mℓ beaker—without organisms—at each concentration for the electrode. This method allowed the initial chlorine demand (Capuzzo 1977) of the filtered seawater to be satisfied and, therefore, the recorded ClO were the concentrations available to the organisms. Microscopic counts were made as often as feasible, with a minimum of two; one at 48 hr and one at 96 hr. Additionally, to test possible recovery capabilities, nutrients (Waine) were added to the Dunaliella cultures at 96 hr and cell concentrations were recorded at 192 hr. Hemocytometers under a compound microscope were used to count the phytoplankters and a binocular dissection scope was used to count relicate 3 mℓ aliquots of the pluteus. Lack of movement in plutei or degeneration in phytoplankters was used as the criterion for death. Percent mortality, or percent decrease in growth in the case of phytoplankters, was calculated for each beaker. These data were summarized graphically by the log-probit method (Litchfield and Wilcoxon 1949) and LC50 values, slope functions, and respective confidence limits were calculated for each organism at each time interval and temperature.

The crustacean bioassay system was similar to that described above except that 2000 mℓ glass beakers, with 12 hermit crabs in each, were substituted for the small beakers. Counts with a hand lens were made at numerous intervals for the first 24 hr after which counts were made every 24 hr. Finally, two species of fish, juveniles of the common mullet Chelon engeli and adults of the cardinalfish Apogon lateralis, were subjected to ClO bioassays at 30.1°C.

The fish bioassays were conducted in aquaria containing 40 ℓ of 1 μ filtered seawater. Four concentrations were used per replicate and four replicates were made for each species. 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 and minimized the time for ammonia excretion. Aeration was suspended for one hour post-NaOCl inoculation to lessen ClO loss. Continuous low-level (8 - 12 microeinsteins$\cdot m^{-2} \cdot s^{-1}$) light from overhead fluorescent panels was maintained throughout the bioassays, because preliminary work indicated that the fish react violently to sudden light-dark changes. Replicate aquaria of exactly the same volume of filtered seawater were set up for each ClO measurement. Fish mortality counts were determined on the same schedule as the hermit crabs, i.e. numerous counts during the first 24 hours. LC50s were calculated by the log-probit method and, for these macroscopic animals, time-percent mortality curves (LT50s) were calculated for nontruncated mortality data for ClO concentrations above the calculated LC50s (Litchfield 1949).

Percent mortality was defined as: $\%\ \text{Mortality} = (1 - \frac{N_e}{N_c}) \times 100$ where N_e is the number of organisms, or concentration of organisms, in the experimental sample and N_c is the number of organisms, or concentration of organisms, in the control samples.

Control mortalities in the fish and hermit crab systems were low (<10%) during the bioassays. Even though starved of nutrients, the phytoplankton control concentrations remained strong and, in some cases, markedly increased. Temperature difference was the most obvious variable when control variations were analysed. Some

ciliate contamination in the pluteus controls were experienced in the latter half of the 96-hr bioassays. Therefore, 48-hr control data were used to calculate the 96-hr percent mortalities. Complete control data for the phytoplankters, urchin larvae, and fish were reported previously (Best et al. 1981).

Salinity was checked with a refractometer and the level of dissolved NH_4^+ was measured with an Orion ammonia probe (95-10) attached to a Beckman expanded scale pH meter. A description of the function of this probe can be found in the appendix of Nelson et al. (1980). Dissolved oxygen and pH were measured with an Orion O_2 probe (97-08) and a triple-purpose Corning probe attached to the Beckman and Corning 135 meters, respectively. Light intensity was measured with a Li-Cor underwater sensor (185-A) and meter.

The physical characteristics for the experimental and control seawater were as follows: salinity ranged from 31.4-34.2°/$_{oo}$; NH_4^+-N was consistently less than 0.2 $\mu g\text{-at}\cdot\ell^{-1}$ for all but the fish assays during which it reached 3.0 $\mu g\text{-at}\cdot\ell^{-1}$; dissolved oxygen ranged from 4.95 to 5.60 ppm; and pH varied from 7.93 to 8.06.

RESULTS

The log-probit dose-effect parameters are summarized in Table I for all test organisms in a matrix of experimental times and temperatures. Generally, the LC50s, or percent decrease in growth for phytoplankters, for the planktonic organisms were inversely related to temperature and duration. Chaetoceros LC50s ranged from 0.16-0.32 ppm ClO (unless specified, ranges do not include confidence limits) compared to the slightly less resistant Dunaliella (0.09-0.19 ppm ClO). The similar slope functions—the fold change in dose required to produce a unit standard deviation change in response along the line—indicate related response patterns (Figure I). The effect of temperature, i.e. faster growth at higher temperature, for Chaetoceros control cultures was very significant (T_s<0.001, N=12), whereas Dunaliella grew more rapidly at 28 than it did at 33°C (table I in Best et al. 1981). This interspecific disparity in phytoplankton control growth accounts for the apparent aberrant response pattern, in Table I, of increased LC50 with increased time for Chaetoceros. Dunaliella test cultures remained depressed after 192 hours following the 96-hr nutrient inoculation.

