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Effects of Loading Density on Golden Shiner Survival during and after Hauling

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Abstract.—Four hauling trips of approximately 6 h each were conducted to investigate effects of loading density on survival of golden shiners *Notemigonus crysoleucas*. Commercially graded golden shiners (mean weight ± SE, 3.3 ± 0.04 g) were transported at densities of 120, 180, and 240 g of fish/L of water in insulated hauling tanks that were filled with fresh well water, chilled with unchlorinated block ice, and aerated with pure oxygen. The criterion for determining success or failure was golden shiner survival. Transportation at a given density was deemed successful if survival both at trip's end and at 18 h postdelivery was at least 99%. At all three hauling densities evaluated, survival exceeded 99% both at trip's end and at 18 h postdelivery. Furthermore, increasing loading density had no effect on whole-body cortisol concentrations, demonstrating that no significant stress response occurred. Un-ionized ammonia concentration increased with loading density (range, 0.05–0.46 mg/L) but had no effect on fish survival. Results of this study indicate that golden shiners can be successfully (≥99% survival) transported for up to 6 h at a density of 240 g/L in well water chilled with unchlorinated block ice and aerated with pure oxygen. Higher loading densities could mitigate the effect of escalating transport cost for commercial farmers.

The golden shiner *Notemigonus crysoleucas* is a deep-bodied, laterally compressed fish having a greenish-olive back and sides with silver or gold luster. Its bright flashing appearance makes the golden shiner excellent live bait (Gray 1988) and an important specialty agriculture crop. Arkansas (1,750 metric tons), Wisconsin (98 metric tons), Mississippi (94 metric tons), and Minnesota (55 metric tons) accounted for 87% of U.S. golden shiner sales (2,281 metric tons, US$17.1 million) in 2005 (NASS 2006).

Most golden shiners are transported from farm to market by trucks similar to those described by Carmichael and Tomasso (1988). Hauling tank water is generally cooled with unchlorinated block ice (Jensen 1990; James Saul, Harry Saul Minnow Farm, Inc., personal communication) and aerated by diffusion of pure oxygen (Carmichael and Tomasso 1988). Loading density typically varies from 120 to 180 g of fish/L of water depending on time of year and duration of the haul (Ronnie Anderson, Anderson Brothers Fisheries, Inc., personal communication; James Saul, Harry Saul Minnow Farm, Inc., personal communication). We hypothesized that loading density could be increased but were concerned that high mortality during transport would occur.

A review of scientific literature provided evidence of extensive investigation into transportation-related stress in warmwater food and game fish but revealed only one published scientific study concerning transportation of golden shiners. Amend et al. (1982) stated that potentially toxic metabolites, such as ammonia

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(NH_3) and carbon dioxide (CO_2), are limiting factors with respect to transportation of live golden shiners. Both of these potentially toxic metabolites, which are affected by water temperature, water pH, loading density, and time in transit, may increase fish stress and reduce survival. Ammonia has a profound effect on the stress response in channel catfish *Ictalurus punctatus* (Tomasso et al. 1981; Small 2004). However, Le Ruyet et al. (1998) determined that short-term exposure of juvenile turbot *Scophthalmus maximus* and gilthead seabream *Sparus aurata* to sublethal exogenous ammonia (150 ng/mL) produced an increase in plasma cortisol levels (indicating a stressful situation) that quickly returned to acceptable levels after a recovery period of less than 1 h.

Several reviews have explored in detail the complexities and consequences associated with the stress response in teleost fishes. Mommsen et al. (1999) examined the physiological control mechanisms associated with the stress response, while Wendelaar Bonga (1997) examined the metabolic and physiological roles of cortisol in the stress response. Davis (2006) noted that stress in fish is often characterized by physiological changes in plasma cortisol, glucose, lactate, and electrolyte concentrations and is quantitatively related to the severity and longevity of the stressor. Readers wishing addition information concerning the physiological changes related to the stress response in fishes, as well as the methods used to assess such changes, are referred to reviews by Adams (1990), Donaldson (1990), and Wedemeyer et al. (1990).

Carmichael et al. (1984) used simulated transportation events to study stress associated with hauling largemouth bass *Micropterus salmoides*. Simulated and actual transportation events were used to investigate plasma cortisol and chloride stress responses in juvenile walleyes *Sander vitreus* during capture, transport, and stocking procedures (Barton et al. 2003) and to show that triploid and diploid rainbow trout *Oncorhynchus mykiss* did not differ in stress response relative to transportation (Leggatt et al. 2006). The corticosteroid stress response of 14 species of warmwater fishes collected by electrofishing and then transported for approximately 2 h was evaluated by Davis and Parker (1986). Minchew et al. (2007) conducted on-farm evaluations of the effects of harvesting and hauling on market-sized channel catfish blood physiology and fillet quality. Each of these investigators observed that plasma cortisol concentrations increased significantly during transport.

