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The Effects of Residual Chlorine, Temperatures and Salinities on the Development of Pacific Oyster *(Crassostrea gigas)*

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Abstract

Chlorination to eliminate oysters and other fouling organisms in the cooling system of a coastal power plant may cause undesirable effects on nearby oyster farming areas. This study was aimed at determining the effect of residual chlorine on the development of Pacific oyster *Crassostrea gigas* under various temperature and salinity regimes. Fertilized eggs at first polar stage and four larval stages - blastula, trochophore, veliger, and D-larva - were exposed to combinations of five initial chlorine concentrations (0 to 2.52 $mg.l^{-1}$) at four temperatures (19° to 28°C) and three salinities (18 to 34 ppt) for one hour. Up to second order multiple regression models were constructed to evaluate and compare the resistance of various life stages of oyster to the residual chlorine. The resistance to chlorine increased with salinity for all stages. Except for first polar eggs and D-larvae, resistance increased with temperature. First polar eggs had the highest resistance to chlorine and trochophore the lowest. Chlorina-tion at 1.392-2.953 $mg.l^{-1}$ eliminated 90% of the oyster fouling in the cooling system of power plants. Chlorine concentration below 0.01-0.028 $mg.l^{-1}$ reduced the impact on oyster culture.

Introduction

Chlorine is one of the antifouling biocides used in power plants to prevent the attachment of fouling organisms in the cooling systems. Stewart et al. (1979) found chloroform, bromoform and bromine caused mortality of American oyster *(Crassostrea virginica)*

larvae at 0.05 mg.l^{-1}. Turner (1948) stated total residual chlorine at 1 mg.l^{-1} caused pumping of blue mussel *(Mytilus edulis)* to stop within 20 to 90 minutes and at 0.18 mg.l^{-1} caused half the mussels to stop opening their shell while total residual chlorine at 0.5 mg.l^{-1} caused immobility of European oyster *(Ostrea edulis)* larvae within two minutes. The toxicity of total residual chlorine increased with temperature and the toxicity of chloramine, a derivative of chlorine and amino acid, was even greater than free chlorine. Capuzzo (1979) found the LC_{50} of seven-day-old American oyster larvae exposed for 0.5 hour at 20°C was 0.12 mg.l^{-1} for free chlorine and 0.01 mg.l^{-1} for chloramine. When those larvae were subjected to increased temperatures of 20 to 25°C within 30 minutes, the LC_{50} was 0.08 mg.l^{-1} for free chlorine and less than 0.01 mg.l^{-1} for chloramine. No study has been conducted on the comparison of mortality of mollusc larvae at various stages when subjected to chlorine under various salinity and temperature regimes.

The effectiveness of chlorine is reduced when salinity and temperature increase. Thus, chlorine dosage has to be increased to kill fouling organisms. However, its environmental impact on the quality of the water from the power plant effluent also increases which may affect the oyster farms located in the vicinity of the Hsien-Da power plant. The prime purpose of this study was to determine the effect of total residual chlorine on larval stages of the Pacific oyster *(Crassostrea gigas)*.

Materials and Methods

Mature oysters collected from Hsien-Da oyster farming area were stocked in the ponds at National Taiwan College of Marine Science and Technology, Keelung. Direct stripping of gonads was employed (Loosanoff and Davis 1963). After pipetting from the gonad, eggs were mixed in 2 l of seawater, and sperm in 200 ml of seawater. The eggs were fertilized with 5 to 10 drops of diluted sperm. Five minutes later, the fertilized eggs were examined under a microscope at 40 x. If too much sperm attached to the egg membrane, the eggs were washed with additional seawater. The egg concentrations were maintained at 20,000 l^{-1} for first polar, blastula and trochophore stages and at 5,000 l^{-1} for veliger and D-larva stages. These were the optimal rearing concentrations recommended by Helm and Millican (1977). Flagellates (*Isochrysis* sp.) were fed to the veligers at 500-5,000 mg.l^{-1}.

Five life stages of the oyster were tested, namely, egg at first polar stage, blastula, trochophore, veliger and D-larva (Calabrese et al. 1977). The incubation time for each stage was usually more than one hour (Table 1) and therefore there was enough time for acute toxicity testing.

Table 1. The incubation time (hours) required to reach five life stages during the development from fertilized egg to D-larva of oyster *Crassostrea gigas* under various temperatures.

Temperature (°C)	Stage				
	First polar	Blastula	Trochophore	Veliger	D-larva
19	1 - 2	6 - 7	12 - 16	26 - 30	48 - 50
22	1 - 1.5	5 - 6	11 - 14	22 - 24	44 - 46
25	0.5 - 1	4 - 5	8 - 12	18 - 20	38 - 40
28	0.5 - 1	3 - 4	6 - 10	16 - 18	24 - 26

The above life stages were tested under six chlorine concentrations at combinations of three salinities (18, 26 and 34 ppt) and four temperatures (19, 22, 25 and 28°C). Except at the first polar stage, the tested chlorine concentrations were 0, 0.168, 0.42, 0.672, 0.84 and 1.68 mg.l^{-1}. Because the preliminary experiment showed that the oysters at first polar stage had higher resistance to residual chlorine than the other four stages, the chlorine concentrations used were 0, 0.42, 0.84, 1.26, 1.68 and 2.52 mg.l^{-1}.

