COO-2532-2

COMBINED TOXICITY EFFECTS OF CHLORINE, AMMONIA, AND TEMPERATURE ON MARINE PLANKTON

Progress Report

for Period September 16, 1975-September 30, 1976

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October 1976

Prepared for

The U. S. Energy Research and Development Administration

Under Contract No. E(11-1)-2532

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Abstract

Research on the combined effects of chlorine, ammonia and temperature on marine plankton have been carried out for 20 months. To date continuous-flow bioassays have been conducted on lobster larvae (*Homarus americanus*), oyster larvae (*Crassostrea virginica*), copepods (*Acartia tonsa*), rotifers (*Brachionus plicatilis*), three juvenile and larval fish, killifish (*Fundulus heteroclitus*), scup (*Stenotomus versicolor*), and winter flounder (*Pseudopleuronectes americanus*), and phytoplankton (the diatom *Phaeodactylum tricornutum*). In addition, studies on zooplankton metabolism, filtration rates, and growth were carried out on exposed organisms. In general, the responses of invertebrates were distinctly different than those of fish: increasing mortality with increasing chlorine dose and greater sensitivity to chloramines than free chlorine in the former, and a threshold level of chlorine and greater sensitivity to free chlorine in the latter. Phytoplankton responses indicate that chlorine effects on primary producers are minimal compared to the serious effects on zooplankton, particularly larval forms that spawn intermittently.

The chemistry of chlorine is exceedingly complex. Bromine compounds are formed upon chlorination of seawater and the disappearance of added chlorine is rapid and occurs in two phases: an organic demand and chemical decomposition of the halites.

The overall conclusion of our studies is that chlorine application at power plants must be carried out with extreme caution and that serious consideration should be given to applying dechlorination at all coastal cooling systems.

Introduction

Research has now been in progress for 20 months on Contract No. E (11-1)-2532 to examine the combined toxicity effects of chlorine, ammonia, and temperature on marine plankton (both zooplankton and phytoplankton). During the first year of work, several key research areas, not identified in the original proposal, were included for intensive study during the second year. These included work on the chemistry of chlorine in seawater, long-term responses of zooplankton and phytoplankton to short-term chlorine and/or temperature stress, and field studies at local power plants to gauge the relevancy of the laboratory bioassays. This new work was in addition to the standard bioassay studies on a variety of marine plankton that have been carried out during the entire course of the study.

The results to date on the various topics have led us to the conclusion that chlorine useage at power plants must be carried out with extreme care. Not only is chlorine extremely toxic to many larval species (many of commercial importance), but there still remain several unanswered questions regarding the fate and potential toxicity of chlorinated compounds (both inorganic and organic) formed upon chlorine addition to seawater.

In this report we summarize our findings to date, and include as an appendix copies of our research papers written this past year. In addition, our study plan for the balance of the project (until January 31, 1977) is outlined.

Updated Literature Review

To maintain a proper perspective of our research goals and achievements, it is necessary to keep abreast of the current literature on chlorine toxicity. Reviews were included in the original proposal and in last year's progress report (COO-2532-1). During the past year a number of new papers have come to our attention and are briefly reviewed below.

Brungs (1976) recently reviewed the entire literature on chlorine toxicity since 1973 when his earlier review was published (Brungs, 1973). His overall conclusions are very similar to our own: chlorine toxicity to certain aquatic organisms is severe at residual levels below the limits of analytical detectability, and the complex and, as yet, poorly understood chemistry of chlorine in seawater makes it difficult to assess the full impact of chlorination on marine life.

The proceedings of a conference on the environmental impact of water chlorination, held in Oak Ridge, Tenn. on October 22-24, 1975 was published (Jolley, 1976). The proceedings were divided into four parts: aqueous chemistry of chlorine, biomedical effects of chlorination, environmental transport and effects, and modeling and prediction. Conspicuously absent from the papers were any new data on toxicity effects on marine organisms, although Davis and Middaugh (1976) reviewed previous work in this area. Carpenter and Macalady (1976) did, however, present some preliminary data on chlorine chemistry in seawater, indicating that Br ions react with chlorine to form bromine compounds and that

these compounds are the main oxidants present. Eppley *et al.* (1976), using fluorescein dye, showed that bromine compounds were definitely formed upon chlorination of seawater. Dove (1970), in a comprehensive theoretical and experimental study on chlorine reactions in seawater also showed that bromine compounds were formed in seawater and, that in the presence of nitrogen compounds the dominance of chloramines or bromamines depended on the relative amounts of chlorine, bromide and ammonia. Sugam and Helz (1976) developed equilibrium models of chlorinated seawater and concluded that hypobromite and bromamines are more dominant than their chlorine analogs when the salinity is greater than about $0.3^{o}/oo$. They also made the very important points that traditional chlorination nomenclature such as "free, total or residual chlorine" is misleading in marine studies because the active halogens are mostly bromine derivatives. Johnson (1976) also discussed problems related to the formation of bromine and bromamine compounds in seawater and concluded that the persistence of the haloamines was a kinetic phenomenon: bromamine compounds appear to be highly unstable.

Improved techniques for measuring halogens in seawater were discussed by Manabe (1974), Marinenko (1976), Eppley *et al.* (1976), and Midgley (1976). All are variations of the standard amperometric titration technique, the procedure recommended for its analytical sensitivity in Standard Methods (A.P.H.A., 1974). Although the sensitivity of the technique is good down to <0.01 mg/ℓ chlorine, in seawater significant "noise" occurs, probably due to the presence of

oxidized metal ions (Fe^{+3}, Cu^{+2}) that react with I^-, liberating I_2, thus contributing to the "recovered" oxidants (Eppley *et al.*, 1976). Hence, below about 0.05 mg/ℓ titrated chlorine, results in seawater may be an over-estimation.

Perhaps, the most serious shortcoming in our understanding of chlorine reactions in seawater is the rapid disappearance of titrateable halogens, which cannot all be accounted for by the oxidation of organics present (Eppley *et al.*, 1976). Capuzzo *et al.* (1976), in fact, showed that seawater treated for dissolved and particulate organics removal had a 12-hour chlorine demand (both free and combined) of about 80% of the applied chlorine up to chlorine dosages of 8 mg/ℓ. Eppley *et al.* (1976) found, though, that in short term decay studies (<100 min.) the rate of chloramine decay was considerably less than for free chlorine. This finding is extremely important because, it suggests that in coastal receiving waters (where ammonia levels are usually high) residual halogen compounds will persist longer and thus represent a more serious threat to marine life than in the absence of ammonia.

Studies on phytoplankton response to chlorine in marine waters were reported by Stone *et al.* (1973), Briand (1975), Eppley *et al.* (1976) and Gentile *et al.* (1976). Stone *et al.* (1973) looked at phytoplankton photosynthetic rates of natural populations from central San Francisco Bay to chlorinated, unchlorinated and dechlorinated wastewater and found, as did Krock and Mason (1971) in an earlier and

similar study, that chlorine at residual levels of about 0.06 mg/ℓ significantly inhibited photosynthesis. Briand (1975) looked at species diversity in the intake and discharge of two southern California coastal power plants and claimed that there were measurable changes in species distributions after entrainment. Carpenter *et al.* (1974), in contrast, indicated that unless large numbers of plankton tows were taken in the vicinity of power plant discharges, it was virtually impossible to estimate with statistical confidence that there were changes in both standing crop and diversity.

Eppley found that chloramines were more toxic than free chlorine to phytoplankton photosynthetic rates at identical chlorine dosages. They attributed this to the fact that chloramines are more persistent in seawater. In 24 hour ^{14}C incubation studies they were unable to measure any recovery. Gentile looked at short term exposures (0-1200 seconds) of a diatom *Thalassiosira pseudonana* to varying levels of chlorine dose and found time-dose dependent responses in both photosynthetic rate and post exposure growth rates. They also compared the relative effects of chlorine on 11 species of marine phytoplankton and found over 3-fold variations in response among species.

To date, the major shortcoming of most of the phytoplankton studies is that they have been restricted to short-term responses after chlorine exposure; virtually no consideration has been given to the actual impact on the receiving water where the dynamic factors of water quality and hydrodynamics play crucial roles in determining

the ultimate effect on the marine environment.

Several articles published in the past year have dealt with the effects of chlorine on aquatic animals including freshwater, estuarine, and marine species. Several of these have been review articles of past literature summarizing the basic points to date on chlorine toxicity. Brooks and Seegert (1976) reviewed the influence of a variety of environmental conditions on the toxicity of chlorine to freshwater organisms. The major factors influencing toxicity are temperature, exposure time, and the chemical nature of receiving waters. Davis and Middaugh (1976) summarized previous studies in the marine and estuarine ecosystems and current EPA work on chlorine toxicity. Their conclusions were that much still needs to be known concerning the chemistry and toxicity of chlorine in marine waters. Whitehouse (1975) also reviewed the literature concerning chlorine effects on aquatic organisms. He reiterated the importance of environmental conditions on the toxicity of chlorine but stressed the importance of additional work to fully understand the impact of chlorination practices on aquatic ecosystems.

Several research papers have also been published in the past year, the majority of which have dealt with fish. Tompkins and Tsai (1976) determined the survival time and lethal exposure time for blacknose dace, *Rhinichthys atratulus*, exposed to free chlorine and chloramine. They concluded that chloramine was more toxic (in terms of median survival time or median lethal exposure time) than

free chlorine at high concentrations of total chlorine but less so at lower concentrations. Thatcher *et al.* (1976) investigated the effects of chlorine and temperature on brook trout, *Salvelinus fontinalis*. They found that temperature had a significant synergistic effect on chlorine toxicity only at 20°C, whereas no effect of temperature was apparent at 10° and 15°; they also observed no delayed mortality of brook trout exposed to chlorine as was evident in similar studies with crayfish. Katz (in press) demonstrated a reduction of acute chlorine toxicity to mosquito fish, *Gambusia affinis*, in the presence of various nitrogenous compounds including amino acids, nucleotides, phosphocreatine and urea. Hoss *et al.* (in press) observed a decreased tolerance of young-of-the-year estuarine fish with increases in both temperature and exposure time. The results of these four studies confirm earlier findings that the toxicity of chlorine to fish is affected by temperature, exposure time, and water quality.

The toxic action of chlorine, however, is still a mystery. Bass and Heath (1975) as well as earlier authors (Ellis, 1937; Dandy, 1972; Rosenberger, 1971) have suggested that the primary result of chlorine toxicity in freshwater fish is gill damage, leading to increased mucous production and impairment of respiratory exchange at the gill surface. Fobes (1971), however, found no decrease in oxygen consumption of excised gill tissue from white suckers, *Catastomus commersoni* exposed to a lethal concentration of residual chlorine. Differences in these studies may be due to differences in the actual chlorine toxicants pro-

duced and different modes of action of the toxicants. Grothe and Eaton (1975) suggested that the toxic action of chloramine on fathead minnows, *Pimephales promelas*, was an oxidation of red blood cells, resulting in the conversion of hemoglobin to methemoglobin; as methemoglobin concentrations increased, sufficient O_2 could not be supplied to tissues and death was a result of asphyxiation. Bass and Heath (1975), however, found no change in the methemoglobin levels of fish exposed to free chlorine; their conclusions were that the respiratory epithelium was damaged and again death was a result of asphyxiation. Our own results with juvenile marine fish are indicative of a reduction in oxygen consumption with exposure to either free chlorine or chloramine, suggesting interference with essential metabolic reactions.

Studies dealing with the effects of chlorine to aquatic invertebrates have been restricted to only a few species. Roberts *et al.* (1975) investigated the toxicity of chlorine to a number of estuarine species and concluded that bivalve larvae (*Crassostrea* and *Mercenaria*) and the copepod *Acartia tonsa* were the most sensitive species tested with 48h-LC_{50} values <0.005 mg/ℓ. Latimer *et al.* (1975) in a study on the freshwater copepods *Limnocalanus macrurus* and *Cyclops bicuspidatus thomasi* observed a significant number of sluggish, inactive animals in addition to dead animals following a 30 minute exposure to varying concentrations of free chlorine. Whereas, inactive animals are not included in percent mortality data, they may be regarded as "ecologically dead" because of their reduced chance for survival in natural ecosystems.

