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Effects of loading density during transport on physiological stress and survival of Sacramento-San Joaquin Delta fishes

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Truck transportation from the Bureau of Reclamation's Tracy Fish Collection Facility in California's Sacramento-San Joaquin Delta is the final phase of a multi-component process that results in the capture and release of >50 fish species, thereby preventing entrainment at a downstream water pumping facility. Fish-transport tables (termed Bates Tables) developed in 1955 do not take into consideration the fish loading process, commonly transported sensitive species, or physiological effects of elevated densities. To investigate suitability of the Bates Tables, effects of loading and transport at recommended and twice-recommended transport densities on physiological stress and survival of threadfin shad (*Dorosoma petenense*), Chinook salmon (*Oncorhynchus mykiss*), and striped bass (*Morone saxatilis*) were tested. Density did not have a significant effect on fish survival or physiological stress, as indicated by blood plasma constituent levels. At both densities, mean post-transport (168 hour) survival of all species was high (>98%), and ammonia and carbon dioxide levels increased in transport water as a result of fish metabolism, but levels remained below lethal levels. Among all species tested blood cortisol, glucose, and lactate levels followed a predictive adaptive response, with levels tending to peak immediately following transport and returning to basal levels within 24 hours.

Key words: blood plasma constituents, water quality, adaptive stress response, metabolic rate

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The truck transportation and release of multiple species and life-stages of fish is the ultimate operation at the Bureau of Reclamation's (BOR) Tracy Fish Collection Facility (TFCF; Byron, CA) necessary to return fish to their natal Sacramento-San Joaquin Delta and prevent entrainment into the Delta-Mendota Canal. Fish transport from the TFCF consists of hauling fish in a cylindrical tank (1.2-m deep, 4.4-m long, mean volume post-transport = 6,455 l) provided continuous pure oxygen via pressurized cylinder and diffusing airstones,

over a maximum distance of 49.9 km (Sutphin and Wu 2008). There is temporal variation in the species of fish transported, water quality during transport, and density of fish salvaged and transported from the TFCF (Sutphin and Wu 2008, CDFG 2013). There are >50 species of fish historically salvaged at the TFCF, and temperatures can approach species-specific lethal levels (Brett 1952, Olson and Foster 1957). Mean transport duration is 59.4 minutes (Craft et al. 2008, Sutphin and Wu 2008). Design, operation, and continued research at the multi-component TFCF is intended to maximize fish salvage while minimizing fish mortality (BOR 2013). However, operations at the TFCF result in the salvage of a constant influx of fish, and there is currently no efficient method to control the amount of fish entering the facility. As a result, elevated densities of fish may sometimes be transported.

To maintain fish health and maximize long-term survival, stressors common during loading and transport operations including handling, confinement, unfavorably high densities, and degraded water quality conditions must be considered (Piper et al. 1982, Berka 1986, Sutphin and Wu 2008). Fish loading and transportation, particularly at high densities, can result in mechanical abrasion, poor water quality conditions, and physiological stress, which can contribute to reduced survivability (Ross and Ross 1999, Urbinati et al. 2004, Carneiro et al. 2009). Exposure to stress elicits a general adaptive physiological and behavioral stress response in most fishes (Pickering 1981), consisting of primary and secondary levels and, if the stressor persists, a tertiary level (Schreck 1981, Bonga 1997, Barton et al. 2002). The primary stress response, as a result of capture, loading, and transport, is generally exhibited as the release of circulating catecholamines and corticosteroids (i.e., cortisol) by activation of the hypothalamus-pituitary-interrenal axis (Barton et al. 2003, Urbinati et al. 2004). As a result, cortisol is commonly measured as an indicator of fish primary stress response (Bonga 1997). The secondary stress response, among other physiological processes, can result in increased blood glucose and lactate, as well as increased heart rate, blood flow, and metabolic rate (Barton and Iwama 1991, Mommsen et al. 1999, Barton et al. 2002), making blood glucose and lactate, as well as measures of metabolism (oxygen consumption (MO_2) and ammonia production (M_{TAN}) common means to measure the secondary stress response in fish (Barton and Schreck 1987, Barton et al. 2002). The secondary stress response elicited in fish during fish-loading and transport, and glucose production in particular, is a response to fish energetic requirements, but can coincide with elevated MO_2, carbon dioxide production (MCO_2), and M_{TAN} rates, accelerating the rate of water quality decline (Bonga 1997, Barton et al. 2002). In truck transport systems, accumulated fish metabolic and excretory byproducts can result in toxic levels of CO_2, total ammonia nitrogen (TAN), and unionized ammonia (NH_3), which can impair performance, health, and survival of fish (Meade 1985, Russo and Thurston 1991). Similarly, elevated MO_2 can result in low O_2 levels, leading to a hypoxic state, which can also contribute to deteriorating health and mortality (Wedemeyer 1996). As a result, maintenance of appropriate water quality is often a limiting factor during fish transport, and is generally considered when developing fish transportation tables (Berka 1986, Emata 2000).

During the initial phases of TFCF development, fish transportation tables, termed Bates Tables, were designed based on simple unreplicated experiments during which the number of juvenile striped bass (*Morone saxatilis*) that can be maintained in stagnant water provided compressed air was estimated (BOR 1955). The activity report provided by BOR (1955) provides minimal experimental detail, but apparently doesn't consider stressors associated with actual transport, only considers the response of one species and,

presumably, doesn't incorporate the additional stressor of fish loading. Because multiple acute disturbances (i.e., capture, loading, transport, and release) tend to result in a cumulative stress response (Barton et al. 1986, Mesa 1994), and the survival of millions of fish annually transported from the TFCF is based on transport tables designed with simple, unreplicated, and vaguely described experiments on a single species, the primary research objective was to quantify effects of loading and transporting fish at recommended Bates Table levels on transport water quality, physiological stress, and post-transport (168 hour) survival of striped bass, Chinook salmon (*Oncorhynchus tshawytscha*), and threadfin shad (*Dorosoma petenense*). To determine if higher fish densities could be transported when deemed necessary by TFCF management, the secondary objective was to evaluate the effects of fish transport at densities twice the level recommended by the Bates Tables on the same parameters.

Materials and Methods

Selection of test species.—The species tested represent a phylogenetically diverse group with distinct life histories, and all have incurred precipitous declines in population abundance in recent decades (Yoshiyama et al. 1998, Moyle 2002, Feyrer et al. 2007). Threadfin shad were selected for testing because they are a Pelagic Organism Decline (POD) species (Sommer et al. 2007), have historically been the most abundant fish in the TFCF salvage, and, because they are a shoaling species, are often salvaged in high densities (CDFG 2013). Striped bass are also defined as a POD and are frequently salvaged at the TFCF, but were also selected because the Bates Tables were developed based on data collected with striped bass (BOR 1955). Of the four distinct populations of Central Valley Chinook salmon, winter-run are classified and protected as endangered and spring-run are classified as threatened under the Endangered Species Act (NMFS 1997). Their current listing status, as well as their use as a key species when considering initial design and development of the TFCF, warranted their use as a species during testing (Bates and Visonhaler 1957). Though all runs of Chinook salmon are salvaged at the TFCF, Fall- and Spring-run are generally the most abundant (Aasen 2011, 2012, 2013). Therefore, fall-run were selected for testing, and were intended as a representative surrogate for all salmon runs.

