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Characterization of the cortisol response following an acute challenge with lipopolysaccharide in yellow perch and the influence of rearing density

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Two experiments were performed to characterize the corticosteroid response of yellow perch *Perca flavescens* following an intraperitoneal injection of lipopolysaccharide (LPS) and determine if sustained differences in rearing density alter this response. In the first experiment, yellow perch were injected with LPS ($3\,mg\,kg^{-1}$), saline, or handled without receiving any injection. Concentrations of cortisol in plasma were elevated in all groups relative to non-disturbed fish at 1·5 and 3 h after handling but by 6 h after injection the mean concentration of cortisol in plasma from LPS-injected yellow perch were three to five times higher than fish before injection and significantly larger than groups of fish not treated with LPS. In the second test, yellow perch were held at different rearing densities (9 *v.* 18–19 $kg\,m^{-3}$) for 3, 7 and 14 days before injection with LPS ($3\,mg\,kg^{-1}$). The cortisol response of yellow perch following LPS injection of fish held for 14 days at high density was significantly lower than that of fish held at the low density for the same duration. Additionally a trend of a decreased cortisol response to LPS injection as duration of holding increased was observed among fish held at high density relative to fish held at low density. These data illustrate that the corticosteroid response of yellow perch following LPS injection is distinct from handling alone and that the magnitude of the response is impacted by rearing density.

Key words: combined stressors; immune challenge; *Perca flavescens*; rearing density; yellow perch.

INTRODUCTION

Activation of the hypothalamo-pituitary-interrenal (HPI) axis leading to the production and the release of cortisol into circulation is frequently attributed as the cause of negative manifestations associated with stress in teleosts. Evidence presented by Pickering & Stewart (1984), however, suggests that fishes can acclimate to long-term stressors such as high-rearing density and, that over time, concentrations of circulating cortisol may become indistinguishable among fishes held at lower densities. The consequences of this acclimation are

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poorly understood but alterations in the response to subsequent acute stressors might be anticipated under a chronic stress model proposed by McEwen (1998) that incorporates the concept of allostatic load. In broad terms, allostatic load characterizes chronic stress as a process that imposes a cumulative burden on an organism and this model has only recently been discussed in the context of chronic stress in fishes (Schreck, 2000). The utility of this concept can be appreciated when considering that aquaculture systems can impose sustained stressors, such as chronic confinement, over long time periods.

Models characterizing physiological processes surrounding an inflammatory challenge have been developed using lipopolysaccharide (LPS), a constituent of bacterial cell walls (Schobitz *et al.*, 1994). A LPS challenge results in wide-ranging changes that include increases in the concentrations of adrenocorticotropic hormone (ACTH), glucocorticoids, growth hormone, insulin, glucagon, melanocyte-stimulating hormone, epinepherine, norepinepherine, dopamine and arginine vasopressin (Berczi, 1998). These changes are largely induced and regulated by cytokines produced in response to an immune challenge (Licinio & Wong, 1997).

An acute challenge with LPS results in elevated levels of cortisol in teleosts (Wedemeyer, 1969; Balm *et al.*, 1995). The timing of this cortisol response is not entirely clear from the available literature but injection with LPS is a standardized approach to activate the HPI axis and thus examine the corticosteroid response in closer detail. The activation of the HPI axis, as with all the features of the acute phase response, is considered part of an adaptive suite of responses that in aggregate protect the host from pro-inflammatory conditions while decreasing the likelihood of a proliferating infection (Berczi, 1998). The objectives of the following tests were to first characterize the cortisol response in yellow perch *Perca flavescens* (Mitchill) following LPS injection in contrast to that observed by handling and then to determine if the consequences of a sustained density stressor alter the corticosteroid response to an acute immune challenge.

MATERIALS AND METHODS

FISH

Juvenile yellow perch were obtained from a commercial fish farm (Willow Creek Aquaculture, Berlin, Wisconsin, U.S.A.) and maintained in wet laboratory facilities at the University of South Dakota. The fish tanks were supplied with aerated re-circulating water (18–20° C) under a 12 L : 12 D photoperiod and during maintenance fed twice daily at 1–2% of body mass per day with an artificial salmon diet (Biodiet Grower, BioOregon, Warrenton Oregon, U.S.A.). Institutional animal care and use guidelines were followed throughout the course of these studies.

