Relation of Environmental Calcium and pH to the Stress Response of Striped Bass

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Abstract: Striped bass fingerlings *(Morone saxatilis)* acclimated to hard water (110 mg/liter as calcium carbonate, 41.0 mg/liter as calcium) were exposed for 96 hours at pH 7.7, 6.1, or 4.2 in hard water. In another experiment, striped bass fingerlings acclimated to soft water (32 mg/liter as calcium carbonate, 9.5 mg/liter as calcium) were exposed for 96 h at pH 7.7, 6.1, or 4.2 in soft water. No mortalities occurred during the experiments. The primary stress response and electrolyte disturbance were less severe in fish acclimated to hard water than in fish acclimated to soft water, suggesting that calcium acclimation history may be an important consideration when stocking striped bass into acidic waters.

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Striped bass *(Morone saxatilis)* populations have reportedly decreased in the Chesapeake Bay region over recent years. The buffering capacity (i.e., salinity and alkalinity conditions) of the Bay is generally considered sufficient to neutralize acidic conditions, but this ability is reduced in the tributaries (Richards and Rago 1999). Factors found in the tributaries that have contributed to the decline in the striped bass population include low pH, high aluminum concentrations, and low environmental calcium levels.

The unfavorable environmental conditions in the tributaries of the Chesapeake Bay have led to the practice of capturing wild brood fish and artificially spawning and raising the fry in hatcheries throughout the southeastern United States for release into public water. Water quality characteristics in hatcheries where fry have been cultured are notably different in salinity, hardness, and alkalinity from those in

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the Chesapeake Bay. Fish reared in the hatcheries typically have to acclimatize to water with differing characteristics when they are stocked. Water quality at the stocking locations may be extremely different from that in which fish were cultured and exposure to a change in environmental conditions often induces a stress response in fish.

Calcium ions have long been known to be important in reducing the toxicity of acid waters. Brown (1981) observed that the elevation of environmental calcium reduced the toxic effects of low pH (3.5 to 4.0) to fingerling brown trout *(Salmo trutta)*, as compared to other ions tested (Na+, K+, Mg^{+2}, and Al^{+3}). Studies of the physiological basis of acid stress have indicated the importance of calcium levels on acid-base and osmoregulatory mechanisms. Exposure of rainbow trout *(Oncorhynchus mykiss)* to pH 4.3 in low-calcium water (6 mg/liter) resulted in significant osmoregulatory dysfunction and increased mortality, but only minor acid-base disturbance, whereas the exposure of rainbow trout to low pH in higher calcium (32 mg/liter) resulted in major blood acidosis, but only minor osmoregulatory dysfunction (McDonald et al. 1980, McDonald 1983).

The ecological consequences of acid stress in aquatic ecosystems, especially on fish populations, have generated much interest (Fromm 1980, Brown 1982, Leivestad 1982, Wood and McDonald 1982, McDonald 1983*a*, Wood 1987). The effect of H^+ on osmoregulation in fish depends not only on environmental calcium levels, but also on the calcium acclimation history of the fish. McWilliams (1982) found that the combination of low pH and calcium caused an increase in sodium efflux in brown trout that was substantially more than the efflux caused by low pH alone. However, when fish were first acclimated to low calcium water and then exposed to low pH, the sodium efflux was much less. Using another species, McDonald et al. (1983) found that the chloride efflux was higher in acid-exposed rainbow trout acclimated to soft water (1.2 mg/liter as calcium) than in fish acclimated to hard water (114 mg/liter as calcium). The high sodium efflux was virtually unaffected by the environmental calcium in this experiment.

The primary stress response in vertebrates—elevated cortisol levels (Mazeaud et al. 1977), is often associated with secondary stress responses, including elevated plasma glucose and osmoregulatory dysfunctions (Wedemeyer 1972, Donaldson 1981, Wedemeyer and McLeay 1981). Under acute acid stress (pH 4.0 to 4.5), elevated concentrations of plasma cortisol and glucose have been reported (Mudge et al. 1977, McDonald 1983*b*, Brown et al. 1984, Adams et al. 1985, Brown et al. 1986*a*, 1986*b*, Tam et al. 1988) along with osmoregulatory dysfunctions (McDonald and Wood 1981, Ultsch et al. 1981, Booth et al. 1982, Goss and Wood 1988, Wood et al. 1988).