LC_{50} values at 20°C for *C. b. thomasi* were 5.76 mg/ℓ, including only dead organisms in this determination, and 3.15 mg/ℓ, including both dead and inactive animals. These data are in agreement with our own findings of reduced metabolic activity of lobster larvae (Capuzzo *et al.*, in press) following exposure to chlorine. Heinle (1976) in a field study of several power plants in Maryland concluded that entrainment of several species of estuarine copepods resulted in high mortality as a result of chlorination and not thermal and mechanical stresses of condenser passage.

Gentile *et al.* (1976) studied the effects of chlorine on several species of estuarine copepods in static laboratory bioassays. *Acartia tonsa* showed an increased sensitivity to chlorine with increases in exposure times. Whereas the sensitivity of this copepod was not as great as that of phytoplankton or larval fish, the authors concluded that chlorine stress to this species may have a significant impact on marine or estuarine ecosystems because of the importance of *Acartia tonsa* as a secondary producer.

A major gap in our understanding of chlorine effects on marine organisms, particularly larval species, is that a limited amount of data on sublethal effects exists. As indicated in our earlier progress report, some marine organisms show severe metabolic inhibition at chlorine residuals below the limits of analytical detectability. Their chances for subsequent survival in the environment after entrainment are severely reduced. In addition, long term effects on growth, reproduction, settling, etc. are virtually unknown.

Research Objectives and Protocols

Our overall research objective, as outlined in the original proposal, was to examine the combined effects of chlorine, ammonia, and temperature on marine plankton in continuous flow bioassays. Originally, these efforts were to be restricted to traditional type bioassays (i.e., LC_{50} values in 48 or 96 hr studies), but as our work progressed during the first year it became apparent that certain modifications and additions to the work plan were in order.

First, it quickly became apparent that the chemistry of chlorine in seawater was far more complex than in freshwater. One major perplexing problem was that there was a very significant chlorine demand in seawater that could not be attributed singularly to the oxidation of organics. Also, based on the work of others (Dove, 1970; Eppley *et al.*, 1976; Carpenter and Macalady, 1976), it was clear that bromine compounds, which were formed in place of chlorine, were the main toxicants.

We were fortunate to have the assistance of Dr. George Wong and Dr. Peter Brewer of the Chemistry Department at Woods Hole Oceanographic Institution in exploring further these difficult chemistry problems. Dr. Wong, although funded by the Chemistry Department, spent six weeks working on these problems, while Dr. Brewer served in an advisory capacity.

A second addition to our program included long term studies on lobster larvae to examine whether sublethal exposure to free chlorine and chloramines had an effect on growth and development. As in all of our studies, attempts were made to simulate as closely as possible con-

ditions that might occur at a typical power plant, i.e., sudden and short-term exposure to chlorine and/or heat increase. The protocols for these assays are outlined in detail in Progress Report COO-2532-1.

To gauge the validity of our laboratory work, field studies were initiated at three local power plants: two fossil fuel plants, Montaup Generating Station at Somerset, Massachusetts and Cape Cod Canal Generating Station at Sandwich, Massachusetts, and the nuclear plant, Pilgrim Nuclear Station of Boston Edison, at Plymouth, Massachusetts. All three plants use chlorine for fouling control. The main objectives in these studies were two fold: 1) to look at both the immediate and longer term effects on zooplankton passing through the plants during periods of chlorination, and 2) to determine if changes were occurring in phytoplankton speciation after chlorination by growing natural samples from the plants in laboratory batch and continuous cultures.

In addition to these new studies, work was continued on examining the responses of representive zooplankton species to chlorine-heat treatments in the assay systems described in the first progress report. During the first year of study work was confined to lobster larvae and three representative larval and juvenile fish (winter flounder, scup, and killifish). The results of these studies have been prepared in three manuscripts which have been accepted for publication (see publication list at end of report and Papers No. 5-7* in Appendix). During the past year further work was conducted on lobster larvae

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*Paper numbers used through rest of text refer to order of papers in Appendix.

and new work initiated on three additional zooplankton organisms: oyster larvae (*Crassostrea virginica*), adult rotifers (*Brachionus plicatilis*), and adult copepods (*Acartia tonsa*).

No additional work through September 30, 1976 was carried out on juvenile fish beyond that completed in the first year because of our desire to concentrate on the zooplankton species while they were available, and because it appeared from our first year's work that zooplankton species were more sensitive to free and combined chlorine than juvenile fish.

Work on phytoplankton was carried out in two phases. The first phase involved completing a kinetic description of the chrysophyte *Monochrysis lutheri* under phosphorus-limiting conditions. This work was initially planned to establish the baseline data for examining the effects of continuous exposure to sublethal levels of chlorine on the kinetic parameters of growth (half saturation coefficient and maximum growth rate).

However, experimental difficulties in maintaining chlorine in solution under growth conditions that required the continuous aeration of the culture for pH control, and the unexpected duration of the baseline studies, led to an abandonment of this approach. Full attention was then directed towards using the continuous culture as a physical model of a power plant, where, after first establishing a steady state level of phytoplankton biomass, the organisms could be exposed to a sudden and short-term exposure of combinations of chlorine and tempera-

ture. The extent to which the steady state was disturbed could then be used as a measure of the impact of the imposed stress.

In addition to this study, experiments were performed on the effects of temperature on phytoplankton species competition in mass cultures. Although these studies were in part supported under another grant (NOAA Sea Grant No. 04-6-158-44016), the results have direct application to an understanding of the impact thermal discharges may have in altering phytoplankton speciation in receiving waters.

Results Through September 30, 1976

1. Chlorine Chemistry

In our first report we showed a chlorine demand in natural seawater from Vineyard Sound, Cape Cod, Massachusetts (that had been extensively treated for the removal of particulate and dissolved organics) of about 80% of the applied chlorine up to chlorine dosages of about 8 mg/ℓ. These experiments, carried out in the larval assay systems where seawater and chlorine (free or combined) were added simultaneously and allowed to equilibrate for 12 hours, were continued with chlorine dosages up to 130 mg/ℓ. As seen in Fig. 1, once again chlorine recovery in seawater was linear with dosage, but substantially less than the amount recovered when distilled water was used in place of seawater. These results were unequivocal proof that the loss could not be attributed to the oxidation of organics; the organic chlorine demand would have been satisfied with, at most, a few mg/ℓ of chlorine

because there was virtually no organics present in the seawater tested.

To expand on these findings, a series of 24 hour static decay studies were performed on Vineyard Sound seawater filtered in various ways (Table 1) and on artificial seawater media (the same used in the phytoplankton studies) constructed in different ways to determine if the loss of chlorine could be attributed to a particular seawater component (Tables 2-5).

With low-level chlorination (1.0 mg/ℓ) of seawater that had been filtered through a 1 μm spun-cotton cartridge filter, the chlorine demand was so rapid that only about one-half the added chlorine could be recovered within the three minutes it took to extract a sample after the initial dosage and perform the amperometric titration. After 24 hours virtually all the chlorine was lost. With more finely filtered samples (Millipore membrane filters) the initial losses were reduced, but surprisingly more chlorine was lost with the 0.22 μm filters, both initially and after 24 hours, then with the 1.2 μm filters (Table I).

The suspicion that dissolved organics were leaching out of the membrane filters during filtering, (apparently more pronounced in the 0.22 μm filters) was confirmed by the results of the distilled water chlorination studies (Table 2). A significant increase in chlorine demand was observed in distilled water samples passed through 0.22 μm filters than in unfiltered samples.

Adding individual components of the artificial seawater media used in the phytoplankton studies (see receipe in Paper no. 4) to distilled, deionized and activated carbon treated water produced no demand

Table 1. 24 Hour Chlorine Demand of Natural Seawater* (Salinity = 30‰).

Sample	Chlorine Concentration, mg/ℓ		
	Initial*	Final	24 Hour Demand
1.2 μm filtered	0.85	0.15	0.85
0.22 μm filtered	0.78	0.04	0.96
1 μm cartridge filtered	0.58	0.05	0.95

*Theoretical conc. = 1.0 mg/ℓ. Initial measurements made ∿ 3 minutes after added.

Table 2. 24 Hour Chlorine Demand of Distilled Water and Individual Constituents of Artificial Seawater Media.

Sample	Chlorine Concentration, mg/ℓ		
	Initial	Final	24 Hour Demand
Distilled Water (D.W.)	0.44	0.36	0.08
	0.42	0.40	0.02
	0.42	0.35	0.07
	0.41	0.41	0.00
	1.04	0.99	0.05
	1.02	0.92	0.10
D.W. (0.22 μm filtered)	1.07	0.89	0.18
	1.06	0.79	0.27
D.W. & Trace	0.77	0.67	0.10
D.W. & $NaHCO_3$	0.71	0.67	0.04
D.W. & $MgSO_4$	0.71	0.68	0.03
D.W. & KBr	0.40	0.35	0.05
D.W. & NaCl	0.78	0.70	0.08

Table 3. 24 Hour Chlorine Demand of NaCl Plus Other Constituents of Artificial Seawater Media.

Sample	Chlorine Concentration, mg/ℓ Initial	Final	24 Hour Demand
Artificial Sea Water	1.04	0.71	0.33
	0.93	0.71	0.22
	0.91	0.72	0.19
NaCl	0.78	0.70	0.08
NaCl & Trace	1.06	0.83	0.23
	1.13	0.88	0.25
	1.02	0.83	0.19
NaCl & Na_2SiO_3	0.99	0.74	0.25
	1.03	0.85	0.18
	1.00	0.88	0.12
	1.00	0.88	0.12
	1.00	0.88	0.12
NaCl & $MgCl_2$	0.98	0.81	0.17
	1.00	0.80	0.20
	1.00	0.81	0.19
	1.00	0.83	0.17
NaCl & Trace & Na_2SiO_3	1.05	0.72	0.33
	1.13	0.76	0.37
	1.13	0.73	0.40
NaCl & Trace & Na_2SiO_3 & $MgCl_2$	0.96	0.73	0.23
	1.18	0.65	0.53
	1.09	0.70	0.39
	0.95	0.78	0.27
	0.93	0.75	0.18
	0.98	0.80	0.18

Table 4. Effect of Elimination of Artificial Seawater Constituents on 24 Hour Chlorine Demand.

No.	Constituent Eliminated	Chlorine Concentration, mg/ℓ		
		Initial	Final	24 Hour Demand
1	KBr	0.39	0.16	0.23
2	1 + Trace	0.93	0.77	0.16
3	2 + Na_2SiO_3	0.91	0.64	0.25
4	3 + H_3BO_4	0.99	0.81	0.18
5	4 + KCl	0.98	0.79	0.19
6	5 + $CaCl_2$	1.02	0.83	0.19
7	6 + $MgCl_2$	0.96	0.86	0.10
8	7 + $MgSO_4$	1.00	0.88	0.12

Table 5. Effect of Varying Dilutions of Artificial Seawater Media With and Without Bromide on Chlorine Demand.

Sample	Percent Artificial Seawater	Percent KBr	Chlorine Concentration, mg/ℓ		
			Initial	Final	24 Hour Demand
Distilled Water	0	0	0.44	0.36	0.08
	0	26.4	0.43	0.39	0.04
	0	52.8	0.45	0.37	0.08
	0	79.2	0.33	0.37	0
	0	100	0.40	0.35	0.05
Artificial Seawater	0	0	0.42	0.35	0.07
	26.4	0	0.34	0.28	0.06
	52.8	0	0.40	0.22	0.18
	79.2	0	0.42	0.16	0.26
	89.9	0	0.40	0.13	0.27
	100	0	0.39	0.16	0.23
Artificial Seawater	0	0	0.41	0.41	0
	26.4	26.4	0.40	0.31	0.09
	52.8	52.8	0.46	0.32	0.14
	79.2	79.2	0.37	0.22	0.15
	100	100	0.41	0.21	0.20

over distilled water alone (Table 2), but full strength media had a demand of about 0.1-0.2 mg/ℓ chlorine over distilled water (Table 3). Adding salts in varying combinations seemed to have the same effect on chlorine demand as with full component seawater (Table 3), and systematically eliminating all salts but NaCl and $NaHCO_3$ did not seem to change the chlorine demand appreciably (Table 4). These results provide little evidence as to what seawater component is serving as a catalyst or is reacting with chlorine to effect the disappearance of titratable halogen.

