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Effect of Calcium on Cadmium Uptake and Toxicity in Larvae and Juveniles of Striped Bass (*Morone saxatilis*)

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The recent decline in the striped bass fishery on the east coast of the United States has prompted a concern over the effects of anthropogenic pollutants on this species, especially in important spawning grounds such as the Chesapeake Bay. However toxicological information on this species has been difficult to gather, as a result of the sensitivity of the species to laboratory handling and its short-term availability in the field. In the Chesapeake system, eggs and larvae are available from late March until early June.

A number of studies have shown that water hardness may have a sparing action on cadmium toxicity in freshwater fish (e.g. Pickering and Henderson 1966; McCarthy et al. 1978; Carroll et al. 1979; Calamari et al. 1980) and in the estuarine environment several investigators have stressed the role of calcium in an overall salinity effect on cadmium toxicity (Westernhagen et al. 1974; Wright and Frain 1981).

Recent data on striped bass obtained by the U.S. Fish and Wildlife Service (1983) indicate that the toxicity of cadmium to this species is higher in freshwater than in salt water. The current study was undertaken to elucidate the role of calcium in modifying cadmium toxicity in striped bass larvae and juveniles. Toxicity data were obtained from one-day-old, one-week-old and six-weeks-old animals from a single larval batch.

In both fish and crustaceans there is evidence to suggest that cadmium may alter calcium metabolism (Larsson et al. 1976; Wright 1980). In one-week-old larvae calcium uptake was followed for 5 days in cadmium-exposed and cadmium free conditions and at two different calcium levels.

MATERIALS AND METHODS

Striped bass eggs were obtained from the Cedarville Hatchery, Waldorf, Maryland. After hatching they were maintained at 20^{o}C under 12h light/12h dark conditions until use in experiments.

During experiments, animals were placed in aerated test solutions in polycarbonate containers maintained at 17°C. Mortalities were recorded from duplicate containers each containing 25 larvae at a density of 5 larvae l^{-1}. An experimental run was discarded if larval mortality exceeded 30% over a 96h period. Data were corrected for background (control) mortality by the application of Abbots' (1925) formula.

After disappearance of the yolk sac, larvae were fed Artemia nauplii on a daily basis at a density of 500 nauplii l^{-1}. Thus, experiments on one-day-old larvae were conducted without added food. One-week-old larvae and six-eek-old juveniles were fed throughout experiments.

High calcium water consisted of undiluted Solomons tapwater (113.4 mg Ca l^{-1}; 63.2 mg Mg l^{-1}) with calcium chloride added to bring the final calcium concentration to 125 mg Ca l^{-1}. Total hardness of this medium was 572.2 mg l^{-1} ($CaCO_3$ equivalent). Low calcium water was prepared by diluting Solomons tapwater with deionized distilled water. In order to bring other major ions up to the levels found in high-calcium water, NaCl and KCl were added in the form of stock solutions which had been prepared with reagent grade chemicals and run through Chelex-100 (BioRad 100-200 mesh sodium form) to remove trace elements. High and low calcium media had only 8 and 3ug Zn l^{-1}, respectively. Sodium and potassium concentrations in both high an low-calcium media were maintained at 20.6 and 15.7 mg l^{-1}, respectively. Chloride levels were 40 mg l^{-1} in low-calcium and 29 mg l^{-1} in high-calcium media. The final measured calcium and magnesium concentrations in the low-calcium media were 8 and 6 mg l^{-1}, respectively resulting in a total hardness figure of 44.7 mg l^{-1} ($CaCO_3$ equivalent).

At the start of each experiment, pH was buffered to 7.5 using the 0.05M N-Tris (hydroxymethyl) methyl-2-aminoethanesulfonic acid (TES) buffer described by Good et al. (1966).

For selected treatments using one-week-old larvae, extra tanks were run in order to provide specimens for larval calcium and cadmium analyses. Unlike toxicity data, which were only available from duplicate runs, metal analyses were generally made on triplicate groups of 6-10 larvae although fewer animals were available from higher cadmium concentrations and longer exposure times. Animals were wet oxidized with redistilled concentrated nitric acid before suitable dilution and analysis by atomic absorption spectroscopy.

