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Effects of Chlorine-Produced Oxidants on Survival of Larvae of the Oyster *Crassostrea virginica**

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ABSTRACT: Effects of chlorination on two larval stages of the oyster *Crassostrea virginica* (Gmelin, 1790), were studied in flowing estuarine water. Straight-hinge veliger larvae were exposed to concentrations of 0.01, 0.05, 0.1 and 0.2 ppm chlorine-produced oxidants (CPO) for 6, 12, 24 and 36 h, and to 0.05, 0.1, 0.2 and 0.3 ppm CPO for 8, 24, 48, 72 and 96 h. Setting pediveliger larvae were exposed to 0.05, 0.1,0.2 and 0.3 ppm CPO for 6, 24, 48, 72 and 96 h in two experimental trials. Mortality of straight-hinge and pediveliger larvae was directly related to increased concentrations of CPO and extended exposure time. Pediveliger larvae were generally more resistant to CPO than straight-hinge larvae, especially with longer exposure time. Equations for predicting mortality under different conditions of time and CPO concentration were developed using these data.

INTRODUCTION

Chlorine is in widespread use as a disinfectant for industrial and sewage-treatment plant effluent and as a biocide to remove fouling organisms from the condenser tubes of steam-electric generating stations (Brungs, 1973). There is concern about the effects of chlorine-produced oxidants (CPO) on the biota of water receiving the treated effluent. The commercially important adult oyster *Crassostrea virginica* (Gmelin, 1790), survives stressful changes (including exposure to chlorine) in its habitat by closing its valves (Galtsoff, 1946), resorting to anaerobic metabolism and resuming aerobic metabolism when the situation improves (Galtsoff, 1964). Early developmental stages probably do not have this capablity. Their high rate of metabolism and gradual development of shell may make them vulnerable to adverse environmental conditions. Oyster larvae are part of the meroplankton that may live in the vicinity of outfall or power plant discharge structures, thus coming into close contact with chlorinated effluent. Because oyster larvae may be the most sensitive stage in the oyster's life cycle, investigations were conducted to establish their response to exposure to various CPO concentrations for different periods of time.

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MATERIAL AND METHODS

Oysters obtained from hatchery brood stock were induced to spawn in running estuarine water (12–14 ‰ S) by varying the temperature between 25 ° and 30 °C (Loosanoff and Davis, 1963). As soon as spawn release revealed their sex, males and females were separated to avoid premature fertilization. At time 0, gametes of several males and females were pooled in each experiment to provide genetic diversity (Calabrese and Davis, 1970). Each larval brood was reared according to the methods described by Hidu et al. (1969). Experimental larvae were selected at two stages of development: straight hinge veligers (48–60 h old) and pediveligers (over 14 d old).

Experimental Equipment

Morgan and Prince (1977) have described the equipment used to expose organisms to various concentrations of toxicant. All our experiments were conducted

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in estuarine water at 24 °C and 12–14 ‰ S. Water filtered through a 1-μm filter was fed into a constant-head distribution tank which supplied five mixing tanks by gravity flow. A four way peristaltic pump fed four different calcium hypochlorite solutions, contained in black painted bottles, into four mixing tanks. The fifth mixing tank (control) received no toxicant. From the mixing tank, each concentration of toxified estuarine water descended by gravity flow into the distribution boxes and from there into the experimental chambers. These were 30-μm screen-bottomed glass cylinders (8.5 cm wide × 20 cm high) that were higher than the beaker in which they stood, so that the water flowed into the top of the cylindrical cage, passed through the screen at the bottom and out over the edge of the beaker. The use of 1-μm filtered water was necessary because 5-μm filtered water (preferable for raising oyster larvae) contained algae and particles which clogged the screen bottoms of the cylinders and introduced too many artifacts to permit accurate counts under the microscope. Amperometric titrations measured CPO concentrations twice daily at pH 4 (Block et al., 1978).

Procedure

Two series of replicated experiments were conducted on straight-hinge larvae. The first involved approximate CPO concentrations of 0.01, 0.05, 0.1 and 0.2 ppm and exposures of 6, 12, 24 and 36 h. The second involved approximate concentrations of 0.05, 0.1, 0.2 and 0.3 ppm and exposures of 8, 24, 48, 72 and 96 h. For pediveliger larvae, two experiments were conducted at approximate CPO concentrations of 0.05, 0.1, 0.2 and 0.3 ppm and exposures of 4, 24, 48, 72 and 96 h.