LIPOPOLYSACCHARIDE PREPARATION

All LPS preparations were prepared on the date of injection from a strain of *Escherichia coli* (055:B5; Sigma Chemical Co., St Louis, Missouri, U.S.A.) dissolved in 0·8% saline.

CORTISOL ASSAY

Concentrations of cortisol ($ng\,ml^{-1}$) in the plasma were determined using a radio-immunoassay procedure for unextracted fish plasma (Redding *et al.*, 1984) that has been

validated for use in walleye *Stizostedion vitreum* (Mitchill), a closely related percid species (Barton & Zitzow, 1995). Intraassay and interassay CV were determined from repeated measurements of plasma samples from individual fish and were 3–11 and 3%, respectively.

TEST TANK DESIGN

The tanks used to hold fish in the following experiments were $33 \times 36 \times 50$ cm Rubbermaid® containers with the bottoms removed and replaced with plastic netting with a 1·2 cm mesh-size. Each tank was designed to be suspended within a larger volume of water at two different depths so that the total volume of the tanks could be set to contain either 28 or 56 l of water. Thus, rearing densities ($kg\,m^{-3}$) could be increased or decreased without handling the fish. Each tank was supplied with $2\,l\,min^{-1}$ of aerated re-circulating water (18–20° C) throughout testing and contained an air stone supplied to maintain oxygen levels.

TEST 1: YELLOW PERCH RESPONSE TO LPS

Two trials with yellow perch (mean ± S.E. mass 22·0 ± 0·4 g) designed to examine the cortisol response following LPS injection were performed as follows. Before each trial, seven fish were collected from a single tank that served as the source of all fish for the trial to represent undisturbed fish with baseline levels of circulating cortisol. These fish were collected with a net and immediately euthanized in a solution of 200–300 $mg\,l^{-1}$ tricaine methanesulphonate (Finquel®, Argent Labs, Redmond, Washington, U.S.A.). At this concentration, the fish were immobilized within 1 min. Blood samples were then collected in a 250 µl heparinized Natelson tube after severing the caudal peduncle. Blood was transferred into 2 ml polypropylene tubes and plasma separated *via* centrifugation. Plasma was transferred with a pipette into a 2 ml polypropylene tube and all plasma samples were stored at −25° C until assayed for cortisol.

After collecting samples from resting fish, groups of 18 fish were captured and assigned to one of four 56 l tanks representing different sampling intervals: 1.5, 3, 6 and 22 h after injection. Within each tank of fish an equal number of individuals ($n = 6$) was assigned to one of three treatments: LPS-injected, saline-injected and handled but not injected. Each of the saline- and LPS-treated fish was injected with 0·1 ml of the respective solutions into the peritoneal cavity. The concentration of LPS (0·6 $mg\,ml^{-1}$) and the volume of injection resulted in a mean dose of 3 mg LPS kg^{-1} fish mass in the LPS-injected fish. After the injection or handling a pectoral fin was clipped so that fish in all the treatment groups had a unique mark that could be identified later when collecting plasma samples. At each of the assigned sampling intervals, all the fish from the tank designated for that time interval were collected and immediately euthanized in 200–300 $mg\,l^{-1}$ tricaine methansulphonate.

A three-way ANOVA using general linear models and Type III sums of squares (SAS, 1989) was used to determine significant differences ($P < 0{\cdot}05$) in plasma cortisol between replicate trials, among the three treatments, among four sampling intervals, and for all interactions. Where appropriate significant differences among means were determined. Duncan's multiple range test was used to identify specific differences among individual means. In the presence of significant interactions, pair-wise contrasts of least-squared means were used to characterize the interaction after performing a Bonferoni adjustment to the threshold level of significance. Plasma cortisol of undisturbed fish was compared with samples collected from fish of each treatment over all time intervals by two-way ANOVA and means separated using Duncan's multiple range test.

