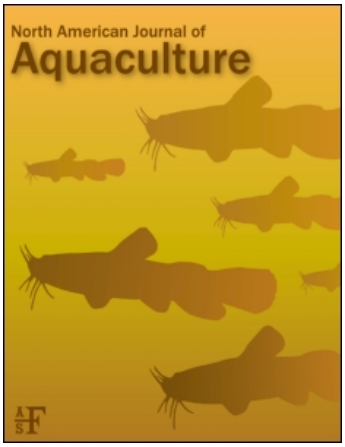

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[Article]

Stress Response and Posttransport Survival of Hybrid Striped Bass Transported with or without Clove Oil

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Abstract.—A study was undertaken to determine whether stress responses and associated mortality in hybrid striped bass (white bass *Morone chrysops* × striped bass *M. saxatilis*) during and after transport could be mitigated by use of anesthesia with low concentrations of clove oil (10 μL/L of water) during transport. Stress indicators, including plasma cortisol, glucose, chloride (Cl^-), sodium (Na^+), potassium (K^+), and calcium (Ca^{++}) concentrations, were determined for hybrid striped bass sampled (1) initially from ponds, (2) after seining (45 min), (3) immediately after a 3-h transport, and (4) at 24 h posttransport (recovery); these values were compared with those of captive control fish. Fish anesthetized with clove oil during transport exhibited prolonged elevation of cortisol concentrations (124.3 ± 13.2 ng/mL, mean ± SE) at 24 h posttransport, while fish not exposed to anesthetic during transport recovered rapidly (to 34.1 ± 13.3 ng/mL) within 24 h. Plasma glucose concentrations in hybrid striped bass exposed to clove oil during transport were significantly ($\alpha = 0.05$, $P < 0.05$) elevated (135.1 ± 5.6 mg/dL) immediately after transport and then decreased by 24 h posttransport (84.3 ± 3.4 mg/dL) compared with the 24-h posttransport level for fish transported without anesthetic (101.0 ± 5.0 mg/dL). Plasma electrolyte (Na^+, Cl^-, and K^+) concentrations indicated that osmoregulatory distress occurred after transport in hybrid striped bass transported with clove oil, while electrolyte changes were less severe in fish transported without anesthesia. No hydromineral imbalance occurred based upon plasma Ca^{++} concentrations in either treatment. High mortality occurred within 7 d posttransport in both treatment groups but was not significantly different ($P < 0.05$) between the treatments. Observed mortality was primarily, if not entirely, due to *Flavobacterium columnare* infection. Hybrid striped bass transported during light clove oil sedation took longer to recover and exhibited no improved survival or disease resistance compared with fish transported without anesthesia. No potential benefit of transporting hybrid striped bass under anesthesia induced by clove oil is evident.

Survival of hybrid striped bass (white bass *Morone chrysops* × striped bass *M. saxatilis*) after stocking in experimental systems and natural waters can be low, and low survival has been attributed to abrupt changes in temperature, a lack of adequate tempering, or handling and transporting stress (Hodson and Hayes 1989; Brewer and Rees 1990; Pitman and Gutreuter 1993). Striped bass and hybrid striped bass are less tolerant of physical, chemical, and biological changes in their environment compared with other commonly stocked species, and when released into a new system they can be subject to acute mortality (Pitman and Gutreuter 1993). Pitman and Gutreuter (1993) found that moronid fingerling survival (0–100%; striped bass mean, 52%; hybrid striped bass mean, 76%) was highly variable and negatively affected by increased transport time, large temperature changes, low pH, and low dissolved oxygen (DO). While maintaining optimal environmental conditions and handling and transporting fish at the lowest practical temperatures is important, many researchers and fish transporters attempt to reduce stress by sedating fish before and during handling (Harrell et al. 1990; Cooke et al. 2004).

Unfortunately, only one fish anesthetic, tricaine methanesulfonate (MS-222), is approved by the U.S. Food and Drug Administration (FDA) for use, and fish exposed to the anesthetic cannot be made available for human consumption for at least 21 d. Thus, the use of MS-222 for put-and-take stocking is prohibited. One chemical receiving considerable attention as a general anesthetic for fish is eugenol or clove oil. Clove oil (95% eugenol, isoeugenol) is used worldwide as a food flavoring as well as a local anesthetic in human dentistry (Borski and Hodson 2003). It is considerably less expensive than other drugs, is widely available, and has a relatively short induction and recovery period

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(Soto and Burhanuddin 1995; Prince and Powell 2000; Sladky et al. 2001). The FDA lists clove oil as "generally regarded as safe" for use as a food additive, but clove oil used as a fish anesthetic is not considered to be a legal food treatment (FDA 2002). Despite this fact, clove oil has gained popularity as a fish anesthetic and has been used as a substitute for traditional FDA-approved anesthetics, such as MS-222 and carbon dioxide (CO_2; Woolsey et al. 2004). The use of clove oil as a fish anesthetic is frequently reported in scientific literature (Prince and Powell 2000; Mulchahy 2003; Woolsey et al. 2004), often for purposes that historically indicate the fish will be released (Prince and Powell 2000; Mulchahy 2003), creating the opportunity for them to be caught and eaten by humans. This has the potential to create the perception that clove oil used as a fish anesthetic is acceptable for field applications.