The LC50s for the Echinometra larvae ranged from 0.20-1.04 ppm ClO. Many abnormal growth forms were observed in the test beakers. Slope-function differences between the urchin bioassays (Table I and Figure I) indicate a more acute narrow-range response for Run 2 (steeper slope) of Echinometra than the other planktonic bioassays.

Table I. Summary of Log-Probit Dose-Effect Parameters for the Test Organisms Exposed to Chlorine-Induced Oxidants.

	26hr 28°C	48hr 28°C	48hr 29°C	48hr 33°C	96hr 28°C	96hr 29°C	96hr 30°C	96hr 33°C	192hr 28°C	192hr 33°C
				Chaetoceros gracilis						
Starting concentration (cells/mℓ)			$10x10^4$	$10x10^4$		$10x10^4$		$10x10^4$		
LC50 (ppm)			0.30	0.25		0.32		0.16		
95% confidence limits			0.20-0.44	0.17-0.37		0.23-0.45		0.11-0.23		
Slope function			2.14	2.27		1.85		1.99		
95% confidence limits			1.61-2.86	1.67-3.09		1.52-2.26		1.45-2.74		
				Dunaliella tertiolecta						
Starting concentration (cells/mℓ)		$7x10^4$		$7x10^4$	$7x10^4$			$7x10^4$	$7x10^4$	$7x10^4$
LC50 (ppm)		0.19		0.17	0.09			0.16	0.15*	0.08*
95% confidence limits		0.14-0.28		0.11-0.25	0.07-0.12			0.10-0.26	0.09-0.25	0.06-0.12
Slope function		1.76		1.74	2.54			2.08	2.43	2.36
95% confidence limits		1.34-2.31		1.30-2.31	1.80-3.59			1.29-3.36	1.45-3.83	1.32-4.47
				Clibanarius humilis						
				$\overline{CL}$ = 5.0 mm						
Number of crabs tested	120	120								
LC50 (ppm)	5.05	3.90**								
95% confidence limits	3.75-6.79	2.87-5.30								
Slope function	1.69	1.72								
95% confidence limits	1.39-2.05	1.27-2.33								

Table 1. Continued

	24hr 28°C	48hr 28°C	48hr 29°C	48hr 33°C	96hr 28°C	96hr 29°C	96hr 30°C	96hr 33°C	192hr 28°C	192hr 33°C
					Echinometra mathaei - Run 1					
					1-day-old-larvae					
Starting concentration (larvae/mℓ)					59			59		
LC50 (ppm)					0.63			0.20		
95% confidence limits					0.50-0.79			0.17-0.24		
Slope function					2.95			2.36		
95% confidence limits					2.50-3.48			2.13-2.62		
					Echinometra mathaei - Run 2					
					1-day-old larvae					
Starting concentration (larvae/mℓ)		25.5		25.5	25.5			25.5		
LC50 (ppm)		1.04		1.00	1.00			0.80		
95% confidence limits		0.97-1.12		0.92-1.09	0.94-1.06			0.66-0.97		
Slope function		1.21		1.25	1.17			2.02		
95% confidence limits		1.15-1.27		1.16-1.35	1.13-1.21			1.60-2.56		
					Apogon lateralis					
					$\overline{SL}$ = 31.5 mm					
Number of fish tested							240			
LC50 (ppm)							0.22			
95% confidence limits							0.18-0.26***			
Slope function							1.23			
95% confidence limits							1.09-1.38***			

Table 1. Continued

	26hr 28°C	48hr 28°C	48hr 29°C	48hr 33°C	96hr 28°C	96hr 29°C	96hr 30°C	96hr 33°C	192hr 28°C	192hr 33°C
					Chelon engeli					
Number of fish tested							240			
LC50 (ppm)							0.31			
95% confidence limits							0.26-0.36***			
Slope function							1.19			
95% confidence limits							1.09-1.29***			

*Nutrients were added to these cultures after 96 hr.