One interesting result did surface from these studies, however. The chlorine demand appeared to be more pronounced in seawater-distilled water mixtures without bromide added than with it included, suggesting that the bromine compounds formed are more persistent than their chlorine analogs.

At this point in our work the chemistry studies were turned over to Dr. Wong. His efforts resulted in two manuscripts, one on the components of the chlorine demand in seawater and the other on the fate of chlorine in seawater. Both papers are found in the Appendix (Papers No. 10 and 11).

2. Zooplankton Studies

a. Lobster Experiments. The major lobster study was completed last year and the manuscript (Paper No. 5) is now in press. Three supplementary studies were carried out during the Spring, 1976 when

larvae were spawned at the Environmental Systems Laboratory. The results of one of these studies are compiled in Paper No. 9. The other two studies are summarized below.

i. Chloramine studies on larvae - Our usual procedure in testing the effect of chloramine on marine zooplankton is to prepare a chloramine solution in distilled water from equimolar concentrations of NH_4OH and NaOCl and add a known concentration to seawater. In this study we compared the mortality of stage I larvae observed after exposure to chloramine prepared in the usual manner and chloramine formed by adding known concentrations of NaOCl to a known concentration of ammonia previously added to seawater. The results of this study are presented in Table 6. No significant difference in mortality was observed between the two situations. Chloramine was more toxic to lobster larvae than either chlorine or ammonia, results identical to those found in the earlier study (Paper No. 5).

ii. Stage IV larvae - Stage IV lobster larvae were exposed to a wide range of concentrations of free chlorine and chloramine prior to their metamorphosis to post-larval forms. Organisms were exposed for 60 minutes at $25^{o}C$; mortality was determined 48 hours after exposure and compared with control organisms. Respiration rates of test organisms were monitored 48 hours after exposure when test organisms had molted to stage V, the first post-metamorphic form. The results from this study were compared to earlier results with stage I larvae (Paper No. 5).

Table 6. Chloramine Toxicity to Stage I Lobster Larvae

Toxicant	Concentration-mg/ℓ	% Mortality
Control	-	10
Applied Chloramine	0.50	35
	1.00	40
	1.50	45
	2.50	65
	5.00	100
Applied Free Chlorine + NH_3 (5:1 by weight)	1.25	30
	2.00	50
	2.50	60
Applied Free Chlorine	1.00	30
	1.50	33
	2.00	35
	2.50	40
	5.00	45

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	2.00	50
	2.50	60
Applied Free Chlorine	1.00	30
	1.50	33
	2.00	35
	2.50	40
	5.00	45

Stage IV larvae were more sensitive to applied chloramine than applied free chlorine; however, they were less sensitive than stage I lobster larvae (Fig. 2). LC_{50} values, for stage IV larvae, estimated by log-probit analysis, were 22.10 mg/ℓ applied free chlorine and 6.50 mg/ℓ applied chloramine compared to 16.30 mg/ℓ free chlorine and 2.00 mg/ℓ chloramine for stage I larvae. The standard respiration rates of organisms exposed to 1.0 mg/ℓ applied free chlorine and 0.5 mg/ℓ applied chloramine were significantly ($P<0.05$) lower than the respiration rate of control organisms (Fig. 3); there was no significant difference between chlorine and chloramine exposed organisms. However, the percent reduction in respiration rate of exposed organisms was not as great as reductions measured in stage I larvae. Hence, older lobster larvae are more tolerant of chlorine stress than the earlier larval stages, but significant metabolic stress is still apparent after exposure to low chlorine levels.

b. Oyster Experiments. After initial difficulties in spawning oyster larvae and maintaining them in the continuous flow bioassays, as outlined in the earlier progress report, the problems were solved and quantitative experiments subsequently carried out. This work is now completed and the results compiled in Paper No. 8.

c. Rotifer Experiments. The rotifer *Brachionus plicatilis*, originally obtained from the laboratory of Reuben Lasker at the National Marine Fisheries Service in San Diego, California, has been kept in culture for over one year in our laboratory. Because of its ready

availability, experiments have been carried out with this organism intermittently when other, more difficult to obtain, species were unavailable. Thus, the studies are not as yet complete.

To date, a series of standard 48 hour bioassays have been performed comparing the effects of free chlorine with chloramine (30 minute exposure), combined with temperature stresses of $\Delta T = 0^{o}$, 5^{o} and $7.5^{o}C$ from an ambient temperature of $20^{o}C$. Adult organisms (75/assay unit), after being exposed to the 30 minute stress, were maintained for 48 hours in the assay chambers under a low feeding regime (the diatom *Phaeodactylum tricornutum*) so as to prevent hatching of a new generation. Live-dead enumeration was made at the end of the study with the neutral red staining technique.

The results indicate that chloramine was more toxic than free chlorine and that temperature increase had a synergistic effect on the toxicity of both chlorine forms (Fig. 4). Studies are currently underway to determine the effects of sublethal chlorine exposure on subsequent egg production, and should be completed shortly. Preliminary results indicate that the reproductive rates of surviving organisms are not affected.

d. Copepod Experiments. Work with the copepod *Acartia tonsa* has been carried out since last winter. Because the organism has a wide tolerance to temperature and is found in waters like Vineyard Sound from late June through January, major emphasis has been placed on comparing seasonal variations in the organism's response to chlorine-

temperature stress. Copepods were originally collected at 8°C in Vineyard Sound and maintained in the laboratory at 10°C. Several studies were carried out on these organisms.

Standard 48 hour bioassays with chlorine and chloramine exposure for 30 minutes were carried out. Temperature shock ($\Delta T = 0^{\circ}, 5^{\circ}, 10^{\circ}C$) was also included. Live-dead determinations were made after 48 hours by neutral red staining. The results, shown in Figs. 5 and 6, indicate that chloramines (as true for all the other invertebrates tested) was more toxic than free chlorine. Temperature increases up to $\Delta 10^{\circ}C$ from the ambient temperature of 10°C had no effect on the results, suggesting that temperature effects only become important when the organism's thermal tolerance limit is approached. Identical experiments will be carried out with organisms acclimated at 20°C to determine if temperature begins to act synergistically with chlorine when the thermal tolerance limit is reached at 25°-30°C.

Also shown in Figs. 5 and 6 is the response of the copepod to chlorinated seawater that was dechlorinated just prior to addition of the organisms. Clearly, a 11toxicity was removed upon dechlorination.

In another experiment filtration rates were determined at 10°C 48 hours after exposure to combinations of temperature and free chlorine or chloramine stress. The results, shown in Fig. 7, indicate that filtration rates increased significantly after exposure to either toxicant. A trend of increasing filtration rate with increased temperature

shock was evident for chloramines and definitely obvious at 20°C for free chlorine.

To determine if copepod biomass was a factor influencing the chlorine dose-response relationships found in Figs. 5 and 6, assay units were stocked with 10, 20, 30, 40, 50, 60 and 75 animals and exposed to a chlorine dosage of 5 mg/ℓ ($\sim LC_{50}$ for free chlorine from Fig. 5). There were no significant differences in mortality, indicating that total biomass was not a factor influencing toxicant response.

3. Phytoplankton Studies

Phytoplankton studies were carried out in three phases. The first phase involved completing the kinetic study on *Monochrysis lutheri* in a phosphate-limiting continuous culture. The experiments took an exceedingly long time ($\sim$ 6 months) and it became apparent that this approach for studying the effects of chlorine exposure on phytoplankton would not be particularly fruitful. This was especially true because the steady state approach with continuous dosage of a sublethal level of chlorine was far removed from the realities of power plant chlorination practice. Hence, a new approach was taken, that is, using the continuous culture as a physical model of a power plant where the cycles of initial entrainment, short-term exposure to chlorine-heat combinations, and return to the receiving water could be simulated. This work is completed and the results presented in Paper No. 4. The results of the kinetic study, although not directly applicable to the chlorine studies, are presented in Paper No. 3.

The third phase involved studies on the effect of temperature on species competition in mass cultures of algae. The results, reported in Papers No. 1 and 2, have direct bearing on the types of responses (in terms of species changes) that might occur in heated receiving waters.

4. Field Studies

To compare the results of our laboratory studies with actual field stituations, we began a sampling program in May 1976 at two coastal power stations in Massachusetts: the Montaup Generating Station, Somerset, Massachusetts, and the Cape Cod Canal Generating Station, Sandwich, Massachusetts. Our studies were concentrated on the marine copepod *Acartia tonsa*, the dominant zooplankter in this area during the summer months. Samples of zooplankton were collected using a 316 μm plankton net; two minute tows were taken at the intake channel and at the discharge before and during chlorination; the temperature of each sample was recorded and the residual chlorine level of the chlorinated discharge was determined by amperometric titration. Within one hour after collection, two replicates of twenty specimens of *A. tonsa* were isolated from each sample and placed in 5 ml microrespirometer flasks with 4 ml filtered seawater. Respiration rates

of copepods were measured at 22°C at 15 minute intervals for 90 minutes. The results of these studies are presented in Table 7 and Figs. 8 and 9. Copepods collected in the discharge at the Montaup Plant during May, June, and July survived plant passage, but showed a significant ($P<0.05$) reduction in respiration rate compared to organisms collected at the intake; no significant difference, however, was observed between organisms collected before and during chlorination. The chlorine level never exceeded 0.1 mg/ℓ total residual chlorine and plant passage was ∿10 minutes. No live copepods were collected from the discharge at the Cape Cod Canal plant during July probably because of the high ΔT of discharge waters.

At later sampling dates, samples collected at both power plants were brought back to the laboratory to determine what effect chlorination and plant passage had on the reproductive potential of *A. tonsa*. Five pairs of copepods were isolated from each and placed in 1 liter containers filled with filtered seawater. Copepods were fed daily rations (2 ml - 1×10^6 cells/ml) of *Phaeodactylum tricornutum* for a three week period after which time the samples were preserved and the number of adults and juveniles (nauplii and copepodites) counted. The results are presented in Fig. 10. A significant decrease ($P < 0.05$) in the number of individuals/sample was determined in chlorinated discharge samples from both power plants. No significant decrease was observed between intake samples and non-chlorinated discharge samples.

Table 7. Summary of Field Data at Coastal Power Stations

Power Plant	Date	Location	T°C	Cl_2 mg/ℓ	O_2 Consumption *Acartia tonsa* µℓ O_2/h/animal
Montaup	26/V/76	Intake	20	-	0.09
		Discharge	21	-	0.04
		Discharge	21	0.08	0.04
	9/VI/76	Intake	21	-	0.07
		Discharge	22	-	0.03
		Discharge	22	0.05	0.03
	7/VII/76	Intake	25	-	0.07
		Discharge	25	-	0.03
		Discharge	25	0.07	0.02
	*30/VII/76	Intake	22.5	-	-
		Discharge	32.5	-	-
		Discharge	32.5	0.10	-
	*18/VIII/76	Intake	23.5	-	-
		Discharge	30.5	-	-
		Discharge	30.5	0.06	-
Cape Cod Canal	9/VII/76	Intake	15	-	0.08
		Discharge	31	-	(no live *Acartia*)
		Discharge	31	0.05	"
	28/VII/76	Intake	17.5	-	0.07
		Discharge	33	-	(no live *Acartia*)
		Discharge	33	0.05	"
	*19/VIII/76	Intake	17	-	-
		Discharge	30	-	-
		Discharge	30	0.05	-
	*24/IX/76	Intake	17	-	-
		Discharge	34	-	-
		Discharge	34	0.10	-

*Samples returned to laboratory for determinations of reproduction potential.

From these results, there is an indication that, whereas low level chlorination has no acute effect on the metabolic activity of *A. tonsa*, there may be long-term effects on generation time and reproductive potential. Further investigations in the laboratory and in the field are needed to substantiate these findings.

Several trips were made to the Plymouth Plant, but on each occasion all zooplankton leaving the entrainment were nonviable, thus making it impossible to carry out experiments similar to those performed at the other two plants.