Regardless of the potential of clove oil as a fish anesthetic, previous research does not rationalize its use with moronid fishes. Hybrid striped bass can experience severe stressors during confinement and transport, including crowding, exposure to high concentrations of ammonia and nitrites, low DO, and extreme temperatures (Weirich et al. 1992). Davis and Griffin (2004) evaluated the effectiveness of several anesthetics, including clove oil and AQUI-S® (50% 2-methoxy-4-propenylphenol; isoeugenol), at mitigating the stress response in hybrid striped bass. Their study found that unstressed hybrid striped bass exposed to clove oil or isoeugenol exhibited elevated plasma cortisol concentrations after 15 min of exposure and after 2 h of recovery from the anesthetic. In addition, they found that hybrid striped bass anesthetized with clove oil before being subjected to a low-water stressor exhibited elevated cortisol concentrations at 6 and 24 h postrecovery compared with fish subjected to stress alone.

The study by Davis and Griffin (2004) provides invaluable data, but the fish were only subjected to a short-term (15-min) stressor and short-term clove oil exposure, and all fish were sampled within 24 h; thus, there are gaps in existing data. Further questions need to be explored, including (1) how do hybrid striped bass respond to prolonged clove oil exposure (>1 h), (2) can clove oil be used to mitigate stress effects during transport, (3) what effect does long-term clove oil exposure have on poststocking survival, and (4) can clove oil mitigate stress-induced disease susceptibility? In this study, we examined these questions using a practical stressor encountered in hatchery operations to determine whether stress responses and associated mortality in hybrid striped bass during and after transport could be mitigated by anesthesia with low concentrations of clove oil.

Methods

Experimental fish.—Juvenile hybrid striped bass (N = 500; mean [±SE] weight, 132.1 ± 7.2 g; mean length, 228.9 ± 4.0 mm) were obtained from Keo Fish Farm (Keo, Arkansas) and stocked into two 0.04-ha ponds at the University of Arkansas at Pine Bluff's (UAPB) experiment station. The fish were split into two ponds to avoid multiple seining and handling events during the study. The fish were stocked into the ponds more than 60 d before the transport studies. The ponds were filled with well-derived, surface-held water from a 3.3-ha reservoir. Temperature, DO (YSI 55/12 FT; YSI, Inc., Yellow Springs, Ohio), pH (UB-10 pH/mV meter; Denver Instrument, Denver, Colorado), total ammonia-nitrogen (TAN; DR/890 colorimeter/low range Test'N Tube; Hach Company, Loveland, Colorado), total hardness (EDTA, ManVer method), alkalinity (phenolphthalein/bromocresol green method), and nitrite (Ceric standard method; Hach Model 16900 digital titrator) concentrations in the ponds were monitored and recorded (Table 1). The fish were fed a commercial diet (32% protein, 8% lipid; Arkat Mills, Inc., Dumas, Arkansas) to satiation on a temperature-dependent maintenance schedule. Feed was withheld for 48 h before seining and transport.

Control fish.—Fourteen days before the transport experiment, 18 control fish were removed from each pond population and immediately stocked into each of two 3-m-diameter (1.1-m depth) outdoor pools lined with black polypropylene liners. The pools were operated as flow-through systems with water supplied at 5.7 L/min from a 3.3-ha surface water reservoir; pools were enclosed in a protective overhead bird netting enclosure, and an air stone in each pool maintained supplemental aeration (Table 1). Fish in the control pools were fed to satiation on the same schedule (days and time) as the experimental fish throughout the study. Feed was withheld 48 h before seining the experimental fish and was first offered to all fish at 24 h posttransport. Mortality, disease occurrence, and water quality were monitored and recorded during the 14-d acclimation period.

Blood collection.—Sampled fish were measured for body weight and total length (TL). Blood samples were collected by inserting a sodium-heparinized syringe (5-mL syringe with 20-gauge, 38-mm needle) into the caudal vasculature of anesthetized fish (MS-222 at 200 mg/L) and drawing approximately 1.5 mL of blood. The blood was placed into 15-mL centrifuge tubes and centrifuged at 1,000 × gravity for 5 min. The plasma was then removed by transfer pipette, placed into 1.5-

TABLE 1.—Water quality for each sampling period from ponds, transport tanks, and recovery pools during a study of hybrid striped bass transport with or without clove oil anesthetic. No detectable nitrites were found during any sampling period from any system. Quantifiable un-ionized ammonia was present only in the transport tanks at the completion of the transportation trials (0.007, 0.011, 0.005, and 0.006 mg/L for transport tanks 1, 2, 3, and 4, respectively). Transport tanks and pools 1 and 2 correspond to no-anesthetic treatments, while tanks and pools 3 and 4 correspond to clove oil treatments.