Static methods (APHA 1981) were employed for the experiment, i.e., no aeration and no stirring but the flask used was tightly plugged during the test to prevent volatile haloforms (Stewart et al. 1979).

Each 250-ml Erlenmeyer flask contained 100 ml of seawater at the designated salinity. Eighteen flasks, six for each salinity, were placed in a thermobath at the desired temperature. Sodium hypochloric acid stock solution at the six designated concentrations was then added into the flask. The flasks were tightly plugged with rubber stoppers immediately and plugged after one drop (0.05 ml) of first polar stage or oyster larvae was added. After one hour, 3 to 5 drops of 10 mg.l^{-1} of sodium thiosulfate solution were added to eliminate the residual chlorine (Brooks and Seegert 1977) and all mortalities were recorded.

A filtering device was used to concentrate the tested larvae from 100 ml to 5 or 10 ml. The device was made of plankton net of mesh size 600 μm plated in a 77-mm diameter plastic frame (Stewart et al. 1979; Martin et al. 1981). One ml was then sampled onto a plankton plate counter under a microscope to assess mortality rate. For the

first polar stage, mortality was defined as when the cell stopped cleaving. For the other stages, the larvae were considered dead if they stopped moving and the cilia were immobile. The average of two counts was used for regression analysis.

Multiple regression analysis was used to show the relationship of mortality *vs.* temperature, salinity and residual chlorine concentration. A second order equation was constructed: $M = B_0 + B_1T + B_2S + B_3C + B_4TS + B_5TC + B_6SC + B_7T^2 + B_8S^2 + B_9C2$, where M is mortality (%); T, temperature (°C); S, salinity (ppt); C, total residual chlorine (TRC) ($mg.l^{-1}$); B_1-B_9, the regression coefficients. For each stage, among 511 (2^9-1) models, an optimal model was selected by stepwise process (Neter and Wasserman 1974). The significance level of the regression coefficient selection criteria was set at 5%.

Results

First Polar Stage

The optimal regression model was $M = 533.177 - 32.276T - 11.6074S + 63.9049C + 0.2248TS - 0.9738SC + 0.5530T^2 + 0.1159S^2$ ($R^2 = 0.9429$, MSE = 52.7273). When the other independent variables were held constant, if TRC increased by 1 $mg.l^{-1}$, the mortality changed by (63.9049 - 0.9728S)%; at salinity 34 ppt, increase of TRC by 1 $mg.l^{-1}$ resulted in 33% more mortality.

Lethal concentration (LC) generally increased with salinity. When all temperatures were pooled, the average LC_{50} ($mg.l^{-1}$) increased with salinity: 0.852 at 18 ppt, 1.2727 at 26 ppt and 1.4245 at 34 ppt. LC_{50} *vs.* temperature curves showed that at 18, 26, 34 ppt when temperature was lower than 24°C, the resistance of first polar eggs to TRC increased with salinity (Fig. 1). When temperature was higher than 27°C, LC_{50} at 34 ppt was slightly lower than that at 26 ppt.

When all salinities were pooled, the average LC_{50} ($mg.l^{-1}$) was highest at 28°C (1.0701), followed by 25°C (1.3223 $mg.l^{-1}$), 22°C (1.3029 $mg.l^{-1}$) and 19°C (1.0307 $mg.l^{-1}$). There was no apparent correlation between LC_{50} and temperature over all salinities (Fig. 2). In general, first polar egg had higher resistance to chlorine at 22 and 25°C than at 19 and 28°C with salinities at the range of 22 to 34 ppt. At a low salinity of 18 ppt, LC_{50} at 19°C was the lowest. But at high salinity, 34 ppt, LC_{50} at 28°C was the lowest.