This investigation focused on the effect of loading density on survival of golden shiners hauled for approximately 6 h in fresh well water chilled with unchlorinated block ice and aerated with pure oxygen. The criterion for determining success or failure was golden shiner survival. Transportation at a given density was deemed successful if survival both at trip's end and at about 18 h postdelivery was at least 99%.

Methods

Hauling trials.—Four hauling trips of approximately 6 h each were conducted in 2007 on May 29 and June 1, 5, and 8 (hauling days 1–4). Commercially graded golden shiners were purchased from Harry Saul Minnow Farm, Inc., De Valls Bluff, Arkansas, and were transported from the farm to the U.S. Department of Agriculture, Agricultural Research Service, Aquaculture Systems Research Unit in Pine Bluff, Arkansas, on each trip.

All trials used a trailer-mounted hauling system consisting of six 68-L insulated hauling tanks, two 4.5-m^3 compressed oxygen cylinders, one oxygen regulator, 6.35-mm oxygen hoses, manifolds, flowmeters, and glass-bonded stone diffusers. Hauling tanks were set in two rows of three tanks each and were separated by a walkway. There was one tank on either side of the trailer for each treatment. On each trip, tanks 1 and 4 were loaded at 120 g/L, tanks 2 and 5 were loaded at 180 g/L, and tanks 3 and 6 were loaded at 240 g/L. Hauling tanks loaded at the respective densities contained 6.6, 8.8, or 11.3 kg of golden shiners in approximately 50, 48, or 46 L of fresh 18°C well water and 3.0 kg of unchlorinated block ice and were aerated with pure oxygen. No sodium chloride or other additive intended to affect osmoregulation was used in the hauling tanks.

The lid of each hauling tank was immediately closed and secured after the tank was loaded at a specified density, iced, and topped off with fresh well water. Researchers assumed that the lowest water temperature occurred after the tanks were secured, so the reduction in water temperature caused by introducing ice was mathematically obtained using calorimetry equations described by Serway and Faughn (1995). The problem-solving approach was tested by comparing computed results with data obtained empirically by James Saul (personal communication) and currently used by employees of Harry Saul Minnow Farm. Hauling tank water temperature and dissolved oxygen concentration at the end of the trial were measured with a YSI Model 550 meter (YSI, Inc., Yellow Springs, Ohio).

A fish sample was collected from one vat of commercially graded golden shiners before load-out on each hauling trip. Graded golden shiners were crowded into a small volume of the vat, and one dip-net sample was collected, quickly euthanized by immersion in a 3-g/L solution of tricaine methanesul-

fonate, sealed in a labeled plastic bag, and placed on ice. Twenty fish from each preserved sample were arbitrarily selected and weighed. Mean (±SE) fish weight (3.3 ± 0.04 g) was based on 80 individual fish weights ($n = 20$ from each dip-net sample).

Posthaul mortality was determined by holding 1 kg of golden shiners from each hauling tank overnight (approximately 18 h) in a labeled cylindrical vat. Each vat contained approximately 75 L of 18.6 ± 1.0°C dechlorinated municipal water aerated with atmospheric air. A mechanical chiller reduced water temperature to 16.8 ± 1.0°C during the night. Mortalities were collected and recorded (by treatment) and weighed the following morning. Vats were drained, cleaned, and refilled the day before each trial.

Water quality analysis.—One well water sample, one sample of holding vat water, and one sample of water from each hauling tank (at trip's end) was collected on each hauling day. Total ammonia nitrogen (TAN) was determined by using the salicylate–cyanurate method (serial dilutions, 1:2, 1:4, and 1:8) and the low range method (0.00–2.50 mg/L) with a Hach DR/890 colorimeter test laboratory (Hach Company, Loveland, Colorado). An electrode was used to measure pH (Denver Instruments, Colorado), and un-ionized ammonia (UIA) was calculated from TAN and pH measurements (Durborow et al. 1997).

Transportation stress.—A second fish sample was collected from the vat of commercially graded golden shiners before load-out on each of the four hauling trips. The fish were collected according to the procedure previously described but were preserved by freezing with dry ice. Golden shiners initially frozen with dry ice were stored at −20°C pending cortisol extraction and analysis. After being thawed, fish were arbitrarily selected from the labeled plastic storage bags. Fish from each preserved vat sample ($n = 10$) and from each preserved hauling tank sample ($n = 10$) were subjected to whole-body cortisol analysis to determine baseline and final cortisol concentrations, respectively.