The effect of chlorination on phytoplankton speciation is currently under full investigation. During the past two months samples have been taken before and after chlorination at both the Montaup and Cape Cod Canal plants, brought back to the laboratory, and maintained in continuous cultures for extended periods of time. Recovery of phytoplankton growth and changes in speciation have been measured. Although the results have not as yet been analyzed, it appears that temperature shock by itself has had little effect on productivity. At both plants ambient water temperatures during September-October, 1976 have been $\sim 16^{\circ}$-18°C and $\Delta T \stackrel{\sim}{=} 15^{\circ}$-$17^{\circ}$C. These results are strikingly similar to those from the laboratory study reported in Paper No. 4. Recovery from chlorine exposure is slow but does occur, indicating severe but not total destruction of entrained cells. There does not appear to be a clear pattern of shifts in dominant species after chlorination, but repeated sampling and growth in the laboratory cultures will be required to substantiate that preliminary observation.

Basic Findings to Date

The results of the zooplankton bioassays, are summarized in Table 8 and in Fig. 11, and for comparative purposes, a summary of the assay work on juvenile fish, taken from last years report, is presented in Table 9 and Fig. 11.

The overall conclusions from our work to date are:

1. The chemistry of chlorine in seawater is perhaps the most important unanswered question remaining regarding the impact of this toxicant on marine biota. As shown by Wong and Davidson in their two enclosed papers, the dissipation of titratable chlorine in seawater is very rapid and occurs in two distinct phases. The first phase is directly attributable to a true organic chlorine demand and the second probably due to the rapid formation of hypobromite followed by its subsequent inorganic decomposition to, as yet, unknown products. The consumptive capacity of seawater for chlorine and the quickly formed hypobromite appears to be limitless, as evidenced by the results shown in Fig. 1.

In trying to assess the true impact of chlorine on marine organisms it is vital that we be able to identify the reaction products of chlorine and seawater, as the potential toxicity of these compounds is unknown. Clearly though, the use of chlorine residuals alone as a measure of toxicity (as is common practice in the environmental field) is, by itself, a useless parameter with out some knowledge of the mode of chlorine dissipation. The kinetics of chlorine dissipation are the most important factor influencing the true impact. The presence of

Table 8. Summary of Chlorine - Chloramine Toxicity to Marine Invertebrates.

Species	Tx °C	Exposure min.	Chlorine Form	LC_{50} - Total Cl_2 mg/ℓ Applied	Residual
Acartia tonsa	10	30	Free chlorine	4.80	0.82
adults	15			4.80	0.82
acclimated at 10°C	20			4.80	0.82
	10	30	Chloramine	2.10	0.34
	15			1.50	0.23
	20			1.50	0.23
Brachionus plicatilis	20	30	Free chlorine	1.20	0.18
adults	25			0.70	0.09
acclimated at 20°C	27.5			0.20	0.01
	20	30	Chloramine	0.35	0.02
	25			-	-
	27.5			-	-
Crassostrea virginica	20	30	Free Chlorine	0.86	0.12
7 day larvae	25			0.63	0.08
acclimated at 20°C	30			-	-
	20	30	Chloramine	0.15	0.01
	25			0.10	<0.01
	30			-	-
Homarus americanus	20	60	Free chlorine	-	-
stage I larvae	25			16.30	2.90
acclimated at 20°C	30			2.50	0.40
	20	60	Chloramine	4.10	0.70
	25			2.00	0.30
	30			0.55	0.05
Stage IV larvae	25	60	Free chlorine	22.10	3.95
acclimated at 20°C	25	60	Chloramine	6.50	1.13

Table 9. Summary of Chlorine - Chloramine Toxicity to Juvenile Marine Fish[a]

Species	T°C	Chlorine Form	100% Mortality		Stress Observed	
			Applied[b]	Residual[b]	Applied[b]	Residual[b]
Pseudopleuronectes americanus	25	free	1.35	0.55	0.70[c]	0.20
		chloramine	2.95	2.55	1.70[c]	1.50
Stenotomus versicolor	25	free	1.50	0.65	1.20[c]	0.50
		chloramine	3.60	3.10	2.50[c]	2.20
Fundulus heteroclitus	25	free	4.00	0.65	2.00[d]	0.32
		chloramine	7.00	1.20	4.00[d]	0.65
	30	free	1.50	0.25	-	-
		chloramine	5.00	0.85	-	-

[a]From Capuzzo *et al.* (In press) *Est. Coast. Mar. Sci.*

[b]Units of mg/ℓ Total Chlorine.

[c]Stress determined as behavioral aberrations during exposure to the toxicants.

[d]Stress measured as significant changes ($P < 0.05$) in standard respiration rates during exposure and 48 hours after exposure to the toxicants.

ammonia and organic compounds magnifies the problem tremendously. The chloramines, bromamines, and trace organochlorine (and possibly organobromine) compounds that are formed are all more persistent than free chlorine, thus increasing their toxicity potential. Organochlorine compounds in particular are a highly serious threat, as so little is known about their fate, transport through the food chain, and degree of toxicity.

2. There appears to be a general pattern of differences in the modes of chlorine toxicity to marine invertebrates and vertebrates, as clearly seen in Fig. 11. Juvenile fish are more susceptible to free chlorine than chloramine and respond in a step or threshold fashion-that is, no death up to a certain toxicant level and complete death beyond that applied level. On the other hand, the various invertebrate forms tested so far are, as a group, more sensitive to chloramines and the response to both halogens has been more classical: a more gradual increase in mortality with increases in applied halogen levels. The mode of action of the two chlorine forms on the test organisms appears to be some form of metabolic inhibition, but the actual mechanisms are still unknown.

The complex fate of chlorine and bromine (and the ammoniated derivatives) combined with the differential effects of these compounds on invertebrates and fish adds strong support to our need to integrate and expand our understanding of the chemical and biological effects of chlorination.

3. The synergistic role of temperature increase on chlorine toxicity is complex. It appears that as long as the organism is not subjected to a temperature shock great enough so that the thermal tolerance limit is reached, the effect is hardly measurable. When the thermal limit is approached then the synergism becomes readily apparent. This effect seems to be true with the zooplankton as well as the phytoplankton.

4. Biomass plays a crucial role in determining the chlorine dose-response of phytoplankton, but has virtually no effect on zooplankton responses. Differences in the mode of toxic action may be the key factor. Resistance to chlorine toxicity does, however, appear to be related to organism maturity. For example Stage IV lobster larvae are more resistant to comparable chlorine doses than are stage I larvae.

5. Chlorination effects on phytoplankton at power plants appear to be a minimal problem compared to effects on larval zooplankton: entrained phytoplankton exposed to chlorine represent only a small fraction of the standing crop, phytoplankton recovery can be fairly rapid and chlorine dissipation in the receiving water is relatively fast. In contrast, populations of larval species that spawn intermittently could be seriously threatened by chlorination. This is particularly true when considering the fact that metabolic activity is seriously affected at chlorine residual levels below the level of detectability (<0.01 mg/ℓ). In the presence of more persistent and more toxic chloramine compounds the effects of power plant chlorination could be widely felt in the receiving as well as entrained water.

6. Reproductive rates of zooplankton and growth rates of larvae appear to be seriously retarded after exposure to sublethal chlorine concentrations. Phytoplankton growth rates of recovered cells, on the other hand, do not appear to be permanently affected. The implications of these findings are self-evident and represent perhaps the strongest condemnation of excessive chlorination use at power plants. The practice of dechlorination immediately after entrainment should be given serious consideration for all coastal cooling systems.

Research Plan for Balance of the Project Year

1. Larvae, juvenile fish, and zooplankton studies. For the balance of this project year, we plan to continue our research on the differential effects of free and combined chlorine to marine larval and juvenile forms. Specific experiments currently in progress or planned for the near future are as follows:

a. Long-term effects of acute exposures to chlorine or chloramine on juvenile scup, *Stenotomus versicolor*, are currently under investigation. Parameters being monitored are growth rates, breathing rates, and osmoregulation.

b. *Acartia tonsa*, a dominant copepod in Cape Cod waters from June to December, has a wide thermal tolerance ranging from $5^{o}C$ to $30^{o}C$. In experiments with copepods acclimated to $10^{o}C$ (this report), there was no significant synergistic effect of temperature on the toxicity of free chlorine and only a slight effect on the toxicity of chloramine. It is our hypothesis that temperature exhibits a synergistic

effect on chlorine toxicity to an organism when that organism is at the upper limits of its thermal tolerance. To test this hypothesis, we will repeat our chlorine-chloramine experiments with copepods acclimated to 20°C with $\Delta 0°$, $\Delta 5°$, and $\Delta 10°$C increases in temperature. Standard respiration rates, filtration rates, and reproduction rates will also be monitored.

c. Our field work at Montaup Generating Station and Cape Cod Canal Generating Station will continue until the end of November, when these power plants cease their chlorination practices until April.

d. The sublethal effects of free chlorine and chloramine toxicity to the rotifer *Brachionus plicatilis* will be investigated. Parameters to be monitored will be reproduction rates and respiration rates.

e. Chlorine-chloramine toxicity to larval winter flounder, *Pseudopleuronectes americanus*, will be investigated. In addition to assessing toxicity and the effect of temperature, we will investigate the comparative responses of different aged larvae and sublethal effects on metabolic activity.

2. Phytoplankton Studies

a. Sampling at the Montaup and Cape Cod Canal Power plants will continue until chlorination practice ends. Intake and discharge phytoplankton species will be identified and enumerated. Samples will be used as inocula for laboratory continuous cultures and long term

growth will be observed. The objective is to determine the effects of heat and heat plus chlorine dose on the speciation of developing phytoplankton populations.

b. During the winter months the continuous culture units will be used to repeat the *Phaeodactylum* experiments (Paper No. 4) with other species (*Skeletonema costatum, Chaetoceros* sp., *Dunaliella teriolecta, Thalassiosira pseudonana*) in both mono and mixed cultures to determine the relative responses of different organisms and to see if chlorine can alter the outcome of species competition.

Project Personnel

The principal investigators, Joel C. Goldman and John H. Ryther have devoted approximately 100% and 25% respectively of their time to the project and this time allotment will continue until the project ends on January 31, 1977.

Dr. Judith Capuzzo, after completing her Postdoctoral Fellowship in June 1976, joined the project as a Postdoctoral Investigator.

The permanent project staff includes John Davidson, Research Assistant II and Helen Quinby, Research Assistant III, who replaced Sarah Lawrence when she returned to graduate studies in September, 1976.

Travel to Date

1. Joel Goldman and Judith Capuzzo (on Fellowship support) attended the "Chlorine Workshop" at the University of Maryland, Solomons, Maryland, March 15-18, 1976.

2. Joel Goldman, Judith Capuzzo (on Fellowship support) and John Davidson attended the Annual conference of the American Society of Limnology and Oceanography in Savannah, Georgia, June 20-24, 1976. Dr. Capuzzo presented a paper at the meeting.

3. Joel Goldman and Judith Capuzzo (on Fellowship support) attended the Water Pollution Control Federation Annual Meeting in Minneapolis, Minnesota, October 3-7, 1976.

Publication Record

1. Manuscripts in press:

Capuzzo, J. M., S. A. Lawrence, J. A. Davidson. Combined toxicity of free chlorine, chloramine and temperature to stage I larvae of the American lobster *Homarus americanus*. Water Research.

Capuzzo, J. M., J. A. Davidson, S. A. Lawrence, and M. Libni. The differential effects of free and combined chlorine on juvenile marine fish. Estuarine and Coastal Marine Science.

Capuzzo, J. M., J. C. Goldman, J. A. Davidson and S. A. Lawrence. Chlorinated cooling waters in the marine environment: Development of effluent guidelines. Marine Pollution Bulletin.

2. Manuscripts submitted for publication:

Goldman, J. C. Biomass production in mass cultures of marine phytoplankton at varying temperatures. Marine Biology.

Goldman, J. C. Temperature effects on phytoplankton growth in continuous culture. Limnology and Oceanography.

Goldman, J. C. Steady state growth of phytoplankton in continuous culture: comparison of internal and external nutrient models. Journal of Phycology.