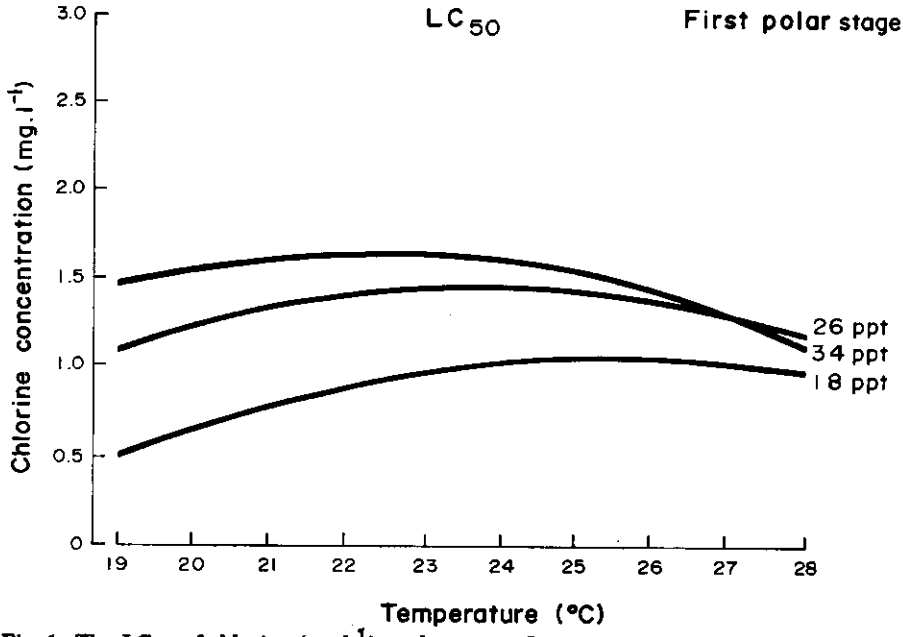

Fig. 1. The LC_{50} of chlorine ($mg.l^{-1}$) to the oyster *Crassostrea gigas* fertilized eggs at first polar stage subjected to three experimental salinities and various temperatures. (Curves drawn from the relevant regression equation.)

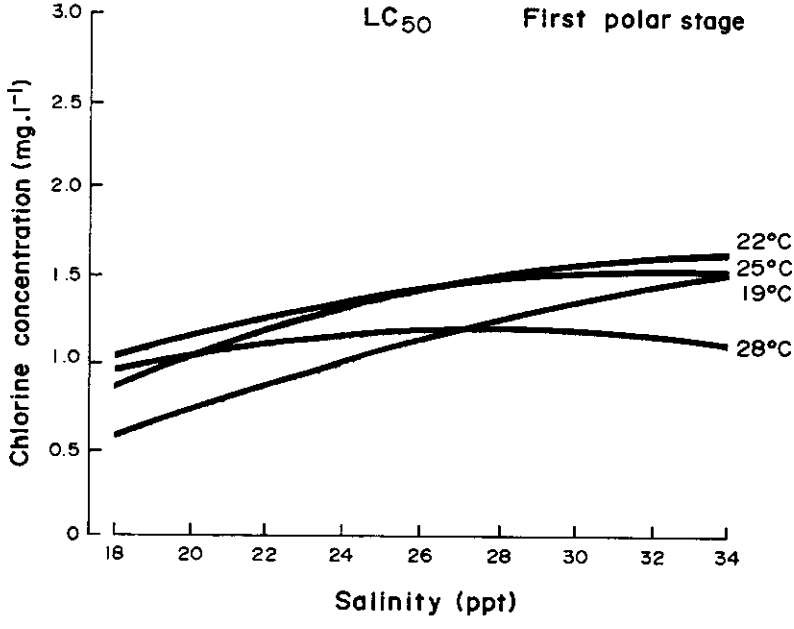

Fig. 2. The LC_{50} of chlorine ($mg.l^{-1}$) to the oyster *Crassostrea gigas* fertilized eggs at first polar stage subjected to four experimental temperatures and various salinities. (Curves drawn from the relevant regression equation.)

Blastula Stage

The optimal regression model was $M = 41.9227 - 0.5995S + 93.1724C - 0.0368T^2 - 26.3359C^2$ ($R^2 = 0.9021$, MSE = 81.1401).

There was a first order negative correlation between mortality and salinity. When the other variables were held constant, the mortality decreased by 0.6% if salinity increased by one ppt. Therefore, LC of chlorine increased with salinity. When all temperatures were pooled, the average LC_{50} increased with salinity, highest at 34 ppt (0.6505 $mg.l^{-1}$), followed by 26 ppt (0.5713 $mg.l^{-1}$) and 18 ppt (0.4584 $mg.l^{-1}$). LC_{50} increased with salinity over all temperatures tested (Fig. 3). Therefore, the resistance of oyster larvae at blastula stage increased with increasing salinity.

The LC_{50} of blastula larvae increased with temperature since there was a second order negative correlation between mortality and temperature. When all salinities were pooled, LC_{50} ($mg.l^{-1}$) was highest at 28°C (0.6543 $mg.l^{-1}$), followed by 25°C (0.6057 $mg.l^{-1}$), 22°C (0.5237 $mg.l^{-1}$) and 19°C (0.4564 $mg.l^{-1}$). LC_{50} increased with temperature for all salinities (Fig. 4) indicating that the toxicity of chlorine to blastula larvae decreased with temperature.

