Comparative Sensitivity of Eggs, Larvae and Adults of the Estuarine Teleosts, *Fundulus heteroclitus* and *Menidia menidia* to Cadmium

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INTRODUCTION

Several recent studies have demonstrated the toxic effect of cadmium to specific life history stages of marine teleosts. EISLER (1971), GARDNER and YEVICH (1969, 1970) and JACKIM et al. (1970) conducted toxicity tests with adult mummichogs, Fundulus heteroclitus. ROSENTHAL and SPERLING (1974) determined the sensitivity of eggs of the herring, Clupea harengus L., to cadmium, and WESTERNHAGEN et al. (1975) observed the toxic effect of cadmium to eggs of the garpike, Belone belone L.

The present study measured the sensitivity of two common species of estuarine fish, the mummichog, Fundulus heteroclitus, and the Atlantic silverside, Menidia menidia, to cadmium at specific stages in their life histories. Developing eggs were tested because of the proven sensitivity of this life stage to cadmium toxicity in marine fish.

Three age groups of larvae, 1-, 7- and 14-days old were tested to determine if changes in sensitivity occur during the first few weeks after emergence. Bioassays were also conducted with adults for comparison with data from egg and larval bioassays.

The duration of tests was limited to 48 hrs so that comparisons of sensitivity to cadmium could be made for specific developmental stages (ages) of each species.

MATERIALS AND METHODS

Acquisition of Eggs and Larvae

Adult Fundulus used as spawning stock were collected from We Creek, Wadmalow Island, South Carolina during May, 1973. Fish were isolated according to sex in acrylic plastic flow-through aquaria

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(volume 18 l) which received 40 l/hr of unfiltered seawater. Water temperature ranged from 19-23°C and salinity from 22-26 ppt. Fluorescent 'cool white' lamps remained on 24 hrs/day (1400 lux). Fish were fed chopped shrimp, Peneaus setiferus, daily.

Intramuscular injections of 50 I.U. of Haver Lockhart chorionic gonadotropin (code 0160)[2] per fish were administered anterior to the anal fins for three consecutive days. Eggs were stripped into glass culture dishes (containing 20 and 30 ppt salinity water) 48 hrs after the third (final) dose of hormone. Milt was then stripped into the culture dishes. After 5 minutes, the demersal eggs were placed in MPC hatchery jars (Midland Plastics, Inc.). Salinities of 20 and 30 ppt and temperatures from 21-23°C were maintained for respective hatchery jars. A Dyna-Flo^R filter system containing activated charcoal and glass wool was used to circulate water through the hatchery jar and a 40 l glass aquarium.

A simultaneous hatch of Fundulus eggs was induced on the 15th day after fertilization by siphoning 2 liters of seawater from the hatchery jar and replacing it with unfiltered seawater of identical temperature and salinity. The mechanism for this response is unknown but it allowed us to acquire larvae of uniform age for use in bioassays. Fundulus larvae were fed Artemia nauplii each day beginning immediately after emergence.

Sexually mature Menidia were collected from Botany Island, South Carolina from April through July 1973; water temperature 16 to 25°C, salinity 27 to 31 ppt. Eggs from ripe females were stripped into glass culture dishes containing 20 and 30 ppt salinity water. Milt was then stripped into the culture dishes.

Fertilized Menidia eggs form gelatinous threads which bind them together. The gelatinous egg mass was attached to a 250 mm length of nylon twine and suspended in a 20 l glass aquarium containing filtered aerated seawater at the fertilized salinity and 21-23°C.

Menidia larvae emerged 6 to 10 days after fertilization. They were fed Artemia nauplii daily. For a detailed account of methods for spawning and rearing Menidia refer to MIDDAUGH and LEMPESIS (1976).

Bioassays

Forty-eight hour acute toxicity bioassays with developing eggs, larvae and adults were conducted by techniques outlined in Standard Methods (APHA, 1971). A stock solution of 1.0 g/l of reagent grade (A.C.S.) cadmium as $CdCl_2 \cdot 2\frac{1}{2}\ H_2O$ prepared in distilled water was

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[2]Mention of trade names does not imply endorsement by the U. S. Environmental Protection Agency.

used for exposing experimental organisms. A minimum of 5 exposure concentrations were used in each bioassay. Checks of seawater-cadmium solutions in bioassay containers were made with a Perkin-Elmer Model 403 Atomic Absorption Spectrophotometer. Less than 6 percent error was detected between nominal (32, 18, 10, 5.6, 3.2, 1.0, 0.56, .032, 0.10 mg/l), and measured cadmium concentrations at the beginning of exposure intervals. There was no detectable loss of cadmium from the test containers during the 48 hr exposures. Bioassays with all three age classes (eggs, larvae and adults) were conducted in filtered 20 and 30 ppt salinity seawater at 20±1°C.