TEST 2: EFFECTS OF REARING DENSITY ON RESPONSE TO LPS

The following test was performed for three different durations: 3, 7 and 14 days. For each duration, nine tanks were initially stocked with a number of yellow perch so that the total mass of fish per tank for each test was *c.* 500 g (9 $kg\,m^{-3}$; Table I). The fish were

TABLE I. The numbers of fish, mean ± S.E. mass, mean ± S.E. total length and rearing densities of fish held for three different durations at low (LD) and high (HD) densities and sampled before and after a subsequent intraperitoneal challenge of 3 mg LPS kg^{-1} fish. Numbers of fish that appear in parentheses indicate the numbers sampled before injection (BI) and 6 h after LPS or saline (Sal) injection in each tank. Length data were not gathered during the test lasting 14 days (ND)

Test duration (days)	Fish per tank (BI, LPS, Sal)	Mean ± S.E. mass (g)	Mean ± S.E. L_T (mm)	Density ($kg\,m^{-3}$) LD	HD
3	7 (3, 4, 0)	70·4 ± 1·8	172 ± 1	8·6	17·9
7	8 (2, 3, 3)	59·0 ± 1·2	162 ± 1	8·8	19·1
14	11 (3, 4, 4)	45·6 ± 0·8	ND	8·8	18·1

allowed to acclimate to these conditions for 2 weeks and fed twice daily at a feeding rate of 2 g of feed per day. At the end of the acclimation period, the volumes of six tanks were reduced to create rearing densities of 18–19 $kg\,m^{-3}$ in the high-density tanks. Yellow perch were held at these densities for durations lasting 3, 7 and 14 days. At the end of each of these durations, the volumes of three of the high-density tanks were increased to return the fish to low-density conditions for 24 h before sampling. Thus, three groups (three tanks per group) were established for each test using the following rearing treatments: low density (LD), high density (HD) and high density with a 24 h recovery at low density (HDR).

At the end of each of these test durations, groups of fish from each tank were captured simultaneously with a dip-net, blood was collected, and plasma samples prepared to serve as a baseline or pre-treatment measurement for cortisol. All of the blood samples from yellow perch after the 3 day test were collected by decapitating the fish and then collecting the blood from the heart with a heparinized 250 µl Natelson tube. For tests lasting 7 and 14 days, all the fish were collected with a dip-net and immediately euthanized with 200–300 $mg\,l^{-1}$ tricaine methanesulphonate. Blood was then collected from the caudal blood vessels using ammonium-heparinized syringes with 21-G needles inserted ventrally (Houston, 1990). Plasma samples were prepared as described above in the previous test and vials of plasma stored at −80° C until assayed for cortisol.

After collecting the pre-treatment blood samples, the remaining fish in each tank were captured, weighed (g), and then injected intraperitoneally with the appropriate volume to deliver a dose of 3 mg LPS kg^{-1} fish mass or saline using a 1 ml syringe with a 25-G needle. For the 3 day test, all of the remaining fish were injected with a dose of 3 mg LPS kg^{-1} fish mass and immediately returned to the original tanks (Table I). For tests lasting 7 and 14 days, half of the remaining fish were injected with 3 mg kg^{-1} LPS and half were injected with saline using an injection volume based on the LPS dosing schedule (Table I). The pectoral fins of saline- and LPS-injected fish were uniquely clipped following injection so they could be distinguished at sampling. Once injected and marked, the fish were returned to their original tanks. Six hours after injection the fish were simultaneously captured, sampled for blood, and plasma prepared and collected as described above for the pre-treatment sample.

Two-way ANOVA was performed to determine differences in the concentrations of cortisol of fish held at the different density treatments and among groups of fish sampled before any treatment, after saline injection, and after LPS injection. When significant differences among main effects were observed using ANOVA, Duncan's multiple range test was used to determine differences among individual means. Type III sums of squares were used for all ANOVA (SAS, 1989) to determine significant differences ($P < 0{\cdot}05$). In the presence of significant interactions, pair-wise contrasts of least-squared means were used to characterize the interaction after performing a Bonferoni adjustment of the

threshold of significance. The responsiveness to an LPS challenge among high-density fish was further characterized in the two high-density treatments (HD and HDR) by expressing the concentrations of cortisol as a proportional response relative to that of fish held at low density (LD). Differences in the relative responses of these two high-density groups over time were determined using an analysis of covariance (ANCOVA) and least-squared means were compared to characterize differences. The pattern over time demonstrated by the two high-density groups was then illustrated using values for the *y*-intercept and slope determined by the ANCOVA.