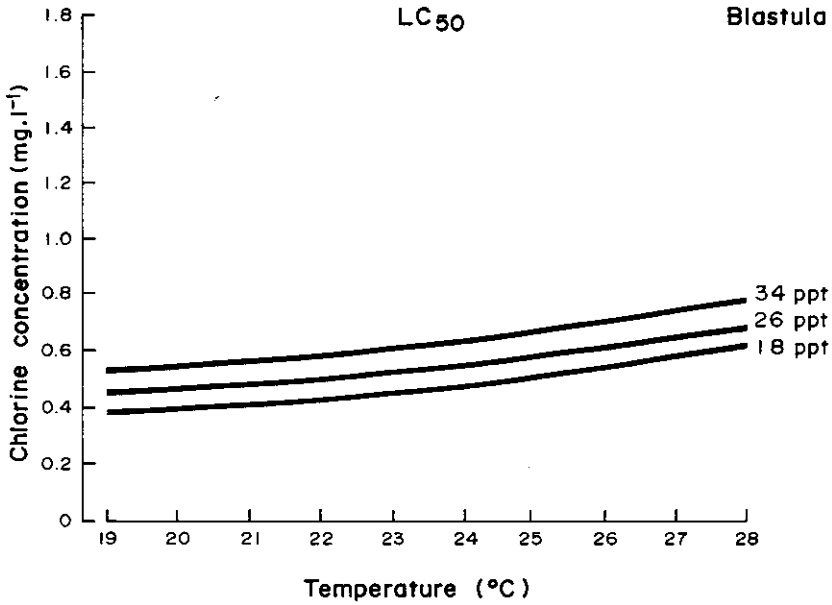

Fig. 3. The LC_{50} of chlorine ($mg.l^{-1}$) to the oyster *Crassostrea gigas* larvae at blastula stage subjected to three experimental salinities and various temperatures. (Curves drawn from the relevant regression equation.)

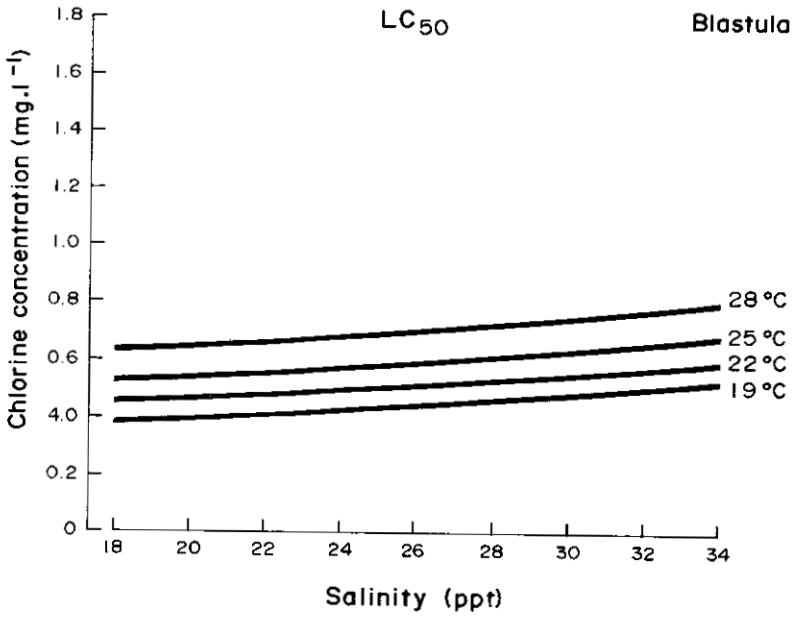

Fig. 4. The LC_{50} of chlorine ($mg.l^{-1}$) to the oyster *Crassostrea gigas* larvae at blastula stage subjected to four experimental temperatures and various salinities. (Curves drawn from the relevant regression equation.)

Trochophore Stage

The optimal regression model for this stage was $M = -264.819 + 25.0622T + 201.956C - 3.867TC - 1.6785SC - 0.5664T^2$ ($R^2 = 0.9246$, MSE = 69.2222).

The regression model indicated that salinity coupled with chlorine concentration had a negative correlation with salinity. When chlorine concentration was held constant, chlorine toxicity decreased when salinity increased. When all temperatures tested were pooled, average LC_{50} ($mg.l^{-1}$) by salinity was highest at 34 ppt (0.6767 $mg.l^{-1}$), followed by 26 ppt (0.5244 mg/l) and 18 ppt (0.4295). When all temperatures were pooled, LC_{50} was also highest at 34 ppt and lowest at 18 ppt (Fig. 5). All these indicate the resistance of trochophore to chlorine increases with salinity.

When all salinities were pooled, average LC_{50} ($mg.l^{-1}$) by temperature was highest at 28°C (0.9260 $mg.l^{-1}$), followed by 25°C (0.5018 $mg.l^{-1}$), 19°C (0.3841 $mg.l^{-1}$) and 22°C (0.3623 $mg.l^{-1}$) (Fig. 6). Although average LC_{50} at 19°C was greater than that at 22°C, the difference was insignificant. At all salinities, LC_{50} was proportional