Sample period	Sample source	Temperatures (°C)	Dissolved oxygen (mg/L)	pH	Total ammonia-nitrogen (mg/L)	Total hardness (mg/L)	Total alkalinity (mg/L)	Nitrate (mg/L)
Initial	Pond 1	16.5	8.7	7.6	0.001	115	184	0.572
	Pond 2	13.9	12.5	7.9	0.000	122	152	0.010
Start of transport	Transport tank 1	14.3	10.9	7.6	0.001	115	184	0.572
	Transport tank 2	14.3	Saturated	7.6	0.001	115	184	0.572
	Transport tank 3	17.3	8.8	7.8	0.000	123	150	0.015
	Transport tank 4	17.4	6.8	7.9	0.001	114	148	0.015
End of transport	Transport tank 1	14.1	13.1	7.6	0.692	120	164	0.463
	Transport tank 2	14.6	Saturated	7.7	0.853	124	152	0.423
	Transport tank 3	18.0	Saturated	7.6	0.534	120	152	0.012
	Transport tank 4	18.0	9.6	7.7	0.492	124	152	0.016
Start of recovery	Control pool	13.7	12.2	7.2	0.032	99	127	0.588
	Control pool	18.3	6.1	7.7	0.020	120	150	0.206
	Pool (transport) 1	13.6	11.3	7.4	0.001	114	113	0.001
	Pool (transport) 2	13.9	11.0	7.4	0.000	106	102	0.001
	Pool (transport) 3	18.7	10.6	7.4	0.000	127	142	0.000
	Pool (transport) 4	17.9	15.5	7.4	0.000	110	134	0.000
24 h (recovery)	Control pool	14.2	11.9	7.2	0.040	102	125	0.456
	Control pool	14.8	9.2	7.7	0.018	118	127	0.265
	Pool (transport) 1	14.1	10.8	7.4	0.021	119	115	0.000
	Pool (transport) 2	14.3	11.2	7.4	0.028	99	104	0.000
	Pool (transport) 3	14.8	10.7	7.4	0.028	120	140	0.001
	Pool (transport) 4	14.7	10.8	7.3	0.020	110	130	0.000

mL cryotubes, and frozen at −40°C until analyses were performed. All sampled fish were euthanized after their blood was acquired.

Initial sampling (resting).—Ten fish were removed from each pond and sampled to determine initial blood plasma chemistry and cortisol concentrations after the pond temperature was within the recommended transport temperature range (<20°C) for hybrid striped bass. The initial fish were sampled by angling using heavy gear. The fish were hooked, landed, and immediately placed into a solution of 200-mg/L MS-222 before hook removal. Time of hooking to deep anesthesia (complete loss of equilibrium, greatly reduced or halted opercular movement, and lack of response to external stimulus) was less than 3 min. Water quality variables, including water temperature (°C), DO (mg/L), TAN (mg/L), total hardness (mg/L), total alkalinity (mg/L), and nitrate (mg/L), were recorded.

Transport.—Approximately 24 h after sampling the initial fish, water quality was again measured from each of the ponds and remaining fish in the ponds were seined. All seining, holding, loading, and transport events were timed and maintained within ±5 min between the ponds. The fish were seined and held confined within the nets for 45 min to simulate the seining duration at a production facility. At the conclusion of the seining period, 10 fish were indiscriminately removed from each seine and anesthetized, and blood samples were obtained for analyses.

Fish were then stocked into identical, replicate, insulated transport tanks located on the cargo bed of the transport vehicle. Each transport tank was supplied with compressed oxygen to maintain supplemental aeration. Fish were dipnetted from the seines and stocked at a density of 118.2 ± 3.3 g/L into each of two insulated transport tanks (internal dimensions for each tank, 64.8 cm long × 64.8 cm wide × 77.5 cm high) for the control (no anesthetic) treatment. For the second treatment, fish were dipnetted and stocked (density, 115.9 ± 2.7 g/L) into each of two insulated tanks containing 10-μL/L clove oil (Sigma-Aldrich, St. Louis, Missouri) emulsified 1:10 in ethanol (Sigma-Aldrich). The clove oil concentration used was determined from the literature (Cooke et al. 2004; Davis and Griffin 2004) and previous experimentation. The clove oil dosage was chosen to induce light anesthesia, in which fish were able to maintain equilibrium, position in the water column, and opercular movement, while respiration rate (as opercular beats per minute, approximately 40% rate reduction) and evasion response to external stimuli were reduced. The loading process was timed and spread over 15 min. The fish were held in the tanks for an additional 15 min before the start of transportation.