Cortisol assays were conducted using whole-body extract (Sink et al. 2007a) validated for use with enzyme-linked immunosorbent assays and radioimmunoassay (RIA) for golden shiners (Sink et al. 2007b), as modified by Sink and Lochmann (2008). This procedure yielded a lipid extract containing cortisol, which was stored at −80°C pending RIA. Cortisol in the whole-body extract was analyzed using the Coat-A-Count cortisol RIA kit (Siemens Medical Solution Diagnostics, Los Angeles, California). Whole-body cortisol concentration (ng cortisol/g body weight) was obtained by multiplying the cortisol concentration in lipid extract (ng cortisol/mL lipid extract) by the total volume of lipid extract (mL) and dividing the resulting product by fish mass (g).

Statistical analysis.—Statistical comparisons were conducted using the Statistical Analysis System version 9.1.3 (SAS Institute 2002). Assumptions for homogeneity of variance and normality of the data were tested by examination of correlation between absolute residuals and predicted values and the Shapiro–Wilk test for normality. Survival data were arcsine-transformed, and whole-body cortisol data were log transformed before analysis of variance (ANOVA) to meet assumptions. The effects of loading density on posthaul survival, overnight (18 h posthaul) survival, whole-body cortisol, TAN, and UIA were analyzed using the ANOVA mixed-model procedure with loading density as the fixed effect and hauling day and loading density × hauling day as random effects. When significant differences were found using ANOVA, pairwise contrasts using Fisher's least-significant-difference test were used to identify differences at the 0.05 significance level.

Results and Discussion

During late May and early June, natural foods present in production ponds are typically supplemented by a daily ration of commercially prepared fish feed. Selected locations in ponds scheduled for harvest are also baited with commercially prepared feed for several days, including the day of harvest (William Saul, Harry Saul Minnow Farm, personal communication). Specific information regarding the feeding regimen for fish used in this study was not provided. Generally, golden shiners are seined, transported to a holding facility, and held in a rectangular concrete vat from about 1000 hours on a given day until about 0600 hours the following day, when they are graded. Fish are not fed while they are in the vat, before load-out, or during transport. Golden shiners transported on a given hauling day represented a graded subset of a mixed population that was harvested from an unspecified production pond.

Increasing hauling density from 120 to 240 g/L had no effect ($P = 0.51$) on hauling survival or survival of 1-kg subsets of fish transferred to tanks and held for 18 h postdelivery ($P = 0.46$; Table 1). Furthermore, there was no effect of day for any of the variables analyzed ($P > 0.05$). Maximum ambient air temperature for hauling days 1–4 was 28, 30, 32, and 29°C, respectively (NWS 2007a, 2007b). Mean hauling tank water temperature for days 1–4 was 16.9, 16.8, 16.4, and 17.7°C, respectively. Ending water temperatures of 20.3°C in one replicate tank loaded at 120 g/L on day 2 and 22.0°C in a 240-g/L replicate on day 4 were not included in calculation of the mean values. Water loss

TABLE 1.—Mean (±SE) hauling survival of golden shiners, survival of 1 kg of fish transferred to tanks and held for 18 h after hauling, whole-body cortisol, total ammonia nitrogen (TAN), and un-ionized ammonia (UIA) before and after hauling at three loading densities (g of fish/L of water). Different letters within a column indicate treatment means that were significantly different (analysis of variance [ANOVA]: $P \leq 0.0001$).

Treatment	*N*	Survival (end of trip)	Survival (18 h postdelivery)	Cortisol (ng/g)	TAN (mg/L)	UIA (mg/L)
Before hauling	4	NA	NA	15.7 ± 1.6	1.68 ± 0.20 z	0.06 ± 0.01 z
120 g/L	8	99.5 ± 0.1	99.9 ± 0.1	15.0 ± 1.3	5.64 ± 0.30 y	0.10 ± 0.02 y
180 g/L	7[a]	99.5 ± 0.1	99.9 ± 0.02	17.5 ± 2.3	8.93 ± 0.20 x	0.13 ± 0.01 x
240 g/L	8	99.4 ± 0.1	99.9 ± 0.02	19.9 ± 2.1	10.66 ± 0.04 w	0.20 ± 0.04 w
ANOVA *P*		0.51	0.46	0.27	<0.0001	<0.0001

[a] On day 1 (May 29), one replicate of the 180-g/L treatment had 32% mortality due to a low dissolved oxygen concentration (0.4 mg/L) attributed to equipment failure; this replicate was not included in the statistical analysis.

during highway travel was the expected cause for the elevated temperatures. The calculated minimum water temperature was between 12.0°C and 12.5°C. Differences among the calculated minimum and the measured ending water temperatures showed an increase of approximately 5°C during each 6-h trip.