**Time was actually 43 hr for these 48 hr 28°C data.

***Confidence limits corrected for significant heterogeneity (Litchfield and Wilcoxon 1949).

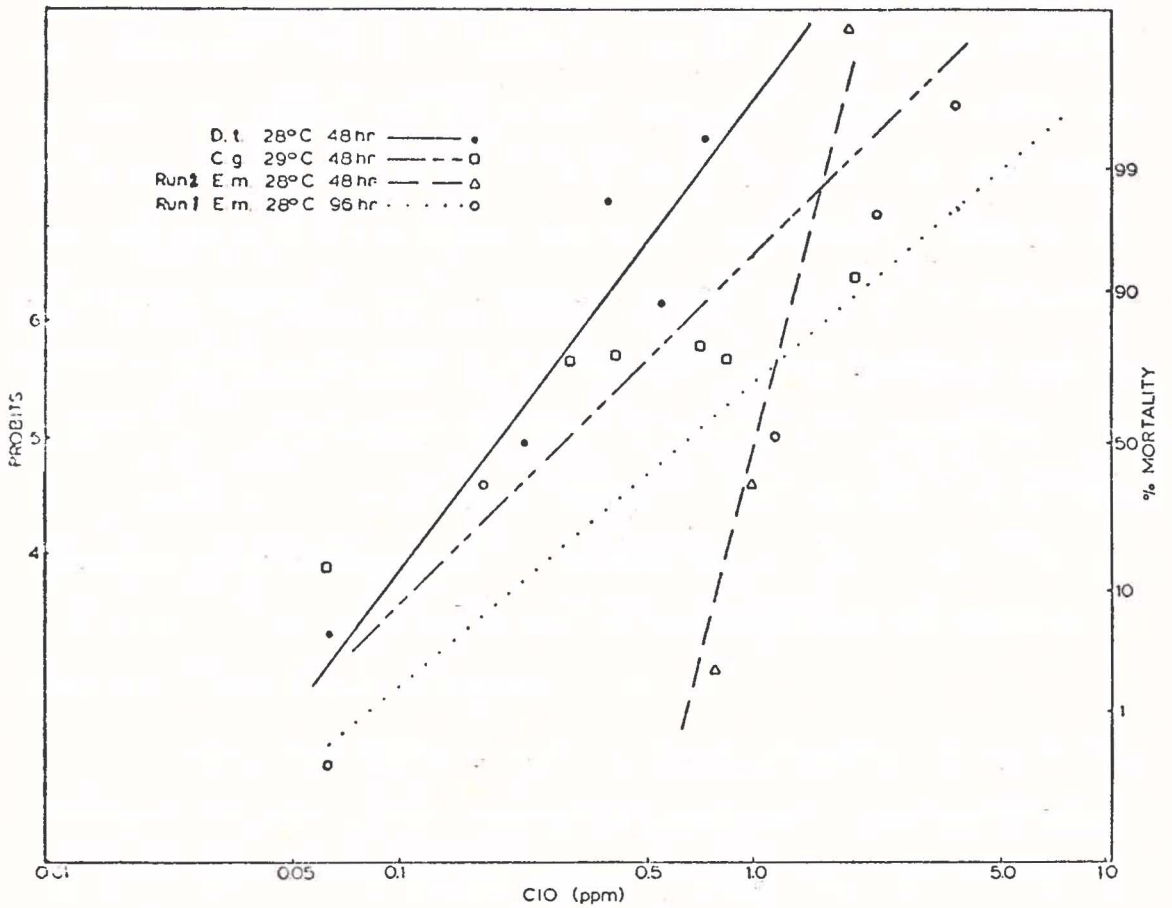

Figure 1. Dose-Motality Curves for Chaetoceros graciiis, Dunaliella tertiolecta, and plutei of Echinometra mathaei.

The resistance of the hermit Clibanarius to ClO varied inversely with temperature. The 28°C LC50s ranged from 5.05 ppm ClO at 26 hr to 3.90 ppm at 43 hr and dropped to approximately 2.4 ppm at 96 hr. Data are listed in Appendix 2. The 28°C Clibanarius response patterns were significantly ($p<0.05$) parallel (Litchfield and Wilcoxon 1949) for the 26- and 43-hr LC50s (Figure 2). Mortality increased markedly at 31.6°C and at the 96-hr 28°C count for the hermit crabs but was so quantal, i.e. all dead or all alive, that statistical calculations were not attempted. Log-probit time-percent mortality data and curves are presented in Table 2 and Figure 3, respectively (Litchfield 1949). The inverse relation of mortality versus time and temperature for Clibanarius clearly emerges. LT50s ranged from 5.5 to 41.6 hr for 27.8 and 31.6°C nontruncated data accumulated from 3.3 and 9.1 ppm ClO concentrations.

As noted from Tables 1 and 2 and the representative curves in Figures 2, 4, and 5, the response of these fish to ClO was acute. The 30°C 96-hr LC50 for the apogonid assays was 0.22 ppm ClO (C.L.; 0.18-0.26 ppm). The mullet were slightly more resistant with a 30°C 96-hr LC50 of 0.31 ppm (C.L.; 0.26-0.36 ppm). The fish LC50s were incipent values (Sprague 1969) because essentially no fish died after 12 hours.