The fish were then transported for 180 min along a

predetermined route consisting of both state and county roads. Upon return to UAPB, mortalities were noted and five fish were removed from each tank, anesthetized with MS-222, and sampled for blood. The remaining fish from each transport tank ($N = 18$ fish/tank) were placed into individual 3-m-diameter (1.1-m depth) outdoor pools corresponding to the individual transport tanks. Twenty-four hours later, five fish from each control pool and each pool containing transported fish were netted, anesthetized, and sampled for blood. Thereafter, the remaining fish in each pool ($N = 18$) were monitored for infections and mortality for 7 d.

Blood plasma cortisol and chemistry analyses.—Plasma samples were thawed at ambient room temperature 1 h before analysis. Plasma cortisol concentrations were determined by enzyme-linked immunosorbent assay (BQ078S; BioQuant, Inc., San Diego, California) as validated for use in fish by Sink et al. (2008) and partially validated by the authors for use in hybrid striped bass (intra-assay coefficient of variation, 8.9%; interassay coefficient of variation, 7.6%; mean recovery of spiked samples, 97.1%). Plasma glucose concentrations were determined with the glucose oxidase colorimetric method (GAGO20-1KT; Sigma-Aldrich). Plasma chloride (Cl^-) concentrations were determined with the mercuric nitrate colorimetric method (DICL-250; BioAssay Systems, Hayward, California). Free plasma calcium (Ca^{++}) concentrations were measured by means of the phenol-sulphonephthalein dye method (DICA-500; BioAssay Systems). Plasma potassium (K^+) concentrations were measured by the S-adenosylmethionine synthase enzymatic method of determination (DZ113B-K; Diazyme Laboratories, Poway, California). Plasma sodium (Na^+) concentrations were measured with the β-galactosidase enzymatic activity determination method (DZ114A; Diazyme Laboratories). All assays were conducted as specified by the manufacturers, except that the K^+ and Na^+ assays were conducted in 96-well microtiter plates rather than in cuvettes.

Statistical analysis.—All statistical analyses were conducted using the Statistical Package for the Social Sciences version 11.0 (SPSS, Inc., Chicago, Illinois). Differences in mortality and disease occurrence between the two experimental treatments (no anesthetic or clove oil) were determined using paired Student's *t*-tests and contrast statements. Analysis of variance (ANOVA) and Tukey's post hoc tests were used to determine differences among fish in the two treatments for plasma cortisol, glucose, and ion concentrations. In instances where concentrations were statistically different ($P < 0.05$) for both the initial and control fish, such as with Ca^{++} concentration, the rate of change for each sampling period was determined and the rate of change was compared by means of an ANOVA and Tukey's post hoc tests.

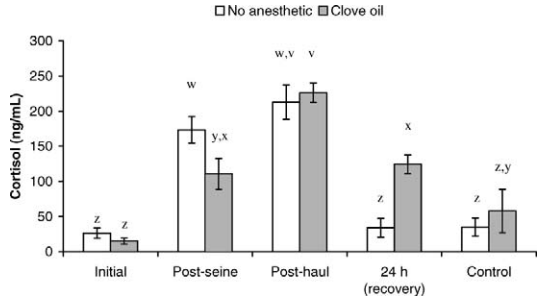

FIGURE 1.—Mean (±SE) plasma cortisol concentrations of hybrid striped bass at each sampling period (initial, 0 min; postseine, after 45 min of confinement in seines; posthaul, after 3 h of transport; at 24 h posttransport [recovery]; and control) during a transport study with or without clove oil anesthetic. Sample means with different lowercase letters were significantly different ($\alpha = 0.05$).

Results

The mean weight (±SE) of fish sampled from pond 1 was 131.8 ± 11.8 g and from pond 2 was 128.8 ± 9.0 g. The size of the fish at each sampling period was relatively uniform, ranging from 103.4 to 167.6 g in weight and from 21.1 to 24.8 cm in TL. Temperature varied by 5.1°C among all sampling periods, and all other water quality conditions were within acceptable limits for hybrid striped bass transport during the study (Table 1). No detectable nitrites were found during any sampling period from any system. Quantifiable unionized ammonia was present only in the transport tanks at the completion of the transportation trials (0.007, 0.011, 0.005, and 0.006 mg/L for transport trials 1, 2, 3, and 4, respectively).

