

**PHYSIOLOGICAL STRESS RESPONSES TO HANDLING IN
PADDLEFISH (*POLYODON SPATHULA*) WITH FRESHWATER AND
SALINE-WATER RECOVERY**

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Introduction

Paddlefish (*Polyodon spathula*), a once abundant chondrosteian fish of the Mississippi-Missouri River system, are an attractive species for aquaculture because of their high growth rate and delicate flesh (Kroll et al. 1994). Although the ecology and life history of the paddlefish have been studied to varying extents (Dillard et al. 1986; Reed 1992; Rosen and Hales 1982), the physiology of the paddlefish is minimally understood. For example, in a recent review, Barton and Iwama (1991) found very limited published information on corticosteroid stress responses in paddlefish. In this experiment, we investigated several physiological effects of handling. Also, as aquaculturists often use salts to improve survival and health of stressed fish (Wedemeyer, in press), we evaluated saline-water remediation of the stress response.

The objectives of our experiments were threefold. First we wished to determine both the magnitude and the time course of physiological responses to a brief handling stress. Second, we monitored the effect of adding salt to the recovery medium on these stress-induced changes. Finally, the fish were subjected to 1 h of chasing to determine the limits of their physiological stress responses. We measured changes in hematocrit, and plasma cortisol, glucose, and chloride in both experiments.

Methods and Materials

The juvenile paddlefish used in this experiment were hatched from eggs collected from wild adults, and were reared indoors at Gavins Point National Fish Hatchery. The fish were held in 1.8-m-diameter circular tanks containing 1320 L and having a flow-through of 39 L/min. For the handling experiment, the water supply (16°C) was pumped from Lewis and Clark Reservoir, passed through a drum filter, and disinfected with ultra-violet irradiation. For the chasing experiment, well water (13°C) at a flow-through of 21 L/min

was used. Fish were left undisturbed and unfed for 24 h preceding each experiment.

Handling Experiment with Salt Recovery. Juvenile paddlefish (mean weight, 157 ± 4.4 g) were removed from stock tanks, held in dip-nets for 30 s, and placed into tanks containing either fresh water (FW), or saline-water (SW, 0.5% noniodized NaCl). The tanks were well aerated by airstones delivering compressed air. The fish were divided into groups of 10-12 in separate tanks for sampling at 1, 3, 6, and 24 h so that sampling would not disturb the remaining fish. In addition, fish were sampled from the stock tanks at 0 h to provide prestress control values, and at 3, 6, and 24 h to provide resting-level control values.

Chasing Experiment. Paddlefish (187 ± 7.3 g each) in the two experimental tanks, which were located apart from the remaining two tanks, were subjected to continuous and vigorous chasing with intermittent dip-netting for 1 h. At 0, 1, 2, 4, and 24 h after the end of the chasing, five fish from each experimental tank were sampled (for $N = 10$). In addition, five fish were sampled from all tanks before chasing to provide prestress control values, and five fish were sampled from the control tanks at 4 and 24 h to provide resting-level control values; these tanks were "sham-sampled" (same sampling disturbance but without removing fish) at the other sample times.

Sampling and Analysis. To obtain blood samples, fish were anesthetized by immediately placing them in a lethal concentration (400 mg/L) of tricaine methanesulfonate (MS-222). This method has been shown to effectively arrest physiological stress responses in salmonids (Wedemeyer et al. 1990). Blood was collected from the caudal vasculature with a heparanized syringe and needle that was inserted ventrally immediately behind the anal fin (Houston 1990). Capillary tubes containing blood were centrifuged for 5 min, and the hematocrit was read as percent packed cell volume (% PCV). The remaining blood was centrifuged for 10 min, and the plasma was stored at -20°C for later analysis. Plasma glucose was determined by a spectrophotometric measurement of ortho-toluidine reaction (Wedemeyer et al. 1990). Plasma chloride was measured on a Corning Model 925 chloride analyzer using 20 μL samples. Plasma cortisol was measured in the unextracted plasma by radioimmunoassay (Foster and Dunn 1974). Standards were prepared by adding known amounts of cortisol (Sigma Chemicals, St. Louis, Missouri) to standard diluant made with bovine serum albumin. Data from all treatments were subjected to analyses of variance followed by Tukey's test ($P < 0.05$) to determine differences among means.