RESULTS

TEST 1: YELLOW PERCH RESPONSE TO LPS

The mean concentration of plasma cortisol from undisturbed yellow perch was $8{\cdot}28\,\mathrm{ng\,ml^{-1}}$. At 1·5 h after handling and injection, the plasma cortisol was elevated in all treatment groups and had risen to levels three to five times higher than in pre-stressed fish (Fig. 1). The average concentrations of plasma cortisol from yellow perch following treatment differed significantly among the three treatments ($P < 0{\cdot}0001$), among the time intervals sampled ($P < 0{\cdot}0001$). The overall mean concentrations of plasma cortisol for each trial differed significantly, 36·2 *v.* $26{\cdot}2\,\mathrm{ng\,ml^{-1}}$ ($P = 0{\cdot}0019$). No significant interactions, however, were observed between the main effect of replicate with time or with treatment which illustrates that while the overall means differed between replicate trials,

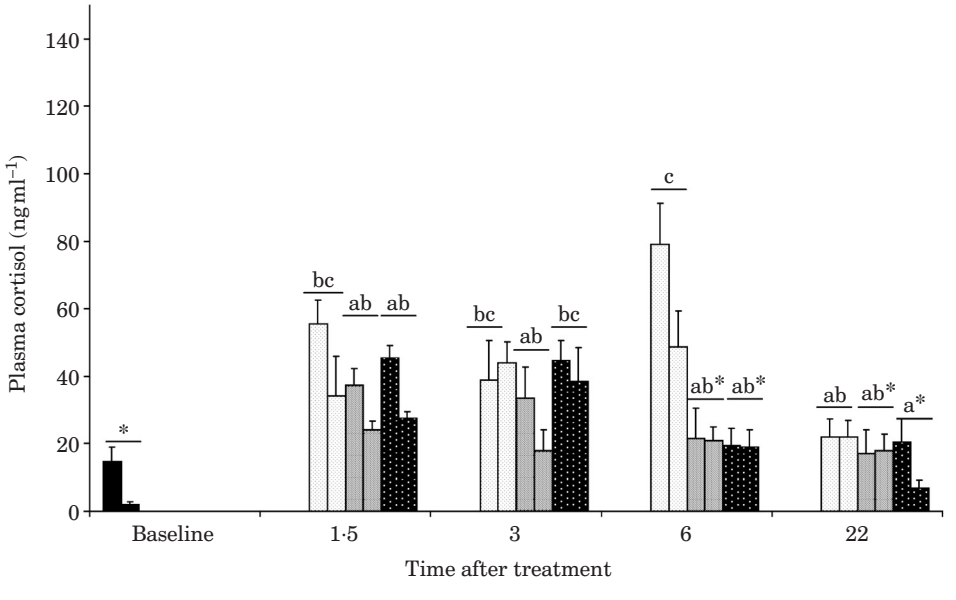

FIG. 1. Mean + S.E. ($n = 4$–7) concentrations of cortisol in plasma from replicate trials where yellow perch were injected with $3\,\mathrm{mg\ LPS\,kg^{-1}}$ (□), saline (▨) or handled but not injected (■) and sampled 1·5–22 h after treatment. Letters above the bars represent results of pair-wise contrasts of the overall means for both trials for each time and treatment; means that share letters in common do not differ significantly ($P > 0{\cdot}05$). Bars accompanied with an asterisk (*) represent mean values similar to those in undisturbed baseline fish (■).

the relative cortisol response over time for each treatment was consistent across both trials. A significant interaction between treatment and time sampled ($P = 0{\cdot}0005$) was also evident. The source of the significant interaction was revealed by *post hoc* pair-wise contrasts of means and results from a different pattern in LPS injected animals *v*. the other two treatment groups (Fig. 1). The mean concentration of cortisol in the plasma from yellow perch 6 h after injection with LPS was $66{\cdot}9\,\mathrm{ng\,ml^{-1}}$, significantly larger than that from fish injected with saline ($19{\cdot}3\,\mathrm{ng\,ml^{-1}}$) and those handled without receiving an injection ($21{\cdot}3\,\mathrm{ng\,ml^{-1}}$). By 6 h after injection the concentrations of cortisol in the plasma from fish that were not injected with LPS were at levels resembling those in undisturbed fish (Fig. 1).