Fish source and care.—Juvenile Chinook salmon (Central Valley Fall-run) were acquired from the Mokelumne River Fish Hatchery (California Department of Fish and Wildlife, Clements, California), juvenile striped bass were acquired from Keo Fish Farm, Inc. (Keo, Arkansas), and adult threadfin shad were obtained from Hermann's Fish Farm (Robstown, Texas) for testing. All test species were truck-transported in 550-l tanks to BOR's Technical Service Center (TSC; Denver, CO), where they were maintained in two to three continuously aerated 870-l circular tanks. Following arrival at the TSC, fish were maintained at transport water temperatures and provided a daily 2–3 hour prophylactic salinity (NaCl at 6–8 g/l) and paracide green/formalin (22.5 ml) bath for four days to minimize likelihood of pathogenic infection and promote internal osmotic balance that can be compromised as a result of handling and transport procedures (Piper et al. 1982, Wedemeyer 1996). Approximately 7 days following arrival, fish were exposed to gradual changes in temperature, not exceeding 1.0°C/day, until target test temperatures were achieved. Fish were maintained under a natural photoperiod (37° 44' 23" N) with a combination of natural and halogen light sources. Chinook salmon were fed slow sinking pellets (1.5 mm, Bio Oregon®, Longview, Washington), striped bass were fed floating pellets (1.5 mm, Skretting USA, Tooele, Utah),

and threadfin shad were fed a mixture of crumbled dry feed (Skretting USA, Tooele, Utah) and Hikari® dry plankton feed (0.4–0.6 mm, Kyorin Co., Ltd., Japan). Threadfin shad were provided supplemental feedings of live brine shrimp (INVE Aquaculture, Inc., Salt Lake City, Utah). All species were fed at 2–3% body weight per day**.**

Density level and thermal regime.—Species-specific transport densities and thermal regime used during testing were selected to represent the worst-case-scenario for fish transport operations as currently outlined in the Bates Tables. This was ultimately selected as the highest water temperature each species would likely be exposed to during TFCF fish-transport, based on historic TFCF fish salvage data (1992–2009), and the corresponding maximum allowable transport density as defined by the Bates Tables (Craft et al. 2008). Elevated water temperatures were assumed to contribute to the worst-case-scenario during fish transport because elevated temperatures, particularly those approaching lethal levels, result in decreased gas solubility (i.e., lower dissolved oxygen levels), increased metabolic rates (i.e., increased dissolved oxygen intake and ammonia excretion), and immunosuppression (Wedemeyer et al. 1976, Colt 1984). Also, basal conditions, and magnitude and duration of stress response can be affected by thermal regime, often increasing at extremes (Wydoski et al. 1976, Barton and Schreck 1987, Davis and Parker 1990). Therefore, test temperature ranges selected for Chinook salmon, striped bass, and threadfin shad were 20 to 21°C, 26 to 27°C, and 23 to 24°C, respectively.

The Bates Tables isolate species into two categories, Chinook salmon and "other", and do not recommend specific transport densities (i.e., g fish/l), but indicate the percent of a load (up to 100%) as a total number of salvaged fish within a particular size class represents. For example, full loads (100%) of Chinook salmon >7.6 cm transported at 20–21°C, threadfin shad 6.4 to 11.4 cm transported at 26–27°C, and striped bass >11.4 cm transported at 23–24°C, are approximately 9,000 fish, 4,500 fish, and 3,500 fish, respectively, in a 3,785-l truck (original TFCF truck volume). To convert number of fish per unit volume to density (g/l), length-to-weight regression relationships developed from fish captured at the TFCF, were used to estimate weights of Chinook salmon ($y = 0.0074\ x^{3.12}$), threadfin shad ($y = 0.000004\ x^{3.27}$), and striped bass ($y = 0.0092\ x^{3.01}$) at 80, 100, and 150 mm fork length, respectively. Calculated Chinook salmon (11.33 g), threadfin (6.82 g), and striped bass (31.85 g) weights were multiplied by total number of fish, based on a full load (100%) as indicated by the Bates Tables, then divided by the volume (3,785 l) of the TFCF fish-transport truck, to calculate the following recommended maximum densities targeted during testing (Bates) for Chinook salmon, threadfin shad, and striped bass: 26.9, 8.1, and 29.5 g/l. These experimental densities were intended to determine if the maximum densities recommended by the Bates Tables are suitable. Double the recommended maximum densities were also tested to determine if higher transport densities could be used, as deemed necessary or appropriate by facility managers (Bates×2).

Fish marking and pre-treatment holding.—Prior to testing, fish were marked using a fluorescent microsphere solution (New West Technologies, Santa Rosa, California) and isolated as a function of treatment condition (species × density level) into individual 190-l conical holding tanks (76 cm diameter × 61 cm high), intended to simulate the TFCF fish haul-out bucket. Twenty fish of each species were also marked and transferred to 340-l post-transport survival-tanks to serve as a control. Marking fish prior to testing permitted an accurate estimate of transport density, and allowed consolidation of treatment and control fish during post-transport survival assessment. This marking method was deemed minimally

invasive compared to other external marking techniques and, coupled with a 7 days post marking holding period, presumably did not affect test fish stress response (Sharpe et al. 1998, Hayes et al. 2000). Species-specific feeding regime before experimentation and 7 days following marking was the same as outlined during fish holding**.** Test fish were not provided food for ~12 hours prior to experimentation. Water temperature (°C), pH (standard units), total ammonia nitrogen (TAN, mg/l), and dissolved oxygen (DO; mg/l) were measured daily using a YSI Pro-Plus multi-parameter instrument (YSI Inc., Yellow Springs, Ohio), and CO_2 (mg/l) was measured daily using a Oxyguard Carbon Dioxide Analyzer (Water Management Tech, Baton Rouge, Louisiana). The YSI Pro-Plus TAN electrode was calibrated daily during testing. All other probes were calibrated once at the initiation of each experimental period.

Experimental methodology.—To initiate fish loading from a 190-l fish holding tank into a fish transport container, water was drained from an external side 3.8-cm valve until ~30 l water and fish remained in the holding tank. During this process, two 1,000 mL-graduated beakers were partially filled and pre-transport water °C, DO, salinity (NaCl, g/l), pH, TAN, and CO_2 were measured. A fish transport container (30.5 cm long × 25.4 cm diameter, 15.1 l), sealed on the ends with 25.4 cm rubber cap and containing an internal microbubble oxygen diffuser (Point 4 System Inc., Coquitlam, British Columbia, Canada), was oriented below the conical holding tank, filled with tank water (permitting flushed fish to transfer directly to water and not an empty transport tank), and a 7.6-cm valve was opened, permitting remaining water and all fish to pass through a 7.6-cm rubber tube into the fish transport container. Drop height of water and fish from the holding tank to the transport container, was 24.1 cm. One hundred ten grams of non-iodized salt (North American Salt Co., Overland Park, Kansas) was added to each container to achieve salinities near 8 g/l, a value targeted during transport of fish from the TFCF. A 7.6-cm plug was immediately inserted to restrict water and fish spillage, and the transport container was transferred to an enclosed rear-section of a vehicle where the internal microbubbler diffuser was immediately supplied pure oxygen via a pressurized cylinder. Minimal fluctuation in temperatures occur within the transport tanks of trucks at the TFCF (Sutphin and Wu 2008); during testing, atmospheric temperature inside the vehicle was monitored and adjusted in an effort to maintain temperature similar to targeted test temperature. During transport, a 1.9-cm valve was partially opened to permit degassing, a similar practice used during truck transport of fish from the TFCF. Target transport duration was 60 minutes, similar to those recorded during fish transport at the TFCF (Sutphin and Wu 2008).