Mean plasma cortisol concentrations (Figure 1) in hybrid striped bass were not different during the initial sampling period (26.4 ± 7.1 and 15.2 ± 4.2 ng/mL for ponds 1 and 2, respectively), posttransport (212.2 ± 24.3 and 226.2 ± 13.8 ng/mL for no anesthetic and clove oil, respectively), or for the control pools (35.1 ± 13.0 and 57.9 ± 30.9 ng/mL for control pools 1 and 2, respectively). Hybrid striped bass transported without anesthetic exhibited greater mean plasma cortisol concentrations after seining (173.6 ± 19.0 ng/mL) than those exposed to clove oil during transport (110.5 ± 22.0 ng/mL before exposure to clove oil). However, fish exposed to clove oil during transport exhibited prolonged elevation of plasma cortisol concentrations (124.3 ± 13.2 ng/mL) at 24 h posttransport (recovery), while fish that were not exposed to anesthetic during transport recovered rapidly, with

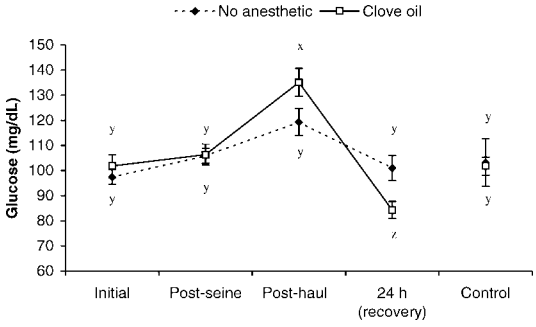

FIGURE 2.—Mean (±SE) plasma glucose concentrations of hybrid striped bass at each sampling period (initial, 0 min; postseine, after 45 min of confinement in seines; after 3 h of transport; at 24 h posttransport [recovery]; and control) during a transport study with or without clove oil anesthetic. Sample means with different lowercase letters were significantly different ($\alpha = 0.05$).

cortisol concentrations (34.1 ± 13.3 ng/mL) returning to control levels within 24 h.

There were no differences in mean plasma glucose concentrations (Figure 2) for hybrid striped bass from initial samples (97.4 ± 2.9 and 101.8 ± 4.6 mg/dL for ponds 1 and 2, respectively), postseining (105.9 ± 3.0 and 106.4 ± 4.2 mg/dL for no anesthetic and clove oil, respectively), or in the control pools (103.2 ± 9.5 and 101.8 ± 3.6 mg/dL for control pools 1 and 2, respectively). Plasma glucose concentrations were slightly elevated but not significantly different post-transport (119.3 ± 5.4 mg/dL) in fish not exposed to anesthetic, while plasma glucose concentrations in fish exposed to clove oil during transport were substantially elevated (135.1 ± 5.6 mg/dL). Plasma glucose concentrations decreased more slowly within 24 h posttransport for fish transported without anesthetic (101.0 ± 5.0 mg/dL) than for fish transported with clove oil (84.3 ± 3.4 mg/dL).

Mean plasma Ca^{++} concentrations (Figure 3) were different between fish from the two holding ponds for all sampling periods. Therefore, the rate of change between sampling periods was analyzed instead of absolute value, and no significant differences in rate of change were found at any of the sampling periods. Mean plasma Cl^{-} concentrations (Figure 3) generally decreased for both treatments during transport and at 24 h posttransport (recovery). Chloride concentrations were not different for the initial sample (100.2 ± 8.5 and 107.8 ± 8.1 milliequivalents [mEq]/L for ponds 1 and 2, respectively), posttransport sample (96.0 ± 6.1 and 88.8 ± 5.4 mEq/L for no anesthetic and clove oil, respectively), or for the control fish (106.4 ± 7.8 and 93.8 ± 7.7 mEq/L for control pools 1 and 2, respectively). Chloride concentrations for pond 1

