Divergence of endocrine and metabolic responses to stress in two rainbow trout lines selected for differing cortisol responsiveness to stress

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Running title: Stress response in selected rainbow trout

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Abstract. Rainbow trout (*Oncorhynchus mykiss*) of two lines selected for low (LR) and high (HR) cortisol stress-responsiveness were subjected to confinement for a period of 336 hours. Endocrine (plasma cortisol, hepatic cortisol binding) and metabolic (plasma glucose, lactate, amino acids; hepatic glycogen and alanine aminotransferase levels) indices of stress were measured at intervals in confined and unconfined fish of both lines. During confinement plasma cortisol concentration reached maximum values earlier in HR fish (2 hours) than in LR fish (6 hours) returning to control values within 336 hours in both lines. Paradoxically, although both HR and LR lines displayed a characteristic metabolic stress response, these changes were more pronounced in LR fish. Plasma glucose and lactate levels increased during confinement in both lines but to a significantly greater extent in LR fish. Confinement significantly elevated plasma amino acids to a greater extent in LR fish than in HR fish. Liver glycogen concentration was depleted most rapidly in LR fish but was significantly higher in confined fish of both lines than controls at the end of the experiment. No significant changes were observed in hepatic alanine aminotransferase activity during confinement. Confined fish of both lines displayed a decrease in hepatic cortisol receptor abundance within 24 h and this was more sustained in HR fish. The more pronounced disturbance of a broad range of indicators of stress in confined LR fish, compared to HR fish, throws doubt on the magnitude of the cortisol response being the primary driver of these differences.

Key Words: stress, cortisol, selective breeding, metabolic response, rainbow trout, *Oncorhynchus mykiss*.

1. INTRODUCTION

The stress response is characterized by activation of the neuroendocrine system resulting in a cascade of physiological and metabolic changes that enable an animal to cope with a potentially hostile environment (van der Boon et al., 1991; Pickering, 1992; Wendelaar Bonga, 1997). The hormones that are primarily associated with this response are the catecholamines, adrenaline and noradrenaline, and the corticosteroids. Accurate measurement of the first presents difficulties because of the sensitivity and rapidity with which the catecholamine response to stressors occurs. The cortisol response is slower and is therefore more easily quantified. Consequently, cortisol has been widely employed as an indicator of stress in actinopterygian fishes (Pickering, 1992; Sumpter, 1997; Arends et al., 1999; Belanger et al., 2001). Cortisol release is mediated by activation of the hypothalamic-pituitary-interrenal (HPI) axis (Wendelaar Bonga, 1997; Rotllant, et al., 2000) in response to a stressor. In metabolic terms, the effect of the cortisol response can be considered to be broadly catabolic. Although complex, and not fully defined, the metabolic role of cortisol in fish seems to be significant (see review by Mommsen et al., 1999) and includes the promotion of pathways that increase glucose levels in the blood (Leach and Taylor, 1980; Vijayan et al., 1997; Diouf et al., 2000). Therefore, glycogenolysis and gluconeogenesis in which metabolites such as amino acids (Milligan, 1997) and lactate (Young and Cech, 1993, 1994) are used as substrates, are activated during stress.

Cortisol release during exposure to stressful conditions has an adaptive role in the short term, but when cortisol levels are elevated over a prolonged period cortisol may increase susceptibility to disease, depress growth rates and interfere with reproduction (Pickering, 1992). During prolonged exposure to a stressor a number of mechanisms may help to reduce the deleterious effects of elevated blood cortisol levels. These include: (i) negative feedback

of cortisol on the release of the hypothalamic and pituitary hormones that promote its secretion (Barton et al., 1987); (ii) desensitisation of the interrenal tissue to ACTH stimulation (Barton and Iwama, 1991; Rotllant et al., 2001); (iii) increased cortisol degradation rate (Vijayan and Leatherland, 1990); (iv) desensitisation of target tissues to cortisol via a reduction in the abundance of cortisol receptors (Pottinger, 1990). There is considerable inter-individual variability in stress-induced cortisol levels and this has a genetic basis (Fevolden et al., 2002). Lines of rainbow trout (*Oncorhynchus mykiss*) with divergent low and high cortisol responses to stressors have been produced by selective breeding, and these offer potential for optimising performance of fish under intensive rearing conditions in aquaculture (Fevolden et al., 1993, 2002; Pottinger and Carrick, 1999a). However, little is known as yet of the functional benefits or costs of being a high- or low-responder to stressors.

The aim of this study was to establish whether modifying the cortisol response to stressors by selection has any effect on the magnitude or duration of stress-induced metabolic changes (linked to intermediary metabolism) in rainbow trout and, if evident, whether such differences are mediated exclusively by the divergent cortisol response to stressors or in part by differences in target tissue sensitivity.