Following experimental transport, two fish from each replicate treatment were removed from their respective transport container. One fish was immediately immobilized and sampled for blood, and one was transferred to a 9.5-l black bucket, containing ~4.7 l water at the same temperature as pre-transport holding, provided continuous aeration via an air pump, and covered with a black lid. Fish were maintained in quiescent conditions for 1 hour, post transport, to determine if a short-duration holding period would allow fish time to recover from loading and transport stress. Post-transport water quality was also measured. Pre- and post-transport water quality conditions permitted the calculation of species- and density-dependent static M_{TAN} and $M_{CO2,}$ indicators of fish metabolic rate and response to stress (Alsop et al. 1999, Randall and Tsui 2002), as well as Δ°C, ΔpH, maximum TAN (NH_3) and CO_2 levels, and verification of transport NaCl levels. Unionized ammonia levels (NH_3) were calculated based on the NH_3 fraction of TAN (Francis-Floyd et al. 2012).

The remaining fish in each transport container were transferred (water to water) to a 340.7 l survival tank, with each survival tank housing a replicate for Bates, Bates×2, and control. Each survival tank was covered with shade cloth to promote quiescent conditions, and was only partially opened during blood sampling, feeding, and water quality measurements. Fish were removed from survival tanks (two/treatment) using a fine-mesh dip-net, at 24 and 168 hours for blood sampling. Water quality measurements and feeding regime through 168 hours post-transport were the same used during pre-treatment holding. Following 168 hour post-transport survival assessment, all fish were euthanized with a lethal dose of tricaine methanesulfonate (MS-222, Argent Chemical Laboratories, Inc., Redmond, Washington; 200 mg/l) weighed (±0.01 g) and measured for standard and total length (±1 mm) using an electronic balance and measuring board.

Due to the difficulty in capturing a known number of fish during fish loading (e.g., draining from conical tanks into fish transport containers) without causing additional harm or stress to other test fish, effects of the fish flushing process on blood plasma constituents (not metabolic rates or survival) were evaluated independently 7 days following the completion of the previously described treatment conditions for each species tested. To assess effects of flushing, fish were marked and provided the same conditions and care during pre-treatment holding described for subsequent treatment conditions. Following flushing from the holding tank (Flush), two fish were immediately captured, sampled for blood, and processed (weighed and measured) using the same methods described for previous treatments. These fish were sampled to assess if the fish loading or flushing process contributed to elevated stress, allowing the differentiation between effects of fish loading and fish transport on stress. However, it is important to note when interpreting flush data that flushed fish were tested independently and after all other treatment conditions.

To sample blood following loading (Flush), transport (1 hour), 1 hour post transport (2 hour), 24 hours, and 168 hours post transport, captured fish were quickly transferred to a water bath containing a lethal dose of MS-222 (200 mg/l), which resulted in rapid immobilization (<20 sec). Elevated MS-222 dose, coupled with sampling fish within 2 minutes following capture, likely inhibited stress-related increases in plasma cortisol, and ensured measured cortisol levels were a result of treatment conditions and not an artifact of handling and sampling techniques (Barton et al. 1986; Barton 2000, 2002). Immobilized fish were wrapped in Kimwipes® to ensure residual water did not contaminate blood samples, and the caudal peduncle was immediately severed with a scalpel. Heparinized microhematocrit capillary tubes (40-µl) were used to collect blood from the caudal vein and artery, and were immediately centrifuged using a microhematocrit centrifuge (Clay-Adams Autocrit Ultra 3, Franklin Lakes, New Jersey) for five minutes at 12,000 rpm to separate plasma and packed red blood cells. Packed red blood cell volume (Hematocrit, Hct) was measured immediately following centrifuging. The volume of red blood cells was discarded, and the remaining blood plasma was transferred into plastic cryogenic freezing vials and stored in a -80°C freezer. Weights (±0.01 g) and lengths (SL, TL ±1 mm) of each fish were obtained following sampling. Cryogenic vials were later thawed for plasma glucose, lactate, and cortisol measurements. Glucose and plasma were measured using a polarographic analyzer (YSI 2700 Select, YSI Inc., Yellow Springs, Ohio), plasma cortisol levels were measured at Mapes Veterinary Endocrinology Laboratory (University of California, Davis) using a modified enzyme immunoassay. Small volumes of blood recovered from threadfin shad precluded cortisol measurement.

Statistical analyses.—The majority of data did not meet the assumptions of parametric statistics; therefore Two-Way ANOVA on ranks was used to test species and treatment (density, time post transport) differences between morphometrics (weight, length), transport conditions (duration, density, water quality post-transport), metabolism (M_{TAN}, M_{CO2}), blood plasma constituents (Hct, cortisol, glucose, and lactate), and 168 hour post-transport survival. Tukey's Test was employed for all pairwise comparisons. All statistical analyses were conducted using SigmaStat 3.5 software (Systat Software Inc., Richmond, California); the significance level (α) for all analyses was set at 0.05.

Results

Wet weights of test fish were significantly different, for all treatment conditions, across species tested (Table 1; $F_{2,6} = 337.81$, $P < 0.001$). Threadfin shad and striped bass weights were not different across treatment conditions (Tukey's Test, $P > 0.05$). However,

Table 1.—Fish lengths, weights, sample size, and number of fish used per replicate during testing. Lengths and weights are reported as means ± 1 standard deviation.

Species	Treatment	Sample Size (*n*)	#fish/ replicate	Standard Length (mm)	Total Length (mm)	Wet Weight (g)
Chinook Salmon	Control	12	20 - 21	123.8 ± 13.7	141.6 ± 15.2	25.5 ± 9.3
	Flush	12	2	114.9 ± 12.4	131.6 ± 13.6	18.11 ± 6.3
	Bates	12	13 - 21	119.3 ± 13.3	136.7 ± 14.8	21.9 ± 7.8
	Bates × 2	12	22 - 40	124.3 ± 13.8	142.2 ± 15.4	25.6 ± 9.2
Striped Bass	Control	12	20	97.3 ± 4.8	115.8 ± 5.3	17.5 ± 2.7
	Flush	12	2	100.1 ± 4.6	118.6 ± 4.7	18.5 ± 2.2
	Bates	12	24 - 37	98.0 ± 5.4	116.5 ± 6.6	18.1 ± 3.6
	Bates × 2	12	50 - 68	97.3 ± 5.8	115.6 ± 6.9	17.8 ± 3.7
Threadfin Shad	Control	8	20 - 21	72.3 ± 14.7	89.7 ± 18.1	7.2 ± 4.9
	Flush	8	2	83.6 ± 12.5	102.6 ± 15.8	10.1 ± 4.6
	Bates	8	14 - 16	77.3 ± 12.5	94.9 ± 15.3	8.4 ± 4.2
	Bates × 2	8	31 - 38	72.8 ± 11.5	89.3 ± 14.1	7.2 ± 3.7

