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Cortisol responses of pallid sturgeon and yellow perch following challenge with lipopolysaccharide

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Plasma cortisol responses of pallid sturgeon *Scaphirhynchus albus* and yellow perch *Perca flavescens* following injection with equal doses of lipopolysaccharide were compared. Concentrations of cortisol in plasma from pallid sturgeon did not change following injection (6·0–11·0 *v.* 6·4 ng l^{-1} pre-stress) while in yellow perch plasma they were shown to increase up to 6 h (117·0 *v.* 9·8 ng l^{-1} pre-stress) after the injection. These results are consistent with other reports for pallid sturgeon that illustrate a reduced cortisol response following other applied stressors relative to teleosts and suggest differences in the expression and regulation of their inflammatory responses.

Key words: cortisol; lipopolysaccharide; stress; sturgeon; yellow perch.

The physiological response of fishes to stress is an integrated process which includes the activation of the hypothalamo-pituitary-interrenal (HPI) axis leading to transient increases in the amounts of cortisol in the plasma (Sumpter, 1997). Standardized handling stress studies have been performed on a variety of species to examine the activation of the HPI axis in fishes by measuring cortisol in the plasma at intervals following an aerial exposure for 30 s (Barton & Zitzow, 1995; Barton *et al.*, 1998). Handling studies like these have been extended to include several species within the order Acipensiformes and a review by Barton *et al.* (2002) illustrates that the cortisol response 1 h after a 30 s aerial net stressor among three members of the Acipenseridae, including pallid sturgeon *Scaphirhynchus albus* (Forbes & Richardson), is reduced relative to the teleost, the yellow perch *Perca flavescens* (Mitchell).

Injection with lipopolysaccharide (LPS), a constituent of bacterial cell walls, is an alternative type of stressor. A wide variety of metabolic effects following

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inflammatory challenge including the activation of the HPI axis have been observed following the application of LPS (Wedemeyer *et al.*, 1968; Wedemeyer, 1969). Pro-inflammatory cytokines produced in response to LPS challenge probably initiate the secretions of corticotropin-releasing hormone leading to transitory increases in plasma cortisol (Balm *et al.*, 1995; McCann *et al.*, 2000). This corticosteroid response to an inflammatory challenge appears to be a conserved trait across a wide range of vertebrate taxa (Berczi, 1998) and is one phenomenon among a cascade of events that follow a pathogen invasion or inflammatory challenge referred to in aggregate as the acute phase response (Kushner, 1982; Berczi, 1998). These phenomena are presumed to be expressions of an adaptive response that protect the host from inflammatory processes while creating an internal environment that reduces the likelihood of a proliferating infection (Berczi, 1998). For example, circulating corticosteroids in particular have long been attributed to protecting the vertebrate host from endotoxin shock (Dougherty, 1949; Flower *et al.*, 1986).

Recent experiments with yellow perch have characterized the time course of the corticosteroid response associated with LPS treatments relative to handling and saline injections (Haukenes & Barton, 2004). By contrasting the cortisol responses of yellow perch and pallid sturgeon following inflammatory challenge it will be confirmed if the reduced cortisol response in pallid sturgeon attributed to handling observed by Barton *et al.* (2000) infers a reduced response to metabolic stressors like an inflammatory challenge. Moreover, differences between these two species to an LPS challenge would be suggestive of differences in the expression and regulation of the inflammatory response between sturgeon and teleosts.

Pallid sturgeon (mean ± S.E. mass, 237 ± 14 g, $n = 19$) derived from the artificial spawning of captive broodstock at the Gavins Point National Fish Hatchery, Yankton, SD, U.S.A. were used in this study. These fish represented production in excess of the stocking programme goals and scheduled to be destroyed. Fish were maintained at Gavins Point National Fish Hatchery in tanks supplied by Missouri River water (17–18° C). A LPS solution (1 mg ml^{-1}) was prepared on the date of injection from a strain of *Escherichia coli* (055:B5; Sigma Chemical Co., St Louis, MO, U.S.A.) dissolved in 0·8% saline. Before injecting pallid sturgeon with LPS, a group of six fish were collected with a net from the tank and euthanized in a bath of the anaesthetic tricaine methanesulphonate (200–300 mg l^{-1}; Finquel, Argent Chemical, Redmond, WA, U.S.A.) and sampled for blood within 3 min of capture from the caudal vasculature using a 3 ml syringe with a 21-gauge needle (Houston, 1990). After these samples from non-treated fish were collected a second group of fish were individually weighed and then injected with the appropriate volume of the LPS solution into the peritoneal cavity to yield a dose of 3 mg kg^{-1} of fish and were placed back into the water. At 1·5 ($n = 5$), 3 ($n = 5$) and 6 h ($n = 5$) and at 1 day ($n = 4$) after injection groups of pallid sturgeon were captured and euthanized in lethal concentrations of tricaine methanesulphonate as described previously and sampled for blood.

On the same date as the pallid sturgeon were injected, yellow perch (mean ± S.E. mass, 60 ± 3 g, $n = 18$) were treated with same dose and solution of LPS. Throughout the day the LPS solution was either stored refrigerated or in a cooler containing ice during transit (<1 h) between Gavins Point National

Fish Hatchery and the University of South Dakota, Vermillion, SD, U.S.A. Juvenile yellow perch were obtained from a commercial fish farm (Willow Creek Aquaculture, Berlin, WI, U.S.A.) and maintained in tanks at the University of South Dakota. Tanks were supplied by aerated re-circulating water (18–20° C). Twelve yellow perch were collected with a net, weighed, and then injected with the appropriate volume of LPS into the peritoneal cavity to yield a dose of 3 mg kg^{-1}. After injection, groups of six fish were placed into separate tanks designated to be sampled at intervals of 3 and 6 h. Six yellow perch were also collected from the same tank that provided all the yellow perch described in this test and immediately euthanized in a bath of the anaesthetic tricaine methanesulphonate and plasma was collected to provide a sample representing non-disturbed fish. This sampling schedule was based upon results of an earlier study with yellow perch that characterized the time course of the plasma cortisol response of yellow perch following LPS injection, saline injection and handling without injection (Haukenes & Barton, 2004). At each sampling interval, fish were collected and euthanized in the anaesthetic bath as described previously and blood collected and plasma samples prepared. All procedures conformed to protocols approved by the University of South Dakota Institutional Animal Care and Use Committee.