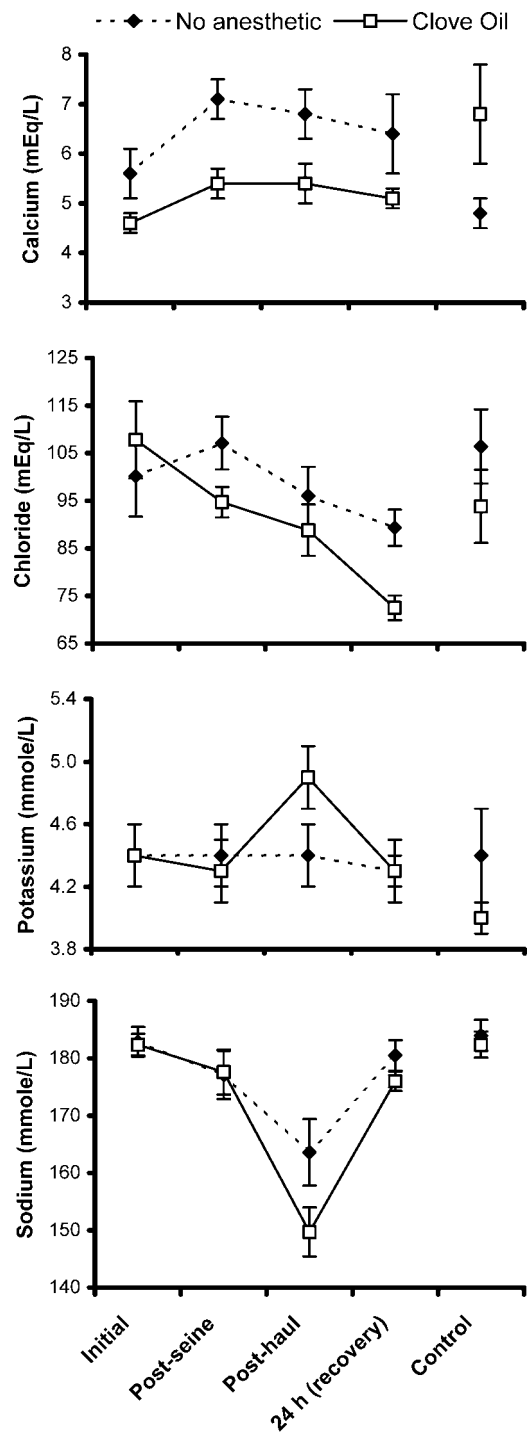

FIGURE 3.—Mean (±SE) plasma calcium (milliequivalents [mEq]/L), chloride (mEq/L), potassium (mmol/L), and sodium (mmol/L) concentrations of hybrid striped bass at each sampling period (initial, 0 min; postseine, after 45 min of confinement in seines; posthaul, after 3 h of transport; at 24 h posttransport [recovery]; and control) during a transport study with or without clove oil anesthetic.

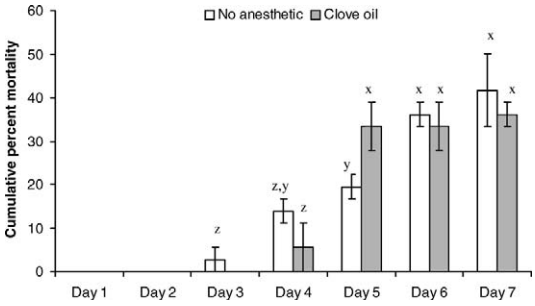

FIGURE 4.—Mean (±SE) cumulative percent mortality of hybrid striped bass during a 7-d recovery period after 3 h of transport with or without clove oil anesthetic. Sample means with different lowercase letters were significantly different ($\alpha = 0.05$).

increased after seining (107.1 ± 5.5 mEq/L) and were greater than postseining Cl^- concentrations from fish in pond 2 (94.7 ± 3.2 mEq/L). Recovery Cl^- concentrations in fish that were transported with clove oil (72.5 ± 2.6 mEq/L) continued to decrease and were lower than those for fish transported with no anesthetic (89.3 ± 3.8 mEq/L) at 24 h posttransport.

Mean plasma K^+ concentrations (Figure 3) were stable for fish not exposed to anesthesia for initial samples (4.4 ± 0.2 mmol/L), postseining (4.4 ± 0.2 mmol/L), posttransport (no anesthetic; 4.3 ± 0.1 mmol/L), and 24 h posttransport (recovery; 4.4 ± 0.3 mmol/L). Potassium concentrations for fish treated with clove oil were not different from those for fish transported without anesthesia for initial samples (4.4 ± 0.2 mmol/L), postseining (4.3 ± 0.2 mmol/L), or 24 h posttransport (clove oil; 4.3 ± 0.2 mmol/L). However, plasma K^+ concentrations increased after transport in fish anesthetized with clove oil (4.9 ± 0.2 mmol/L) compared with those from fish transported without anesthetic.

Mean plasma Na^+ concentrations (Figure 3) were not different for initial samples (182.9 ± 2.6 and 182.4 ± 1.9 mmol/L for ponds 1 and 2, respectively), postseining (177.1 ± 4.2 and 177.6 ± 3.9 mmol/L for no anesthetic and clove oil, respectively), at 24 h posttransport (180.5 ± 2.7 and 176.0 ± 1.7 mmol/L for no anesthetic and clove oil, respectively), or for the control pools (184.0 ± 2.7 and 182.4 ± 2.3 mmol/L for control pools 1 and 2, respectively). Plasma Na^+ concentrations decreased from the postseining sampling period for fish transported without anesthetic (163.6 ± 5.8 mmol/L) and for fish transported with clove oil (149.7 ± 4.3 mmol/L). Plasma Na^+ concentrations were significantly lower posttransport for hybrid striped bass transported with clove oil than for those transported without anesthetic.