Bioassays with developing Fundulus eggs were conducted in 500 ml glass jars containing 300 ml of seawater. Thirty newly fertilized eggs were placed in each test container. No aeration was used. Dissolved oxygen levels in test containers remained above 5 mg/l. After 48 hrs of exposure to cadmium, eggs were removed, triple rinsed in clean seawater and placed in containers of clean 20 and 30 ppt salinity water. On the 15th day after fertilization, 100 ml aliquots of seawater were removed from each container and replaced with an equal amount of unfiltered seawater at the test temperature and salinity. This procedure stimulated larval emergence. Observations for total percentage mortality (non-emergence) were made on the 16th day after fertilization.

Bioassays with developing Menidia eggs were conducted in 4 l glass jars containing 3 l of seawater. Newly fertilized eggs were separated into groups of 40 to 80 eggs. Individual groups were attached to a length of nylon twine and suspended in test containers. Gentle aeration maintained dissolved oxygen levels above 5 mg/l in each container. After 48 hrs of cadmium exposure, eggs were triple rinsed in clean seawater and resuspended in containers of clean 20 and 30 ppt salinity seawater.

Larval bioassays with Fundulus and Menidia were performed with three age groups, 1-, 7- and 14-days after hatching. Twenty larvae (two groups of 10), were exposed to cadmium in 500 ml glass jars containing 300 ml of seawater. The 48-hr LC50 and 95 percent confidence intervals for each bioassay were calculated by the probit analysis method (LITCHFIELD and WILCOXON, 1949).

Adult Fundulus and Menidia were held in 80 l acrylic plastic aquaria at 20 and 30 ppt salinity and 21-23°C for 5 days prior to use in bioassays. Fundulus were fed chopped shrimp, Peneaus setiferus and Menidia live Artemia daily. Bioassays were conducted in 20 l glass jars containing 16 l of seawater. Ten adults of each species (unfed for 24 hrs) were tested at each cadmium concentration. Gentle aeration maintained dissolved oxygen levels above 5 mg/l in each container. Mortality data were subjected to probit analysis.

RESULTS

Results of tests with developing Fundulus eggs indicate that they are not very sensitive to cadmium toxicity during the first 48 hrs after fertilization. In tests at both 20 and 30 ppt salinity, the maximum mortality (non-emergence) was 54 percent for the highest exposure concentration of 32 mg/l cadmium (Table 1). A similar resistance to cadmium was observed for developing Menidia eggs. Mortality for eggs exposed to 32 mg/l cadmium was 66 percent at 20 ppt salinity and 60 percent at 30 ppt salinity (Table 1).

Control mortalities in excess of 10 percent for each of the bioassays with developing Fundulus and Menidia eggs prevented statistical treatment (probit analysis) of test results. However, a trend of decreasing mortality with decreased cadmium concentrations was observed in each of the bioassays.

TABLE 1

PERCENTAGE MORTALITY (NON-EMERGENCE) OF LARVAE FROM FUNDULUS HETEROCLITUS AND MENIDIA MENIDIA EGGS EXPOSED TO CADMIUM FOR THE FIRST 48 HRS AFTER FERTILIZATION

Species Tested	Cadmium Concentration mg/liter	Percentage Mortality Salinity ppt 20	30
Fundulus heteroclitus	32.0	54	54
	10.0	40	54
	3.2	30	50
	1.0	27	47
	0.32	20	23
	control	17	33
Menidia menidia	32.0	66	60
	10.0	52	38
	3.2	58	40
	1.0	36	36
	0.32	50	38
	control	36	33

Toxicity data for 3 age groups of Fundulus larvae indicates that sensitivity to cadmium differed with age. In bioassays at 20 ppt salinity, maximum larval sensitivity occurred 7 days after emergency. This trend was statistically significant (Table 2). For

tests conducted at 30 ppt salinity 1-day old Fundulus larvae were significantly less sensitive to cadmium toxicity than 7- or 14-day old larvae (Table 2).

Salinity rarely had any significant effect on the toxicity of cadmium to larval Fundulus. Bioassay data for each age class of larvae were significantly different between 20 and 30 ppt salinity only for 14-day old larvae.