Assay of cortisol concentration in yellow perch plasma was performed using a radioimmunoassay procedure for unextracted fish plasma (Redding *et al.*, 1984) and subsequently found suitable for use in percid plasma (Barton & Zitzow, 1995). Cortisol assays performed on sturgeon plasma were performed similarly except that the standard curve was derived by adding known amounts of cortisol to pallid sturgeon plasma stripped of endogenous steroids with charcoal (Barton *et al.*, 2000). Data from each species were analysed using one-way ANOVA with general linear model (GLM) procedures to determine differences in plasma cortisol among the sampling intervals (SAS, 1989) and significant differences ($P < 0{\cdot}05$) among individual means was determined using Duncan's multiple range test.

Mean concentrations of cortisol in the plasma collected from non-injected yellow perch and pallid sturgeon were 9·8 and 6·4 ng ml^{-1}, respectively (Fig. 1). At 3 h following injection with LPS the mean concentration of cortisol in yellow perch plasma, 58·7 ng ml^{-1}, was significantly higher than that observed in 'resting' animals and by 6 h after injection the mean concentration of cortisol, 117·0 ng ml^{-1}, had increased significantly from that observed 3 h after injection. In contrast, concentrations of cortisol in pallid sturgeon plasma at 1·5, 3, 6 and 22 h after injection with LPS ranged between 6·0 and 11·0 ng ml^{-1} and never differed significantly from that of the observed 'resting' levels.

Yellow perch injected with the same solution of LPS as the pallid sturgeon provide data to illustrate the potency of the LPS solution by responding in a fashion similar to that in earlier research using this species. A previous report for yellow perch illustrated that handled, saline injected and LPS injected fish all responded with initial increases in plasma cortisol attributed to handling, but by 6 h after handling or saline injection the plasma cortisol concentrations had returned to levels resembling that of resting fish while plasma cortisol among LPS treated fish was at the highest point 6 h following injection (Haukenes & Barton, 2004). A response similar to that in yellow perch was

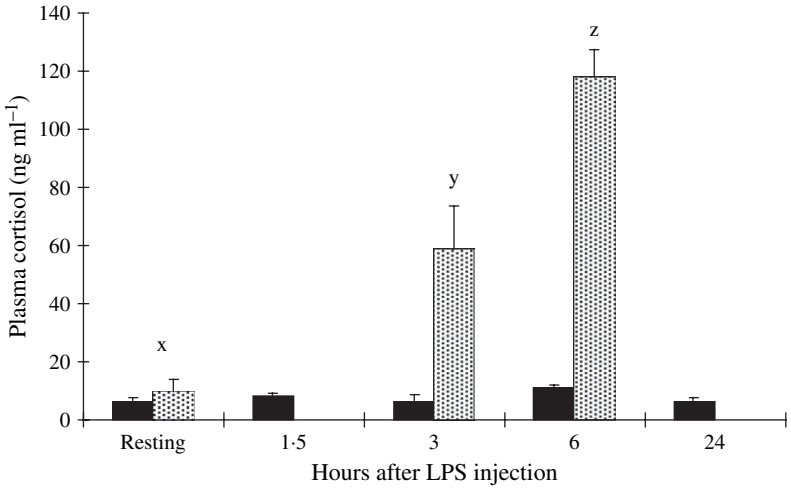

FIG. 1. The profiles of plasma cortisol (mean + S.E.) in plasma after Lipopolysaccharide (LPS) challenge differ between pallid sturgeon (■) and yellow perch (▩). Lower case letters above each of the means corresponding with yellow perch ($n = 6$ per sample) indicate the results of *post hoc* contrasts of individual means; different letters designate significant differences among means. Among pallid sturgeon ($n = 4$–5 per sample), no differences in the concentrations of cortisol in the plasma were observed among the sampling intervals. Resting, for both yellow perch and pallid sturgeon, fishes were sampled on the same day from the source tank that supplied the fish for each species.

also observed in juvenile walleye *Sander vitreus* (Mitchill), a closely related percid species (Haukenes, 2001). The absence of a corticosteroid response in pallid sturgeon held at similar water temperatures and injected with the same dose of LPS as yellow perch is consistent with reports of a reduced cortisol response following other applied stressors for this species. Responses to handling and confinement stressors in pallid sturgeon have been characterized where it was observed that a 30 s aerial net stressor did not induce a measurable increase in plasma cortisol concentrations (Barton *et al.*, 2000).

Observations of changes in yellow perch, walleye (Haukenes, 2001; Haukenes & Barton, 2004) and rainbow trout *Oncorhynchus mykiss* (Walbaum) (Wedemeyer, 1969) indicate that the failure to initiate a robust cortisol response by pallid sturgeon is not typical of the responses observed for those species of teleosts challenged with LPS. The absence of a corticosteroid response in pallid sturgeon to doses of LPS that would lead to an activation of the HPI axis in teleosts illustrates that members of the family Acipenseridae may offer potential as animal models to further characterize the regulation of the vertebrate inflammatory response.

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