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Hatching Success and Larval Mortality in an Estuarine Teleost, *Menidia menidia* (Linnaeus), Exposed to Cadmium in Constant and Fluctuating Salinity Regimes

R. A. Voyer, J. F. Heltsche*, and R. A. Kraus*

U.S. Environmental Protection Agency, South Ferry Road, Narragansett, R. I. 02882

The Atlantic silverside Menidia menidia (Linnaeus), is found along the Atlantic coast of North America. It is an important forage fish throughout its range. Menidia spawn in estuarine areas where developing embryos may be subjected to salinity fluctuations and exposed to various pollutants. Cadmium, a heavy metal, may be found in effluents of electroplating, textile printing, and chemical industries (MCKEE and WOLF 1963) is one such potential pollutant.

Previous experiments conducted to evaluate the influence of salinity on the response of marine fishes to cadmium have involved exposure of test animals to various treatment combinations, each consisting of specific concentrations of the metal and a fixed salinity. Results of one study utilizing this approach show that the survival and viability of herring eggs, Clupea harengus, decreased at salinities of 16 o/oo and lower (VON WESTENHAGEN et al. 1974). Another showed that the viability of winter flounder embryos, Pseudopleuronectes americanus, incubated at 5°C decreased above and below a salinity of about 25 o/oo (VOYER et al. 1977.)

In view of cyclic fluctuations in salinity that may accompany tidal changes in an estuary, we decided to determine the influence of a periodic, fluctuating salinity regime on the hatching success of Menidia embryos exposed to cadmium and on the survival of resulting larvae and to compare these responses with those of embryos and larvae exposed to cadmium at a number of fixed salinities. This undertaking is part of a continuing project to evaluate the influence of environmental factors on the responses of eggs and larvae of marine teleosts to heavy metal poisoning. It represents an attempt to develop an alternative method that may be more useful to regulatory agencies than the presently used standard bioassay methods.

MATERIALS AND METHODS

A schematic of the experimental apparatus is presented in Fig. 1. A cyclic salinity regime fluctuating between 10 and 30 o/oo was provided by intermittent mixing of seawater with deionized tapwater. Mixing periodicity of the two media was controlled with an electronic timer that activated solenoid valves. The salinity in exposure tanks gradually decreased with the inflow of deionized water to a minimum of about 10 o/oo over a 6-hour period; then gradually increased to about 30 o/oo in another 6 hours when the

*Present address: Department of Computer Science and Experimental Statistics, University of Rhode Island, Kingston,RI 02881

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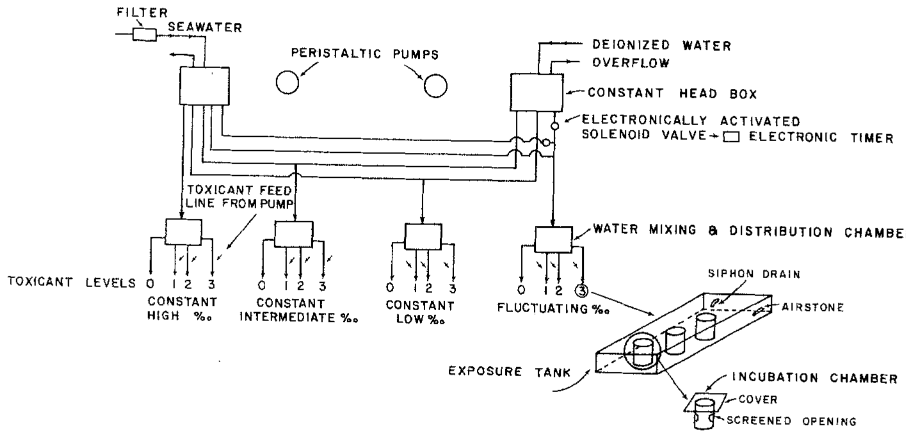

Fig. 1. Schematic of experimental apparatus.

flow of deionized water was stopped. Closing of the valve regulating the flow of the deionized water was synchronized with the opening of a second valve that allowed for the flow of an amount of seawater equivalent to that of the interrupted deionized water. With the alternate opening and closing of the two valves, the pattern of salinity fluctuation depicted in Fig. 2 was achieved. This cyclic pattern was repeated twice every 24 hours during experimentation.