TEST 2: EFFECTS OF REARING DENSITY ON CORTISOL RESPONSE TO LPS

Six hours after injection with LPS the concentrations of cortisol in plasma were significantly higher than from yellow perch sampled before injection regardless of the rearing density in tests lasting 3 and 7 days ($P < 0{\cdot}05$). In tests of 7 and 14 days the concentrations of cortisol in plasma from fish injected with saline were similar to those observed in samples collected before injection regardless of the density treatment applied (Fig. 2). The concentrations of cortisol in plasma from fish held for 3 days at different densities were similar among the density treatments ($P = 0{\cdot}2034$). After 7 days of rearing the mean concentrations of plasma cortisol from fish held for 7 days at high density followed by 1 day of recovery at HDR were significantly higher than both HD and LD fish ($P = 0{\cdot}0227$; Fig. 2). Mean concentrations of plasma cortisol from fish sampled after 14 days differed significantly among the density groups ($P = 0{\cdot}0324$) but a significant interaction between the main effects of density and injection treatment complicates inferences that can be made from these main effects ($P = 0{\cdot}0461$). The source of this interaction appears to be the result of different patterns in the relative amounts of cortisol for fish sampled before and after injection. Pair-wise contrasts performed as a consequence of this interaction illustrate that mean plasma cortisol in LD fish 6 h after injection with LPS ($154{\cdot}7\,\mathrm{ng\,ml^{-1}}$) was significantly larger than HD fish ($83{\cdot}4\,\mathrm{ng\,ml^{-1}}$). No differences, however, were observed among the density treatments in the mean concentrations of cortisol in plasma from fish sampled before treatment or after injection with saline (Fig. 2). Additionally, the mean concentration of cortisol in plasma from HD fish did not differ from samples collected before injection or from samples collected from fish injected with saline. While no differences among saline-injected fish were observed, the similarity in the pattern (highest to lowest) of LPS-injected fish, which was distinct from the pattern of plasma cortisol collected from fish before injection, may also have contributed to the significant interaction noted in the two-way ANOVA.

When all three durations were evaluated in aggregate using ANCOVA, the cortisol response to LPS relative to that observed among LD fish differed significantly over time between the two high-density groups of fish ($P = 0{\cdot}0176$). The least-squared mean for the relative cortisol response for HDR fish was 1·31 times greater than that for LD fish and significantly larger