At least a day before each experiment was scheduled, the constant-flow chlorination apparatus was activated and the experimental chambers were stabilized to the desired CPO concentrations and flow rate. Straight-hinge veliger larvae for experimental use were concentrated into a suspension as close to 100 ml^{-1} as possible. Five 1-ml samples of this suspension were counted under the microscope and the average of these five counts was considered to be the density of larvae ml^{-1}. A volumetric pipet was adjusted to contain approximately 300 individuals per delivery. Injections of the larvae into the experimental chambers were made in as short a time as possible. Pipettes were filled while the stock suspension was agitated (to avoid congregation of larvae on the bottom). At the end of exposure, one control and at least one of the high concentration samples were examined live and compared with a sample of the original brood (kept in 5-μm filtered water) to determine whether the control larvae that were kept in 1-μm filtered water had suffered from starvation (none appeared to have). Immediate examination of high CPO-concentration samples helped to establish the physical appearance of live and dead (disrupted internal organization, etc.) larvae, so that they could be readily distinguished in the preserved samples at a later date. The samples were decanted into 30 ml vials and preserved in 1 % buffered formalin. Counts of the entire samples were made in a Sedgewick Rafter cell and numbers of live and dead larvae at the end of the experiment were recorded.

Preparations for experiments on pediveliger larvae started when the cultured umbo larvae developed eyespots (a sign that setting is imminent). The chlorination apparatus was activated and stabilized for the desired flow and concentration. Microscope slides were placed over the entire bottom of 8" deep fiberglass trays. The entire larval brood was concentrated to more than 500 ml^{-1}, transferred into the trays, after which the trays were filled up with 1-μm filtered saltwater that was changed every 6 h. When a sufficient number of pediveliger larvae had congregated on the slides (but before they had cemented in place) the slides were removed one at a time, numbered, and wiped clean, except for an area of about 4 cm^2 that contained about 100 larvae (average = 93 for all slides used). Each slide was then transferred to an experimental chamber. Live and dead larvae on all slides and those which had detached and were retained in the cages were counted immediately after exposure and without preservation. Criteria of death included no movement and disturbed internal organization.

Data on numbers of live and dead larvae and spat were used to calculate percentage mortalities. Stepwise multiple regression analyses of percentage mortality on CPO concentration and time were performed following the methods of Kennedy et al. (1974a). The data were transformed to arcsine square root percentage mortality to allow the distribution to approximate the normal (Sokal and Rohlf, 1969). First and second order terms for main effects (CPO concentrations and time) and all possible interactions were examined. For straight-hinge larvae, only those terms with $F \geq 3.97$ ($P = 0.05$; *df* 1, 84) were entered in the final equation. For setting pediveliger larvae, $F \geq 4.03$ ($P = 0.05$; *df* 1, 47).

RESULTS

For both larval stages, mortalities in the control treatments remained at low levels during the experiments, although there was a slight increase at 96 h for straight-hinge larvae (Tables 1 and 2). No corrections were made for control mortalities. There was a direct

Table 1. *Crassostrea virginica*. Percentage mortality of straight-hinge larvae under different conditions of chlorine-produced oxidant (CPO) concentrations over time. Control values represent no exposure to CPO. A dash indicates no data available

Time(h)	Control	CPO (ppm) 0.01	0.05	0.1	0.2	0.3
6	6.4	9.0	10.1	14.8	6.3	–
6	2.3	4.3	5.4	6.4	8.9	–
8	0.0	–	3.2	–	–	5.3
8	1.7	–	4.7	3.2	–	7.8
12	4.6	11.8	7.6	28.7	26.4	–
12	1.8	8.0	12.1	31.7	38.5	–
24	2.2	4.2	17.0	24.8	30.4	–
24	5.4	2.2	26.5	15.8	23.1	–
24	1.3	–	2.3	13.2	26.1	28.6
24	3.3	–	3.9	14.4	23.3	30.2
36	7.1	13.7	23.4	28.1	30.9	–
36	5.6	10.2	8.8	12.4	31.8	–
48	3.3	–	10.8	40.0	35.1	66.7
48	8.1	–	8.6	34.3	24.5	61.0
72	8.5	–	26.1	69.4	71.0	60.5
72	7.3	–	28.6	71.7	73.1	78.3
96	23.2	–	53.9	80.7	100.0	100.0
96	12.4	–	39.5	90.9	95.0	83.0

Table 2. *Crassostrea virginica*. Percentage mortality of setting pediveliger larvae under different conditions of chlorine-produced oxidant (CPO) concentrations over time. Control values represent no exposure to CPO