Continuous mixing of appropriate volumes of filtered seawater and deionized water provided constant experimental salinities of 10, 20, and 30 o/oo. Rates of flow of water to each exposure tank of the system were controlled (15 ml/minute) using sections of capillary tubing.

Nominal cadmium concentrations tested ranged from approximately 0.1 to 1.0 mg Cd^{+2}/l. Working stock solutions were prepared by diluting aliquots of a 10 g/l primary stock solution of $CdCl_2 \cdot 2\frac{1}{2}H_2O$ with deionized tapwater. Peristaltic pumps metered cadmium working stock solutions directly into exposure tanks where test media were gently aerated to facilitate mixing of seawater and cadmium stock. Cadmium levels in incubation chambers were measured each working day using an atomic absorption spectrophotometer and routine analytical techniques. The mean standard deviation for all cadmium measurement in Test 1 was 0.02 mg/l and 0.04 mg/l in Test 2.

Mixing and distribution chambers were similar to those described by WHITWORTH (1968) and were fabricated from Plexiglas pipe. Chambers used in conjunction with fixed salinity regimes measured 23.8 cm high x 9.7 cm ID. The chamber used in the fluctuating salinity treatment measured 23.8 cm high x 14.1 cm ID. Media flowing through the latter chamber were mixed by a magnetic stirrer to prevent stratification of deionized water and seawater.

Exposure tanks (Plexiglas boxes 38.5 x 25 x 88 cm) were patterned after culture chambers described by BUCHANAN et al. (1975). Incubation chambers were fashioned from pieces of Nitex[1] screening (250 μ mesh) rolled to form a cylinder that was glued to the bottom of a 50 mm diameter petri dish with silicone cement.

A randomized block design involving 4 concentrations of cadmium (0.0, 0.1, 0.31, and 1.0 mg/l) and 4 salinity regimes (fixed 10, 20, and 30 o/oo; 1 fluctuating between 10 and 30 o/oo) was used in conducting tests. Each of the 16 treatments was repeated twice with 25 eggs exposed per observation.

Procedures used to collect and fertilize Menidia eggs were those described by BARKMANN and BECK (1976). Briefly, eggs were hand stripped and evenly distributed over a Nitex screen immersed in filtered seawater. Eggs were fertilized and then held in filtered flowing seawater at 16-19°C and 30 o/oo salinity 4 to 5 days before exposure. Twenty-five apparently normal eggs, each with a heartbeat evident, were then transferred, either singly or in groups of 6-7 eggs, to each incubation chamber.

Assays were checked daily and the number of eggs that hatched were counted. Larvae that were free of their chorions, without apparent morphological deformities, and able to swim were considered to be viable and were maintained in assay containers from the time of emergence to termination of the experiment. Others were counted as dead and were removed. Tests were continued until all eggs had hatched or until it became apparent that no further hatching would occur. In Test 1 hatching began on the 9th day of exposure and continued for 10 days; in Test 2 hatching commenced after 6 days of treatment and in this case continued for the next 5 days.

Regression analysis was used to describe the relationship of percent larval mortality to cadmium concentration and constant salinity. Prior to analysis, an arc-sine transformation was applied to the total percentage of dead larvae in each observation to stabilize variance. Results of each experiment were analyzed several times using variations of the basic model ($Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1X_2 + b_4X^2_1$, where Y = response in angular units; X_1 = salinity; X_2 = log cadmium concentration) in which selected terms of the equation were deleted. The predictive model having the smallest mean square error was used to estimate concentrations of cadmium lethal to 50% of the larvae (LC_{50}) hatched from the cadmium-exposed eggs at constant salinities. Straight-line graphical interpolation (APHA 1975) was used to estimate an LC_{50} concentration for larval mortality in the fluctuating salinity regime. In both instances, estimates of LC_{50}s were based on measured amounts of cadmium in exposure tanks. Percentages of larvae dying in the various cadmium-salinity treatments were compared using the Duncan-Multiple Range test.