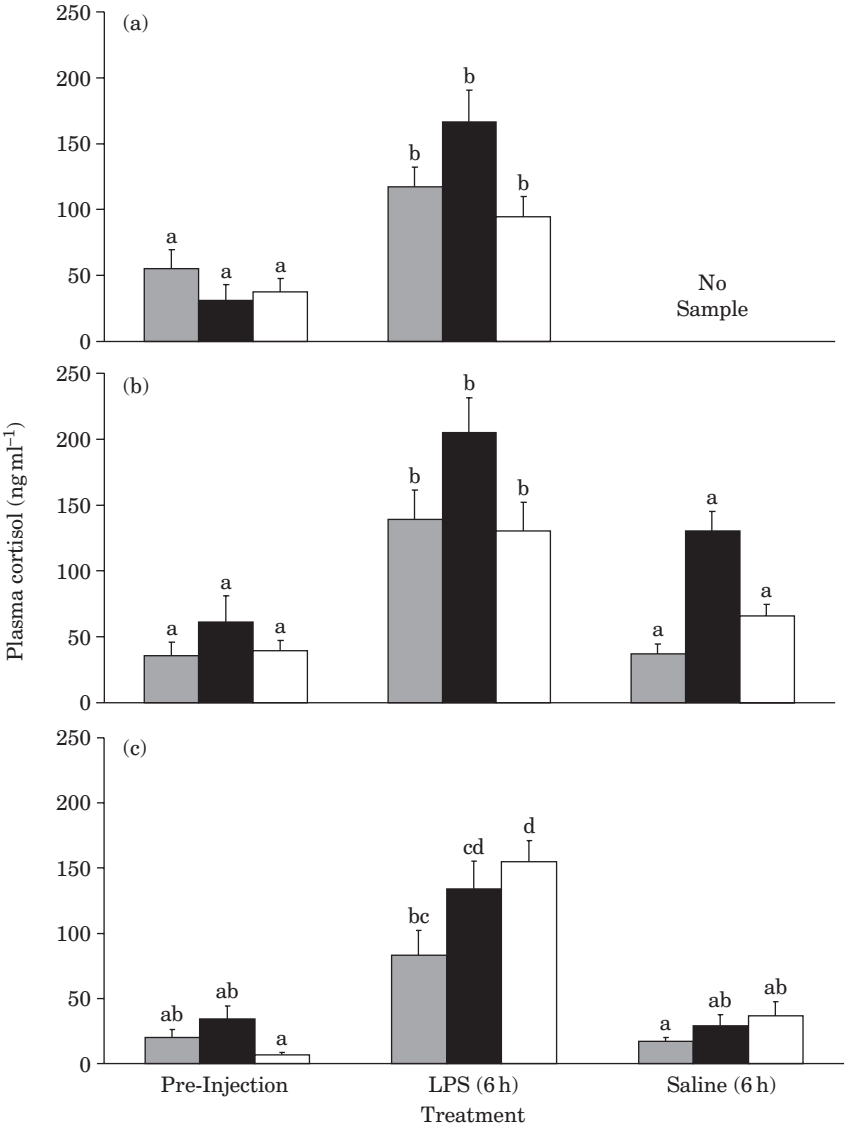

FIG. 2. Mean + S.E. concentrations of cortisol in yellow perch plasma before and after injection with LPS or saline. Fish were held for (a) 3, (b) 7 and (c) 14 days at high density (■), high density followed by a 24 h recovery at low density (■), and low density (□). Letters above the bars demonstrate significant differences within each duration; bars representing means that share letters in common do not differ significantly ($P > 0{\cdot}05$). After 7 days of rearing the main effect of density was determined significant by ANOVA ($P < 0{\cdot}05$) and *post hoc* tests revealed that HDR > HD and LD fish.

than the relative response of HD fish, which was only 0·93 times that observed for LD fish. The covariate, days of rearing, was also significant and is apparent as a general reduction of the LPS-induced cortisol response by 7·4% per day for both HDR and HD groups relative to LD fish ($P < 0{\cdot}0001$; Fig. 3).