The intraspecific effect of fish length on mortality for Apogon was noted in those aquaria with a midrange mortality response (over 60% of the fish responded in an all-or-none mortality pattern). There was a 5 to 8% size effect: larger fish in a given aquarium were slightly more resistant to ClO.

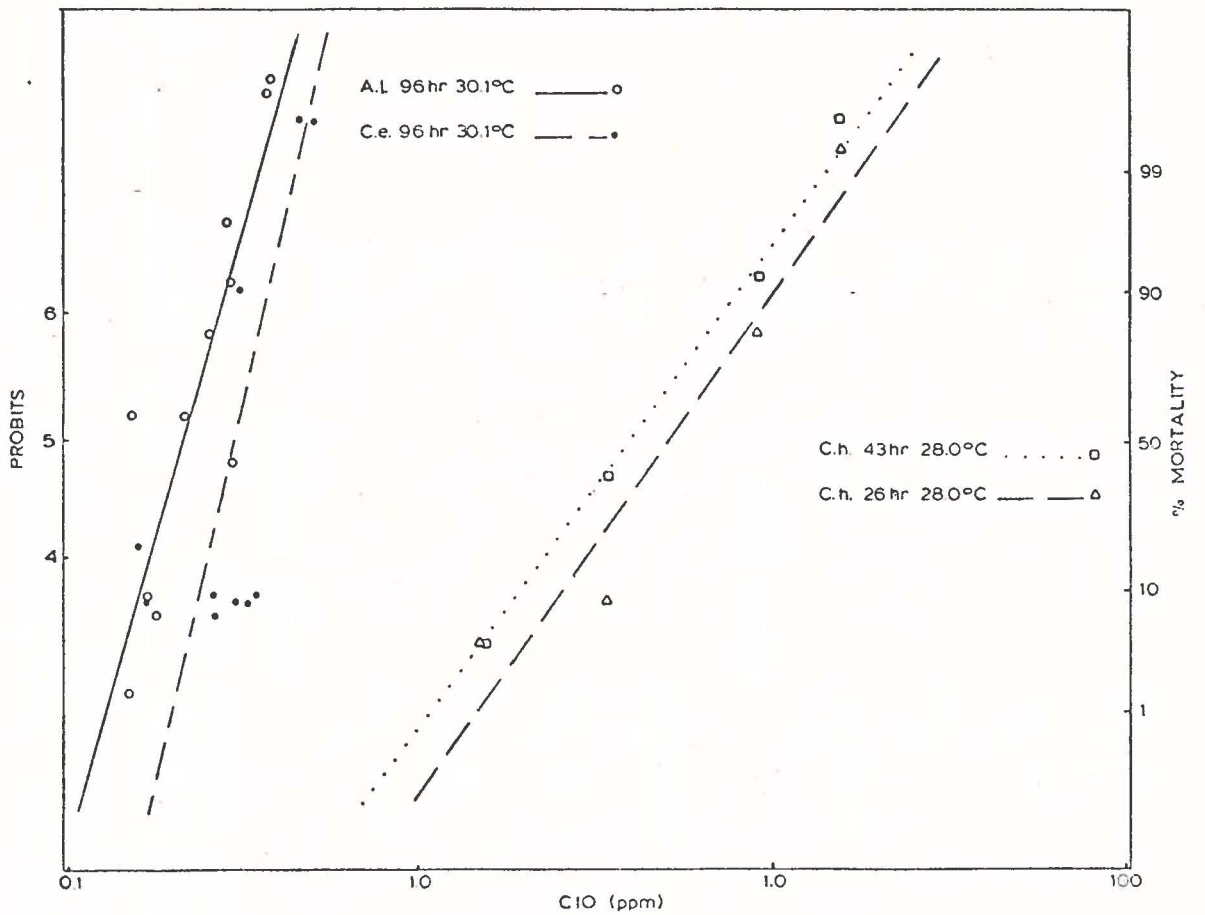

Figure 2. Dose-Mortality Curves for Apogon lateralis, Chelon engeli, and Clibanarius humilis.

Table 2. Summary of Time - Percent Mortality Curve Parameters for Test Organisms Exposed to Chlorine-Induced Oxidants. Selected LT50s were calculated from ClO concentration data that were not truncated. Log-probit method.

