Influence of Sodium and Calcium Chloride on the Stress Response and Survival of Striped Bass During and After Transport from a Hard and Soft Water Hatchery

Patricia M. Mazik, *U. S. Geological Survey/Biological Resources Division, West Virginia Cooperative Fish and Wildlife Research Unit, 322 Percival Hall, West Virginia University, Morgantown, WV 26506–6125*

Bill A. Simco, *Ecological Research Center, Department of Biology, University of Memphis, Memphis, TN 38152*

Nick C. Parker, *U. S. Geological Survey/Biological Resources Division, Texas Cooperative Fish and Wildlife Research Unit, Texas Tech University, Lubbock, TX 79409–2120*

Abstract: Survival and the stress response of striped bass *Morone saxatilis* during transport and for a 1-month recovery period after transport were evaluated in 2 studies. In the first study, fish from Carbon Hill National Fish Hatchery (CHNFH), a hard water hatchery (total hardness, 100 mg/liter as calcium carbonate), were transported and recovered for 1 month at the Southeastern Fish Cultural Laboratory (SFCL), a hard water facility (total hardness, 108 mg/liter as calicum carbonate). In the second study, fish from Warm Springs National Fish Hatchery (WSNFH), a soft water hatchery (total hardness, 26 mg/liter as calicum carbonate), were transported and recovered for 1 month at the Southeastern Fish Cultural Laboratory (SFCL), a hard water facility (total hardness, 108 mg/liter as calicum carbonate). For each study, striped bass were transported and allowed to recover in 1 of 6 salt concentrations: 1.0% sodium chloride; 1.0%, 0.5%, or 0.1% calcium chloride; 0.5% sodium chloride + 0.5% calcium chloride; and fresh water. Results in both studies were similar. The addition of 1.0% sodium chloride to the hauling and recovery waters significant;y increased survival and reduced stress compared to the other treatments. Percent survival of striped bass was inversely proportional to calicum chloride levels in the hauling and recovery medium. The addition of 0.5% sodium chloride + 0.5% calcium chloride increased survival and decreased the stress response compared to fish hauled and recovered in only 0.5% calcium chloride.

Proc. Annu. Conf. Southeast. Assoc. Fish and Wildl. Agencies 54:118–127

The use of additives such as salts or anesthetics have been recommended to reduce stress and increase survival during the handling and transportation of fish. Tomasso et al. (1980) reported that 1.0% sodium chloride (NaCl) significantly reduced stress in hybrid striped bass (white bass × striped bass) and Weirich and Tomasso (1991) reported 0.4% to 1.6% salinity increased survival in red drum *(Sciaenops ocellatus).* Mazik et al. (1991) reported that 1.0% NaCl significantly reduced stress and increased survival in transported striped bass *(Morone saxatilis),* but 0.1% calcium chloride ($CaCl_2$) did not provide a beneficial effect compared to the NaCl or fresh water.

Water hardness has also been identified as a factor in the ability of fish to survive handling and transportation. Wedemeyer (1972) recommended that $CaCl_2$ be added to increase the total hardness of water to at least 50 mg/liter as calcium carbonate ($CaCO_3$) Grizzle et al. (1985) significantly increased survival of striped bass by using $CaCl_2$ to elevate the hardness of pond water from 10 to 70–200 mg/liter as $CaCO_3$. Weirch et al. (1992, 1993) also reported that the addition of at least 80 mg/liter $CaCl_2$ to freshwater increased survival of sunshine bass (female striped bass x male striped bass) to confinement and transport-induced stress and ammonia and nitrite toxicity. Mazik (1989) found that the $CaCO_3$ acclimation history of striped bass was important in their ability to handle stress. Striped bass acclimated in hard water were less stressed when moved to acidic waters than were fish acclimated in soft water.