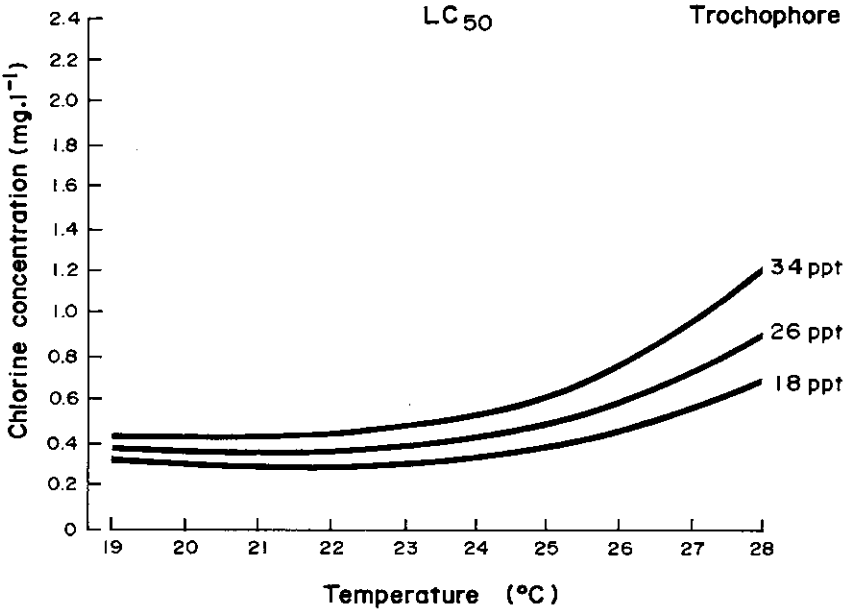

Fig. 5. The LC_{50} of chlorine ($mg.l^{-1}$) to the oyster *Crassostrea gigas* larvae at trochophore stage subjected to three experimental salinities and various temperatures. (Curves drawn from the relevant regression equation.)

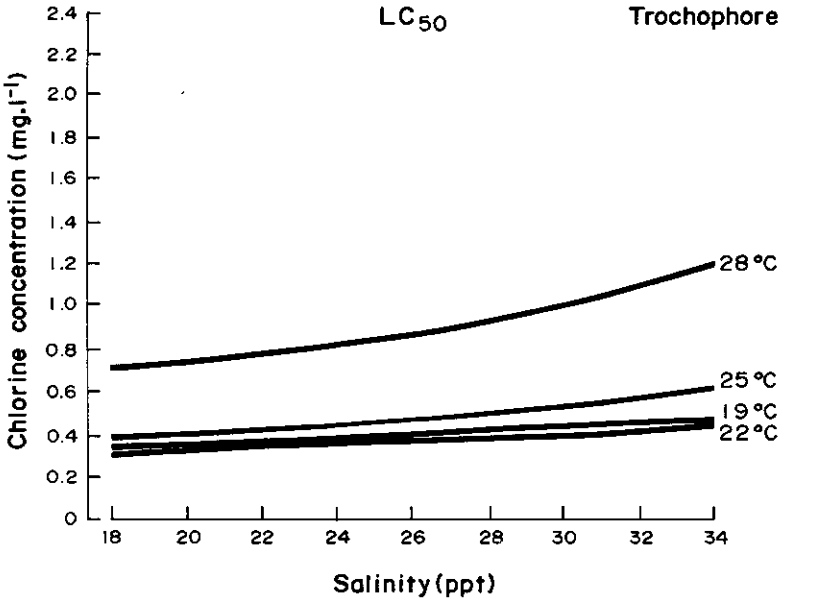

Fig. 6. The LC_{50} of chlorine ($mg.l^{-1}$) to the oyster *Crassostrea gigas* larvae at trochophore stage subjected to four experimental temperatures and various salinities. (Curves drawn from the relevant regression equation.)

to temperature with the exception of 19 to 22°C. However there was only a slight difference of LC_{50} between 19 and 22°C. Therefore chlorine toxicity to trochophore generally decreases when temperature increases.

Veliger Stage

The optimal regression model for this stage was $M = 146.392 - 11.1558T + 121.432C + 1.2521TC - 0.6922SC + 0.2111T^2 + 15.3212C^2$ ($R^2 = 0.9026$, MSE = 77.1913).

When all temperatures were pooled, average LC_{50} ($mg.l^{-1}$) by salinity was highest at 34 ppt (0.8723 $mg.l^{-1}$), followed by 26 ppt (0.7659 $mg.l^{-1}$) and 18 ppt (0.6883 $mg.l^{-1}$) (Fig. 7). At all temperatures tested, LC_{50} also increased with salinity.