TABLE 2

RESULTS OF BIOASSAYS WITH LARVAL FUNDULUS HETEROCLITUS TESTED AT 20 AND 30 PPT SALINITY AND 20±1°C

Larval Age Days	Salinity ppt	48-hr LC50 mg/l Cd	95% C. I.
1	20	16.2	12.7-21.2
7		9.0	6.4-12.5
14		32.0	24.6-41.6
1	30	23.0	19.2-27.6
7		12.0	9.2-15.6
14		7.8	5.6-10.3

Menidia larvae were more sensitive to cadmium than were Fundulus larvae. Older Menidia larvae were more sensitive to cadmium toxicity at both 20 and 30 ppt salinity than young larvae (Table 3). At 20 ppt salinity there was no statistically significant difference in sensitivity with increased age although this trend is obvious. In tests at 30 ppt salinity, 14-day old larvae were significantly more sensitive than 1-day old larvae (Table 3).

There was no significant difference in the toxicity of cadmium for each age class of Menidia tested at 20 and 30 ppt salinity.

Adult Fundulus were apparently, but not statistically, more sensitive to cadmium-toxicity at 30 ppt than at 20 ppt salinity. Adult Menidia which were more sensitive than adult Fundulus showed nearly identical responses to cadmium at 20 and 30 ppt salinity (Table 4).

TABLE 3

RESULTS OF BIOASSAYS WITH LARVAL *MENIDIA MENIDIA* TESTED AT 20 AND 30 PPT SALINITY AND 20±1°C

Larval Age Days	Salinity ppt	48-hr LC50 mg/l Cd	95% C. I.
1	20	3.8	2.7-5.3
7		3.2	1.9-4.7
14		2.2	1.6-2.9
1	30	5.6	4.0-7.8
7		3.4	2.1-4.9
14		1.6	1.1-2.4

TABLE 4

RESULTS OF BIOASSAYS WITH ADULT *FUNDULUS HETEROCLITUS* AND *MENIDIA MENIDIA* TESTED AT 20 AND 30 PPT SALINITY AND 20±1°C

Species Tested	Salinity ppt	48-hr LC50 mg/l Cd	95% C.I.
Fundulus heteroclitus	20	60	40-90
	30	43	33-56
Menidia menidia	20	13	9-20
	30	12	8-16

DISCUSSION

Results of tests with developing *Fundulus* and *Menidia* eggs indicate that they are not very sensitive to cadmium toxicity. The eggs of both *Fundulus* and *Menidia* are found in very rigorous environments (ABLE and CASTAGNA, 1975; HILDEBRAND, 1922). It is possible that the developing embryos were shielded from cadmium toxicity by the chorionic membrane.

In tests with developing eggs of other marine teleosts, WESTERNHAGEN and DETHLEFSEN (1975) also assumed that a major portion of the cadmium accumulated by eggs of the Baltic flounder, Pleuronectes flesus, was fixed in the chorion. On a study with developing eggs of the herring, Clupea harengus L., ROSENTHAL and SPERLING (1974) found that most of the cadmium was accumulated in the chorion, the embryo and newly emerged larvae showed only traces of cadmium.

Larval Fundulus and Menidia were more sensitive to cadmium toxicity than were the eggs or adults. Low sensitivity of 1-day old Fundulus larvae to cadmium in bioassays at each test salinity may be related to a lack of gill function in this age group. Armstrong and Child (1965) found that newly hatched Fundulus larvae showed no coordination between mouth movements and the gills which remained immobile. Coordination of lower jaw and opercular movements was not observed until about 4 days after larval emergence.

For 7-day old larvae tested at each salinity, increased sensitivity may have resulted from the susceptibility of the differentiated and functional gill lamellae to cadmium toxicity. GARDNER and YEVICH (1970) observed epithelial tissue coagulation in the gills of adult Fundulus exposed to cadmium.

A significant increase in the sensitivity of 14-day old Fundulus larvae tested at 30 ppt salinity compared to those tested at 20 ppt salinity was observed. This increased sensitivity at the higher test salinity follows a trend observed by EISLER (1971) in which adult Fundulus were found to be more susceptible to cadmium toxicity at higher salinities.

The sensitivity of Menidia larvae to cadmium increased with increasing age. In a study to determine optimal rearing conditions for Menidia (MIDDAUGH and LEMPESIS, 1976) we found that larvae reared at 30 ppt salinity consistently had better survival rates than those reared at 20 ppt salinity. In the present study a consistent trend of greater resistance to cadmium toxicity was also apparent for larvae tested at 30 ppt salinity compared to those tested at 20 ppt salinity.

Data from bioassays with adult Fundulus generally agrees with that of EISLER (1971), who determined that this species is slightly more sensitive to cadmium at high salinity regimes than at intermediate salinities. Data for adult Menidia also indicate only slight differences in the response of fish tested at 20 and 30 ppt salinity.

These results emphasize the need for utilization of differing developmental stages of test species when establishing water quality criteria. Values that are based on experiments with adults only may lead to inadequate interpretation and generalizations when considering the impact of a pollutant.

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