Chinook salmon Bates×2 and control treatments, though not different in weight from each other, were significantly heavier than the other treatment conditions (Tukey's Test, $P < 0.001$). Pre-treatment (168 hour acclimation) and post-treatment (168 hour survival assessment) water quality conditions are reported in Table 2. Transport duration was not different, as a function of treatment, within species tested ($F_{1,2} = 1.7$, $P = 0.20$). However, mean transport durations of threadfin shad (Bates=58.8 minutes, Bates×2=59.8 minutes) were significantly shorter than Chinook salmon (Bates = 60.5, Bates×2 = 60.8 minutes) and striped bass (Bates = 60.2, Bates×2 = 60.2 minutes; $F_{1,2} = 11.2$, $P < 0.001$; Table 3). Transport °C ($F_{1,2} = 1.1$, $P = 0.305$), DO ($F_{1,2} = 0.11$, $P = 0.738$), and TAN ($F_{1,2} = 2.0$, $P = 0.162$) levels were unaffected by fish density level (Table 3). However, final transport CO_2 levels increased with increasing density for all species tested (Table 3; $F_{1,2} = 73.9$, $P < 0.001$). As designed (see Methods) transport densities ($F_{1,2} = 596.9$, $P < 0.001$) and temperatures ($F_{1,2} = 130.9$, $P < 0.001$) were different across species, and typically within the predetermined target range (Table 3). Interestingly, NaCl levels were different across species during testing ($F_{1,2} = 38.8$, $P < 0.001$), but differences in means (Table 3) were small enough that they likely were not biologically relevant.

Table 2.—Pre-treatment water quality conditions, as measured in 190-l conical tanks (treatment) and 870-l tanks (control) 1–7 days before fish transport, and post-transport water quality conditions as measured in 340-l holding tanks during 168 hour survival assessment. Temperature is reported as °C, dissolved oxygen (DO), salinity (NaCl), and total ammonia nitrogen (TAN) are reported as mg/l, and pH is reported in standard units. Values are reported as means ±1 standard deviation.

		Pre-Treatment Conditions					*Post-Transport (168 hours) Conditions*			
Species	Treatment	°C	DO	NaCl	pH	TAN	°C	DO	pH	TAN
Chinook Salmon	Control	20.0 ± 1.3	6.8 ± 0.3	0.2 ± 0.0	7.6 ± 0.3	0.4 ± 0.2	21.4 ± 0.7	6.3 ± 0.2	7.8 ± 0.3	0.3 ± 0.1
	Bates	20.1 ± 1.3	6.8 ± 0.4	0.2 ± 0.0	7.6 ± 0.4	0.4 ± 0.2				
	Bates × 2	20.1 ± 1.3	6.6 ± 0.4	0.2 ± 0.0	7.6 ± 0.3	0.4 ± 0.2				
Striped Bass	Control	22.7 ± 1.3	6.2 ± 0.4	0.4 ± 0.3	7.5 ± 0.2	0.7 ± 0.4	23.3 ± 0.5	6.1 ± 0.3	7.7 ± 0.2	0.4 ± 0.2
	Bates	22.8 ± 1.1	6.3 ± 0.3	0.2 ± 0.1	7.7 ± 0.2	0.4 ± 0.2				
	Bates × 2	22.8 ± 1.1	6.1 ± 0.3	0.2 ± 0.1	7.7 ± 0.2	0.4 ± 0.2				
Threadfin Shad	Control	26.8 ± 0.6	5.6 ± 0.3	0.2 ± 0.0	7.9 ± 0.1	0.7 ± 0.4	26.7 ± 0.7	5.6 ± 0.2	7.9 ± 0.2	0.2 ± 0.0
	Bates	27.1 ± 0.3	5.9 ± 0.1	0.2 ± 0.0	8.0 ± 0.2	0.6 ± 0.3				
	Bates × 2	27.2 ± 0.2	5.8 ± 0.1	0.2 ± 0.0	8.0 ± 0.1	0.1 ± 0.0				

Table 3.—Target and actual densities during fish transport, transport duration, and water quality conditions (temperature = °C, dissolved oxygen = DO (mg/l), salinity = NaCl (mg/l), total ammonia nitrogen = TAN (mg/l), unionized ammonia = NH_3 (mg/l), carbon dioxide = CO_2 (mg/l)) in transport containers at the end of transport during testing.

Species	Treatment	Target Density (g/l)	Actual Density (g/l)	Transport Duration (minutes)	° C	DO	NaCl	pH	Max TAN	Max NH_3	Max CO_2
Chinook Salmon	Bates	26.9	24.5 ± 1.5	60.5 ± 0.9	20.5 ± 1.2	24.0 ± 4.3	8.5 ± 0.2	6.8 ± 0.3	5.8 ± 1.3	0.01	7.0 ± 2.0
	Bates × 2	53.8	48.8 ± 1.6	60.8 ± 0.8	20.5 ± 1.2	24.1 ± 2.5	8.8 ± 0.2	6.9 ± 0.3	6.0 ± 1.1	0.02	12.1 ± 4.3
Striped Bass	Bates	29.5	34.7 ± 3.9	60.2 ± 0.6	23.0 ± 1.0	21.4 ± 6.2	8.4 ± 0.1	6.8 ± 0.1	4.8 ± 0.8	0.01	8.3 ± 2.5
	Bates × 2	59	68.4 ± 5.7	60.2 ± 0.3	23.0 ± 1.0	23.5 ± 1.4	8.6 ± 0.2	6.8 ± 0.0	5.1 ± 0.9	0.01	13.7 ± 4.1
Threadfin Shad	Bates	8.1	8.5 ± 0.2	58.8 ± 0.9	25.8 ± 0.9	19.0 ± 3.4	8.2 ± 0.1	7.1 ± 0.1	4.6 ± 0.2	0.04	4.6 ± 0.9
	Bates × 2	16.2	16.5 ± 0.4	59.8 ± 0.8	26.8 ± 0.2	16.5 ± 2.1	8.3 ± 0.2	7.1 ± 0.0	4.8 ± 0.1	0.03	11.6 ± 0.9

Survival for all species tested, through 168 hours following transport, was >98%, not affected by density level, and not different compared to survival of control fish (Table 4; Two-Way ANOVA on Ranks, $F_{2,4} = 2.5$, $P = 0.09$). Ammonia production rates (M_{TAN}) were

Table 4.—Mean (± 1 standard deviation) percent survival (168 hour post transport), change in temperature (Δ°C), and ammonia (M_{TAN}) and carbon dioxide (M_{CO2}) fish production rates measured during testing.

Species	Density Category	Density (g/l)	Survival (%)	Δ °C	M_{TAN} (mg/g/h)	M_{CO2} (mg/g/h)
Chinook Salmon	Bates	24.5 ± 1.5	98.7 ± 4.4	0.3 ± 0.6	0.23 ± 0.06	0.27 ± 0.08
	Bates × 2	48.8 ± 1.6	99.3 ± 1.6	0.4 ± 0.6	0.12 ± 0.02	0.24 ± 0.10
	Control	NA	100.0 ± 0.0			
Striped Bass	Bates	34.7 ± 3.9	100.0 ± 0.0	0.5 ± 0.8	0.13 ± 0.02	0.22 ± 0.08
	Bates × 2	68.4 ± 5.7	100.0 ± 0.0	0.3 ± 1.0	0.07 ± 0.01	0.19 ± 0.06
	Control	NA	100.0 ± 0.0			
Threadfin Shad	Bates	8.5 ± 0.2	99.0 ± 2.7	-0.9 ± 1.0	0.54 ± 0.05	0.54 ± 0.10
	Bates × 2	16.5 ± 0.4	99.2 ± 1.4	-0.2 ± 0.4	0.28 ± 0.01	0.70 ± 0.06
	Control	NA	100.0 ± 0.0			

significantly different across all species tested ($F_{1,2}$ = 184.4, P < 0.001), and decreased with increasing density level (Table 4; $F_{1,2}$ = 130.6, P < 0.001). Threadfin shad M_{CO2} levels were significantly greater at an elevated density (Bates×2) during transport (Tukey's Test, P < 0.001), but transport density did not affect M_{CO2} levels for Chinook salmon (Tukey's Test, P = 0.25) and striped bass (Table 4; Tukey's Test, P = 0.32). Threadfin shad M_{CO2} levels, at both transport density levels, were significantly greater than those for Chinook salmon and striped bass (Table 4; $F_{1,2}$ = 148.6, P < 0.001).