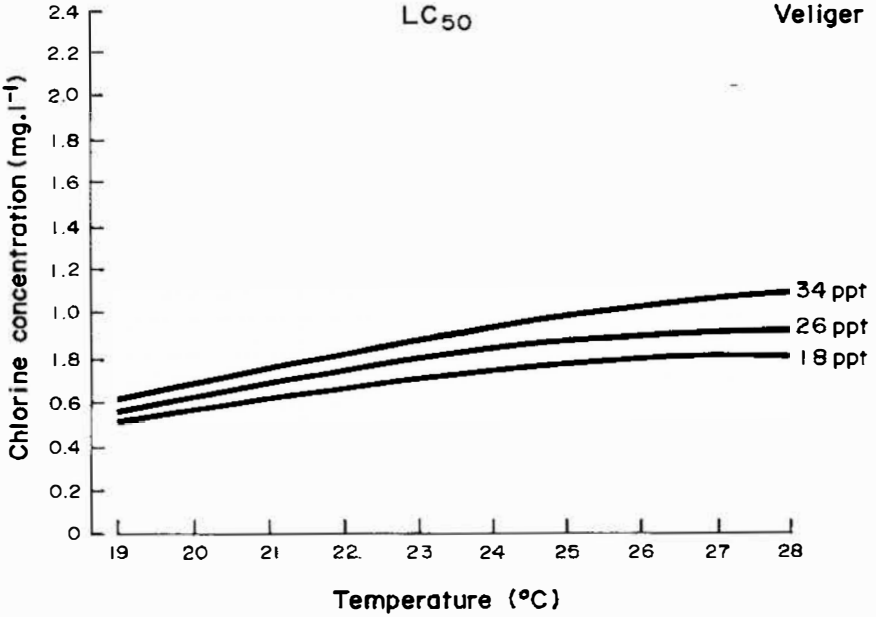

Fig. 7. The LC_{50} of chlorine ($mg.l^{-1}$) to the oyster *Crassostrea gigas* larvae at veliger stage subjected to three experimental salinities and various temperatures. (Curves drawn from the relevant regression equation.)

LC_{50} increased with temperature. When all tested salinities were pooled, average LC_{50} ($mg.l^{-1}$) by temperature was highest at 28°C (0.9497 $mg.l^{-1}$), followed by 25°C (0.8674 $mg.l^{-1}$), 22°C (0.7289 $mg.l^{-1}$) and 19°C (0.5560 $mg.l^{-1}$) (Fig. 8). The results showed chlorine toxicity decreased when temperature increased.

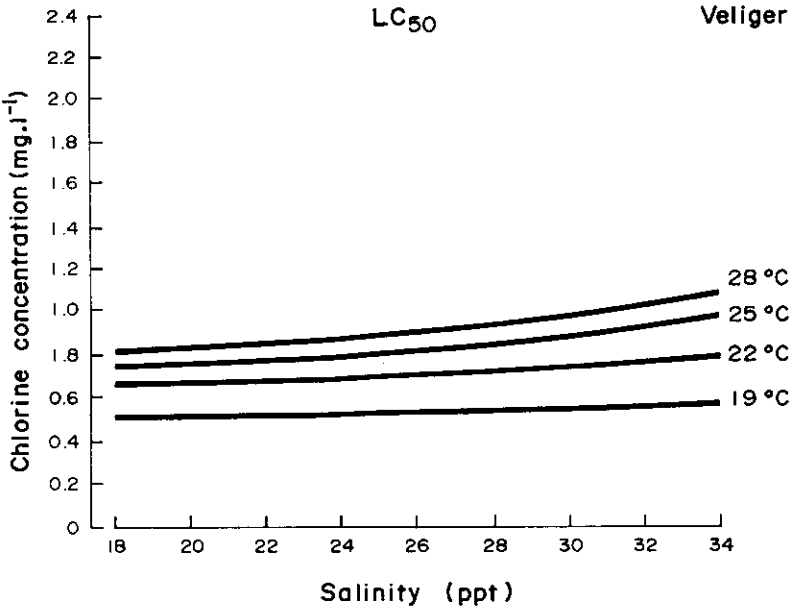

Fig. 8. The LC_{50} of chlorine ($mg.l^{-1}$) to the oyster *Crassostrea gigas* larvae at veliger stage subjected to four experimental temperatures and various salinities. (Curves drawn from the relevant regression equation.)

D-Larvae Stage

The optimal regression model for this stage was M = -1.9638 + 0.3748T + 109.047C - 0.732SC - 25.7364C2 (R^2 = 0.9236, MSE = 62.8147).

LC_{50} of D-larvae increased with salinity. When all tested temperatures were pooled, average LC_{50} was highest at 34 ppt (0.6375 $mg.l^{-1}$), followed by 26 ppt (0.5728 $mg.l^{-1}$), and 18 ppt (0.5251 $mg.l^{-1}$) (Fig. 9). When the mortality was less then 50%, salinity had little effect on LC_{50}.

LC_{50} increased when temperature decreased. Positive correlation between mortality and temperature in the model showed that when temperature increased by 1°C, mortality increased by 0.4%. When all tested salinities were pooled, average LC_{50} ($mg.l^{-1}$) by temperature was highest at 19°C (0.6070 $mg.l^{-1}$), followed by 22°C (0.5891 $mg.l^{-1}$), 25°C (0.5688 $mg.l^{-1}$) and 28°C (0.5502 $mg.l^{-1}$). Similar results were obtained when LC_{50} increased at decreasing temperature and salinity (Fig. 10).