[1] Use of trade names does not imply endorsement by the United States Environmental Protection Agency.

RESULTS AND DISCUSSION

Percentage total hatches in control treatments at constant salinities were always at least 90% (Table 1). Mean total hatches among metal-exposed eggs in the two tests increased with each incremental increase in test salinity, from a low of about 45% the 10 o/oo - 0.96 mg Cd/l to a high of about 95% for all levels of cadmium at 30 o/oo salinity.

Percentage of larval mortality at the constant salinities was higher among larvae from cadmium-exposed eggs than in control treatments, and was consistently greatest at 10 o/oo and least at 30 o/oo salinity (Table 1). Statistical analyses of these data show that they are best represented by first order equations (Table 2). The pattern of response denoted by these equations contrasts with one exhibited by winter flounder, *P. americanus*, indicating that the eggs and larvae of the two species respond differently to similar ranges of cadmium and salinity. Methods used in treating flounder eggs in the earlier study (VOYER et al. 1977) were essentially identical to those reported here for *Menidia*. Regression equations that best described the responses of flounder included a quadratic term for salinity effects, and show that the survival of larvae from cadmium-exposed eggs increased from a minimum at 10 o/oo salinity to a maximum at about 25 o/oo, then decreased again at 30 o/oo salinity. As indicated by the regression equations presented here no similar decrease in the survival of *Menidia* larvae was evident at 30 o/oo salinity in this series of experiments.

The LC_{50}s based on the computed regression equations are presented in Table 2 and represent another estimate illustrating the linear relationship between salinity increases and increased larval survival. Mean concentrations of cadmium lethal to 50% of the larvae from cadmium-exposed eggs in the two experiments were <0.15, 0.63, and >1.12 mg/l at salinities of 10, 20, and 30 o/oo, respectively.

Percentages total hatches among controls in the fluctuating salinity averaged 93% for the two tests (Table 1). Mean total hatches in cadmium-treated eggs in the fluctuating salinity regime averaged at least 90% in each of the two low metal concentrations and was about 70% at the high cadmium concentration. Mean percentage larval mortality was approximately 63% at 0.97 mg Cd/l, 32% at 0.33 mg Cd/l, and 20% at 0.16 mg Cd/l. Results of the Duncan-Multiple Range tests (Table 2) show that, in terms of percentage larval mortality, responses at the fluctuating salinity were significantly different ($P < .05$) from those at the constant 10 and 30 o/oo salinity regimes. Results of the fluctuating salinity were similar, however, to those at the constant 20 o/oo salintiy.

The chorions of teleosts are apparently permeable to water (HOLLIDAY 1969). The comparative nature of responses in the fluctuating salinity reported here may be due simply to a passive movement of water through the chorions of developing eggs which paralleled the periodic increases and decreases in the salinity of the surrounding water. Information presented in Fig. 2 shows that eggs reared in the fluctuation salinity experienced the extremes

TABLE 1

Percentage response of the Atlantic silverside exposed to cadmium in combination with constant and fluctuating salinity regimes at 15-19°C. Each datum for % Total Hatch is the mean response of 2 groups of 25 eggs. The % Larval Mortality was calculated from the total number of cadmium-exposed eggs hatching in each observation. Larvae were maintained in exposure containers following emergence. Levels of cadmium are the means of measurement recorded daily during the treatment period.

Response Parameter and Test No.	Fluctuating o/oo S				10 o/oo S				20 o/oo S				30 o/oo S			
1																
mg Cd/l	0.0	0.16	0.33	0.94	0.0	0.15	0.36	0.94	0.0	0.17	0.39	0.75	0.0	0.20	0.38	0.97
% Total Hatch	92 96	84 88	96 96	82 96	96 100	92 96	80 84	56 60	80 100	100 100	92 96	72 76	96 100	96 100	100 100	76 100
% Larval Mortality	4 8	32 38	25 33	59 63	13 28	57 71	80 90	50 93	5 8	12 28	29 30	56 78	4 8	4 4	0 4	5 16
2																
mg Cd/l	0.0	0.15	0.32	1.00	0.0	0.15	0.34	0.98	0.0	0.16	0.31	0.74	0.0	0.20	0.42	1.12
% Total Hatch	84 100	100 100	88 100	48 56	84 96	72 80	48 56	24 40	84 96	88 96	84 100	76 80	96 100	96 100	92 100	76 100
% Larval Mortality	4 10	4 4	28 41	50 79	0 10	65 67	75 100	80 83	4 5	0 5	14 20	58 65	0 0	0 0	0 0	12 16