Time (h)	Control	CPO (ppm) 0.05	0.1	0.2	0.3
6	3.4	1.1	15.3	12.9	25.0
6	11.3	6.9	21.1	13.8	22.4
24	2.0	4.0	10.6	6.2	12.3
24	2.3	10.6	21.6	8.2	36.6
48	2.7	2.6	16.0	27.9	67.9
48	6.6	13.9	14.3	28.9	48.6
72	8.0	10.1	22.7	54.6	47.7
72	5.2	28.4	17.2	21.5	38.9
96	0.0	15.4	12.9	27.8	46.1
96	7.1	25.6	37.1	36.1	32.4

Table 3. *Crassostrea virginica*. Analysis of variance of multiple regression of percentage mortality on chlorine-produced oxidant concentrations and time for straight-hinge and setting pediveliger larvae

Source of variation	Straight-hinge larvae df[a]	MS[b]	Pediveliger larvae df[a]	MS[b]
Regression	2	4.151[c]	3	0.518[c]
Residual	83	0.014	46	0.015

[a] df: Degrees of freedom
[b] MS: Mean Square
[c] Significant at the 0.001 level

relationship of mortality with increased concentrations of CPO and time of exposure (Tables 1 and 2). In general, straight-hinge larvae were more sensitive to CPO than were pediveliger larvae, especially with increased period of exposure. However, after 6 h exposure to CPO concentrations of 0.1 and 0.2 ppm, pediveliger larvae had slightly higher mortalities than did straight-hinge larvae.

Estimating equations for percentage mortality (Y) for each larval stage (within the bounds of the experimental conditions) are as follows (C = CPO concentration; T = time in hours):

(A) Straight-hinge larvae

$$Y = 13.6650 + 6.2452\,CT - 13.5791\,C^2T$$

Coefficient of determination = 87.4 %

(B) Pediveliger larvae

$$Y = 11.3961 + 37.8668\,C + 1.9710\,CT - 0.0516\,C^2T^2$$

Coefficient of determination = 69.8 %

These equations provide estimates of percentage mortality that are in transformed form, which must be converted to untransformed values (Sokal and Rohlf, 1969). For both stages, the final empirical models appear to be good predictive equations (Table 3).

Figures 1 and 2 were constructed using these equations and selected values of CPO concentration and time. Equation A can be used to calculate LC 50 values for straight-hinge larvae, e. g., 0.3 ppm CPO at 48 h, 0.08 ppm at 72 h, and 0.06 ppm at 96 h. No LC50 values for setting larvae are calculated because they did not reach 50 % mortality within the bounds of our experiments.

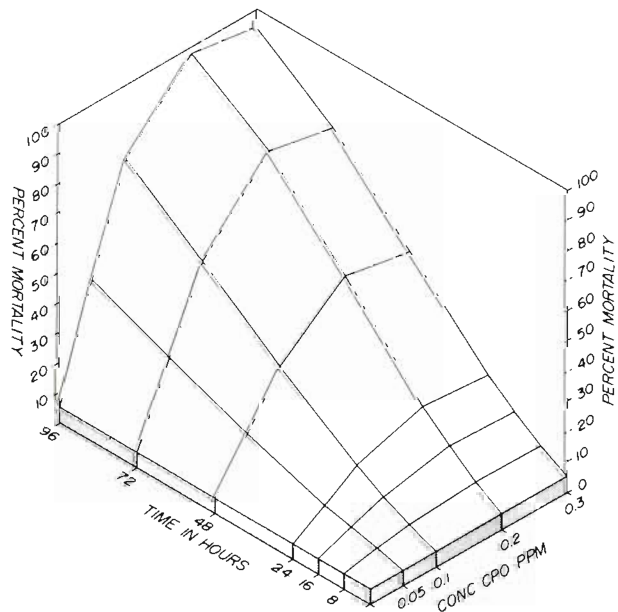

Fig. 1. *Crassostrea virginica*, straight-hinge larvae. Response surface generated from multiple regression analysis of percentage mortality on CPO concentration and time

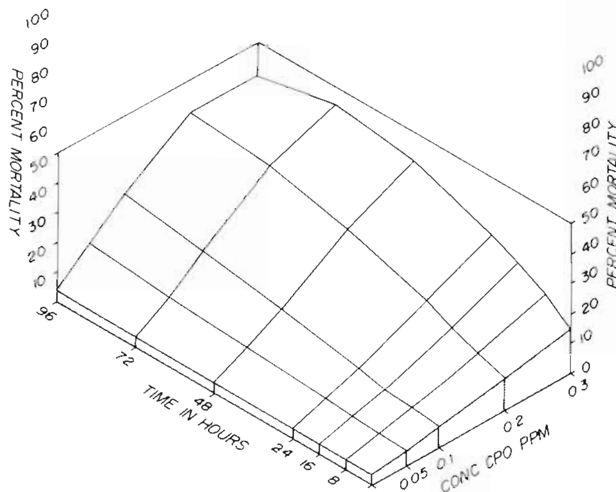

Fig. 2. *Crassostrea virginica*, pediveliger larvae. Response surface as in Figure 1