RESULTS AND DISCUSSION

Tables 1 and 2 show toxicity data from larval fish, respectively before and after the disappearance of the yolk sac. Table 1 records larval mortality in low-calcium media up to 52h (control mortalities in high-calcium media exceeded 30% after 48h, probably due to an aeration failure, and were discarded). In these very young larvae cadmium-dependent mortality was only seen in 20ug Cd l^{-1}.

Table 1. Cadmium toxicity in 1-day-old striped bass larvae (low calcium medium; 8 mg Ca l^{-1}; total hardness 44.7 mg $CaCO_3$ equivalent l^{-1}). Figures are mean percentage mortalities from duplicate tanks.

Cd concentration (ug l^{-1})	Time (h) 16 h	36 h	52 h
Control	0	15	15
	5	5	5
1	2 (0)*	6 (0)	12 (2)
	2 (0)	5 (0)	15 (6)
2	0 (0)	5 (0)	12 (2)
	2 (0)	5 (0)	18 (9)
5	6 (3)	6 (0)	9 (0)
	6 (3)	8 (0)	10 (0)
10	2 (0)	4 (0)	7 (0)
	6 (3)	8 (0)	12 (2)
20	2 (0)	72 (69)	89 (88)
	6 (3)	48 (42)	73 (70)

*Numbers in parentheses are corrected for background (mean control) mortality using Abbott's (1925) formula.

Week-old larvae (Table 2) showed mortalities after 120h exposure to low-calcium media having cadmium concentrations as low as 5ug l^{-1}. Exposure to 10ug Cd l^{-1} for 51h resulted in mortalities of 78% in low-calcium media (mean of 2 experimental runs) although there were no significant mortalities in high-calcium media at these cadmium levels. One-day-old larvae in low-calcium medium showed no significant mortalities after 52h exposure to 10ug Cd l^{-1} indicating a possible greater resistance in yolk sac larvae than post yolk sac larvae. However toxicity tests using six-week-old juveniles show considerably greater resistance than larval stages (Table 3). In low-calcium medium containing 20ug l^{-1}, 100% mortality was reached between 268 and 290h, whereas in the corresponding high calcium medium mortality had only reached 22% after 290h.

Results of total body calcium and cadmium analyses of 1-week-old larvae are presented in Figure 1. From figure 1A it appears that, after 120h, the degree of cadmium accumulation from 10ug Cd l^{-1} is inversely related to the calcium concentration of the medium. Cadmium concentrations were calculated as ng. Cd per larva although batches of larvae were weighed at the beginning and end of the experiment to enable concentrations to be placed on an approximate dry weight basis. For example, at the end of the bioassay the mean larval weight was 0.225 mg. Applying this

