Physiological Stress Responses of Cutthroat Trout to Loading by Fish Pump, Conveyor, or Dip Net

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ABSTRACT. The physiological stress response of cutthroat trout, *Oncorhynchus clarki*, to three methods of loading into truck tanks for transport was evaluated. All loading methods caused a significant decrease in chloride and increase in plasma cortisol levels, relative to the baseline sample of unstressed fish. Based on plasma chloride changes, the conveyor method appeared the least stressful to the fish, while fish pump and dip netting were not significantly different from each other. Differences in plasma cortisol responses suggested that the conveyor and dip net method were less stressful than the fish pump. Plasma glucose concentrations in the fish were not elevated after loading and did not differ among loading methods. Overall, the conveyor method was the least stressful method of loading, but the differences were not of sufficient magnitude to preferentially use one method over another.

INTRODUCTION

For several reasons, a substantial amount of research in the last 20 years has evaluated the effects of stress upon fish. Stress affects osmotic balance, causing hemodilution in fresh water and hemoconcentration in sea water (Mazeaud et al. 1977). Stress creates an additional drain on the energy reserves of the fish, which use this

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energy to recover from the disturbance (Schreck 1982). Stress also alters behavior. For example, Sigismondi and Weber (1988) found that stressed chinook salmon, *Oncorhynchus tshawytscha*, took longer to reach cover and were thus more susceptible to predation. Olla and Davis (1989) and Järvi (1989) both found that juvenile salmonids showed a decreased ability to avoid predators after being stressed. The immune system of fish can also be negatively impacted by stress, leading to greater susceptibility to disease (Pickering et al. 1989). Clearly, stress reduction has the potential to improve the survival of stocked fish.

Several studies have assessed the stress associated with fish transport (Barton et al. 1980; Davis and Parker 1986; Robertson et al. 1987). Nikinmaa et al. (1983) demonstrated the stress-reducing benefits of salt in the transport tank water. Use of anesthetics at sedating doses during transport has also helped improve survival (Mishra et al. 1983). Carmichael et al. (1984) increased survival of largemouth bass, *Micropterus salmoides*, after hauling by treating the fish for diseases before loading, by not feeding the fish for 72 hours before loading, and by hauling the fish in cooler temperatures and in physiological concentrations of salts with an antibiotic and a mild anesthetic.

The most stressful part of the stocking operation is loading the fish at the hatchery (Barton et al. 1980; Maule et al. 1988). Several methods are used to load fish into hauling trucks, three of which were evaluated in this study in order to determine if one is superior to another in reducing selected physiological stress responses.

METHODS

The Bear Lake Bonneville strain of cutthroat trout, *O. clarki utah*, (mean length = 232 mm; mean weight = 102 g) used for this experiment was raised at the Mantua State Hatchery, Mantua, Utah. The experiment was conducted on October 23, 1990. Prior to loading, a baseline sample of 20 fish was taken, combining fish sampled from the upper and lower portions of the raceway. Blood was collected in heparinized syringes from the caudal vein. The needle was removed prior to ejection of the blood into microcentrifuge tubes. The blood was kept in an ice water bath until it was centrifuged (5 minutes at 13,000 g), and the plasma was transferred to 1.5-ml microcentrifuge tubes. Plasma samples were transported to the lab on dry ice, then frozen at −79°C until clinical analysis was performed. Water quality parameters prior to loading were: temperature, 8.3°C; carbon dioxide, 25 mg/L; pH, 7.8; total alkalinity, 205 mg/L; total hardness, 256 mg/L.

Fish were loaded into each of two identical fish stocking trucks equipped with four fiberglass tanks (1,700 L capacity per tank) each. The Aqua Life[1] fish pump (Magic Valley Heli-Arc and Manufacturing, Twin Falls, Idaho) was used first to fill one tank on each truck. The loading of the first truck was then completed using the Sartorius conveyor (Sartorius Company, San Francisco, California) to fill the second tank, and dip netting to fill the third tank. Total loading time was about one hour. The second truck was then filled in the same sequence, taking about 1.5 hours from start to finish of loading. All tanks on both trucks were filled with fish from the same raceway, with the exception of the fish dip netted for the second truck. There were insufficient fish in the first raceway to load all the tanks, so additional cutthroat trout were obtained from a raceway serially upstream from the first raceway. About 409 kg of fish were loaded per tank.

After transport from Mantua to Strawberry Reservoir, Wasatch County, Utah, 20 fish from each tank were removed and anesthetized with 60 mg/L ethyl *m*-aminobenzoate methanesulfonate (MS-222). The order in which the tanks were sampled was random, with the tanks of one truck being completely sampled before those of the second truck were sampled. Time from loading until sampling ranged from 3.5 to 5.0 hours.

Plasma samples from the cutthroat trout were tested for chloride ion concentration with a Haake-Buchler chloridometer. Plasma glucose levels were analyzed by the ortho-toluidine method (Wedemeyer and Yasutake 1977). Plasma cortisol was radioimmunoassayed according to the modifications by Redding et al. (1984) of the procedure of Foster and Dunn (1974). The standards used in the radioimmunoassay were prepared from salmonid plasma stripped of endogenous steroids with activated charcoal.

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1. Use of trade or manufacturer names does not imply endorsement.