DISCUSSION AND CONCLUSIONS

As expected, increased concentrations of chlorine-produced oxidants and increased length of exposure to a single concentration of CPO led to increased mortalities of both early life-history stages of *Crassostrea virginica*. A similar situation prevailed for straight-hinge and pediveliger larvae of the soft clam *Mya arenaria* tested under comparable conditions (Roosenburg et al., 1980).

The generally greater sensitivity of the younger straight-hinge larvae to CPO compared with the older setting pediveliger larvae parallels the greater sensitivity of younger life-history stages of bivalves to environmental factors such as temperature (Pelseneer, 1901; Loosanoff and Davis, 1963; Kennedy et al., 1974a, b). This difference in sensitivity was also found for *Mya arenaria* larvae exposed to CPO (Roosenburg et al., 1980). Further, although our techniques and experimental designs differed from those of Roberts and Gleeson (1978), their study of chlorine toxicity in relation to 4 h old *Crassostrea virginica* larvae supports this generalization. Their lethal concentration value for 50 % of their population after 48 h of exposure was 0.026 ppm. Thus, these even younger larval stages demonstrated greater sensitivity to chlorine addition than did our older stages.

In conclusion, given the demonstrated susceptibility of young oyster larvae to chlorine addition to their environment, it appears important that industries and utilities which release chlorinated effluent into an area in which oyster larvae might be growing should carefully monitor that effluent to prevent excessive amounts of these biocides from killing the larvae.

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LITERATURE CITED

Block, R. M., Burton, D. T., Gullans, S. R., Richardson, L. B. (1978). Respiratory and osmoregulatory responses of white perch (*Morone americana*) exposed to chlorine and ozone in estuarine waters. In: Jolley, R. L. (ed.) Water chlorination. Environmental impact and health effects. Vol. 2. Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan, pp. 351–360

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

Calabrese, A., Davis, H. C. (1970). Tolerances and requirements of embryos and larvae of bivalve molluscs. Helgoländer wiss. Meeresunters. 20: 553–564

Galtsoff, P. S. (1946). Reaction of oysters to chlorination. U. S. Dept. of the Interior, Res. Rep. U. S. Fish. Wildl. Serv. 11: 1–28

Galtsoff, P. S. (1964). The American oyster, *Crassostrea virginica*. Fish. Bull. U. S. 64: 1–480

Hidu, H., Drobeck, K. G., Dunnington, E. A., Jr., Roosenburg, W. H., Beckett, R. L. (1969). Oyster hatcheries for the Chesapeake Bay region. N. R. I. Special Report No. 2, Contribution No. 382, Natural Resources Institute, University of Maryland

Kennedy, V. S., Roosenburg, W. H., Zion, H. H., Castagna, M. (1974a). Temperature-time relationships for survival of embryos and larvae *of Mulinia lateralis* (Mollusca: Bivalvia). Mar. Biol. 24: 137–145

Kennedy, V. S., Roosenburg, W. H., Castagna, M., Mihursky, J. A. (1974b). *Mercenaria mercenaria* (Mollusca: Bivalvia): Temperature-time relationships for survival of embryos and larvae. Fish. Bull. U. S. 37: 1160–1166

Loosanoff, V. L., Davis, H. C. (1963). Rearing of bivalve molluscs. Adv. mar. Biol. 1: 1–136

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

Pelseneer, P. (1901). Sur le degré d'eurythermie des certaines larves marine. Bull. Acad. r Belg. Cl. Sci. 1901: 279–292

Roberts, M. H., Gleeson. R. A. (1978). Acute toxicity of bromochlorinated sea water to selected estuarine species with a comparison to chlorinated sea water toxicity. Mar. Env. Res. 1: 19–30

Roosenburg, W. H., Rhoderick, J. C., Block, R. M., Kennedy, V. S., Vreenegoor, S. M. (1980). Survival of *Mya arenaria* larvae (Mollusca: Bivalvia) exposed to chlorine-produced oxidants. Proc natn. Shellfish. Ass. 70

Sokal, R. R., Rohlf, F. J. (1969). Biometry: The principles and practice of statistics in biological research, W. H. Freeman, San Francisco

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