Goldman, J. C. and J. A. Davidson. A physical model of marine phytoplankton chlorination at coastal power plants. Environmental Science and Technology.

Capuzzo, J. M. and S. A. Lawrence. The toxicity of chlorine, chloramine and temperature to larvae of the American oyster *Crassostrea virginica*. Marine Biology.

Capuzzo, J. M. The effects of chlorine and chloramine on growth rates and respiration rates of larval lobsters (*Homarus americanus*). Water Research.

Wong, G. T. F. and J. A. Davidson. The "chlorine demand" of seawater. Environmental Science and Technology.

Wong, G. T. F. and J. A. Davidson. The fate of chlorine in seawater. Water Research.

3. Manuscripts in preparation:

Goldman, J. C., J. M. Capuzzo, and G. T. F. Wong. Biological and Chemical Effects of Coastal Power Plant Chlorination - A Review.

Capuzzo, J. M., S. A. Lawrence, and J. A. Davidson. The toxicity of free chlorine and chloramine to the rotifer *Brachionus plicatilis* and the sublethal effects on metabolic activity and fecundity.

Capuzzo, J. M., S. A. Lawrence, and J. A. Davidson. The effect of temperature on the toxicity of free chlorine and chloramine to the copepod *Acartia tonsa* and the sublethal effects on metabolic activity, filtration rate, and generation time.

Capuzzo, J. M. Comparative physiological responses of marine organisms to chlorine.

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Capuzzo, J. M., S. A. Lawrence, and J. A. Davidson (in press) Combined toxicity of free chlorine, chloramine and temperature to stage I larvae of the American lobster *Homarus americanus*. Water Research.

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Figure Legends

Fig. 1. Relationship of total applied chlorine to total residual chlorine in distilled water and sea water; circles - free chlorine in seawater, triangles - free chlorine in distilled water; ----- theoretical 1:1 line.

Fig. 2. Percent mortality of larval lobsters 48 hours after 60 minute exposure at 25°C to applied free chlorine or chloramine; circles - stage I larvae; triangles stage IV larvae; open symbols - control mortality.

Fig. 3. Respiration rates of larval lobsters 48 hours after 60 minute exposure at 25°C to applied free chlorine or chloramine; A - control - stage I and stage V (post-larval); B - 1.0 mg/ℓ applied free chlorine; C - 0.5 mg/ℓ applied chloramine.

Fig. 4. Percent mortality of *Brachionus plicatilis* 48 hours after 30 minute exposure to applied free chlorine or chloramine. Each point represents the mean value of three replicate samples; circles - 20°C; triangles - 25°C; squares - 27.5°C; open symbols - control mortality.

Fig. 5. Percent mortality of *Acartia tonsa* 48 hours after 30 minute exposure to applied free chlorine; open circles - control mortality; open half-circles - dechlorinated before addition of test organisms.

Fig. 6. Percent mortality of *Acartia tonsa* 48 hours after 30 minute exposure to applied chloramine; open circles - control mortality; open half-circles - dechlorinated before addition of test organisms.

Fig. 7. Filtration rates of *Acartia tonsa* at 10°C 48 hours after 30 minute exposure to applied free chlorine or chloramine (1) control, exposed to 10°, 15°, and 20°C for 30 minutes, maintained at 10° for 48 hours; (2) 1.0 mg/ℓ free chlorine exposed at 10°; (3) 1.0 mg/ℓ free chlorine exposed at 15°; (4) 1.0 mg/ℓ free chlorine exposed at 20°; (5) 0.5 mg/ℓ chloramine exposed at 10°; (6) 0.5 mg/ℓ chloramine exposed at 15°; (7) 0.5 mg/ℓ chloramine exposed at 20°. Ranges are ± 1 standard error.

Fig. 8. Temperature of intake (circles) and discharge (triangles) water at Cape Cod Canal Generating Station (A) and Montaup Generating Station (B).

Fig. 9. Respiration rates of *Acartia tonsa* at 22°C collected from the intake and discharge of Montaup Generating Station; A - Intake; B - Discharge; C - Discharge during chlorination.

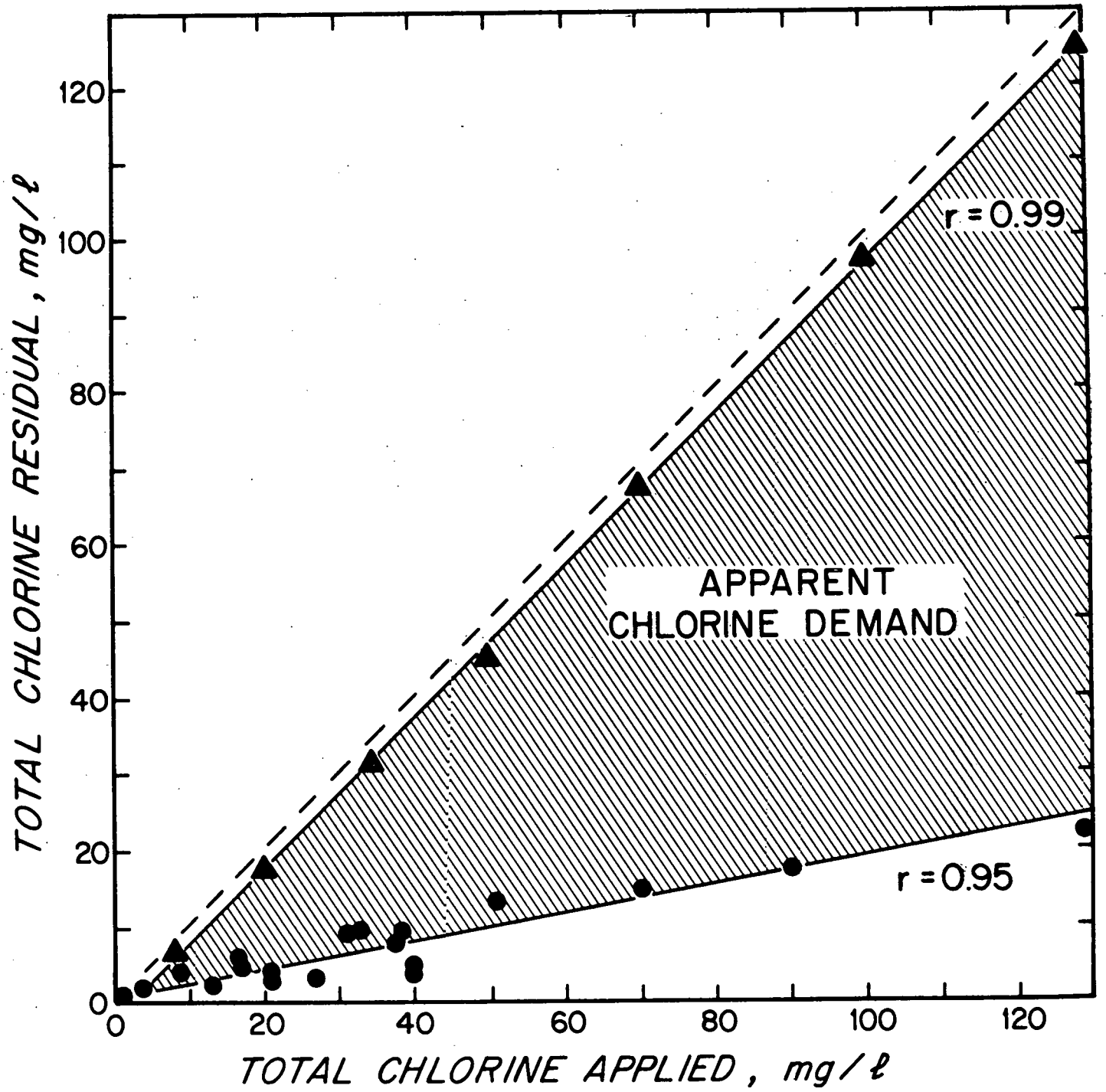
Fig. 10. Number of individuals produced from five pairs of *Acartia tonsa* collected at the intake and discharge of Cape Cod Canal Generating Station (A) and Montaup Generating Station (B);

Fig. 10 (continued):

A - Intake; B - Discharge; C - Discharge during chlorination; white - number of adults; black - number of juveniles (nauplii and copepodite stages).

Fig. 11. Summary of chlorine - chloramine toxicity. A, A^1 - *Crassostrea virginica*, 7 day larvae, 30 minute exposure at 20°C; B, B^1 - *Brachionus plicatilis*, adults, 30 minute exposure at 20°C; C, C^1 - *Acartia tonsa*, adults, 30 minute exposure at 10°C; D, D^1 - *Homarus americanus*, stage I larvae, 60 minute exposure at 25°C; E, E^1 - *Pseudopleuronectes americanus*, juveniles, 30 minute exposure at 25°C; F, F^1 - *Stenotomus versicolor*, juveniles, 30 minute exposure at 25°C; G, G^1 - *Fundulus heteroclitus*, juveniles, 30 minute exposure at 25°C.

Fig. 1


TOTAL CHLORINE RESIDUAL, mg/ℓ
TOTAL CHLORINE APPLIED, mg/ℓ
0
20
40
60
80
100
120
r = 0.99
r = 0.95
APPARENT
CHLORINE DEMAND