Observations at the Southeastern Fish Cultural Laboratory have indicated that striped bass stocked from a hatchery with hard water generally survive better than fish stocked from a soft water hatchery. Because previous research (Mazik et al. 1991) showed no significant differences in survival or the stress response between striped bass in the fresh water and 0.1% $CaCl_2$ treatments from a soft water hatchery, further research was needed to determine if even higher levels of $CaCl_2$ would be beneficial.

The objective of this research was: 1) to determine the effects of $CaCl_2$ and NaCl on the stress response and survival of striped bass transported from Carbon Hill National Fish Hatchery (CHNFH) and recovered for 1 month at the Southeastern Fish Cultural Laboratory (SFCL), 2) to determine the effects of $CaCl_2$ and NaCl on the stress response and survival of striped bass transported from Warm Springs National Fish Hatchery (WSNFH) and recovered for 1 month at the Southeastern Fish Cultural Laboratory (SFCL). For both studies, plasma cortisol, the dominant corticosteroid in fish (Donaldson 1981) was measured as a primary stress indicator. Plasma glucose and chloride concentrations were measured as secondary indicators of stress.

We thank the hatchery managers, John Breland of Carbon Hill National Fish Hatchery, Carbon Hill, Alabama, and Greg Looney of Warm Springs National Fish Hatchery, Warm Springs, Georgia, for their support and the striped bass for this project. J. Jenkins, J. Morrison, N. Parker, and J. Tomasso critically reviewed the manuscript.

Methods

Carbon Hill National Fish Hatchery Study

Phase II striped bass were transported from the CHNFH to the SFCL. Mean weight of fish were 37.4 ± 1.5 g. Phase II striped bass are fish grown for 5 to 9 months and to a size of 7.6 to 25.4 cm. Water quality characteristics for CHNFH and SFCL, respectively, were: pH 7.4, 7.3; hardness (mg/liter as $CaCO_3$) 100, 108; and alkalinity (mg/liter as $CaCO_3$) 104, 105. Dissolved oxygen levels were ≥ 7.0 mg/liter and ammonia and nitrite levels were ≤0.01 mg/liter at all times.

In accordance with standard procedures at CHNFH, phase II striped bass were harvested from ponds 24 hours prior to transport and were held overnight in concrete raceways. Fish were handled in a solution of 1.0% NaCl. Immediately prior to transport, 100 fish were stocked into each of 12 208-liter barrels containing 170 liters of hatchery water. Fish were loaded at a density of ≤0.1 kg/liter into each barrel. During the 5-hour transport, fish were held in 6 duplicated treatments: (1) 1.0% NaCl, (2) 0.1% $CaCl_2$, (3) 0.5% $CaCl_2$, (4) 1.0% $CaCl_2$, (5) 0.5% NaCl + 0.5% $CaCl_2$, and (6) fresh water from the hatchery. Water quality characteristics during transport were: temperature 10 C; pH 7.4; dissolved oxygen >8.0 mg/liter; nitrite and ammonia ≤0.01 mg/liter.

Warm Springs National Fish Hatchery Study

Phase II striped bass were transported from the WSNFH to the SFCL. Mean weight of fish were 102 ± 3.4 g. Water quality characteristics for WSNFH and SFCL, respectively, were: pH 7.2, 7.3; hardness (mg/liter as $CaCO_3$) 26, 108: and alkalinity (mg/liter as $CaCO_3$) 29, 105. Dissolved oxygen levels were ≥7.0 mg/liter and ammonia and nitrite levels were ≤0.01 mg/liter at all times.

In accordance with standard procedures at WSNFH, phase II striped bass were harvested from ponds 24 hours prior to transport and were held overnight in concrete raceways. Fish were handled in a solution of 0.5% NaCl plus 0.02% Ca Cl_2. Immediately prior to transport, 100 fish were stocked into each of 12 208-liter barrels containing 170 liters of hatchery water. Fish were loaded at a density of ≤0.1 kg/liter into each barrel. During the 5-hour transport, fish were held in 6 duplicated treatments: (1) 1.0% NaCl, (2) 0.1% $CaCl_2$, (3) 0.5% $CaCl_2$, (4) 1.0% $CaCl_2$, (5) 0.5% NaCl + 0.5% $CaCl_2$, and (6) fresh water from the hatchery. Water quality characteristics during transport were: temperature 12 C; pH 7.2; dissolved oxygen >8.0 mg/liter nitrite and ammonia ≤0.01 mg/liter.