The objective of this study was to determine the effects of environmental calcium and pH on the stress response and survival in striped bass fingerlings in freshwater.

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Methods

Striped bass fingerlings (104±3 g; mean + SE) were maintained at the Southeastern Fish Cultural Laboratory for at least 4 weeks in 1200-liter fiberglass tanks supplied at a flow rate of 2 turnovers per hour with aerated well water here termed ambient hard water (23 C, pH 7.5, hardness 110 mg/liter as calcium carbonate, calcium 41.0 mg/liter, alkalinity 108 mg/liter as calcium carbonate, dissolved oxygen >6.0 mg/liter, chloride 1.06 mEq/liter, and nitrite and ammonia <0.01 mg/liter). The fish were fed commercial fish food (40% protein) daily, feeding was discontinued 48 hours before the fish were transferred into experimental aquaria or tanks for acclimation to soft or hard water. Fish continued to be fed commercial food while in the acclimation tanks.

Fish Acclimation

Striped bass were transferred into 1200-liter fiberglass tanks supplied with water circulated through a biological filter at a flow rate of 19 liter/minute. One group of fish was maintained in the ambient hard well water (110 mg/liter as calcium carbonate) with the previously described characteristics for 1 month. Another group was acclimated to a slow decrease in water hardness over a 2-week period and acclimated for 1 month to soft water (23 C, pH 7.1, hardness 29 mg/liter as calcium carbonate, calcium 9.5 mg/liter, alkalinity 28 mg/liter as calcium carbonate, dissolved oxygen >6.0 mg/liter, chloride 0.05 mEq/liter, nitrite and ammonia <0.01 mg/liter).

Experimental Design

Striped bass acclimated to hard water (110 mg/liter as calcium carbonate, 41.0 mg/liter as calcium) were exposed to 3 levels of pH for 96 hours. Striped bass acclimated to soft water (32 mg/liter as calcium carbonate, 9.5 mg/liter as calcium) were exposed to 3 levels of pH in soft water for 96 hours. The methodology for both experiments was the same: 20 fish were stocked into each of 6 80-liter glass aquaria. The pH levels (in duplicate) of 7.7 (ambient), 6.1, and 4.2 were maintained by using a peristaltic pump (Technicon Instrument Corp., Tarrytown, N.Y.) to deliver a 1:1 combination of nitric and sulfuric acid diluted as needed with deionized water. Water quality characteristics were measured every 24 hours. The pH levels were maintained as follows throughout the experiment: 7.7±0.2, 6.1±0.3, and 4.2±0.2. Five fish from each aquarium were sampled at 24, 48, 72, and 96 hours. The 0-hour specimens were taken immediately before stocking into aquaria and were considered control fish. All fish were anesthetized in a 0.02% solution of tricaine methanesulfonate before they were bled.

Sample Collection and Analytical Procedures:

Blood was collected in ammonia-heparinized syringes from vessels in the caudal peduncle. Sampling was completed within 5 minutes after initial disturbance and no fish was bled more than once. Blood was centrifuged and the plasma stored at –20

C until analyzed. Plasma concentrations were determined for total corticosteroids by radioimmunoassay with a commercially prepared kit for cortisol (Serono Diagnostics, Norwell, Mass.), glucose with a clinical kit (Sigma Chem. Co., St. Louis, Mo.), chloride by amperometric-coulometric titration (Am. Instrument Co., Silver Spring, Md.), sodium by a flame photometer (model 51C, Perkin-Elmer, Norwalk, Conn.), and potassium and calcium by atomic absorption spectrophotometry (model 372, Perkin-Elmer, Norwalk, Conn.).