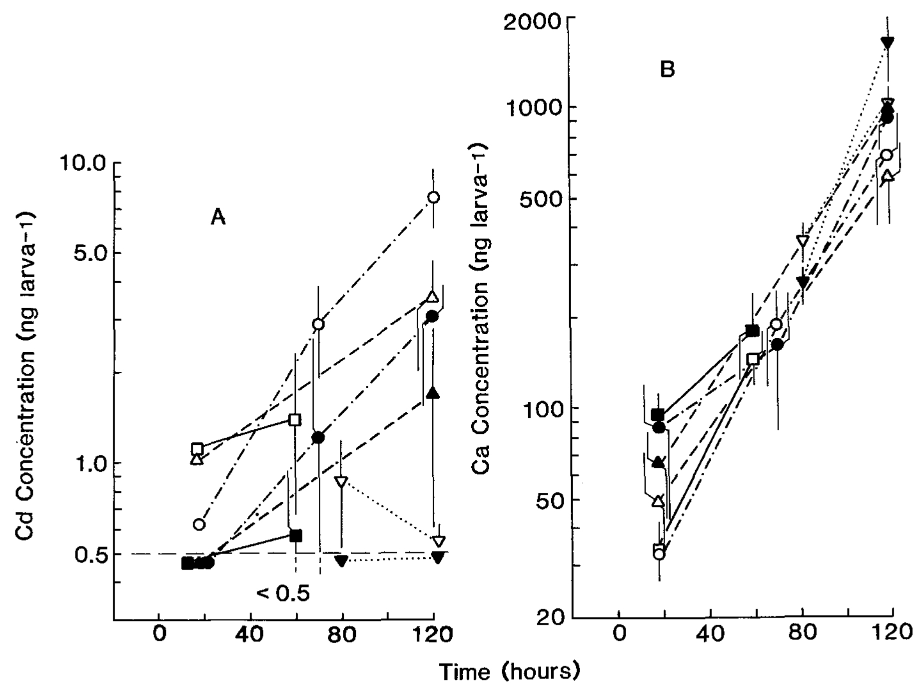

Figure 1. Cadmium (A) and Calcium (B) uptake in selected high-calcium (solid symbols and low calcium (hollow symbols) media containing different concentrations of cadmium. High-calcium medium: 125 mg Ca l^{-1}, total hardness 572.2 mg $CaCO_3$ equiv. l^{-1}. Low calcium medium: 8 mg Ca l^{-1}, total hardness 44.7 mg $CaCO_3$ equiv. l^{-1}. Vertical lines indicate ±I.S.D. ▼ - high Ca controls, ▲ - high Ca, 5ug, Cd L^{-1}, ● - high Ca, 10ug, Cd L^{-1}, ■ - high Ca, 20ug, Cd L^{-1}, ▽ - low Ca controls, Δ - low Ca, 5ug, Cd L^{-1}, o - low Ca, 10ug, Cd L^{-1}, □ - low Ca 20ug, Cd L^{-1}.

weight to the 120h cadmium data from 10ug Cd l^{-1} gives a cadmium concentration of 34.2ug Cd g dry wt^{-1} for low-calcium medium and 13.8ug Cd g dry wt^{-1} for high-calcium media. Calcium levels in the same larvae are shown in figure 1B. In both high and low calcium media analyses of variance reveal significant differences between control and cadmium treated animals. After 120h larvae exposed to 5ug Cd l^{-1} under low calcium conditions had significantly lower calcium levels ($p<0.05$) than controls in high calcium media, larvae exposed to 10ug Cd l^{-1} had lower calcium concentrations relative to the appropriate controls ($p<0.05$).

Cadmium uptake rates found in the current study tend to exceed those obtained from investigations of salt-water fish when external cadmium levels are taken into account (Dethlefsen et al., 1975; Middaugh et al. 1975).

Calcium levels in selected larvae from these experiments are shown in Figure IB. Transformations of calcium concentrations to a "dry weight" basis, as performed above on cadmium data, result in figures of 480 $\pm$ 236ug Ca g dry wt^{-1} (low-Ca medium) and 667 $\pm$ 189ug Ca g dry wt^{-1} (high-Ca medium) after 18h (5ug Cd l^{-1}). Calcium concentrations in control animals after 120h were 4502 $\pm$ 738 ug Ca g^{-1} and 7151 $\pm$ 1738 ug Ca g^{-1} in low-Ca and high-Ca media, respectively. Larval calcium levels were apparently dependent to some extent on the calcium concentration of the external medium and there is some evidence to suggest that body calcium levels in low-calcium media may be lower in the presence of cadmium.

The two calcium concentrations investigated here represent levels which could be found in very hard and soft waters within the natural environmental range. Clearly calcium exerted a considerable effect on cadmium toxicity although the overall toxicity levels varied according to the developmental stage. By comparison with week-old larvae, recently hatched animals are somewhat more resistant, although six-week-old juveniles are significantly more resistant than early larvae. We could find no evidence of cadmium-induced changes in water quality parameters over the range of cadmium concentrations used here and no large changes in cadmium concentrations over the experimental period. It therefore seems that the sparing action of calcium seen under current conditions is largely physiological in nature.

A feature of the present study was the very sensitive nature of striped bass larvae. In soft water, cadmium-induced mortality in 1-week-old larvae was seen after 120h exposure to 5ug Cd l^{-1} (Table 2) and in 10ug Cd l^{-1} (Table 2) mortality exceeded 50% after 24h (but slowed thereafter). Given differences in experimental conditions these figures are highly comparable with recent mortality data obtained by the U.S. Fish and Wildlife Service (1983) who found 96h LC50 values for striped bass larvae in "reconstituted" water (hardness = 39mg $CaCO_3$ l^{-1}) and well water (hardness = 285mg $CaCO_3$ l^{-1}) of 4ug l^{-1} (95% confidence interval, 3-6ug l^{-1}) and 10ug l^{-1} (95% confidence interval, 6-16ug l^{-1})

Table 2. Cadmium toxicity in 7-day-old striped bass larvae. Figures are percentage mortalities from duplicate tanks.