Upon completion of transport in both studies, the fish were stocked into identical barrels containing water for SFCL with the same additives as the water in which they were transported. One-half of the volume of each barrel was replaced every day to ensure water quality parameters remained within acceptable levels. Water quality characteristics, measured throughout the 1-month recovery, varied only slightly from those typically found at the SFCL. Over the 4-week study, temperature gradually increased from 11 to 19 C. Fish were not fed during the 1-month recovery.

For each study, 10 fish were bled before loading (pre-haul sample) for transport.

Upon arrival at SFCL, 5 fish were bled from each recovery tank at 0, 3, 6, 9, 12, 24, 48, 72, and 96 hours, and at 1, 2, 3, and 4 weeks. Blood was obtained from fish that had been anesthetized in a 0.02% solution of tricaine methanesulfonate. Sampling was completed within 5 minutes of the initial disturbance and blood samples were taken from each fish only once. Blood was collected into heparinized syringes from blood vessels in the caudal peduncle and the plasma was stored at −20 C until analyzed. Plasma levels were determined for cortisol by radioimmunoassay (Serono Diagnostics), glucose with a clinical kit (Sigma Chemical Co.) and chloride with a chloridometer (Am. Instrument Co.).

The data are presented as mean ± SE unless stated otherwise. Analysis of variance and Duncan's multiple-range tests (procedure GLM, SAS Inst. 1985) were used to test for significant differences in each study. The level of significance established in all tests was $P \leq 0.05$.

Results

Carbon Hill National Fish hatchery Study

No mortalities occurred during transport; however, survival did vary during the recovery period (Fig. 1). Striped bass hauled and recovered in a 1.0% NaCl had 100% survival. All other treatments had 0% survival by the 4th week of recovery. The duration of survival throughout the recovery period was inversely proportional to $CaCl_2$ concentrations.

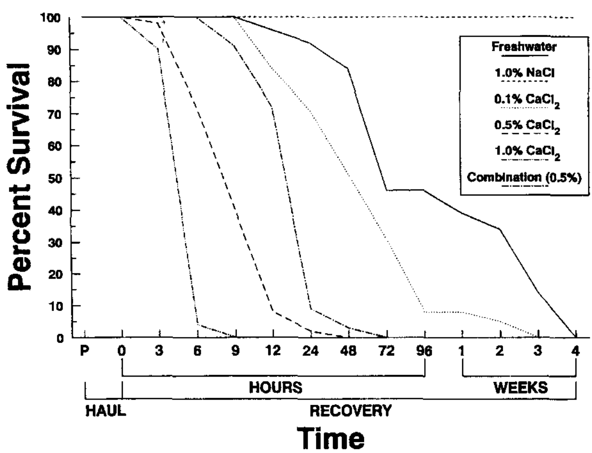

Figure 1. Percent survival of CHNFH striped bass during hauling and recovery for 1 month in either 1.0% NaCl, 0.1% $CaCl_2$, 0.5% $CaCl_2$, 1.0% $CaCl_2$, 0.5% NaCl + 0.5% $CaCl_2$, or fresh water in duplicate. Fish were hauled at a density of ≤0.1 kg/liter in hard water (100 mg/liter hardness) and allowed to recover in hard water (108 mg/liter hardness). Temperature during the haul was 12 C and gradually increased to 19 C during the 4-week recovery period. P represents pre-haul conditions.