The data are presented as means ±SE. Analysis of variance, followed by Duncan's multiple range test, was used to test for treatment effects where appropriate. The level of significance used for all tests was $P \leq 0.05$.

Results

Water quality characteristics remained within acceptable ranges throughout the experiments and did not significantly vary from those described for hard water and soft water. No fish died during the experiments.

Plasma in striped bass acclimated in soft water differed significantly from that of fish acclimated in hard water at 0 hours (Table 1). In striped bass acclimated in hard water, plasma ion disturbance was generally reduced compared to that of fish acclimated in soft water.

Plasma cortisol in fish acclimated in hard water did not vary significantly among the 3 pH levels during the 96-hour experiment (Fig. 1). Plasma cortisol in fish acclimated to soft water did not vary significantly from control levels at pH 7.7. Plasma cortisol levels in fish acclimated to soft water were significantly higher than control levels at pH 6.1 and 4.2, except at 24 hours (pH 6.1).

Plasma glucose levels in fish acclimated in hard or soft water did not significantly differ from control levels at pH 7.7 (Fig. 2). In fish acclimated to hard water, plasma glucose levels were significantly higher than control levels at pH 4.2, except at 24 hours. In fish acclimated to soft water, plasma glucose levels were significantly higher than control levels at pH 4.2 and 6.1, except at 24 hours.

Table 1. Plasma for striped bass acclimated to hard water (110 mg/liter as calcium carbonate, 41.0 mg/liter as calcium) or acclimated to soft water (32 mg/liter as calcium carbonate, 9.5 mg/liter as calcium) at 0 hours (mean±SE, N=20). Asterisk denotes significant differences between values.

Variables and unit	Hard water	Soft water
Cortisol (ug/dliter)	6.1±0.7	6.5±0.2
Glucose (mg/dliter)*	94.0±3.4	125.8±4.6
Chloride (mEq/liter)	93.3±2.4	97.6±3.4
Sodium (mEq/liter)*	116.3±2.4	101.7±2.9
Potassium (mEq/liter)*	4.7±0.3	8.8±0.5
Calcium (mEq/liter)	4.2±0.1	4.2±0.1

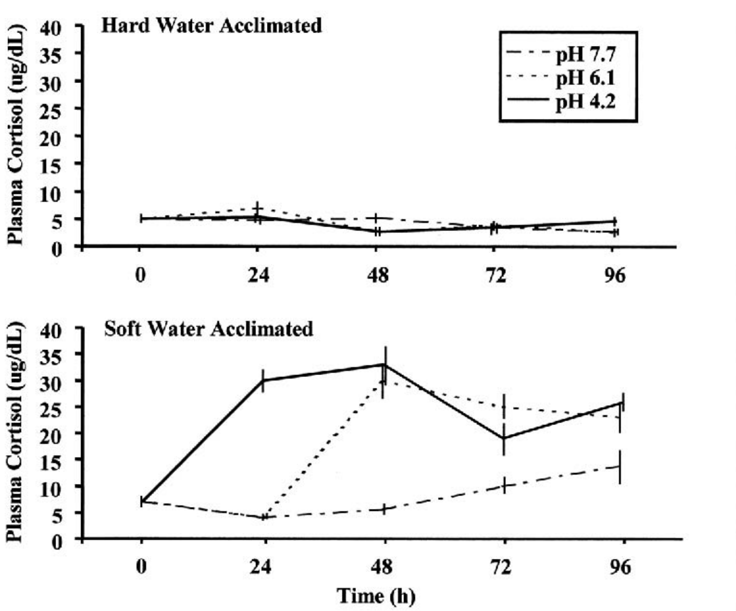

Figure 1. Plasma cortisol (mean±SE) in striped bass (*N*=10) exposed to 3 pH levels in fish acclimated in hard and soft water.