Ending dissolved oxygen concentration among hauling tanks averaged 15.0 ± 1.2 mg/L (mean ± SE) over the four hauling trips. On day 1, ending dissolved oxygen concentration averaged 6.5 ± 2.9 mg/L. One replicate of the 180-g/L treatment had a dissolved oxygen concentration of 0.4 mg/L and 68% survival, but survival rates in the remaining five tanks were greater than 99%. Equipment failure appeared to be the cause for the low dissolved oxygen concentration and high mortality in this 180-g/L replicate. For this reason, the high mortality replicate was considered an outlier and not included in statistical analyses. On hauling days 2–4, ending dissolved oxygen concentrations averaged 17.2 ± 1.7, 19.0 ± 0.5, and 15.8 ± 0.8 mg/L, respectively.

Analysis of water samples collected from hauling tanks at the end of each trip yielded a mean pH of 7.73 ± 0.04 and indicated that TAN increased with loading density ($P < 0.0001$; Table 1). The range for TAN in the 120-, 180-, and 240-g/L treatments was 4.3–7.0, 7.7–9.6, and 9.7–11.0 mg/L, respectively. A World Health Organization (WHO) study of the effect of ammonia in the environment on aquatic organisms (WHO 1986) yielded mean 48- and 96-h lethal (causing 50% mortality) UIA concentrations of 0.56–2.48 mg/L for freshwater fish. Furthermore, Durborow et al. (1997) stated that short-term (a few days) exposure to UIA concentrations of 0.6 mg/L or higher can be lethal in freshwater culture ponds. Carmichael et al. (1984) reported that a UIA concentration of 0.2 mg/L was responsible for the 88% mortality observed in largemouth bass subjected to simulated hauls of up to 30 h in untreated water. The ranges for UIA in the 120-, 180-, and 240-g/L treatments (0.06–0.26, 0.09–0.17, and 0.08–0.46 mg/L, respectively) were below the value of 0.56 mg/L set by WHO (1986) and the 0.6 mg/L suggested by Durborow et al. (1997) but included the concentration that was found to be lethal in largemouth bass (0.2 mg/L; Carmichael et al. 1984). There was a significant effect of hauling density on UIA ($P < 0.0001$; Table 1). When golden shiners were hauled at 240 g/L, UIA was twice that recorded for the 120-g/L treatment.

Analysis of lipid extract obtained via the whole-body cortisol procedure showed that increasing the loading rate did not significantly ($P = 0.27$) elevate whole-body cortisol, although there was a trend toward increasing cortisol with increasing density (Table 1). The lack of a significant cortisol response suggests that the fish were not stressed as loading density increased. Observed increases in ammonia could prove to be limiting if loading densities greater than 240 g/L are attempted. Long-term exposure to elevated ammonia concentrations has been demonstrated to increase circulating cortisol concentrations in fish (Tomasso et al. 1981; Small 2004). Ammonia control systems might abate this response and allow for greater loading densities. Zhang et al. (2004) provided the foundation for the design of ammonia control systems for commercial fish hauling trucks by examining the effects of temperature and size on golden shiner ammonia excretion in recirculating systems; however, more research is needed to determine effectiveness and economic feasibility.

In this study, we demonstrated that live golden shiners can be successfully transported at a density of 240 g/L in fresh well water chilled with unchlorinated block ice and aerated with pure oxygen for up to 6 h. Each hauling event depicted the outbound leg of a delivery trip that can be completed in one workday by one commercial motor vehicle driver. The time budget included approximately 1 h for load-out, 4 h of driving time, and 1 h to unload the fish. If a 4-h driving time is included for the return leg, then the trip can be

conducted in compliance with Federal Motor Carrier Safety Administration Hours-of-Service Regulations (FMCSA 2008).

Study results indicate that fish farmers can offset the increasing cost of highway diesel fuel by transporting more golden shiners and less water. In a hypothetical example, a baitfish farmer owns one diesel-powered tractor–trailer combination, which is used exclusively for transporting golden shiners to a market located 241 km from his farm. If highway diesel fuel costs $1.05 per liter (EIA 2008) and the truck tractor travels 2.5 km/L of fuel, then the fuel expense for the 482-km round trip is $203. At a loading density of 120 g/L, the truck's maximum legal cargo capacity of 11,340 kg consists of 1,215 kg of golden shiners and 10,125 kg of water. If loading density is increased to 240 g/L, then 2,195 kg of golden shiners and 9,145 kg of water can be carried. The fuel cost per kilogram of golden shiners would then be $0.17 at a density of 120 g/L versus $0.09 at a density of 240 g/L.

This study demonstrates that live golden shiners can be successfully transported at 240 g/L for up to 6 h in fresh well water chilled with unchlorinated block ice and aerated with pure oxygen. Survival (>99%) of golden shiners transported at 240 g/L, and of a 1-kg subset (13 g/L) of these fish held for approximately 18 h postdelivery supports this conclusion. In addition, increased loading density did not result in a significant cortisol stress response. Application of these results to large-scale operations could mitigate the effect of increasing highway diesel fuel expense.

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