LT50 (hr) (95% confidence limits)	Slope function (95% confidence limits)	ppm ClO	Temp. (°C)
	Apogon lateralis		
0.37 (0.30-0.44)	1.40 (1.22-1.60)	0.29	30.1
0.19 (0.17-0.20)	1.17 (1.10-1.25)	0.38	30.1
0.41 (0.33-0.50)	1.46 (1.24-1.67)	0.39	30.1
2.50 (1.91-3.28)	1.61 (1.32-1.96)	0.31	30.1
	Chelon engeli		
0.25 (0.22-0.29)	1.27 (1.15-1.40)	0.47	30.1
0.22 (0.20-0.25)	1.23 (1.14-1.33)	0.51	30.1
0.31 (0.26-0.36)	1.34 (1.19-1.51)	0.52	30.1
0.17 (0.15-0.18)	1.14 (1.08-1.20)	1.08	30.1

Table 2. Continued

LT50 (hr) (95% confidence limits)	Slope function (95% confidence limits)	ppm ClO	Temp. (°C)
	Clibanarius humilis		
41.6 (36.8-47.0)	1.24 (1.14-1.35)	3.35	27.8
26.5 (22.1-31.8)	1.38 (1.21-1.57)	3.35	31.6
17.5 (14.0-21.9)	1.49 (1.29-1.72)	9.10	27.8
5.50 (4.51-6.71)	1.42 (1.24-1.63)	9.10	31.6

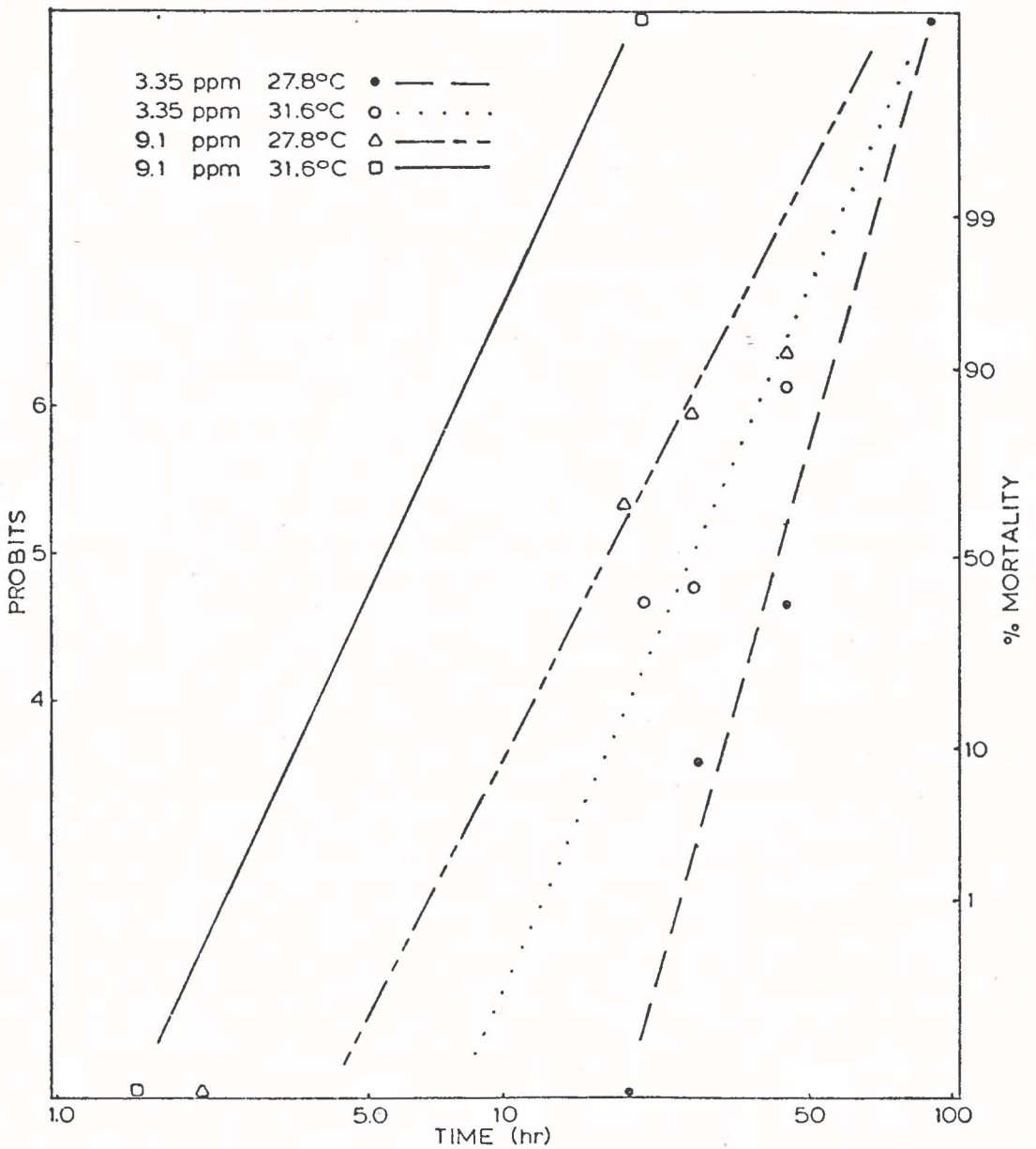

Figure 3. Time-Mortality Curves for Clibanarius humilis.