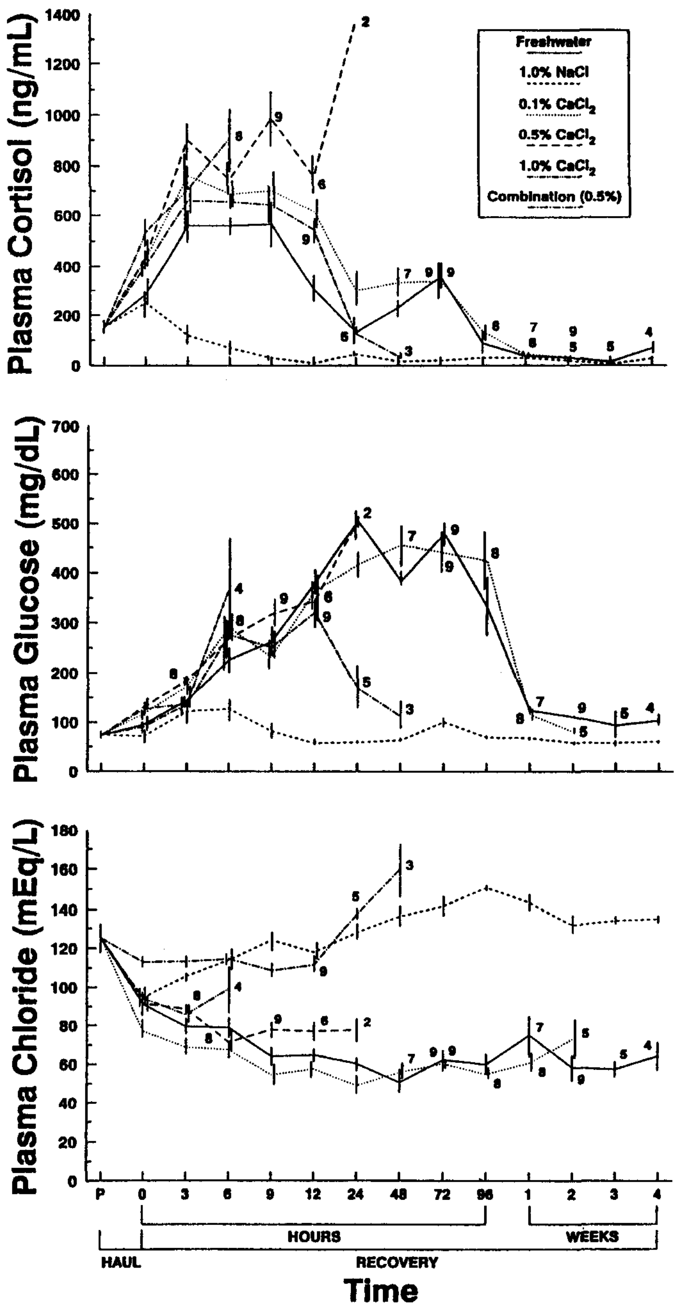

Figure 2. Plasma cortisol, glucose, and chloride (mean ± SE; $N = 10$ unless stated otherwise) in CHNFH striped bass during hauling and recovery for 1 month in either 1.0% NaCl, 0.1% $CaCl_2$, 0.5% $CaCl_2$, 1.0% $CaCl_2$, 0.5% NaCl + 0.5% $CaCl_2$, or fresh water in duplicate. See Figure 1 caption for details of experimental conditions.

Plasma cortisol levels in striped bass hauled and recovered in 1.0% NaCl were significantly higher than pre-haul levels at 0 hours recovery, but returned to pre-haul levels by 3 hours recovery (Fig. 2). Cortisol concentrations in all other treatments were significantly higher than pre-haul levels and did not return to these levels until after 24 hours recovery, with the exception of 0.1% $CaCl_2$ which returned to pre-haul levels after 72 hours recovery.

Plasma glucose levels in striped bass hauled and recovered in 1.0% NaCl were significantly lower than pre-haul concentrations, with the exception of slight increases at 3, 6, and 72 hours (Fig. 2). All other treatments were significantly higher than pre-haul levels with 0.1% $CaCl_2$ and fresh water returning to pre-haul levels within 1 week after transport. Fish hauled and recovered in 1.0% NaCl had significantly lower plasma glucose levels compared to the other treatments, with the exception of the 0- and 3-hour samples.