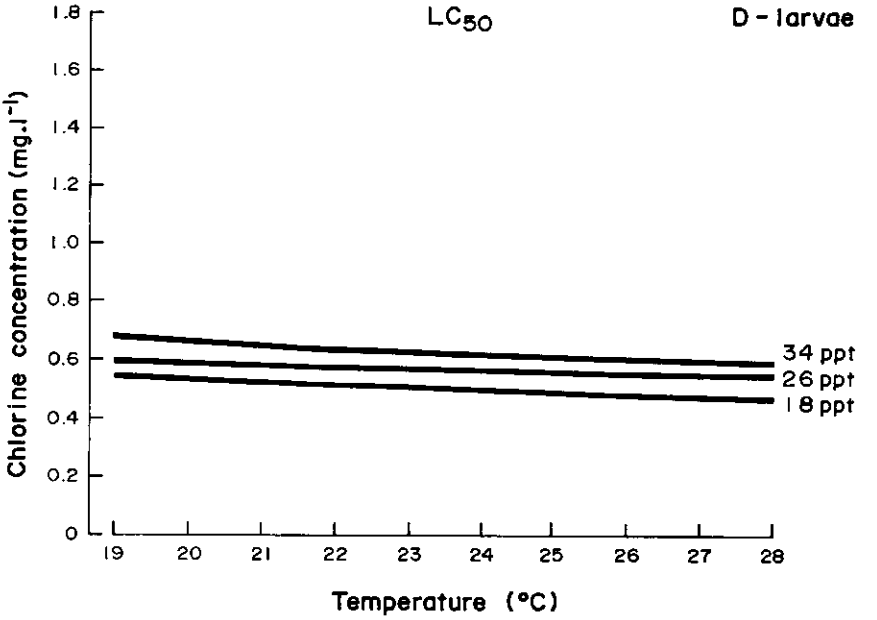

Fig. 9. The LC_{50} of chlorine ($mg.l^{-1}$) to the oyster *Crassostrea gigas* larvae at D-larvae stage subjected to three experimental salinities and various temperatures. (Curves drawn from the relevant regression equation.)

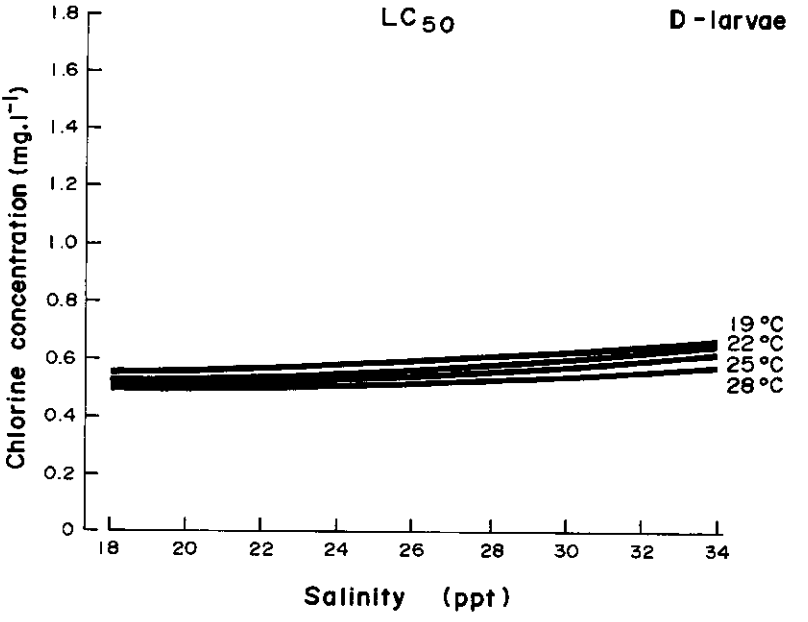

Fig. 10. The LC_{50} of chlorine ($mg.l^{-1}$) to the oyster *Crassostrea gigas* larvae at D-larvae stage subjected to four experimental temperatures and various salinities. (Curves drawn from the relevant regression equation.)

Comparison of LC_{50} at All Life Stages

The comparisons of LC_{50} at various temperatures and salinities among all life stages are summarized in Table 2. LC_{50} for first polar eggs was higher than any larval stage at almost all temperatures and salinities. Except at 28°C or at 34 ppt, the trochophore had the least resistance to chlorine.

Table 2. The comparison of average LC_{50} of various oyster larval stages (a) at various temperatures when all salinities were pooled and (b) at various salinities when all temperatures were pooled. S1: Fertilized eggs at first polar stage, S2: Blastula, S3: Trochophore, S4: Veliger, S5: D-larvae.

(a) 19°C	
	S1 (1.0307) > S5 (0.6070) > S4 (0.5560) > S2 (0.4564) > S3 (0.3841)
22°C	
	S1 (1.3092) > S4 (0.7289) > S5 (0.5891) > S2 (0.5237) > S3 (0.3623)
25°C	
	S1 (1.3223) > S4 (0.8674) > S2 (0.6057) > S5 (0.5688) > S3 (0.5018)
28°C	
	S1 (1.0701) > S4 (0.9497) > S3 (0.9260) > S2 (0.6547) > S5 (0.5502)
(b) 18 ppt	
	S1 (0.8520) > S4 (0.6883) > S5 (0.5251) > S2 (0.5713) > S3 (0.4295)
26 ppt	
	S1 (1.2727) > S4 (0.7659) > S5 (0.5738) > S2 (0.5713) > S3 (0.5244)
34 ppt	
	S1 (1.4245) > S4 (0.8723) > S3 (0.6767) > S2 (0.6565) > S5 (0.6375)

Discussion

In general, when temperature increased, the toxicity of TRC decreased and hence LC_{50} for the larvae stages of oyster increased except for the first polar eggs and D-larvae. It was conceivable that the concentration of TRC decreased with increasing temperature (Chou 1986). Therefore, the chlorine remaining in the water at high temperature was lower than that at low temperature.