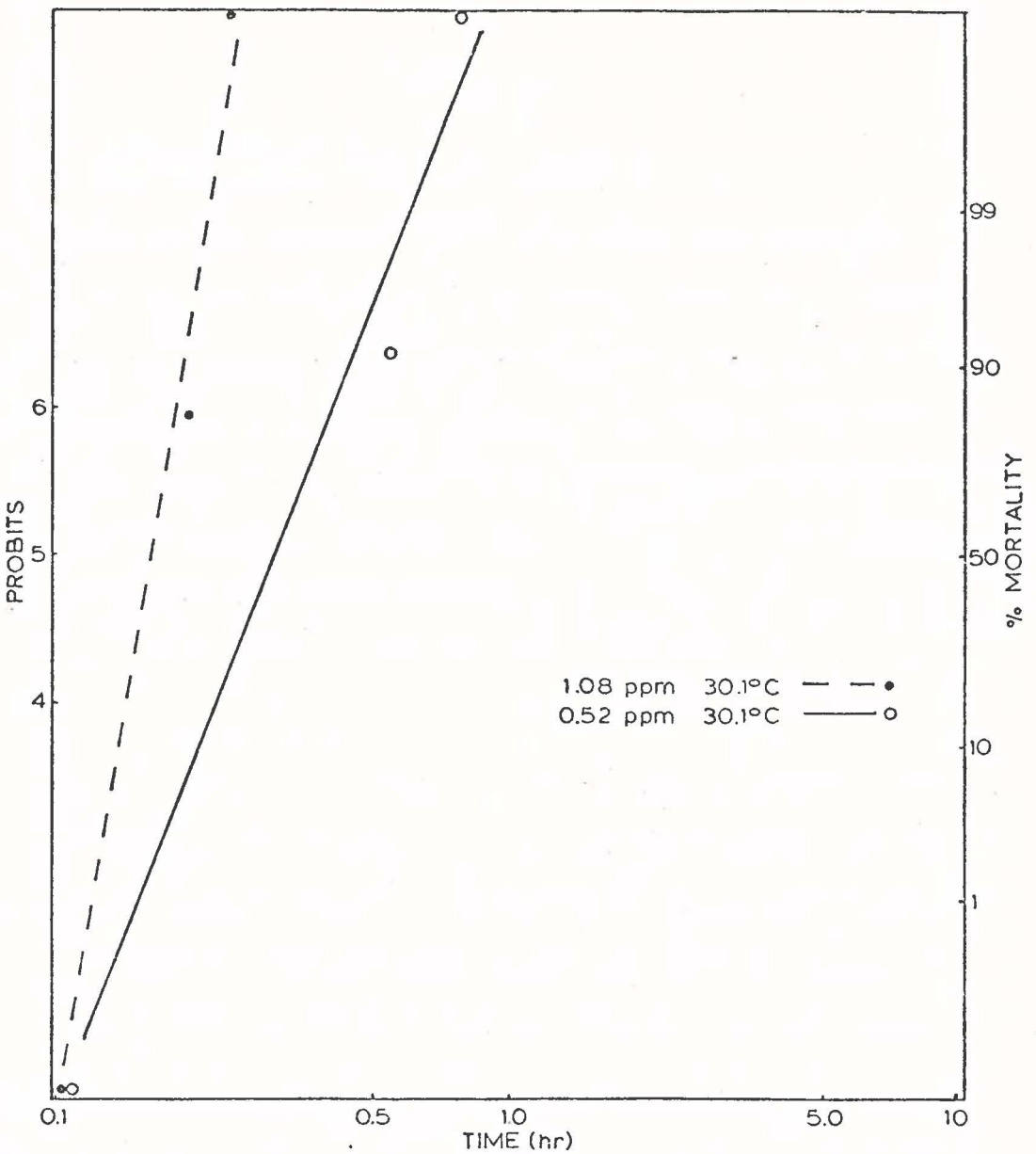

Figure 4. Time-Mortality Curves for Chelon engeli.