Chinook salmon, striped bass, and threadfin shad mean Hct levels ranged from 37.4 to 42.1, 28.6 to 37.5, and 38.4 to 49.0% packed cell volume, respectively, and species ($F_{3,6}$ = 92.81, P < 0.001) and treatment ($F_{3,6}$ = 14.74, P < 0.001) specific differences are reported in Figure 1. Chinook salmon, striped bass, and threadfin shad basal Hct levels were

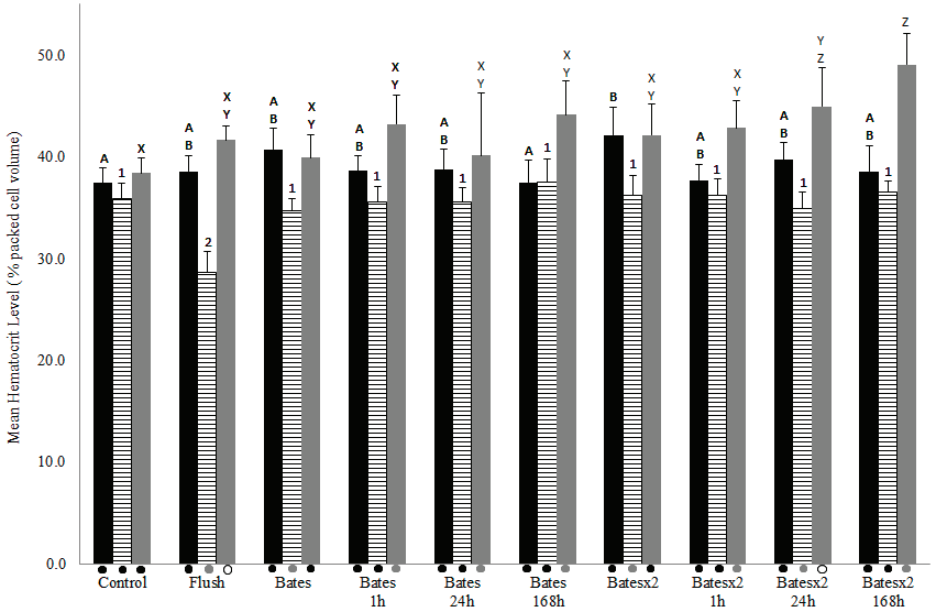

Figure 1.—Mean (±2 *SE*) blood hematocrit levels (% packed cell volume) of Chinook salmon (black), striped bass (horizontal stripes), and threadfin shad (grey) before (control, flush) and after transport at two different density levels (Bates, Bates×2). Different letters (Chinook salmon = A,B; threadfin shad = X,Y,Z) and numbers (striped bass = 1,2) above error bars indicates significant differences across treatment, but within each species only (Two-way ANOVA on Ranks, Tukey's Test). Different colored circles (black, grey, white) below graphs and above treatment titles indicate significant differences across species, but within each treatment condition (Two-way ANOVA on Ranks, Tukey's Test).

indicative of healthy levels as reported for salmon (Mazur and Iwama 1993, Martinelli et al. 1998), as well as other freshwater species (Davis and Parker 1990, Wedemeyer et al. 1990), suggesting fish had not been exposed to chronic stress, and were healthy during testing. Transport density had no effect on Chinook salmon or striped bass blood cortisol level at any sampling period following transport (Figure 2; $F_{9,9}$ = 17.48, P < 0.001). Chinook salmon and striped bass cortisol levels (ng/ml) were significantly higher immediately following

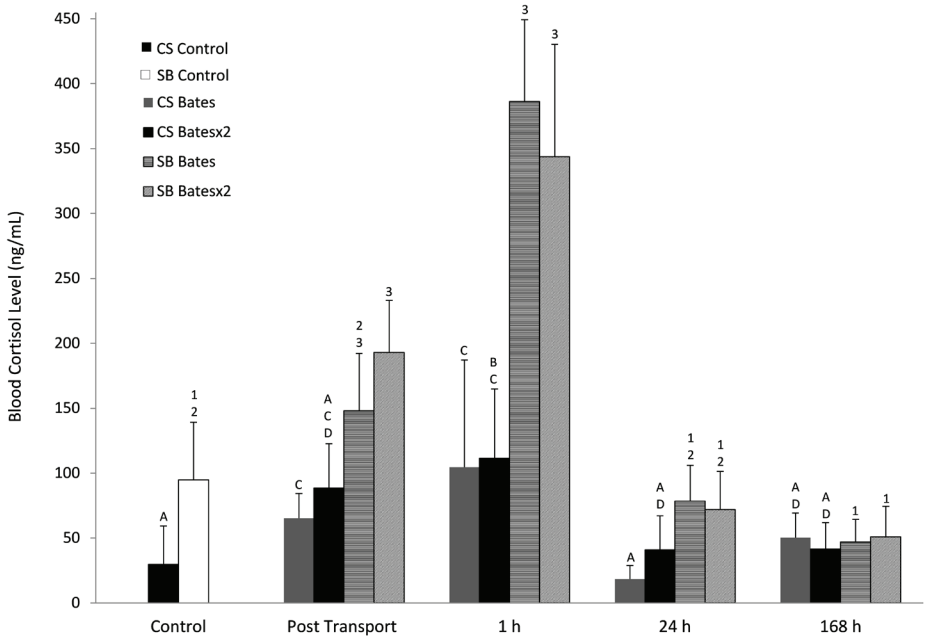

Figure 2.—Mean (±1 *SE*) blood cortisol levels (ng/ml) for Chinook salmon and striped bass before (control) and after transport at two different density levels (Bates, Bates×2). Mean (±1 *SD*) Bates (Chinook salmon = grey, striped bass = horizontal bars) and Bates×2 (Chinook salmon = black, striped bass = perpindicular bars) densities during transport for Chinook salmon and striped bass were 24.5 ± 1.5 and 48.8 ± 1.6, and 34.7 ± 3.9 and 68.4 ± 5.7, respectively. Mean (±1 *SD*) water temperatures during transport for Chinook salmon and striped bass were 20.5 ± 1.2 and 23.0 ± 1.0°C, respectively. Different letters (Chinook salmon) and numbers (striped bass) above error bars indicates significant differences across treatment, but within each species (Two-way ANOVA on Ranks, Tukey's Test).

transport, peaked one hour post-transport, but returned to basal (control) levels within 24 hours post-transport (Figure 2; Tukey's Test, $P < 0.05$). Striped bass cortisol levels were significantly higher than Chinook salmon levels at all sample periods except 168 hours post-transport (Figure 2; Tukey's Test, $P = 0.80$).