Plasma chloride levels in striped bass hauled and recovered in the combination and 1.0% NaCl treatments were not significantly lower from pre-haul levels, with the exception of the NaCl fish at 0 and 3 hours (Fig. 2). Fish in all other treatments had plasma chloride levels significantly lower than pre-haul concentrations and were also significantly lower compared to the combination and NaCl treatments.

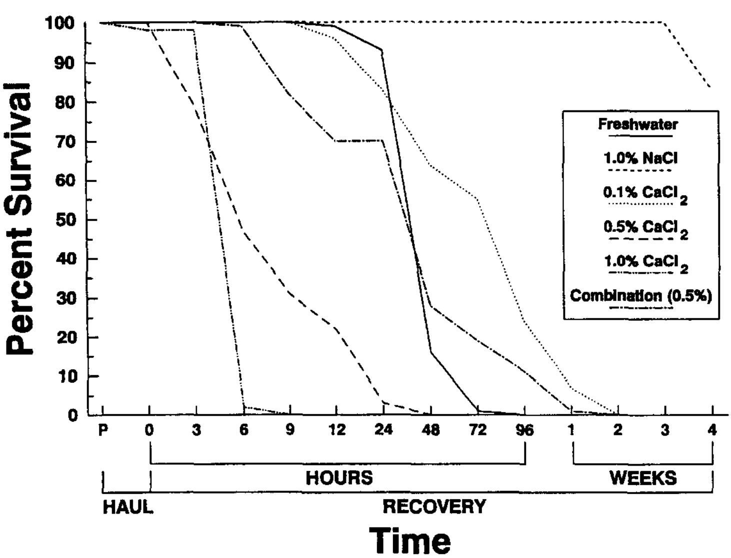

Figure 3. Percent survival of WSNFH striped bass during hauling and recovery for 1 month in either 1.0% NaCl, 0.1% $CaCl_2$, 0.5% $CaCl_2$, 1.0% $CaCl_2$, 0.5% NaCl + 0.5% $CaCl_2$, or fresh water in duplicate. Fish were hauled at a density of ≤0.1 kg/liter in soft water (26 mg/liter hardness) and allowed to recover in hard water (108 mg/liter hardness). Temperature during the haul was 10 C and gradually increased to 19 C during the 4-week recovery period. P represents pre-haul conditions.