TABLE 2

Ranking of overall mean percentage mortality of larvae (% LM) from eggs exposed to cadmium at constant and fluctuating salinity regimes. Ranking based on results of Duncan-Multiple range tests with means tabulated in decreasing order of magnitude. Means connected by vertical line are not significantly ($p < .05$) different. Regression equations (Y = larval mortality in angular units; X_1 = salinity X_2 = log Cd concentration) were used to estimate LC_{50} levels at the constant salinities; straight-line graphical interpolation was used to estimate median tolerance concentrations at the fluctuating salinity. Estimated LC_{50}'s are based on concentrations of cadmium measured in exposure chambers. Experimental temperatures ranged from 15-18°C in Test 1, and from 17-19°C in Test 2.

Test No.	Salintiy Regimes (o/oo)	Overall Mean % LM by o/oo Regime	LC_{50} % LM in mg Cd/1
1	12.7 (12-13)	60	< 0.16
$\hat{Y} = 1.622 - 0.040\,X_1 + 0.14\,X_2$	Fluctuating (10-30 o/oo)	32 |	0.65
	20.3 (19-23)	30 |	0.54
	29.9 (27-30)	5	> 0.97
2	10.4 (8-14)	60	< 0.15
$\hat{Y} = 1.879 - 0.0530\,X_1 + 0.256\,X_2$	Fluctuating (10-30 o/oo)	27 |	0.57
	20.3 (18-23)	21 |	0.73
	30.4 (27-30)	3	> 1.12

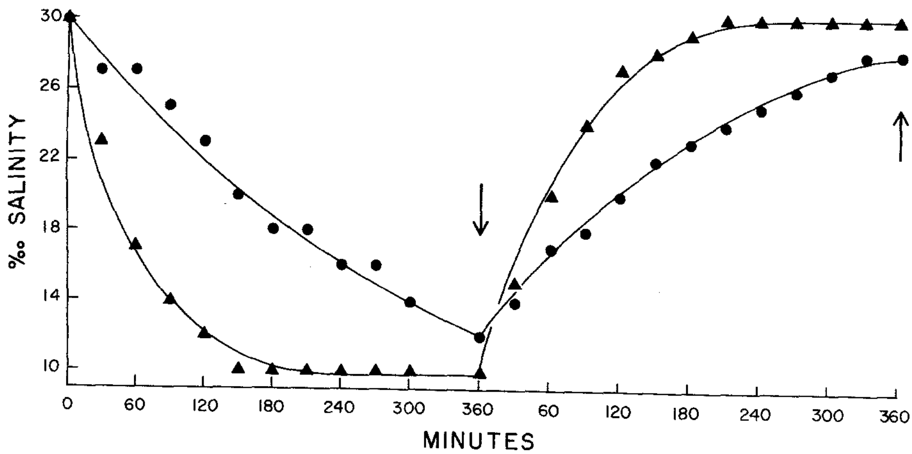

Fig. 2. Graphical representation of salinity changes with time in fluctuating salinity regime. Arrows indicate times at which deionized water was turned off and on. indicate salinity of water flowing through water-mixing-and distribution chamber. indicate salinity in incubation chamber.

of the cyclic regime (10, 30 o/oo) twice and the intermediate salinity four times each 24 hours of treatment. This Figure also indicates that the median salinity in the cyclic regime was approximately 20 o/oo. This interpretation suggests that eggs reared in the fluctuating salinity responded to the influence of that range of salinities most frequently encountered during cadmium exposure.

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