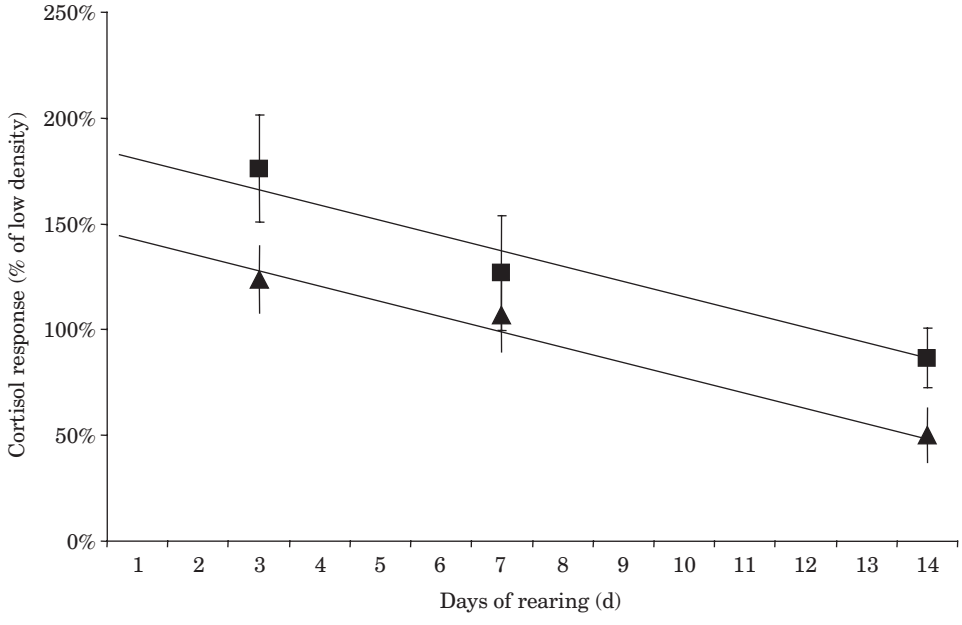

FIG. 3. Mean ± S.E. cortisol response to an intraperitoneal injection of LPS into yellow perch held continuously at high density (HD, ▲) and fish held for high density followed by 1 day at low density (HDR, ■). Data are expressed as a percentage of the equivalent response in low density-reared fish (LD). The curves, illustrating the response over time, were determined using the slope and intercepts determined by the ANCOVA. HD: $y = -7{\cdot}364x + 144{\cdot}240$ and HDR: $y = -7{\cdot}364x + 189{\cdot}681$.

DISCUSSION

Yellow perch injected with LPS demonstrated a pattern of sustained elevation in the amount of cortisol in circulation that differed from fish treated with a saline injection or handling without injection. All of the treatments of yellow perch led to increases in the amount of cortisol measured in the plasma from fish samples at 1·5 and 3 h after treatment relative to the amounts observed in plasma from undisturbed fish. Transient increases in plasma cortisol are routinely observed in a wide variety of teleosts following a standardized 30 s aerial net stress (Barton, 2002). At 6 h after treatment, however, fish injected with LPS had the highest concentrations of cortisol, which is in contrast with fish injected with saline or handled without injection. Saline injected and handled fish had concentrations of cortisol in plasma that resembled pre-injection levels by 6 h after treatment. Other researchers evaluating the corticosteroid response in yellow perch and walleye have also indicated a rapid and vigorous response to a handling stressor (Barton & Zitzow, 1995; Head & Malison, 2000). Experiments performed in the laboratory have shown that 1 h after a 30 s aerial net stress the mean concentration of cortisol in the plasma from yellow perch was 25 times higher than that from undisturbed fish (Haukenes, 2001). The data presented in these experiments concur with these other observations that handling leads to a cortisol response but it is clear that the profile for plasma cortisol following LPS injection differs from handling alone. The highest amounts of

cortisol were observed at intervals when the amount of cortisol in plasma from fish not treated with LPS did not differ from those collected from an undisturbed population.

The exact mechanism for the activation of the HPI axis by LPS is unclear but presumed to be the result of the actions of cytokines such as interleukin 1 (IL-1), and tumour necrosis factor (TNF) that are produced in response to this immune challenge (Dunn, 1992; McCann *et al.*, 2000). For example, IL-1 produced in response to an immune challenge has central actions in other vertebrate species leading to increased corticotropin-releasing hormone (CRH) release, in turn leading to increases in the concentrations of cortisol (McCann *et al.*, 2000). Further understanding of the cytokines as they interact with the HPI axis is limited by the lack of reagents necessary to quantify these compounds in fishes. While research into fish cytokines is rapidly providing a better understanding of the role of these compounds in fishes, the studies have been limited to a small number of species for which only a few of these chemical messengers are fully characterized (Manning & Nakanishi, 1996; Secombes *et al.*, 2001).