In general, transport density had no effect on species-specific blood-glucose level as a function of sampling period before or after transport. The exception: glucose levels of threadfin shad 1 hour post transport, exposed to Bates×2 density levels were significantly greater than threadfin shad at the same time period following transport and exposed to Bates density (Figure 3; $F_{9,18} = 2.87$, $P < 0.001$). At both transport density categories, peak measured blood-glucose levels occurred at 0 (immediately following transport) to 1 hour following transport for all species, and returned to, or below, basal levels within 24 hours post transport (Figure 3). Basal glucose levels were significantly different across all species (Tukey's Test, $P < 0.02$), and striped bass glucose level, transported at Bates density level at 1 hour post transport, were greater than other species tested (Tukey's Test, $P < 0.03$). When transported at Bates×2 density levels and measured at 1 hour post transport, striped bass and threadfin shad glucose levels, though not different from each other, were greater than Chinook salmon levels (Tukey's Test, $P < 0.02$). Striped bass glucose levels at both transport densities and 168 hours post transport were greater than Chinook salmon and threadfin shad levels (Tukey's Test, $P < 0.03$).

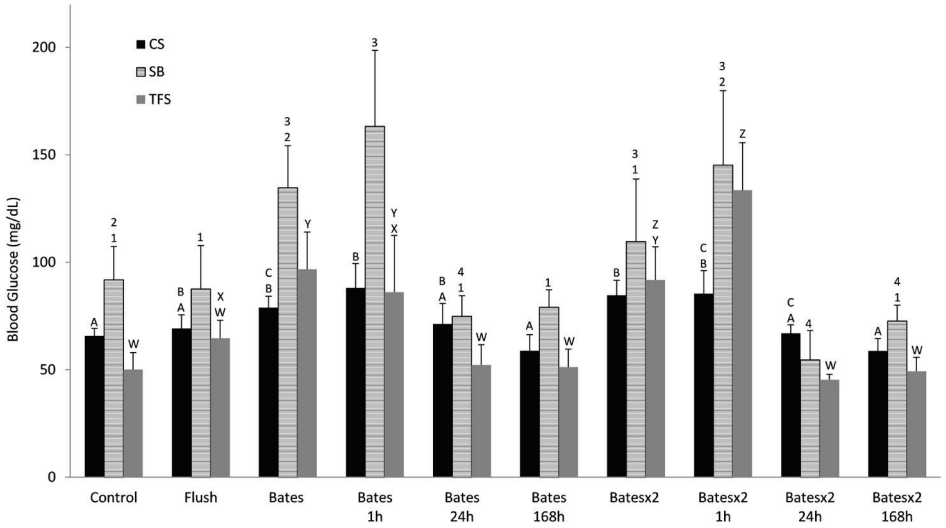

Figure 3.—Mean blood glucose levels (± 2 *SE*) for Chinook salmon (CS, black), striped bass (SB, horizontal bars), and threadfin shad (TFS, grey) before (control, flush) and after transport at two different density levels (Bates, Bates×2). Mean (±1 *SD*) Bates and Bates×2 densities during transport for Chinook salmon, striped bass, and threadfin shad were 24.5 ± 1.5 and 48.8 ± 1.6, 34.7 ± 3.9 and 68.4 ± 5.7, and 8.5 ± 0.2 and 16.5 ± 0.4, respectively. Different letters (CS = A,B,C; TFS = W,X,Y,Z) and numbers (SB = 1,2,3,4) above error bars indicates significant differences across treatment, but within each species only (Two-way ANOVA on Ranks, Tukey's Test).

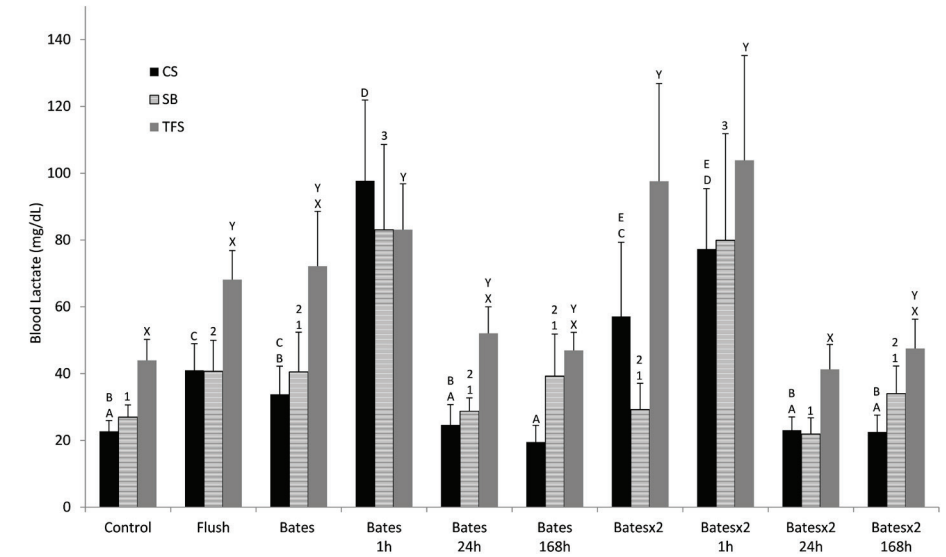

Figure 4.—Mean blood lactate levels (±2 *SE*) for Chinook salmon (CS, black), striped bass (SB, horizontal bars), and threadfin shad (TFS, grey) before (control, flush) and after transport at two different density levels (Bates, Bates×2). Mean (±1 *SD*) Bates and Bates×2 densities during transport for Chinook salmon, striped bass, and threadfin shad were 24.5 ± 1.5 and 48.8 ± 1.6, 34.7 ± 3.9 and 68.4 ± 5.7, and 8.5 ± 0.2 and 16.5 ± 0.4, respectively. Different letters (Chinook salmon = A,B,C,D,E; threadfin shad = X,Y) and numbers (striped bass = 1,2,3) above error bars indicates significant differences across treatment, but within each species only (Two-way ANOVA on Ranks, Tukey's Test).

Similar to what was observed for species-specific cortisol and glucose levels, transport density had no effect on blood lactate levels for any of the species tested, across any sample period, following transport (Figure 4; $F_{9,18} = 29.50$, $P < 0.001$). For all species, and across both density categories evaluated, measured lactate levels were greater than basal levels and peaked at 1 hour post transport, then returned to basal levels by 24 hours post transport (Tukey's Test, $P < 0.05$). Basal lactate levels of threadfin shad were greater than those measured for Chinook salmon and striped bass (Tukey's Test, $P < 0.001$), which were not significantly different from each other (Tukey's Test, $P = 0.98$). At measured peak levels (1 hour post transport), and across both density categories tested, there was no difference in species-specific lactate level. However, at 168 hours following transport and at both density categories, threadfin shad and striped bass lactate levels were not different (Tukey's Test, $P > 0.08$), but were both greater than Chinook salmon levels (Tukey's Test, $P < 0.03$).