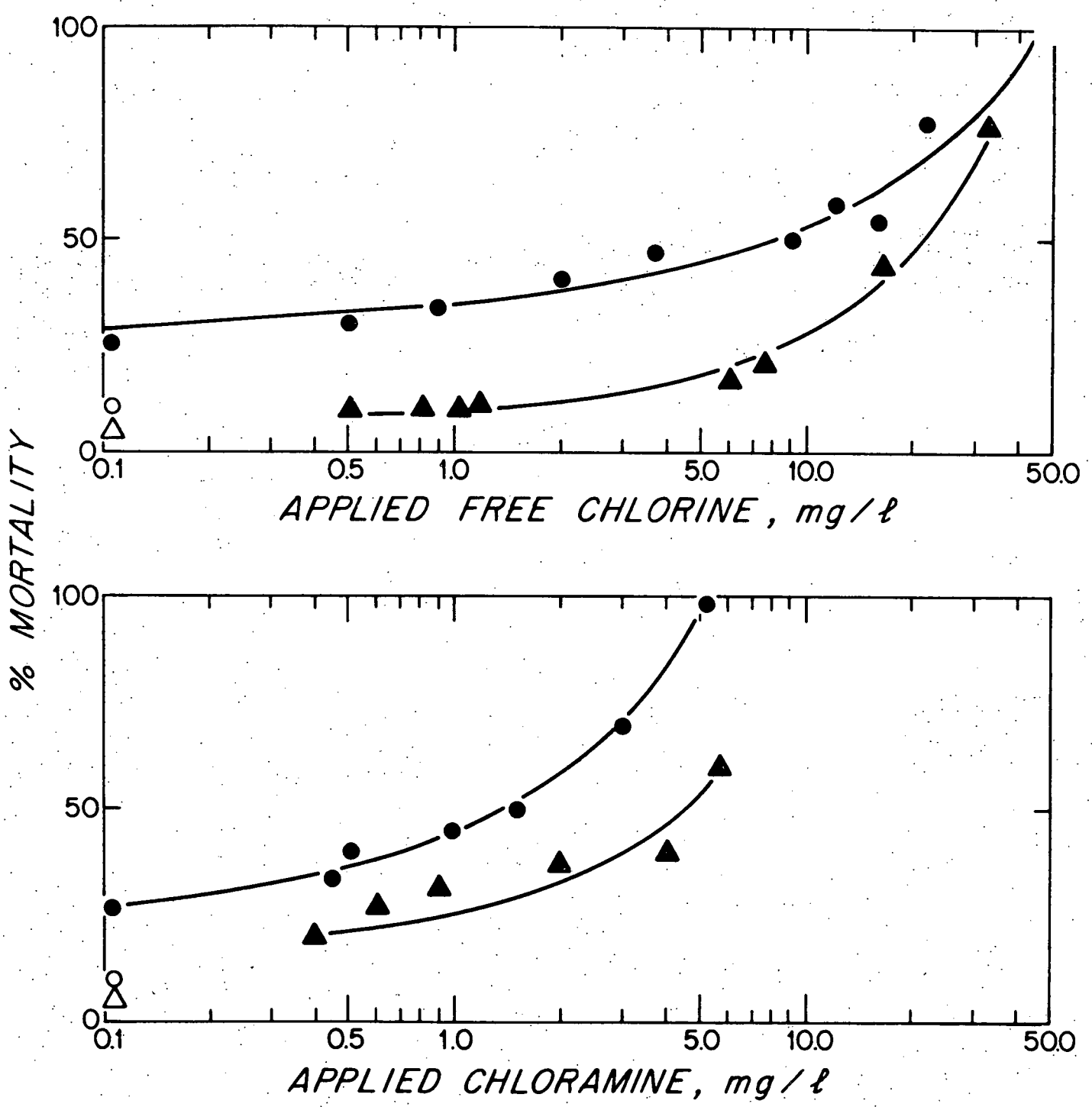
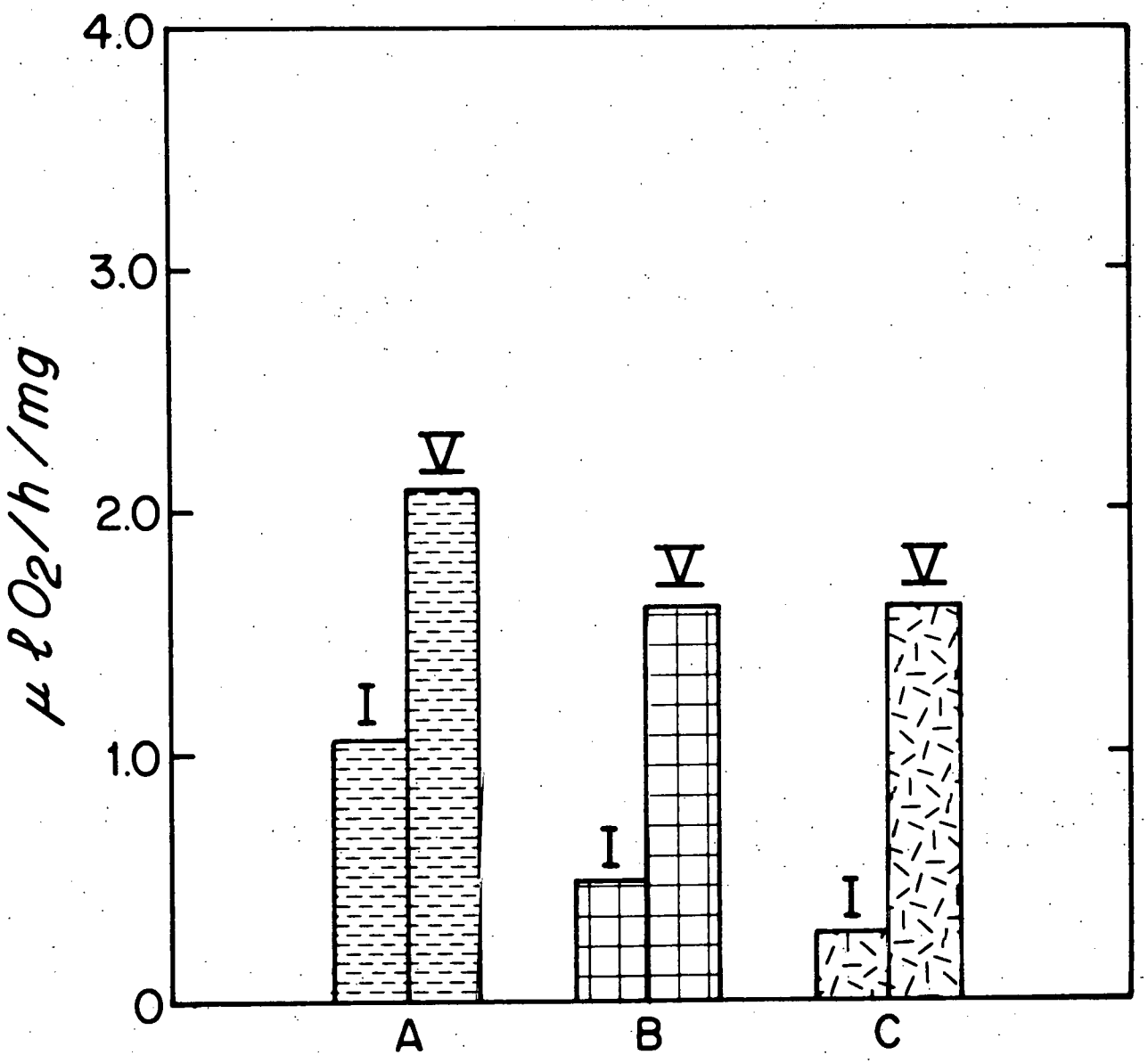

100
50
0
0.1
0.5
1.0
5.0
10.0
50.0
APPLIED FREE CHLORINE, mg/ℓ
% MORTALITY
100
50
0
0.1
0.5
1.0
5.0
10.0
50.0
APPLIED CHLORAMINE, mg/ℓ

Fig. 2

Fig. 3



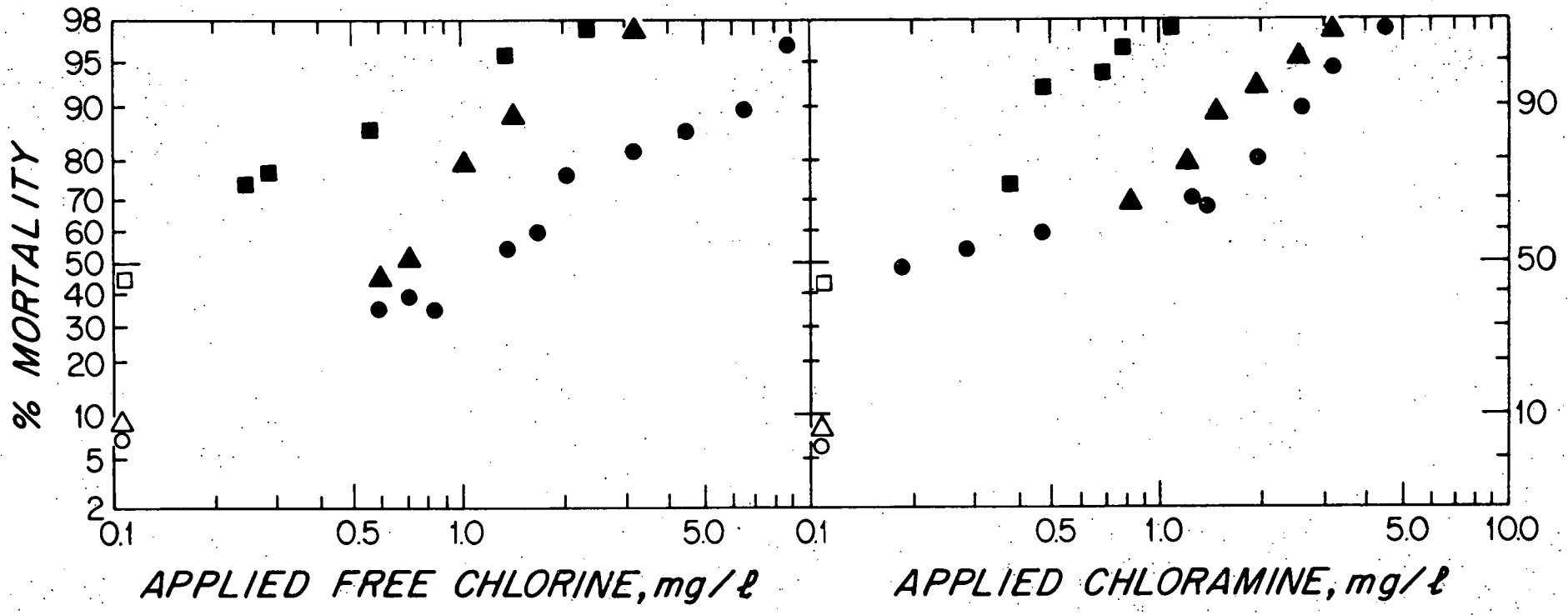

% MORTALITY
98
95
90
80
70
60
50
40
30
20
10
5
2
0.1
0.5
1.0
5.0
0.1
0.5
1.0
5.0
10.0
90
50
10
APPLIED FREE CHLORINE, mg/ℓ
APPLIED CHLORAMINE, mg/ℓ