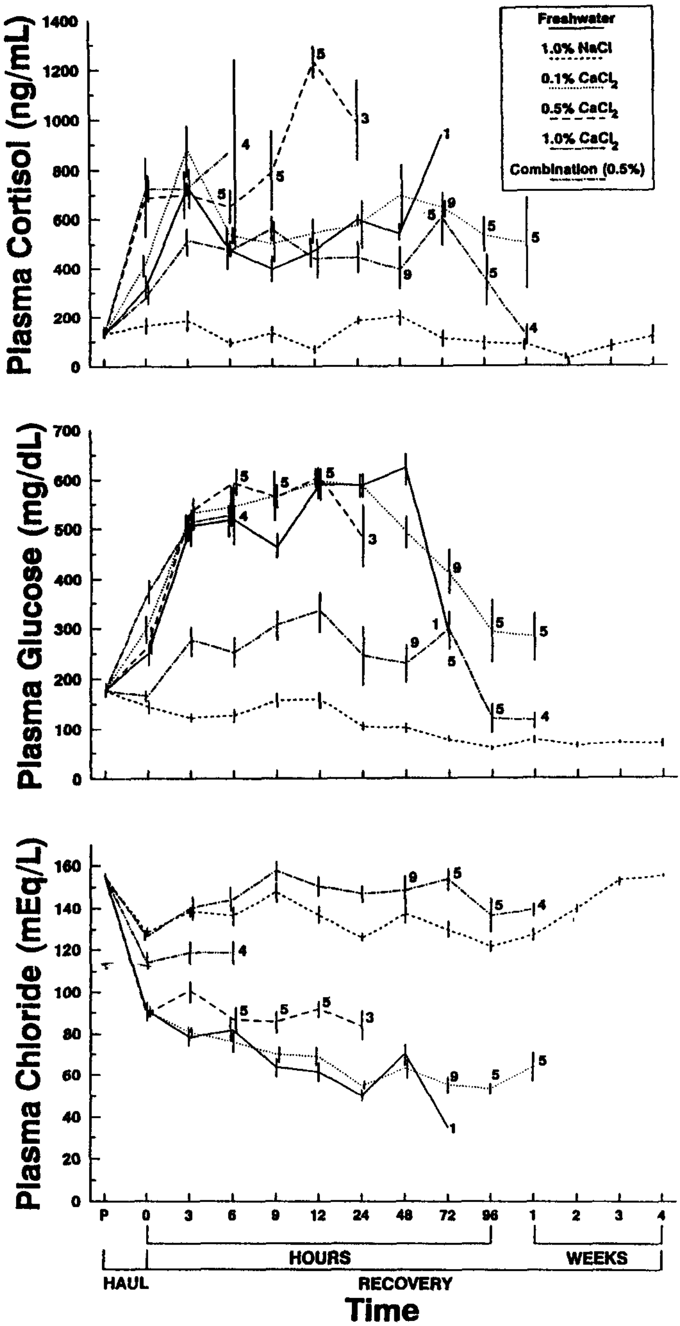

Figure 4. Plasma cortisol, glucose, and chloride (mean ± SE; $N = 10$ unless stated otherwise) in WSNFH striped bass during hauling and recovery for 1 month in either 1.0% NaCl, 0.1% $CaCl_2$, 0.5% $CaCl_2$, 1.0% $CaCl_2$, 0.5% NaCl + 0.5% $CaCl_2$, or fresh water in duplicate. See Figure 3 caption for details of experimental conditions.

Warm Springs National Fish Hatchery Study

Five percent mortality occurred in the 1.0% $CaCl_2$ treatment during transport. No mortalities occurred during transport in all other treatments: however, survival did vary during the recovery period (Fig. 3). Striped bass hauled and recovered in 1.0% NaCl had $\geq$83% survival. All other treatments had 0% survival by the 4th week of recovery. The duration of survival from both hatcheries throughout the recovery period was inversely proportional to $CaCl_2$ concentrations.

Plasma cortisol levels in striped bass hauled and recovered in 1.0% NaCl were significantly lower than the other treatments and did not significantly increase throughout the experiment from pre-haul levels (Fig. 4). Cortisol concentrations in fish held in 0.1, 0.5, or 1.0% $CaCl_2$ were significantly higher than pre-haul levels and did not return to pre-haul concentrations throughout the recovery period. Striped bass held in fresh water or the combination treatment (0.5% NaCl + 0.5% $CaCl_2$) had plasma control levels significantly higher than pre-haul levels throughout the study.

Plasma glucose levels in fish hauled and recovered on 1.0% NaCl were not significantly elevated from pre-haul levels throughout the study (Fig. 4). Striped bass hauled and recovered in other treatments had glucose concentrations significantly increased from pre-haul levels. Plasma glucose levels in fish in the 1.0% NaCl treatment were significantly lower compared to other treatments with the exception of the 0-hour sample.

Plasma chloride levels in striped bass hauled and recovered in the combination treatment did not significantly decrease from pre-haul levels, except at 0 hours (Fig. 4). Fish in the combination and 1.0% NaCl treatments did not have significantly different plasma chloride levels, although the NaCl treatment was slightly lower than pre-haul levels. Striped bass on the 3 $CaCl_2$ and the fresh water treatments had chloride levels significantly lower than pre-haul levels and were also significantly lower than the combination and NaCl treatments.