Our results are contrary to some past studies which showed that temperature had a synergistic effect on chlorine toxicity to some aquatic organisms. The study by Brooks and Seegert (1977) found that LC_{50} ($mg.l^{-1}$) of chlorine for rainbow trout was 0.99 ($mg.l^{-1}$) at 10°C, 0.94 $mg.l^{-1}$ at 15°C and 0.60 $mg.l^{-1}$ at 20°C when exposed for 30 minutes. Burton et al. (1979) found chlorine toxicity to striped bass fertilized eggs and larvae increased with temperature. Hall et al. (1981) also found that sudden increase of temperature decreased LC_{50} for white perch juveniles. In the present experiment, however, the resistance of D-larvae to chlorine toxicity was decreased when temperature increased. First, with the use of the static method, TRC decreased with time while those studies were conducted under overflow system in which chlorine concentration remained constant over time. Second, in the optimal regression model, with an absence of temperature and chlorine concentration interaction term (TC), the temperature acted independently and had a relatively slight effect on mortality (regression coefficient = 0.3748). Therefore the temperature had no synergistic effect on chlorine toxicity on D-larvae.

The resistance to TRC of first polar eggs was higher than any other larval stage. No literature was found on the comparison of chlorine LC_{50} among molluscan larval stages. Past studies indicated that fish fertilized eggs had higher tolerance to TRC than larval stages; for example, Morgan II and Prince (1978) for eggs and larvae of five fish species in Chesapeake Bay; Johnson et al. (1977) for spotted seatrout; and Burton et al. (1979) for striped bass.

Unlike in fish, tolerance to chlorine did not increase with the development of larvae of oyster. As for the hatched fry, Morgan II and Prince (1978) pointed out that tolerance to chlorine decreased with the development of the fish fry. Anderson (1974) found that larger plaice fry were more resistant to chlorine than younger fry. LC_{50} of each larval stage of oyster varied with temperature and salinity, but generally LC_{50} at veliger stage was the highest among all larval stages, first polar eggs excluded.

According to the results of this experiment, fertilized eggs at first polar stage had the highest resistance to chlorine. At this stage LC_{50} after 1 hour ranged from 1.392 to 2.953 $mg.l^{-1}$, depending on temperature and salinity. Therefore, to eliminate 90% of the oyster larval fouling, 1.392 to 2.953 $mg.l^{-1}$ of chlorine should be added in the cooling system. Assuming it takes one hour until the cooling water flows through the cooling system out to sea, the residual chlorine concentration will range from 0.165 to 0.168 $mg.l^{-1}$. At such concentration, it will cause 10 to 40% mortality of trochophore, which is least resistant to chlorine toxicity among all larval stages. Natural mortality of trochophore can be obtained by setting the chlorine concentration in the regression model to 0 $mg.l^{-1}$ as no free chlorine in the water. At 19°C and 34 ppt salinity the natural mortality was the lowest, 6%, and at 28°C and 34 ppt salinity the natural mortality was the highest, 18%. LC_1 and LC_5 have been set as safety standards by Sprague (1971). Adding 1 to 5 % to the lowest and highest natural mortality as safety standards for chlorine for one-hour duration for trochophore stage, the safety concentrations of chlorine should be $LC_{7\text{-}19}$ ranging from 0.01 to 0.028 $mg.l^{-1}$ and $LC_{11\text{-}23}$ 0.051 to 0.138 $mg.l^{-1}$.

Latimer et al. (1975) conducted a 30-minute chlorine toxicity experiments on copepods *(Limnocalanus macrurus* and *Cyclops bicuspidatus thomasi)* and set a safety concentration at 0.5 $mg.l^{-1}$ since no mortality resulted from this concentration. However, to set a safety concentration standard of power plant effluent, the effects of chlorine on various organisms, especially those with commercial value such as oyster, in the nearby water must be considered. Pacific salmon juvenile mortality occurred when they were exposed to 0.05 $mg.l^{-1}$ residual chlorine for 23 days (Holland 1960). Residual chlorine also caused reduction of primary production from phytoplankton (Carpenter et al. 1977). When marine phytoplankton was continuously exposed to 0.1 $mg.l^{-1}$ residual chlorine, primary production was reduced to 70% and when it was exposed to 0.2 $mg.l^{-1}$ residual chlorine for 1.5 hours, primary production was reduced to 25%. The pumping activity of oysters decreased with residual chlorine at 0.01 to 0.05 $mg.l^{-1}$ and even discontinued at 1.0 $mg.l^{-1}$ (Galtsoff 1946).

From the previous and present study, residual chlorine at 0.01 $mg.l^{-1}$ has already caused ill-effects on marine organisms. The USEPA (1976) criterion of 0.01 mg.l-1 for chlorine was consistent with this study. Therefore the safety standard for chlorine should be set at

0.01 to 0.028 mg.l^{-1}. Dechlorination is required for power plant effluent with residual chlorine concentration exceeding this standard so that the impact on the environment can be lessened.

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