The effects of different loading techniques on cortisol, glucose, and chloride levels of cutthroat trout were tested with a general linear model (GLM procedure; SAS Institute, Inc. 1988), blocking for the two replicates. Replicates were treated as random variables in the model. Mean comparisons were tested with Fisher's LSD test. The F_{max} test was used to evaluate the assumption of equal variances (Kirk 1982). One-way analysis of variance (ANOVA) was used to test the null hypothesis of equal treatment means, if the effects of the replicates and treatment-replicate interactions were not significant (SAS Institute, Inc. 1988). A calculation of relative efficiency (Kirk 1982) showed that one-way ANOVA was more efficient than a randomized block design for analysis of the cortisol data. There was a significant treatment-replicate interaction in the chloride data, as well as heterogeneity of variance, so each replicate was analyzed separately in a one-way ANOVA of rank transformed data.

RESULTS AND DISCUSSION

Fish loaded by all three methods had lower ($P \leq 0.05$) mean chloride levels than fish sampled prior to loading (Table 1). Mean plasma cortisol concentrations also increased significantly in response to each of the loading methods (Table 1). This indicates that the process of loading is stressful, as other authors have reported (Barton et al. 1980; Barton and Peter 1982). If no further stressors act upon the fish, transported fish will likely survive. However, since repeated stressors can cause cumulative increases in plasma cortisol (Barton et al. 1986), additional stressors in conjunction with transport could compromise fish survival. Additional stressors with stocking could include large temperature changes (Wedemeyer (1973), changes in pH (Goss and Wood 1988), osmotic differences (Ferraris et al. 1988; Järvi 1989), and the presence of predators (Järvi 1989). Combinations of these variables may act synergistically to affect mortality as well (Järvi 1989).

In this study, the contribution of loading to the physiological stress response was influenced by the loading method used. Fish loaded by the fish pump had higher concentrations of plasma cortisol than those loaded by conveyor or dip net ($P < 0.05$). Mean

TABLE 1. Mean plasma cortisol, plasma glucose, and plasma chloride concentrations ± SD in cutthroat trout at rest or 3.5 to 5 hours after loading by fish pump, dip net, or conveyor. Means in a row followed by the same letter are not statistically different ($P \leq 0.05$).

		Loading Method		
	At rest	Fish pump	Dip net	Conveyor
Cortisol (ng/ml)	25.9±21.1a	105.4±35.6b	90.0±21.9c	91.4±30.6c
Glucose (mg/dl)	89.2±19.9a	83.6±25.3a	91.7±25.2a	94.2±28.0a
Chloride (mEq/l)				
Replicate 1	138.7±3.2a	115.9±9.2b	116.9±3.6b	118.8±5.5b
Replicate 2	138.7±3.2a	117.4±7.0c	120.6±6.3c	127.3±4.7b

plasma chloride concentrations, which decline in response to stress (Wedemeyer 1973), were significantly lower in fish loaded by fish pump and dip net than in those loaded with the conveyor method in the second replicate. In the first replicate, there were no significant differences in plasma chloride concentrations among any of the loading methods.

Although plasma cortisol was significantly lower in fish loaded by dip net and conveyor than in fish loaded by pump, whether these differences are biologically significant is debatable. The mean cortisol concentrations ranged from 90 to 105 ng/ml after loading–a narrow range, given the inherent variability of cortisol among individuals. These values are less than the range of 136 to 170 ng/ml reported for cutthroat trout subjected to 30 seconds of handling, 2 hours of transport, or continual confinement at 9 or 23°C (Barton and Iwama 1991). Poor water quality (a combination of reduced oxygen, elevated free CO_2, and elevated ammonia) has been shown to suppress the stress response in brown trout, *Salmo trutta,* and rainbow trout, *O. mykiss* (Pickering and Pottinger 1987). However, in the present study, dissolved oxygen was maintained at saturation in the truck tanks. Pickering and Pottinger (1987) noted no individual effects of reduced pH (from 7.1 to 6.3), elevated free CO_2, or elevated ammonia on the cortisol response.

The mean plasma glucose concentration (Table 1) in transported fish did not differ significantly from that in unstressed fish in response to any of the loading methods, nor did glucose concentrations differ among fish subjected to any of the loading methods. Glucose concentrations generally increase in fish in response to a stressor (Mazeaud et al. 1977). Plasma glucose and chloride typically require 3 to 10 hours to peak (Mazeaud et al. 1977; Pickering et al. 1982). In this experiment, both glucose and chloride levels had enough time to peak, but only chloride responded to all three loading methods. Results indicated that plasma glucose was a poor indicator of stress for this experiment.

The order of loading did not appear to be a factor. If this were the case, cortisol and chloride values in fish loaded by dip net (last in order) would have been significantly different from values in the fish loaded by the fish pump.

The plasma samples of this study were obtained upon arrival at the stocking site, 3.5 to 5 hours after loading. Plasma cortisol typically peaks after 0.5 to 3 hours and remains high for 4 to 6 hours after loading (Barton and Peter 1982; Thomas 1990). Davis and Parker (1983) observed no significant differences in cortisol or chloride between samples taken at 1 hour and 6 hours from rainbow trout or Atlantic salmon, *S. salar,* but lake trout, *Salvelinus namaycush,* had higher chloride concentrations at 6 hours than at 1 hour. Thus, the sampling time was adequate for evaluating the effects of loading.

Continued sampling over a longer period of time (e.g., 24 hours) would have provided additional information on the dynamics of the stress response and possibly on the added stressors of stocking and novel water quality. However, these responses have been well documented elsewhere (Barton et al. 1980; Davis and Parker 1983;

Carmichael et al. 1984) and would not have provided additional information regarding the response relative to various methods of loading. It is reasonable to assume that the physiological stress responses to additional stressors and return to resting or pre-stress levels would be similar in fish among treatment groups, since all groups were subjected to the same stressors after loading.

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