Discussion

Basal blood hematrocit levels for all species tested were within reported normal levels for fish, suggesting test fish were not likely anemic or diseased prior to testing (Barton et al. 2002). Similarly, basal cortisol levels for Chinook salmon were similar to those generally reported for salmonids (Barton and Iwama 1991). However, striped bass basal cortisol levels were >3× greater than what has been reported for the species (Davis et al. 1982, Davis and Parker 1986). Though basal glucose levels for striped bass were greater than those for Chinook salmon and threadfin shad, all species basal glucose levels were near the typical range reported for fish (Barton et al. 2002). While it is difficult to ascertain the cause of elevated basal cortisol levels in striped bass, normal basal glucose levels and a nearly identical pre-treatment holding environment across tested species would suggest the elevated levels are perhaps a species specific sensitivity to a stressor not perceived by the other species tested or simply elevated basal cortisol levels common in striped bass.

Immediate post-transport and 168 hours post-transport survival of Chinook salmon, striped bass, and threadfin shad exposed to recommended Bates Table, and twice recommended density levels were high (>98%). In general, reported immediate or en route survival of transported fish is high (>90%; Johnson and Metcalf 1982), even at elevated densities (Staurnes et al. 1994 [560 g/l], Hasan and Bart 2007 [400 g/l]) or for longer durations (Carmichael 1984 [30 hours]). However, long-term survival (≥96 hours) of transported fish varies greatly, and is reportedly dependent on a multitude of parameters including, but not limited to, species, capture or loading technique, transport water quality, and duration. Mazik et al. (1991) reported high (100%) survival of striped bass transported 5 hours in freshwater at a density of 180 g/l, then maintained in 1% NaCl solution for four weeks. Carmichael (1984) also reported elevated survival through 168 hours (>85%) when transporting largemouth bass (*Micropterus salmoides*) at a density of 180 g/l with various combinations of pre-, during, and post-treatments, including NaCl concentration near plasma level. However, in the absence of treatments, and transporting bass at the same density in well water, survival was <20%. When transported in well water, with no additives (e.g., no NaCl), at a density of 200, 300, and 400 g/l for 3 hours, rohu (*Labeo rohita)* experienced 28, 35, and 41% mortality through two weeks post transport, respectively (Hasan and Bart 2007). These results support those reported by others, as well as those of the current study, that suggest use of NaCl, at levels between 0.5 and 8%, placate fish stress response

and contribute to increased survivability (Johnson and Metcalf 1982, Mazik et al. 1991, Swanson et al. 1996) of fish during, or immediately following, handling and transport operations. In response to stress, permeability of fish gills generally increase, resulting in osmoregulatory imbalances (Mazeaud et al. 1977). The addition of NaCl to water during stressful situations (i.e., handling, loading, transport) near the internal plasma concentration of fish minimizes the energetic requirements of osmoregulation (Redding and Schreck 1983). However, the use of NaCl as an additive to improve survival of fish during transport is apparently species specific (Gomes et al. 2003, 2006). For example, juvenile matrinxa (*Brycon cephalus)* can tolerate (100% survival) transport for 4 hours at densities between 83 and 206 g/l without the apparent addition of NaCl or other treatments (Urbinati et al. 2004, Abreu et al. 2008). Therefore, care should be taken to consider species-specific needs, and other practices, particularly pre-transport capture and handling, that may limit survival of fish during transport.

In the current study, fish were transferred from holding tank to transport container by water-to-water. As a result, fish were not exposed to physical damage and additional stress, often associated with netting and physical-handling, which could have compromised survival (Barthel et al. 2003). For example, freshwater drum (*Aplodinorus grunniens*) transported at densities of 60 and 120 g/l for 6 hours experienced 70 and 96% mortality, even with the addition of 5% NaCl, through two weeks following transport (Johnson and Metcalf 1982). Ultimately, Johnson and Metcalf (1982) suggested capture and handling (shore seining and hand counting) contributed most significantly to elevated post-transport mortality. Though post-transport survival of fish in the current study was high, it is important to note transport and post-transport water in the current study is different from SSJD water. Sacramento-San Joaquin Delta water reportedly contains elevated levels of pesticides and heavy metals, as well as pathogens and parasites (Lee and Lee 2004). Stress incurred during fish loading and transport can lead to immunodeficiency, and contribute to increased bacterial (Pickering and Pottinger 1989; Iwama et al. 1997) and parasitic (Woo et al. 1987) infection.

Increasing transport densities, from recommended Bates Table density levels to twice the recommended levels, had no effect on Chinook salmon, striped bass, or threadfin shad survival (immediate or long-term) or measured blood plasma constituents in general (hematocrit, cortisol, lactate, or glucose). There is variability in reported species-specific effects of density during fish transport on long-term survivability and physiological stress response. Abreau et al. (2008) reported survival and cortisol response during 4-hour transport of juvenile matrinxa (*Brycon cephalus*) was not density dependent, and survival of walleye (*Sander vitreus*) fry during 4-hour transport is also reportedly not density dependent (Peterson and Carline 1996). Similarly, only at the highest transport density (350 g/l) was there an increase in mortality, cortisol, and glucose concentrations following 4-hour transport of juvenile jundia (*Rhamdia quelen*), but at lower densities (75, 150, and 250 g/l) there was no effect (Carneiro et al. 2009). However, both Hasan and Bart (2007) and Gomes et al. (2006) indicated long-term survival and cortisol response of rohu and matrinxa, respectively, were density dependent. When studying the same species as Gomes et al. (2006), Urbinati et al. (2004) reported an inverse relationship between transport density and matrinxa cortisol response, but no density-dependent effect on long-term survival. Apparently, the physiological response of fish to density during transport isn't ubiquitous, and other environmental factors, such as life-stage or development (Pottinger et al. 1995), rearing environment (Woodward and Strange 1987, Jentoft et al. 2005), evolutionary history (Barton

et al. 2000), schooling nature (Parker, 1973, Iguchi et al. 2002), and water quality (Pickering and Pottinger 1987), amongst other factors (Barton 1988), may ultimately supersede or confound the response to elevated density during transport. This further exacerbates the need to understand species, and life-stage, specific needs during transport.

In general, the duration of stress response, as represented by peaking cortisol levels shortly (0.5 to 1 hour) post-transport and returning to basal levels within 24 hours, is typical across most freshwater teleosts exposed to handling, loading, or short-duration transport operations, then returned to a placid environment (Barton and Iwama 1991, Barton 2000, Barton 2002). Glucose and lactate levels for tested species followed a similar temporal trend, which is also a common glucose (Robertson et al. 1987, Barton 2000, Abreu et al. 2008, Carneiro et al. 2009) and lactate (Pickering et al. 1982, Pottinger 1998, Davis and Schreck 1997) response across fish exposed to similar stressors. Duration of response to fish-loading and transport stress can have important management-level implications. The inability of fish to fully recover from loading and transport-related stress within a very short duration ($\leq$1 hr) would likely preclude the necessity of a short-duration holding period for fish prior to release following large-scale transport operations.

The magnitude of cortisol increase, from basal to peak levels, for Chinook salmon (3.8×) and striped bass (4.1×) are similar to what is reported for other fish following transportation (Davis and Parker 1986, Maule et al. 1988). Elevated release of cortisol in response to loading and transport stress is a neuroendocrine response; stress functions to stimulate the hypothalamic-pituitary-interrenal axis, leading to the circulatory release of cortisol and other corticosteroid hormones (Randall and Perry 1992, Bonga 1997). Maximum post-stress glucose and lactate levels for all species tested were within the general range reported for other species (Barton et al. 2002). When subjected to a stressor, it is generally accepted that elevated glucose levels, or hyperglycemia, is a physiological response to meet energetic demands to respond to the stressor (Mazeaud and Mazeaud 1981), and blood lactate build-up is a result of increased muscular activity, generally associated with heightened activity or swimming (Driedzic and Hochachka 1978). In general, the severity of a stressor is represented by magnitude of a fish's stress response (Barton et al. 1980, Barton and Iwama 1991). The reported experimental results suggest loading and transporting Chinook salmon, striped bass, and threadfin shad at recommended or twice recommended Bates Table densities results in a stress-response similar to other fish-handling and transport operations.