Cd Concentration (ug l^{-1})	High Ca Medium (125 mg Ca l^{-1}; total hardness 572.2 mg $CaCO_3$ eqiv. l^{-1})			Low Ca Medium (8 mg Ca l^{-1}; total hardness 44.7 mg $CaCo_3$ equiv. l^{-1})		
	24 h	51 h	120 h	24 h	51 h	120 h
Control	20	25	25	15	20	30
	25	25	30	25	25	30
5	15 (0)*	20 (0)	35 (10)	20 (0)	20 (0)	60 (43)
	10 (0)	20 (0)	36 (11)	10 (0)	15 (0)	40 (14)
10	20 (0)	23 (0)	23 (0)	70 (63)	80 (74)	95 (93)
	17 (0)	21 (0)	30 (3)	70 (63)	85 (81)	190 (186)
50	45 (29)	65 (53)	80 (72)	60 (50)	75 (68)	100 (100)
	40 (23)	60 (47)	80 (72)	65 (56)	80 (74)	100 (100)
100	67 (57)	70 (60)	87 (82)	90 (88)	100 (100)	100 (100)
	60 (48)	83 (77)	97 (96)	90 (88)	100 (100)	100 (100)
250	70 (61)	93 (91)	100 (100)	93 (92)	100 (100)	100 (100)
	67 (57)	83 (77)	100 (100)	93 (92)	100 (100)	100 (100)

*Numbers in parentheses are corrected for background (mean control) mortality using Abbott's (1925) formula.

Table 3. Cadmium toxicity in 6-week-old juvenile striped bass. Figures are percentage mortalities from duplicate tanks.

Cd Concentration (ug l^{-1})	High Ca Medium (125 mg Ca l^{-1}; total hardness, 572.2 mg $CaCO_3$ equiv. l^{-1})				Low Ca Medium (8 mg Ca l^{-1}; total hardness, 44.7 mg $CaCO_3$ equiv. l^{-1})			
	24 h	144 h	268 h	290 h	24 h	144 h	268 h	290 h
Control	0	0	0	10	0	0	0	0
	0	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0	10
	0	0	0	0	0	0	0	15
2.0	0	0	0	0	0	0	0	15
	0	0	0	0	0	0	0	10
5.0	0	0	5	5 (0)*	0	0	15	40
	0	0	15	25 (21)	0	0	10	35
10.0	0	0	0	25 (21)	0	20	0	80
	0	0	0	15 (11)	0	20	20	70
20.0	0	5	15	20 (16)	0	20	70	100
	0	15	25	35 (32)	20	30	80	100

*Numbers in parentheses are corrected for background (mean control) mortality using Abbott's (1925) formula.

respectively. Such values are consistent with the 96h LC50 value of 1ug Cd l^{-1} reported for striped bass larvae by Hughes (1973) and indicate a degree of susceptibility to cadmium similar to that found in salmonids. For example, McCarthy et al. (1979) reported that brook trout, (Salvelinus fontinalis), reached 100% mortality in 1.5ug Cd l^{-1} in soft water (42 mg l^{-1} as $CaCO_3$ compared with the hardness value of 45 mg l^{-1} in low-calcium water, used here) in under 96h. Six-week-old juvenile striped bass showed considerably greater resistance than larvae (Table 3). Nevertheless the 290h LC50 value of 6ug l^{-1} derived from that assay indicated a high sensitivity for a freshwater fish.

Although calcium data are limited, there is some evidence that calcification may be adversely affected by the presence of cadmium in the medium. In both hard and soft water the calcium levels in animals from cadmium-free media were significantly higher after 120h than in animals from cadmium-dosed media (Fig. IB). In soft water animals these findings may be queried on the grounds that a high mortality had already been reached by that time, and those used for analysis may have been in poor condition. However, high-calcium larvae had virtually no mortality and still showed significantly lowered calcium levels in the presence of cadmium. Although this may not necessarily contribute to acute toxicity, it is consistent with reports of cadmium-induced sketelal abnormalities and hypocalcaemia in Phoxinus phoxinus reported by Bengtsson et al. (1975) and Larsson et al. (1976), and indicates that studies of chronic effects on calcium metabolism by very low cadmium levels would be valuable in developing striped bass.

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