Results and Discussion

Response to 30-s Handling. After 30 s of handling, plasma cortisol significantly increased ($P < 0.05$) from 2.2 ± 0.6 to 11 ± 1.8 ng/mL after 1 h (Figure 1). The response was similar in pattern to that in teleosts, but was much smaller in magnitude (Figure 2) and was also lower than what has been previously documented in many teleost fishes from a variety of disturbances generally (Barton and Iwama 1991). Plasma glucose varied by treatment ($P = 0.015$), but no levels were conclusively greater than controls (Table 1). No significant change in either plasma chloride (mean level of 101 ± 0.6 mmol/L) ($P = 0.12$) or hematocrit ($P = 0.06$) was evident. Hematocrit data is presented in Table 1 for comparison to the 1-h chasing data.

Effect of Saline-water Mitigation. Saline water as a recovery medium did not significantly alter any physiological constituent measured. The mean plasma cortisol for saline-water recovery was slightly lowered, but not significantly so (Figure 1).

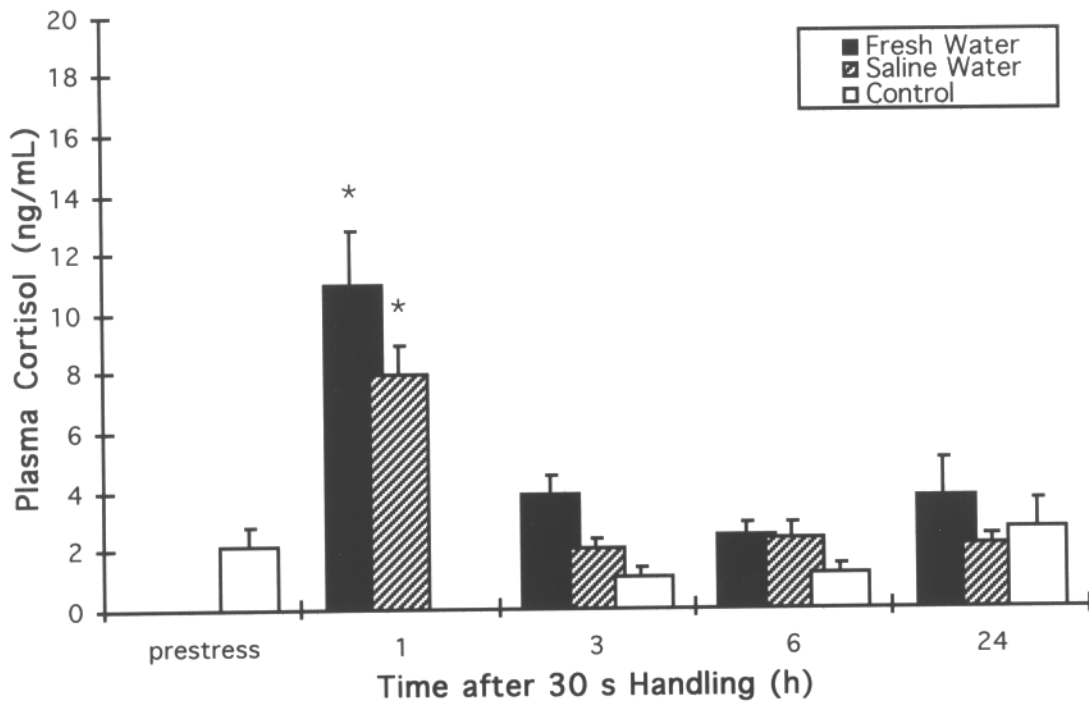


Figure 1. Mean + SE plasma cortisol in juvenile paddlefish subjected to 30 s of handling, with recovery in fresh water (FW) or water containing 0.5% salt (SW). The asterisks mark the values that are significantly different from the prestress value ($P < 0.05$).