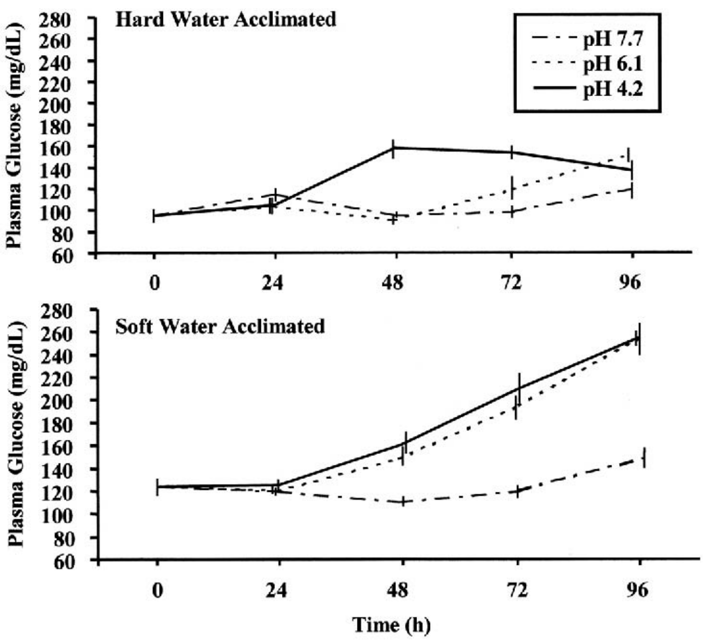

Figure 2. Plasma glucose (mean±SE) in striped bass (*N*=10) exposed to 3 pH levels in fish acclimated in hard and soft water.

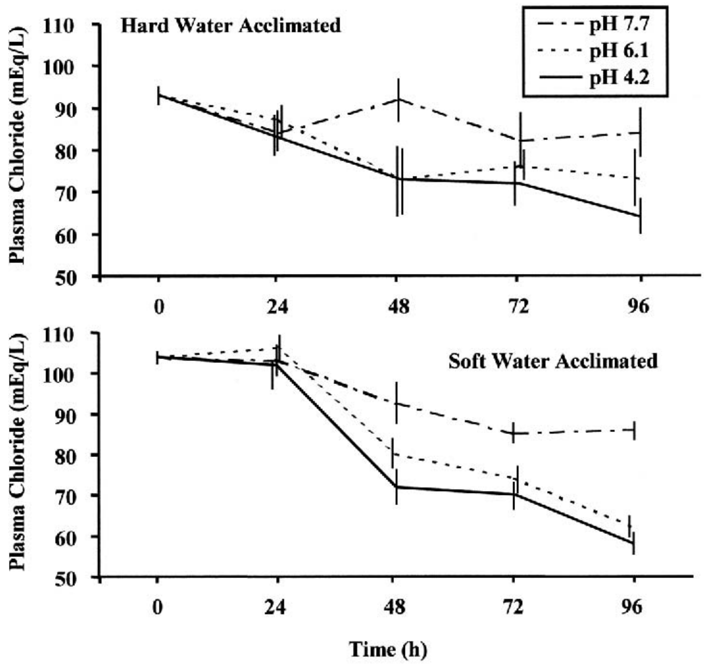

Figure 3. Plasma chloride (mean±SE) in striped bass (*N*=10) exposed to 3 pH levels in fish acclimated in hard and soft water.

Plasma chloride levels in striped bass acclimated to hard water did not significantly differ from control levels at pH 7.7 (Fig. 3). In fish acclimated to hard water, plasma chloride levels were significantly lower than control levels at pH 4.2 and pH 6.1, except at 24 hours. Plasma chloride levels in fish acclimated to soft water were significantly lower from control levels at all pH levels, except at 24 hours.

Discussion

Environmental calcium is an important factor in fish survival in acidic waters (McDonald et al. 1980, Brown 1981, McDonald 1983*b*). Mortalities did not occur when striped bass were exposed for 96 hours to low pH (4.2) in either soft or hard water. McDonald and Wood (1981) reported 29% mortality in rainbow trout exposed to pH 4.3 in water with hardness (32 mg/liter as calcium) similar to that used in our study, however, mortality was 70% in soft (6 mg/liter as calcium) water (McDonald 1983*b*).