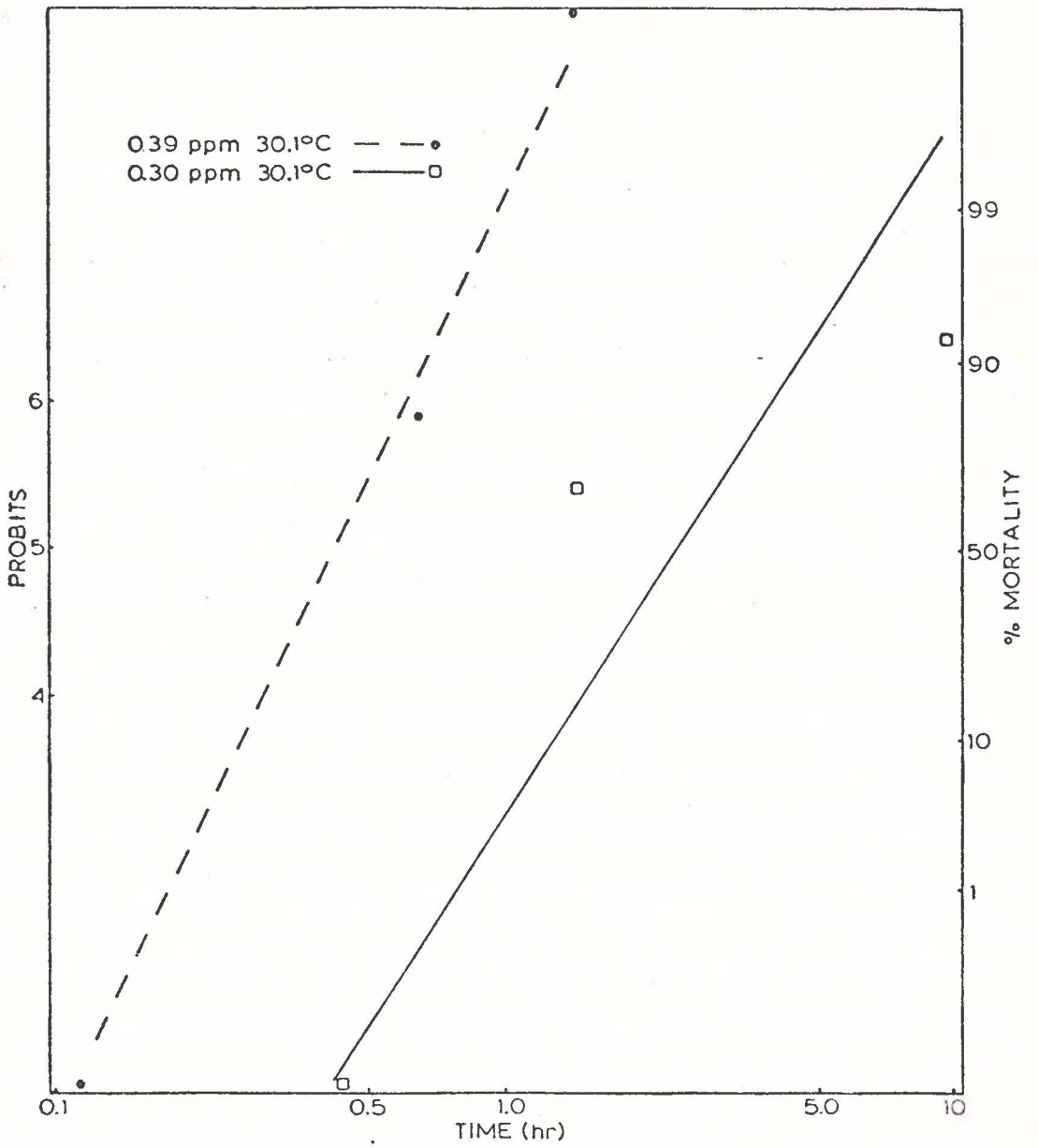

Figure 5. Time-Mortality Curves for Apogon lateralis.

DISCUSSION

Response patterns for the phytoplankton were similar to those reported for other marine studies. Disparities in growth rates for control cultures at different temperatures between species were observed--Chaetoceros grew faster at 33°C while Dunaliella cultures were more productive at 28°C. These control data emphasize the need to incorporate multiple control cultures in the experimental design of a phytoplankton bioassay system to differentiate growth patterns at various temperatures.

The difference in the slopes, and therefore the response, between the Echinometra bioassays could be attributed to different larval densities. The toxic response of Run 2, with less than half the density of plutei, was delayed and acute (Figure 1) compared to the more general response of increased mortality with increased concentration as shown by other planktonic organisms in this study.