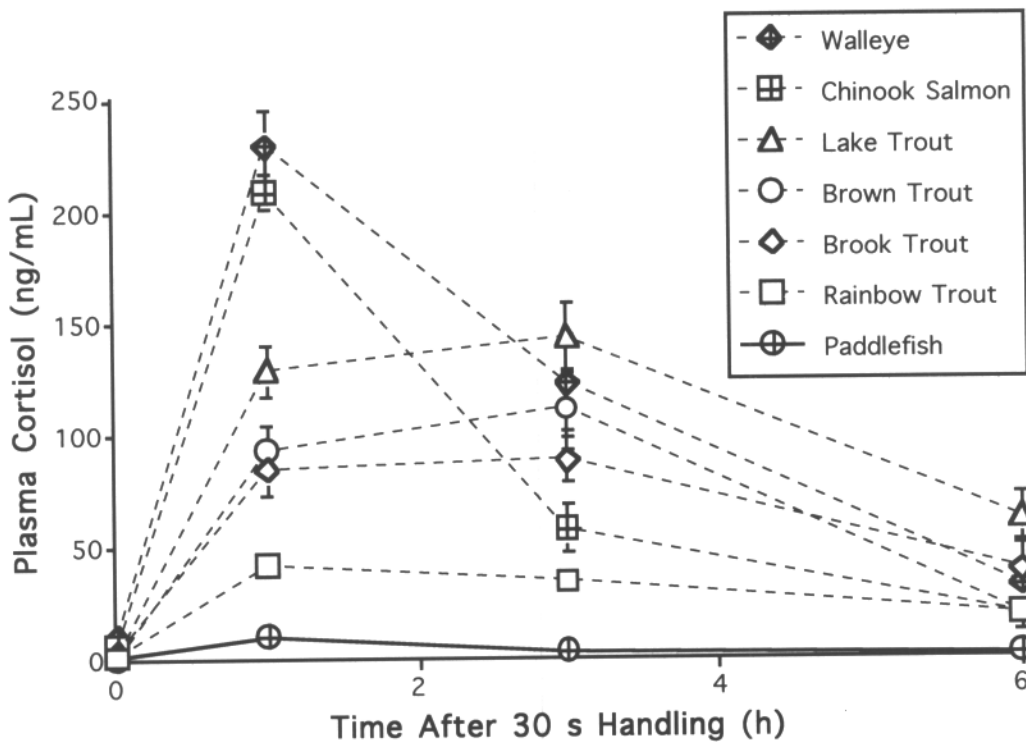


Figure 2. Examples of mean \pm SE plasma cortisol levels in a variety of teleost fishes and paddlefish following 30 s of handling. Walleye (Barton and Zitzow 1995) and chinook salmon (Barton and Schreck 1987) had relatively high cortisol responses, while the trouts and chars (Barton, unpublished data) exhibited a range of responses. In contrast, paddlefish (solid line) showed a relatively low cortisol response.

Table 1. Mean \pm SE plasma glucose and hematocrit in juvenile paddlefish recovering from a 30 s handling stress in fresh water (FW) and saline water (SW). Common letters mark treatments that are statistically indistinguishable ($P > 0.05$).

Time after handling (h)	Glucose (mg/dL)		Hematocrit (% PCV)	
	FW	SW	FW	SW
prestress	71 \pm 6.5	a, b	22.8 \pm 0.85	a
1	63 \pm 4.3	b	72 \pm 4.5	a, b
3	66 \pm 4.9	b	71 \pm 4.6	a, b
6	78 \pm 6.2	a, b	77 \pm 3.2	a, b
24	92 \pm 9.7	a	75 \pm 3.4	a, b

Response to 1-h Chasing Stress. Plasma cortisol increased after 1 h of continuous chasing from 0.19 ± 0.106 to 13 ± 4.8 ng/mL cortisol ($P < 0.05$) (Figure 3). Because 1 h of vigorous chasing left the fish visibly exhausted, we speculate that these values approached the maximum plasma cortisol level attainable from acute stress in paddlefish. No significant differences were found among plasma glucose values. Plasma chloride levels, which averaged 104.7 ± 0.70 mmol/L, did not vary significantly throughout the study. Although ANOVA detected significant variation in the hematocrit data ($P < 0.0001$), Tukey's test found no specific differences (Table 2).