Different rearing densities did lead to different cortisol responses to LPS injection as the duration of the test was increased. After 7 days at the test densities the plasma cortisol in fish held at high density and allowed to recover for 1 day at low density before testing (HDR) was greater than that observed in fish held continuously at the high density for 7 days without recovery (HD) and fish held continuously at low density. While it is known that increased rearing density can lead to elevated levels of cortisol in the plasma (Pickering & Stewart, 1984) the observations in this 7 day test with yellow perch demonstrate that fish that had been relieved from a high-density confinement stressor for 24 h had higher concentrations of circulating cortisol than fish held continuously at both high and low densities. This response illustrates that changing the rearing environment by increasing the absolute volume of water and allowing yellow perch to recover following confinement at higher rearing density can also lead to increased cortisol levels in the plasma measured. It should be noted that fish from these experiments were not handled in order to relieve the density stressor; the water volume of the tank was increased with minimal disturbance to the fish. This release of the constraints of a smaller holding environment thus appears to lead to a heightened responsiveness to additional trauma. The trend towards a diminished capacity to respond to a subsequent immune stressor as the duration of chronic stressor is increased, however, was observed in both high-density groups. When the results of all tests are viewed in aggregate both of the high-density groups showed the same pattern of decreased responsiveness to the LPS stressor as the length of confinement at higher rearing density was increased.

The significantly lower concentrations of plasma cortisol in yellow perch injected with LPS in fish after being held for 14 days continuously at high density, relative to those in low density-held fish, corresponds with a preliminary study with juvenile walleye performed using similar methods as those described in this study. Walleye subjected to identical high-density stressors also showed a reduced response relative to low density-held fish after 14 days (Haukenes, 2001). In this study, when yellow perch were held continuously at 17–18 $kg\,m^{-3}$, and then injected with LPS the amount of cortisol in the plasma

6 h after injection was indistinguishable from fish sampled before treatment or from fish injected only with saline. The explanations for this reduced response include a decrease in the release of CRH, a decreased ACTH response to the CRH signal, a diminished cortisol output by the interrenal cells, or a decrease in the signal to the brain that activates the HPI axis following a LPS challenge. Linthorst *et al.* (1997) examined the impact of long-term applications CRH into the third ventricle of the brains of rats and demonstrated a decreased responsiveness to LPS administration as assessed by central monoaminergic activity. Monoaminergic systems are linked to the response along the HPI axis in fishes (Winberg *et al.*, 1997) as the level of cortisol in circulation increases following the application of serotonin-receptor agonists.

A down-regulation of cortisol output by interrenal cells by endogenous cortisol has been proposed (Bradford *et al.*, 1992). This phenomena does not appear to account for the observations described here as a pattern that links a reduced cortisol response to LPS to the levels of cortisol present before injection was not observed in samples collected in this study. A third mechanism implicates a reduced signal to the brain; LPS results in peripheral inflammation and signals can be carried to the brain *via* nerve transmission. Even in the absence of these afferent pathways, the cytokines produced are observed to induce behavioural and physiological changes (Porter *et al.*, 1998; MohanKumar *et al.*, 2000). The pattern of cortisol responsiveness among fish held for 14 days at different densities (*i.e.* LD > HDR > HD) was similar between saline- and LPS-injected fish. The similar pattern of responses in saline- and LPS-injected fish suggests a general decrease in the responsiveness of the HPI axis rather than a decreased cytokine signal induced by the LPS challenge.

Interrenal dysfunction in yellow perch has been discussed by Hontela *et al.* (1992) but in these cases impaired cortisol production was attributed to long-term exposures to environmental contaminants rather than social stressors. Presently, however, it is not known if interrenal dysfunction associated with toxicant exposure and the altered capacity to respond to immune challenge observed here are the results of similar mechanisms. Effects of some toxicants, such as organochlorine pesticides, include direct action on the interrenal cells leading to a reduced cortisol output (Leblond *et al.*, 2001). Further study on fishes is required to determine if the diminished corticosteroid response observed here is attributable to the capacity of these cells to produce cortisol or is an indication of a more general decrease in the responsiveness of the HPI axis or at the level of perception to subsequent stressors.

The experiments performed here suggest an alternative model for chronic stress that differs from one that describes continuously elevated amounts of cortisol associated with chronic stress. A model proposed by McEwen (1998) suggests that there is a cumulative burden associated with chronic stress. This model, referred to as 'allostatic load' has been discussed in a recent review on chronic stress in fishes but there is a paucity of data collected from fishes that examines this concept of allostatic load (Schreck, 2000). The data collected during the course of these studies suggests that one of the consequences of long-term confinement at increased rearing densities in yellow perch is a decreased corticosteroid response to an acute immune challenge.

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