No immediate transport mortality occurred in fish transported without anesthetic (transport tanks 1 and 2); for fish transported with clove oil, no transport mortality was observed for transport tank 3, but 5.3% transport mortality was observed for transport tank 4. Fish in the control pools ate readily throughout the experiment when offered food. Fish subjected to transport treatments ate readily when offered food 24 h after transport, but appetite quickly diminished by 72 h posttransport as bacterial infections became evident. High mean (±SE) posttransport mortality (Figure 4) occurred during the 7-d posttransport monitoring period in fish transported without anesthetic (41.7 ± 8.3% mortality) and in fish transported with clove oil (36.1 ± 2.8% mortality), although mortality rates were not different between treatments. With the exception of the 5.3% mortality that occurred in transport tank 4 (with clove oil), all posttransport mortalities were associated with severe infections of *Flavobacterium columnare*, the causative agent of columnaris disease. Columnaris growth was visually evident on the majority of fish within 7 d posttransport, although not all infected fish died within the observation period. Columnaris infection was confirmed by microscopic identification of the pathogen from moribund fish.

Discussion

Prolonged elevation of plasma cortisol concentrations, large oscillation in plasma glucose concentrations, greater rates of Na^+ and Cl^- depletion, and elevated plasma K^+ concentrations indicate that prolonged exposure to clove oil during transport induces a longer stress response—if not one of greater magnitude—and slower recovery rates compared with those in hybrid striped bass transported without anesthetic. These responses to prolonged clove oil exposure in the presence of a stressor are similar to those found during short-term exposure of hybrid striped bass to clove oil (Davis and Griffin 2004). Clove oil had no effect on transport or long-term posttransport survival compared with hybrid striped bass transported without anesthetic. Additionally, hybrid striped bass transported under clove oil-induced anesthesia exhibited no reduced susceptibility to disease, either by stress reduction or anti-microbial properties of clove oil.

Fish transported with the clove oil anesthetic exhibited prolonged elevation of plasma cortisol concentrations at 24 h posttransport (recovery). This response is contrary to the rationale for the use of anesthetics during transport, which is to suppress or mitigate stress responses to multiple stressors (Carmichael et al. 1984; Cooke et al. 2004). Davis and Griffin (2004) observed a similar response in hybrid striped

bass. In their study, fish exposed to clove oil alone or clove oil plus a 15-min low-water stressor exhibited elevated plasma cortisol concentrations after 24 h of recovery, compared with control fish or fish subjected to only the low-water stressor.

Among clove oil-sedated fish, elevation of plasma cortisol for at least 24 h posthandling confirms that this anesthetic can exacerbate the stress response in hybrid striped bass (Davis and Griffin 2004). Other studies have demonstrated that simple exposure to anesthetics administered at low sedation concentrations produces a stress response to the anesthetic itself. Hybrid striped bass (Davis and Griffin 2004) and striped bass (Davis et al. 1982) exposed to several anesthetics without the addition of a stressor experienced increased plasma cortisol concentrations after exposure. Similar increases in cortisol secretion in response to clove oil (or its active ingredients) anesthesia can be found in other fish species. Rainbow trout *Oncorhynchus mykiss* exposed to isoeugenol or clove oil (Sink et al. 2007) exhibited increased cortisol concentrations after exposure compared with resting, nonsedated fish, indicating that anesthetics themselves are stressful to this species.

Hybrid striped bass are often exposed to extreme temperatures during transport (Weirich et al. 1992), and temperature can affect the cortisol response in fish (Strange 1980). The temperatures in recovery pools for the clove oil-treated fish were warmer (18.0°C and 18.3°C) at the start of recovery than those for fish transported without anesthetic (13.6°C and 13.7°C). Water clarity fluctuated among individual pools, and differences in water clarity within the tanks probably created the differences in temperature through differential warming.

It is unlikely that differences in pool temperatures were responsible for the slowed recovery rate of fish transported with clove oil, as the fish in Davis and Griffin's (2004) study demonstrated similar responses and slowed recovery when held at the same temperature. Additionally, Davis and Parker (1990) tested the response of striped bass to a confinement stressor at various temperatures. While they determined the stress response was greatest and the recovery rate was slowest in fish held at 5°C and 30°C, the stress response was lowest and the recovery rate was fastest in fish held at 10°C and 16°C. A 6°C difference did not produce major differences in the stress response in Davis and Parker's (1990) study. Based upon these results, it would not be expected that the observed maximum temperature difference of 4.7°C between the pools would produce significantly different results. Recovery pool temperatures were similar (14.1–14.8°C) at the time of sampling for cortisol analysis (24 h posttransport) and were within the 10–16°C temperatures that produced the lowest magnitude of response and fastest recovery rates in striped bass (Davis and Parker 1990). This information indicates that temperatures observed during recovery would not significantly affect the cortisol response or recovery rates.