Table 2. Mean \pm SE plasma glucose and hematocrit in juvenile paddlefish recovering from a 1-h chasing stressor in fresh water (FW) and saline water (SW).

Time after handling (h)	Glucose (mg/dL)	Hematocrit (% PCV)
prestress	82 \pm 7.7	a
0	97 \pm 9.5	a
1	81 \pm 2.9	a
3	87 \pm 6.7	a
4	73 \pm 5.0	a
24	83 \pm 7.5	a

In summary, paddlefish did not exhibit physiological responses to handling similar in magnitude to teleosts. The reasons for this difference are presently not known and investigations are currently in progress to assess differences in interrenal tissue structure between paddlefish and selected teleosts. Adding salt to the medium did not appear to significantly alter recovery time of the stressed fish. This is probably because the principal benefit of a saline recovery medium is to cushion osmoregulatory disturbances, which the paddlefish did not show.

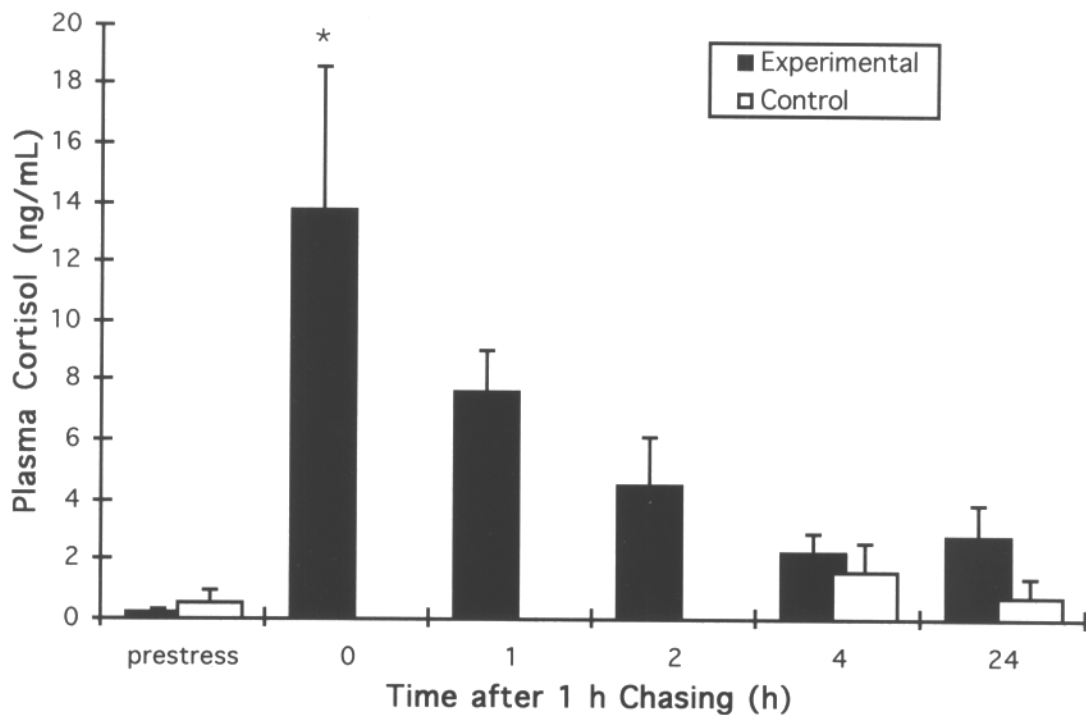


Figure 3. Mean + SE plasma cortisol in juvenile paddlefish after 1 h of chasing. The asterisk marks the bar which is different ($P < 0.05$) from the prestress value.

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