Species-specific differences in the magnitude of physiological response to loading and transport stress are common (Davis and Parker 1986; Barton and Grosh, as reported in Barton and Iwama 1991). Across most sampling periods during testing, striped bass cortisol levels were greater than levels for Chinook salmon. Similarly, basal glucose level of striped bass, as well as the level 1 hour following transport at the recommended Bates Table density, was greater than levels for other species tested. Threadfin shad basal, immediately post-transport, and 24 hours post-transport lactate levels, as well as en route M_{TAN} and M_{CO2} levels, were greater than those for Chinook salmon and striped bass. As designed, there were distinct differences in test temperatures across species. Water temperature, particularly those outside a preferred range, appear to contribute to elevated cortisol and glucose levels in some fish (Strange 1980, Davis et al. 1984, Barton and Schreck 1987). So, it is possible environmental conditions may have influenced results. However, the two species that exhibited elevated cortisol, glucose, and lactate levels during testing (striped bass and threadfin shad), were likely within or close to their preferred temperature range (Coutant et

al. 1984). Therefore, we ultimately attribute species-specific differences in physiological stress response to genetic differences, as is supported by Davis and Parker (1986), Barton and Iwama (1991), and Barton et al. (2002).

Ammonia and carbon dioxide production rates of fish, though indicators of metabolic rate and response to stress (Alsop et al. 1999, Randall and Tsui 2002), were important measured variables to determine what density levels of fish could be transported over short duration without reaching harmful and acutely lethal levels of TAN, NH_3, and CO_2. Elevated NH_3 and CO_2 can be toxic to fish, and are of particular importance during fish transport operations because stress and increased activity during transport can contribute to increased ammonia and carbon dioxide production rates (Wedemeyer 1996, Alsop et al. 1999, Randal and Tsui 2002). Also, stressors and elevated CO_2, often encountered during transport, can increase fish sensitivity to ammonia (Randall and Tsui 2002). Excessive CO_2 results in a reduction of CO_2 gill excretion, leading to hypercapnia and respiratory acidosis. Elevated NH_3 can result in gill corrosion and nerve damage, impairing osmoregulation and central nervous system functionality (Wedemeyer 1996, Portz et al. 2006).

Ammonia production rates of fish following transport in the current study were greater than rates reported for fasted (Altinok and Grizzle 2004) and recently fed fish (Brett and Zala 1975, Jarboe 1995), as well as those for swimming fish (Sukumaran and Kutty 1977). Results of the current study are supported by Randall and Tsui (2002), who suggested M_{TAN} of fish increased in response to stress and activity. Ammonia production rates of all species tested tended to have an inverse relationship with density, similar to what was observed during the transportation of juvenile matrinxa (Urbinati et al. 2004, Abreu et al. 2008). Results from Fromm and Gillette (1968) and Fromm (1970) suggested ammonia excretion rates decline with exposure to increasing ammonia levels. It is likely higher densities of fish during transport result in rapid increases in ammonia, and elevated levels earlier during the transport process. Once ammonia levels reach a critical level, it is possible reduction in ammonia production in fish is a physiological response to minimize likelihood of exposure to lethal levels (Randall and Tsui 2002).

Recommended maximum CO_2 levels for short-term holding or transport and fish culture operations are reportedly 15–60 mg/l and <5–10 mg/l, respectively (Wedemeyer 1996, Timmons et al. 2002, Portz et al. 2006). Short duration exposure to levels >200 mg/l are often used as a fish anesthetic (Sommerfelt and Smith 1990, Ackerman et al. 2005) and, though dependent on the buffering capacity of transport water (i.e., alkalinity level), exposure to CO_2 levels >85 mg/l may affect survival of fish during transport operations (Amend et al. 1982, Grottum and Sigholt 1996). Recommended maximum NH_3 levels for fish culture operations are reportedly 0.01–0.02 mg/l (Westers 1981, Meade 1985, Wedemeyer 1996), whereas acutely toxic NH_3 levels for freshwater fish, Chinook salmon included, range from 0.32–3.10 mg/l (Ruffier et al. 1981, Thurston and Meyn 1984). Results of the current study indicate transport of fish at recommended, and twice recommended, Bates Table densities should permit transport at or below recommended CO_2 and NH_3 levels, and likely not expose fish to acutely lethal CO_2 or NH_3 levels.

Results of the current study support those reported in the scientific literature indicating, when sufficient means are taken to maintain appropriate water quality conditions (e.g., increase NaCl and dissolved oxygen levels) and transport densities, stress incurred as a result of loading and transport is adaptive and functions to reestablish homeostasis and promote survival. In a review of fish transportation operations conducted by federal, state,

tribal, and private entities, Carmichael and Tomasso (1988) indicated transport densities typically range from 24 to 431 g/l. Therefore, maximum Bates Table densities are on the lower end of the spectrum compared to most other fish transportation operations. Moderate transport densities, paired with elevated NaCl levels during transport, likely result in minimal transport induced mortality of fish from the TFCF. Though evaluating the fish loading process was not a project objective, study results compared to those reported in the published literature suggest water-to-water transfer of fish (i.e., draining water and fish from a bucket to a transport tank) results in high survival, and is therefore a technique that is not only efficient, but one that should be explored during other fish transference operations. Tracy Fish Collection Facility standard operating procedures for fish transportation (TFCF SOP#41) require fish be transported, at a minimum, every 12 hours, and every eight hours when delta smelt (*Hypomesus transpacificus*) are present at the facility. Also, there are multiple fish holding tanks that can be employed at the TFCF in an attempt to regulate the density of fish transported. As a result, transportation of exceedingly high densities of fish (i.e., >Bates Table levels) is infrequent. However, pulses of high densities of fish do occur at the TFCF, and that is why the Bates Tables were developed, and the current study was undertaken. When such conditions arise, or if there are upward shifting trends in populations of fish in the SSJD, results of the current study allow TFCF management to permit transport of fish at up to twice the recommended TFCF densities with the confidence that survival of fish will not be compromised.

All three species evaluated during testing are commonly produced during hatchery or private commercial operations. Chinook salmon and striped bass are produced in hatcheries throughout the coastal western and eastern United States, respectively, for artificial propagation, sport fishing, and research activities (Geiger and Parker 1985, Hilborn 1992). Threadfin shad are often produced and stocked in lentic systems as a supplemental prey item (DeVries et al. 1991). Therefore, results of this study expand beyond operations at SSJD state and federal fish collection facilities, and add to the existing knowledge of species-specific fish handling, loading, and transport requirements. Reported survival data can be used to develop or verify species specific fish transport standard operating procedures. However, the current report extends beyond simply evaluating effects of loading and transport on stress and survival, and details effects on metabolic rate (M_{O2}, M_{TAN}, M_{CO2}). Since reduced oxygen levels, and elevated ammonia and carbon dioxide levels can impair chronic health and survival of fish, data from the current study can be used to ascertain adequate densities levels required to maintain adequate water quality conditions during fish holding necessary to support long-term health and survival of fish.

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