Blood plasma Ca^{++} ion concentrations remained relatively stable for fish in both treatments, and the rate of Ca^{++} ion change between the two treatments was not different, indicating that hydromineral imbalance did not occur for either treatment. The water from the ponds, transport tanks, and recovery pools all contained high levels of hardness and alkalinity (Table 1), which may have aided in maintenance of Ca^{++} ion regulation. Several studies have demonstrated the importance of high hardness to the short- and long-term survival of hybrid striped bass (Hodson and Hayes 1989; Brewer and Rees 1990; Parker et al. 1990).

Hybrid striped bass transported with clove oil experienced greater osmotic distress compared with fish transported without anesthetic. Plasma Cl^- concentrations remained relatively stable for hybrid striped bass transported without anesthetic. Interestingly, plasma Cl^- concentrations in fish transported with clove oil were depleted during transport and continued to decrease during recovery. Continued depletion of plasma Cl^- concentrations, coupled with protracted elevation of cortisol concentrations during recovery in fish transported with clove oil, confirms that fish transported with clove oil experienced prolonged stress responses after exposure to clove oil. Unlike the present study, Davis and Griffin (2004) reported no dramatic or consistent change in Cl^- concentrations during stress or recovery of hybrid striped bass anesthetized with clove oil, with or without the application of a stressor. Davis and Parker (1990) ascertained that Cl^- concentrations generally decreased in response to stress, similar to this study, across a range of temperatures in striped bass.

Sodium–ammonium (NH_4^+) ion exchange processes were disrupted in fish transported with clove oil, as plasma Na^+ concentrations continued to be depleted after transport. The depletion of plasma Na^+ concentrations was less severe in fish transported without anesthetic. Sodium and K^+ regulation are linked as Na^+ is exchanged via Na^+-K^+ ATPase to actively transport Na^+ into the blood. Plasma K^+ concentrations in fish transported with clove oil increased posttransport, probably in response to greatly decreased plasma Na^+ concentrations. While plasma Na^+ concentrations in hybrid striped bass transported without anesthetic decreased after transport, plasma Na^+ concentrations remained higher than those in fish transported with

clove oil. Plasma K^+ concentrations in fish transported without anesthetic remained stable throughout the study, and this was attributed to only small decreases in plasma Na^+ concentrations.

Davis and Griffin (2004) and Davis and Parker (1990) reported poststress increases in plasma glucose concentrations in hybrid striped bass and striped bass, respectively, as seen in this study. However, neither publication reports a substantial decline in plasma glucose concentrations after a 24-h period of recovery, as was found in the hybrid striped bass transported with clove oil in this study. Plasma glucose concentrations in hybrid striped bass transported without anesthetic returned to initial concentrations within 24 h posttransport, but glucose concentrations in fish transported with clove oil were lower after 24 h of recovery than initial glucose concentrations, possibly indicating depletion of glycerol stores in the liver. Dramatic fluctuation in plasma glucose concentrations confirmed that fish transported with clove oil were stressed posttransport and for prolonged periods during recovery.

Although high mortality occurred within 7 d (recovery) after transport in both treatment groups, it was not different between the treatments. Pitman and Gutreuter (1993) reported mean survival for fingerling striped bass and hybrid striped bass as 52% and 76%, respectively, at 24 h poststocking. They concluded that observed mortalities were associated with increased transport time, large temperature changes, low DO, and decreased pH. In the present study, survival was 100% at 24 h poststocking, and observed mortality after 24 h was primarily, if not entirely, due to columnaris infection, whereas no fish in the control pools died. Hybrid striped bass in the Pitman and Gutreuter (1993) study were subjected to extreme changes in temperature (mean change [Δ], 15.9°C), DO (mean Δ, 18 mg/L), or pH (mean Δ, pH 2.6) that affected survival. Fish in the present study (maximum Δ, 4.8°C and pH 0.5 between the pond and stocking) were not subjected to these extremes in physical and chemical changes and therefore had enhanced survival during the first 24 h posttransport, but the effects of transport-induced stress and disease began to cause mortalities after 72 h posttransport. Pitman and Gutreuter (1993) did not examine poststocking survival beyond 24 h to determine whether disease-related mortality became problematic. Although *F. columnare* is a secondary pathogen that typically requires mechanical injury before infections occur, it is possible that the stress incurred during transport weakened the immune system of the fish, leading to an increase in infection rates.

Legal or not, the results of this study and others (Davis and Griffin 2004) indicate that clove oil provides no benefit to hybrid striped bass during common handling and transport stressors. Clove oil did not mitigate the cortisol response or electrolyte imbalance. Glucose depletion was more severe and disease occurrence was not moderated in hybrid striped bass transported with clove oil. We suggest that research programs and state agencies that transport hybrid striped bass do not use clove oil as an anesthetic, as no potential benefit is evident from our study.

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