The Clibanarius were relatively hardy when compared to other crustacean bioassays. General behavioral observations of this organism could help explain its resistance to ClO. These clumping crustaceans are the predominate macroinvertebrate on many shallow, windward reef flats of Guam where, at low tide, they endure high salinities and temperatures by clinging to supratidal rocks. This aerial adaptive behavior permits Clibanarius to survive, whereas other benthic fauna must move to deeper moat areas. In the hermit crab bioassay system, mortality was delayed for at least one hour,

even at concentrations as high as 40 ppm. The initial resistant interval might allow the hardy crabs to escape acute environmental changes brought on by extreme low tides or by cloroxing their reef-flat habitat.

The results from these bioassays reveal no obvious difference from the inverse relation between temperature and chlorine toxicity shown by temperate organisms. However, it should be noted that maximum bioassay temperatures in temperate studies seldom were above 30°C. This is near ambient in many tropical environments. For instance, the 30°C LC50s for fish from this study are similar to those of temperate studies by Seegert and Brooks (1978) at 30°C. This suggests similar response patterns between the two geographic regions, although comparison of respective ambient temperature responses indicates fish found in the tropics would most likely be more susceptible to chlorine in their natural environment.

The mechanisms of toxic action of ClO to marine organisms are unclear. Many studies (reviewed in Brooks and Seegert 1978) suggest the gills are the effected tissue in fish. The results of the present study support this hypothesis. Fish reacted in a sterotyped pattern, with erratic movements followed by a gasping behavior terminating in death. This reaction was generally completed in 15-45 minutes following NaOCl inoculation. The juvenile topshell, Trochus niloticus, and shelled veligers of Stylocheilus longicauda, immediately closed up, possibly to protect their vital respiratory tissues. When the toxin had decayed they would reemerge (Best et al. 1981). Therefore, the response of invertebrates to ClO is much delayed compared to the fish.

In summary, this investigation, coupled with other known tropical Pacific studies, indicated that phytoplankton would be acutely affected at chlorine concentrations as low as 0.09 ppm, schooling reef-flat fish at approximately 0.2-0.3 ppm chlorine, followed by sea urchin larvae (0.2-1.0 ppm), larval and juvenile mollusks at 1.95 ppm (Best et al. 1981), reef-flat hermit crabs at concentrations above 2.4 ppm chlorine, and finally, super-resistant planulae at chlorine concentrations of 40.0 ppm (Davis 1971).

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APPENDICES

APPENDIX I

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.

APPENDIX 2

Mortality Data for Clibanarius humilis

Mortality (%)	Initial ClO (ppm)
4.15 @ 27.8°C @ 26 hr	1.49
0 @ 31.6°C @ 26 hr	1.49
4.15 @ 27.8°C @ 43 hr	1.49
0 @ 31.6°C @ 43 hr	1.49
4.15 @ 27.8°C @ 91 hr	1.49
0 @ 31.6°C @ 91 hr	1.49
8.35 @ 27.8°C @ 26 hr	3.35
41.7 @ 31.6°C @ 26 hr	3.35
37.5 @ 27.8°C @ 43 hr	3.35
87.5 @ 31.6°C @ 43 hr	3.35
100 @ 27.8°C @ 91 hr	3.35
100 @ 31.6°C @ 91 hr	3.35
83.4 @ 27.8°C @ 26 hr	9.1
100 @ 31.6°C @ 26 hr	9.1
91.65 @ 27.8°C @ 43 hr	9.1
100 @ 31.6°C @ 43 hr	9.1
100 @ 27.8°C @ 91 hr	9.1
100 @ 27.8°C @ 26 hr	15.7
100 @ 31.6°C @ 26 hr	15.7

APPENDIX 3

192 hr data for Dunaliella tertiolecta. These data were obtained after a 96 hr bioassay (Run 2, Dunaliella tertiolecta, table 2, Best et al. 1981) in which nutrients were added to the experimental and control beakers (after 96 hr) to test for recovery.

Controls

23.8×10^4 cells/mℓ (± 0.57, n=6) at 28.0° C at 192 hr.

37.8×10^4 cells/mℓ (± 2.31, n=6) at 33.0° C at 192 hr.

Mortality

(% of Controls)	Initial ClO (ppm)
18.6 @ 28°C @ 192 hr	0.064
49.6 @ 33°C @ 192 hr	0.064
64.5 @ 28°C @ 192 hr	0.225
86.6 @ 33°C @ 192 hr	0.225
92.4 @ 28°C @ 192 hr	0.390
76.3 @ 33°C @ 192 hr	0.390
86.3 @ 28°C @ 192 hr	0.55
100 @ 33°C @ 192 hr	0.55
98.4 @ 28°C @ 192 hr	0.71
99.1 @ 33°C @ 192 hr	0.71
100 @ 28°C @ 192 hr	1.43
99.5 @ 33°C @ 192 hr	1.43