Fig. 4

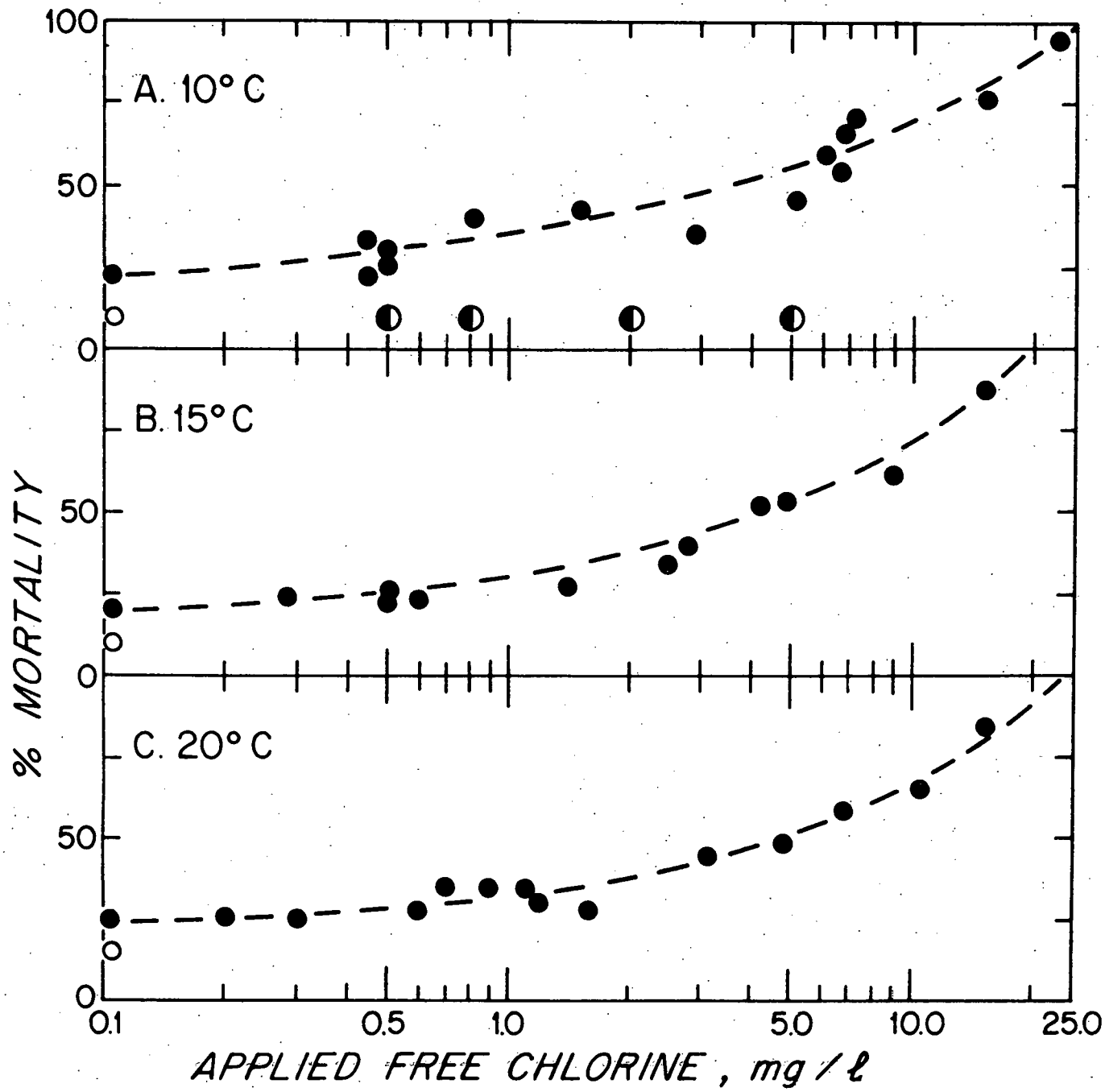

100
A. 10° C
50
0
B. 15° C
50
0
C. 20° C
50
0
% MORTALITY
0.1
0.5
1.0
5.0
10.0
25.0
APPLIED FREE CHLORINE, mg/ℓ

Fig. 5

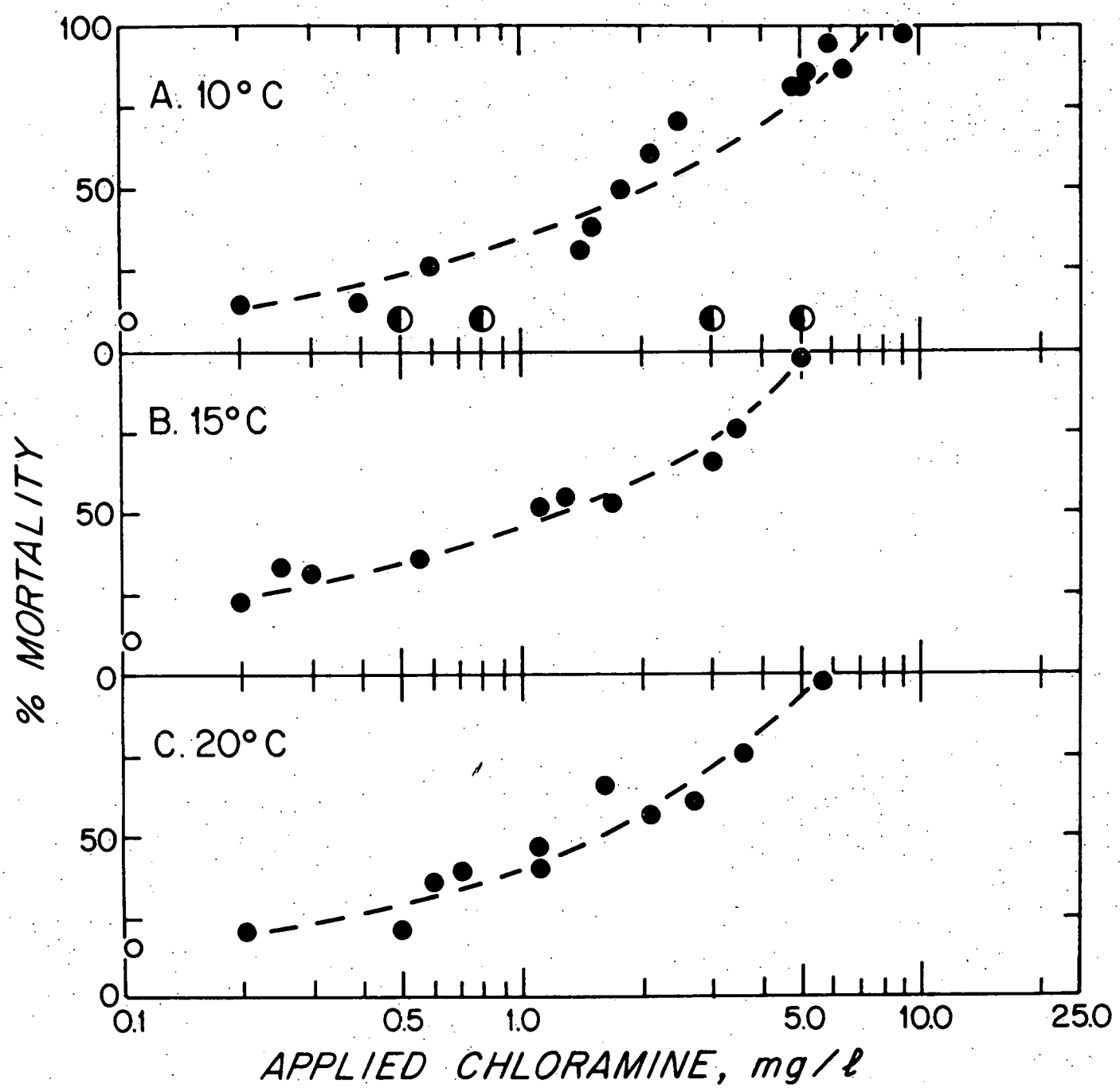

100
A. 10° C
50
0
B. 15° C
50
0
C. 20° C
50
0
% MORTALITY
0.1
0.5
1.0
5.0
10.0
25.0
APPLIED CHLORAMINE, mg/ℓ

Fig. 6

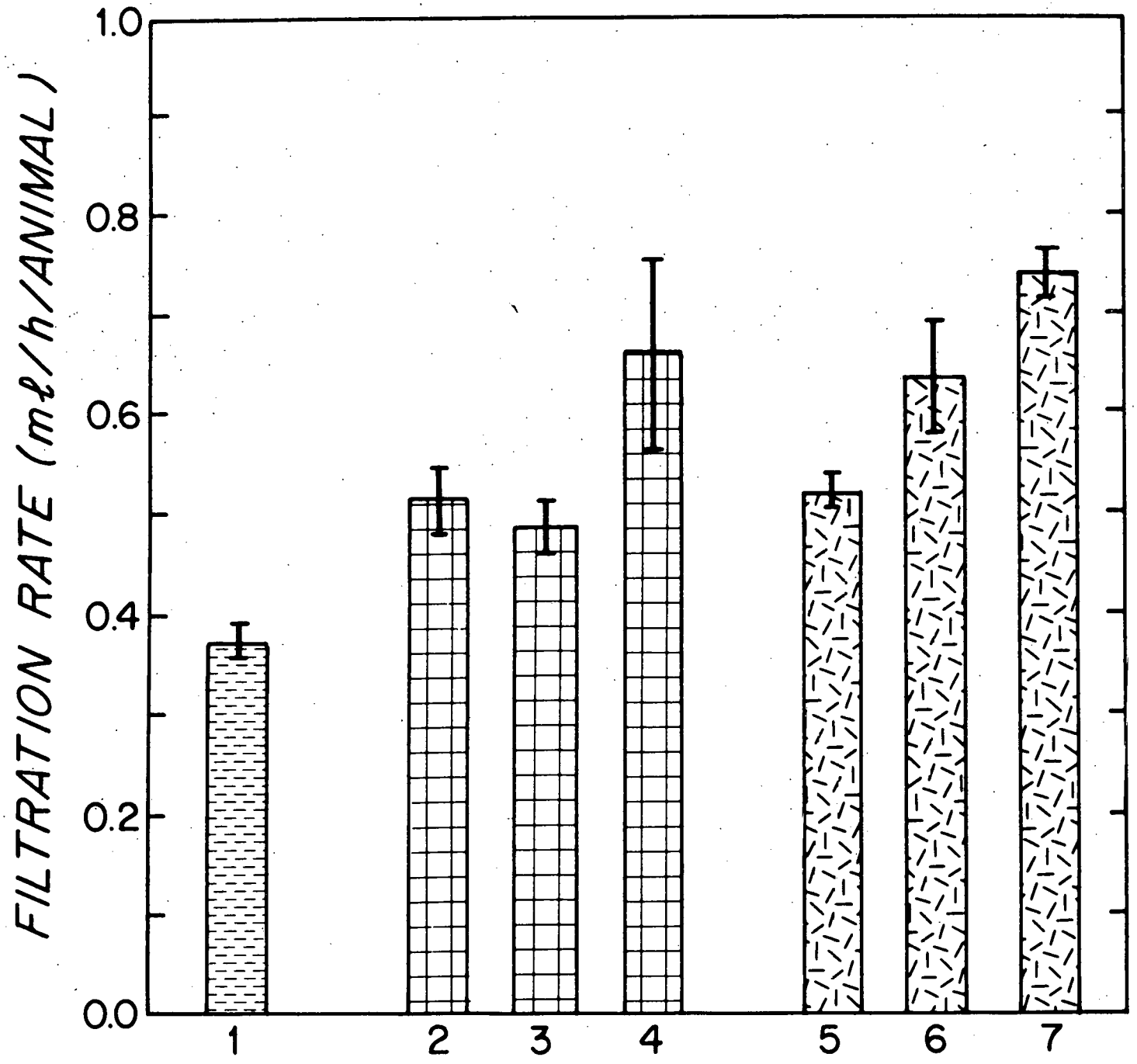


Fig. 7

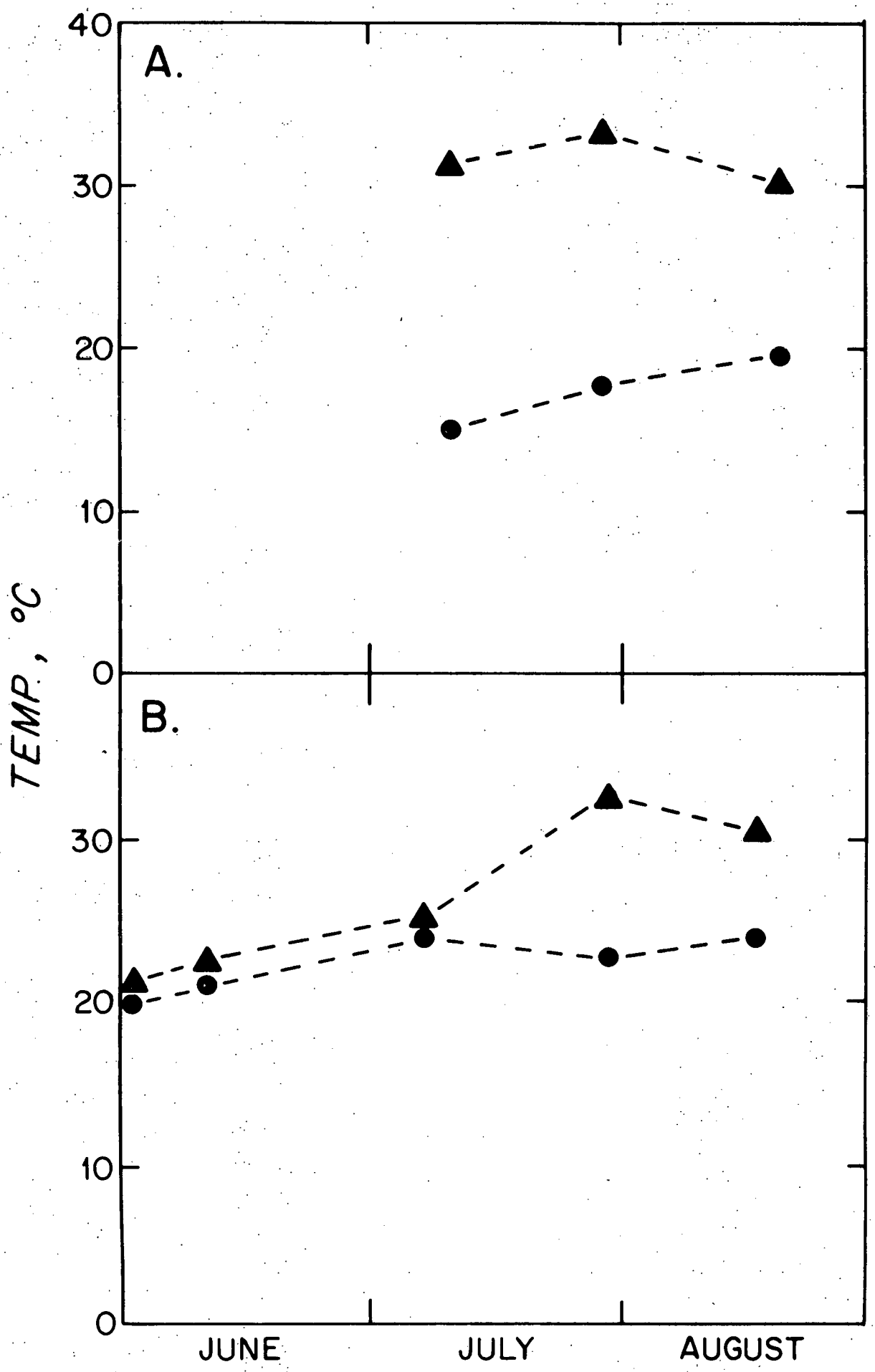


Fig. 8

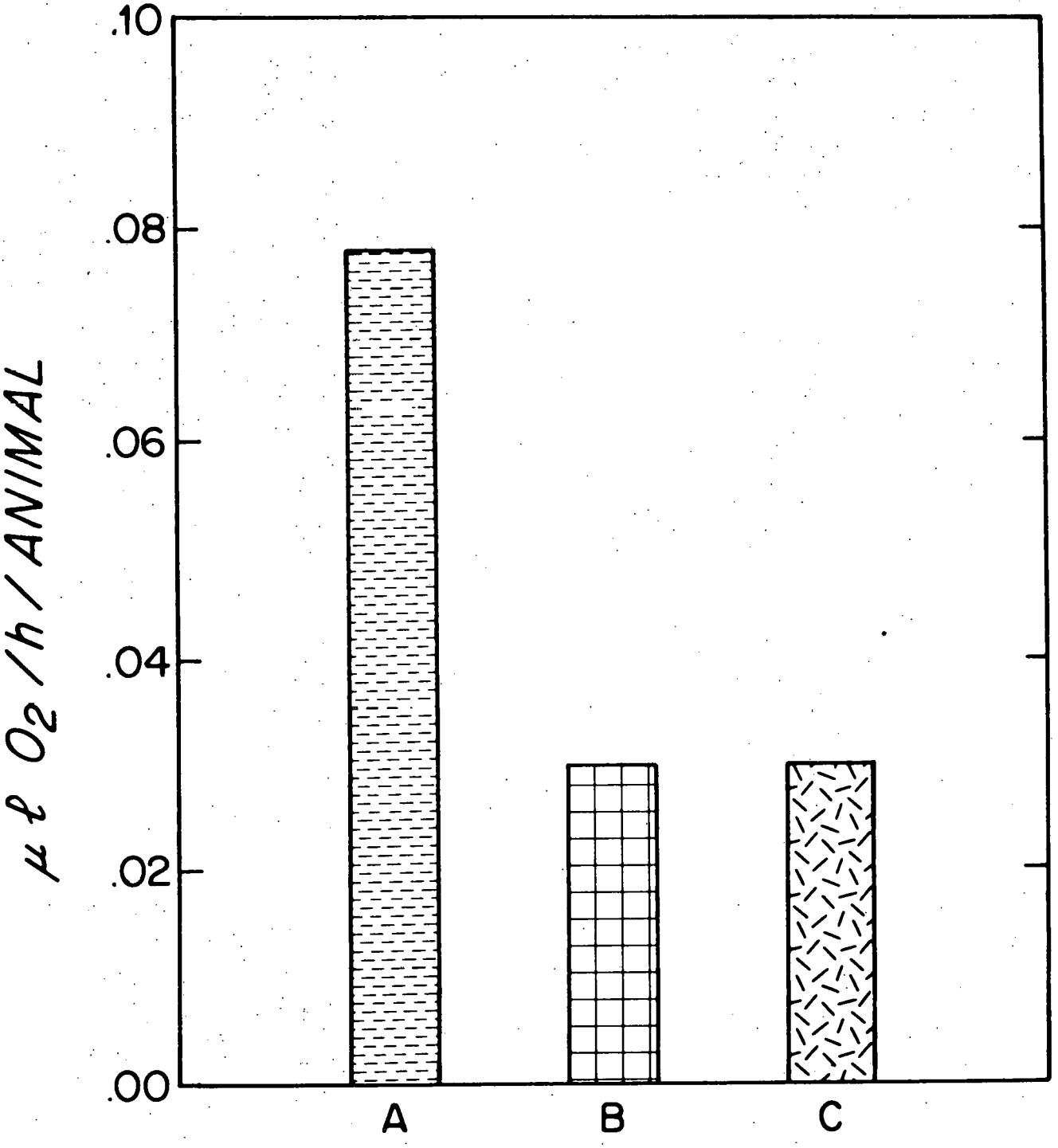