The role of environmental calcium in reducing acid toxicity in fish has been well documented (Brown 1981, Brown 1982, McWilliams 1982, McDonald 1983*b*, McDonald et al 1983), but little research has been done on the influence of environmental calcium and pH on the stress response in fish. Plasma cortisol, which has been reported as a primary stress response in fish (Mazeaud et al. 1977, Donaldson 1981), increased in striped bass exposed to low pH, but was generally lower in fish in high calcium water. Our results thus indicated that environmental calcium may have been an important factor in alleviating the primary stress response of striped bass to acute exposure to acid. Plasma cortisol in rainbow trout (Adams et al. 1985, Goss and Wood 1988) and brook trout *Salvelinus fontinalis* exposed to low pH levels (Mudge et al. 1977) increased transiently, but returned to control levels after 24 hours. Plasma cortisol in striped bass acclimated to hard water did not alter from control levels, however, any transient increase would not have been noticed, since the first sample was taken at 24 hours.

Plasma glucose in fish has been reported to increase with increasing H^+ concentrations (Lee et al. 1983, McDonald 1983, Brown et al. 1984, 1986*b*, Audet et al. 1988, Tam et al. 1988). Our results agree with findings that plasma glucose increased significantly in fish exposed to low pH (4.0 to 4.5), however, when the fish were exposed to less acidic conditions (>4.7), the response required at least 96 hours to occur (Lee et al. 1983, McDonald 1983*b*, Adams et al. 1985, Brown et al. 1986*b*, Goss and Wood 1988).

Our results indicate that environmental calcium may diminish the extent of hyperglycemia (elevated plasma glucose levels) in acid-stressed striped bass. Fish acclimated to hard water tended to have glucose levels lower than fish acclimated to soft water. Research has indicated that hyperglycemia is apparently mediated in part by increasing plasma catecholamine and cortisol (Mazeaud et al. 1977, Leach and Taylor 1980, 1982, Brown et al. 1984). In this experiment, plasma glucose and cortisol were not correlated, indicating that the observed hyperglycemia may not be caused by cortisol increases.

The effects of environmental calcium and pH on plasma ion regulation in fish have been reported in other studies (McDonald et al. 1980, McWilliams 1982, McDonald 1983*b*, Hobe et al. 1984, Audet et al. 1988, Mount et al. 1988). Our results for striped bass agree with reports that plasma chloride levels decrease in fish exposed to low environmental pH (≤4.8), compared to fish held at ambient pH (Mudge and Neff 1971, Ultsch et al. 1981, Booth et al. 1982, Lacroix and Townsend 1987, Fryer et al. 1988).

The effect of low pH on osmoregulation in fish has been reported to depend not only on the concentration of environmental calcium, but also on the acclimation history of the fish (McWilliams 1982). Striped bass in our study that were acclimated to hard water had significantly less ion disturbance as compared to striped bass acclimated to soft water. This relation agrees with previous reports that the calcium acclimation history of fish is important. In rainbow trout acclimated to low calcium, chloride ion efflux at the gills was higher than in fish acclimated to high calcium, furthermore, as environmental calcium decreased, plasma chloride losses increased relative to sodium losses (McDonald et al. 1980, McDonald 1983*a*).

In summary, plasma cortisol, glucose, and electrolyte disturbances increased in striped bass at low environmental pH, though no fish died during the 96-hour exposure. Striped bass acclimated to hard water exhibited more osmoregulatory tolerance to acid-stress conditions than fish acclimated to soft water, indicating that the calcium acclimation history of fish should be an important consideration when stocking fish into acidic waters. Since hatchery-reared fish will probably be stocked largely to provide short-term fisheries in acidic waters, it is worth noting that striped bass acclimated in hard water will tolerate the acid-stress conditions in either soft or hard water better than will fish acclimated in soft water.

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