Discussion

Survival of striped bass hauled and recovered in 1.0% NaCl in both studies was similar to that found by Mazik et al. (1991). In both studies, survival of fish hauled and recovered in $CaCl_2$ was inversely proportional to the $CaCl_2$ level, indicating that increasing levels of $CaCl_2$ may be detrimental possibly due to the osmotic imbalance between the hauling medium and fish. Mazik et al. (1991) reported striped bass hauled and recovered in 0.1% $CaCl_2$ did not have a significantly different stress response compared to fish hauled and recovered in fresh water and postulated that higher $CaCl_2$ levels may be required. The present studies found that the addition of increasing amounts of $CaCl_2$ to the hauling and recovery waters did not reduce the stress response on striped bass as indicated by high concentrations of plasma cortisol. Plasma cortisol levels in striped bass hauled and recovered in 1.0% NaCl were similar to levels reported in striped bass (Mazik et al. 1991), hybrid striped bass (Tomasso et al. 1980), and red drum (Robertson et al. 1988). Pre-haul plasma cortisol levels in

WSNFH and CHNFH striped bass (130.3 ± 21.3 and 149.5 ± 28.1 μg/ml, respectively) were elevated above normal levels (0–125 μg/ml) for striped bass as reported by Tisa et al. 1983. The elevated levels in our study are possibly due to harvesting of the striped bass 24 hours before transportation. The significantly elevated cortisol levels in striped bass hauled and recovered in 0.1%, 0.5%, and 1.0% $CaCl_2$, fresh water, and the combination treatment indicated a severe stress response in these media.

Plasma glucose levels in striped bass hauled and recovered in 1.0% NaCl were comparable to those reported in striped bass transported and hauled in 1.0% NaCl (Mazik et al. 1991), in red drum after transport and recovery in 4% and 32% seawater (Robertson et al. 1988), and in largemouth bass *Micropterus salmoides* transported and recovered in anesthetic and solutions approximating physiological saline (Carmichael et al. 1984). Glucose levels decreased (WSNFH) or did not significantly change (CHNFH) in striped bass hauled and recovered in 1.0% NaCl indicating that these fish were not stressed during transportation or recovery. Pre-haul plasma glucose levels in WSNFH striped bass were higher than the pre-haul levels in CHNFH striped bass indicating the WSNFH striped bass were initially stressed. The soft water acclimation history of these fish may have been a factor in their higher pre-haul stress levels. Mazik (1989) has found that the calcium acclimation history of striped bass is important in how well stressors, such as low pH, are tolerated. Also, the addition of 0.5% NaCl to the combination treatment in both studies was beneficial compared to the 0.5% $CaCl_2$ treatment, indicating that NaCl has a protective effect.

Plasma chloride levels in the WSNFH and CHNFH striped bass indicate that the addition of NaCl, either alone or in combination with $CaCl_2$, provided a beneficial effect compared to the absence of NaCl (i.e, $CaCl_2$ or freshwater treatments). This protective effect may be due to the presence of the sodium and chloride ions. Mazik et al. (1991) found that plasma sodium and chloride decreased at a similar rate in striped bass that were stressed during hauling and recovery. The addition of 0.5% or 1.0% NaCl to the hauling and recovery water in both studies alleviated the stress response, possibly due to the fact that the NaCl levels used were similar to the physiological plasma saline level (0.85%) in fish. Redding and Schreck (1983), using coho salmon *(Oncorhynchus kisutch)*, found that the presence of salt in hauling and recovery waters helps reduce stress by decreasing the osmotic gradient between the plasma and environment, thus reducing the energy cost for osmoregulation. Our results indicate that the addition of 1.0% NaCl to the transportation and recovery water increases survival and reduces the stress response of striped bass compared to $CaCl_2$ or freshwater. We recommend that 1.0% NaCl be added to hauling and recovery waters, regardless of water calcium levels, and $CaCl_2$ be added in soft water to raise the total hardness to at least 50 mg/liter $CaCO_3$ as recommended by Wedemeyer (1972).

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