Fig. 9

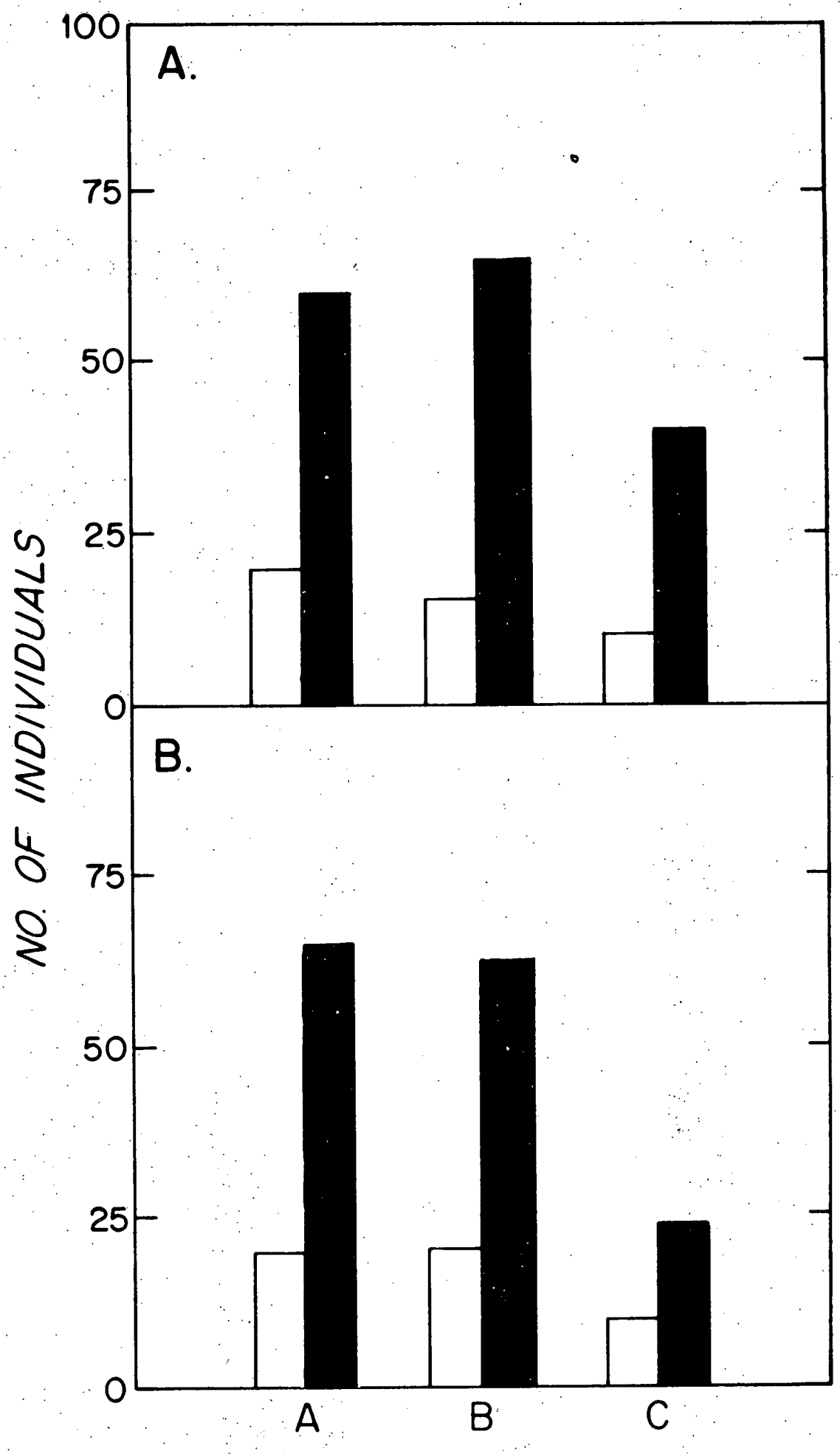
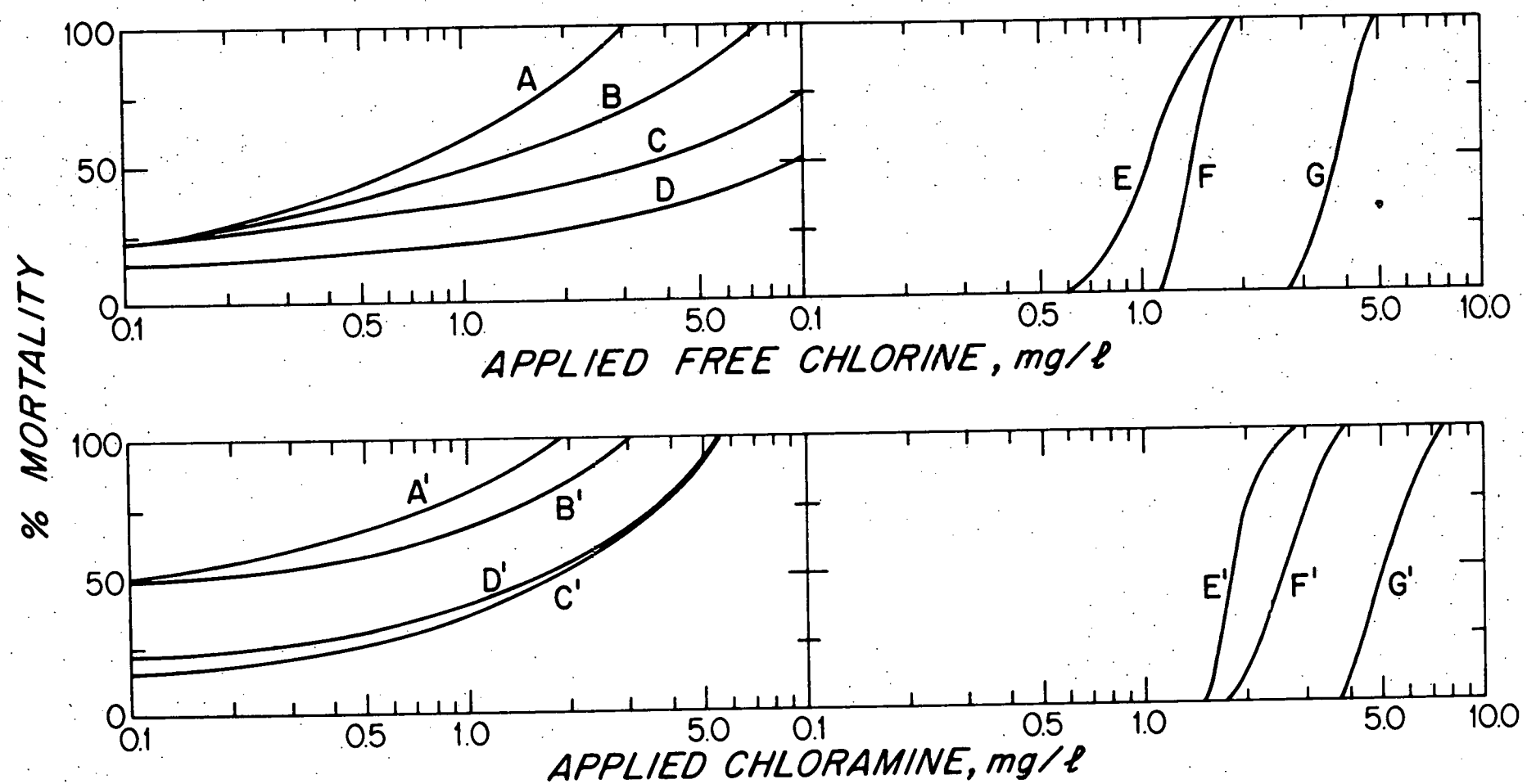


Fig. 10

Fig. 11


100
50
0
% MORTALITY
A
B
C
D
E
F
G
0.1
0.5
1.0
5.0
0.1
0.5
1.0
5.0
10.0
APPLIED FREE CHLORINE, mg/ℓ
100
50
0
A'
B'
D'
C'
E'
F'
G'
0.1
0.5
1.0
5.0
0.1
0.5
1.0
5.0
10.0
APPLIED CHLORAMINE, mg/ℓ

Appendix

Table of Contents

10. Wong, G. T. F. and J. A. Davidson. The "chlorine demand" of seawater.

11. Wong, G. T. F. and J. A. Davidson. The fate of chlorine in seawater.