2. MATERIALS AND METHODS

2.1. Fish

Fish used in the study were rainbow trout lines (Stirling strain; F2 generation; 187 ± 4 g; mean ± SEM, n = 108) with a divergent responsiveness to stressors (low-responding: LR; high-responding: HR; Pottinger and Carrick, 1999a). From hatch, the fish were maintained in circular glass fibre tanks (1500 l capacity), each tank supplied with a constant flow of Windermere lake water (30 l/min) at ambient temperature (ranging from 4^{o}C in March to

17ºC in September) and photoperiod. Fish were fed once daily, five times a week with commercial trout feed (Trouw) at the manufacturers recommended rate.

2.2. Confinement stress and sampling

Fish from LR and HR holding tanks (stocking density approx 20 kg/m^3) were transferred to 50-liter confinement tanks, six fish per tank (density approx 60 kg/m^3), five tanks per line. Each confinement tank was supplied with a constant flow of lake water (15 l/min). To minimise unwanted effects of stock tank environment, each confinement tank received two fish from each of three holding tanks. At each sampling time (2, 6, 24, 168, 336 hours), the fish within one confinement tank were transferred to anaesthetic (2-phenoxyethanol, 1: 2000) and a blood sample was removed within 2-3 minutes from the caudal vessels into a heparinized syringe. Six control fish for tank transfer and confinement for each line were sampled from previously undisturbed holding tanks at 0, 24, 168 and 336 hours in the same way. After blood sampling, fish were killed by a blow to the head and the weight, fork length, and sex of each fish was recorded. At 24, 168 and 336 hours the liver was removed from each fish, weighed and processed as described below.

2.3. Blood and Tissue preparation

Blood samples were kept on ice until centrifugation. After separation, plasma was dispensed in four aliquots (100 μl for cortisol and 150 μl for glucose, lactate and amino acids) that were stored frozen (-70ºC) until required for assays. After weighing, livers were halved; one half was frozen in liquid nitrogen and stored at –70ºC for enzyme and glycogen analysis; the remainder was placed on ice and, within 30 min of removal, was homogenized in ice cold buffer (0.2 M Tris HCl, 1 mM EDTA, 10 mM sodium molybdate and 12 mM monothioglycerol) in the ratio 1:2.5 (weight: volume). The tissue was homogenized on ice

(Ultra-Turrax TP 18/10) and afterwards centrifuged at 20,000 rpm for 120 min at 4ºC. The resultant cytosol was dispensed into capped tubes and frozen at –70ºC until required for assay. Cytosol for alanine aminotransferase (AAT) assays was prepared in a similar manner, using a different buffer (100 mM / 250 mM Tris- sucrose pH 7.6) in a ratio 1: 9 (weight: volume). For glycogen assays, livers were homogenised in 0.05 M acetate buffer in the ratio 1:5 (weight: volume) with no centrifugation step. Both preparations were stored frozen until required for assays.

2.4. Assay procedures

Plasma cortisol levels were determined by a validated radioimmunoassay procedure (Pickering et al., 1987). Plasma glucose concentrations were measured colorimetrically by the Trinder method which couples glucose oxidase to peroxidase reaction (Sigma Diagnostics, Kit. No. 315). Plasma lactate levels were also measured colorimetrically by lactate oxidase coupled to peroxidase reaction (Sigma Diagnostics, Kit No. 735). Amino acids were estimated by the ninhydrin method following the procedure of Rosen (1957). Glycogen in liver homogenates was assayed as glucose following treatment with amyloglucosidase (Roehrig and Allred, 1974). Hepatic alanine aminotrasferase was assayed in liver cytosol by colorimetric measurement of phenylhydrazones derived proportionately from the reaction of 2,4-dinitrophenylhydrazine and the pyruvic acid formed in transaminase activity (Sigma Diagnostics, Kit No. 505). The measured activity was standardized to the tissue total protein concentration, determined by the Biuret-Folin method (Ohnishi and Barr, 1978). The concentration of specific cortisol binding sites was determined by saturation analysis. Aliquots of liver cytosol (200 μl) were incubated in duplicate for 2 h at 4ºC together with 50 μl of [1,2,6,7-^{3}H] cortisol (Amersham; 1.74 TBq/mmol) at 29.14 nM, both in the absence and in the presence of 1000-fold excess of inert cortisol. After incubation, a 200-μl aliquot of

dextran-coated charcoal (0.5 % activated charcoal, 0.1 % dextran, in homogenization buffer) was added to each tube. The tubes were mixed, allowed to stand on ice for 10 min, and then centrifuged at 3600 rpm for 10 min at 4ºC. A 300-µl aliquot of supernatant was transferred to a 5.0-ml scintillation vial together with 4.0-ml Ecoscint A (National Diagnostics), mixed, and counted under standard ^{3}H conditions in a Packard 1900 TR liquid scintillation counter. Sample total protein concentration was determined as above (Ohnishi and Barr, 1978).

2.5. Statistical Analysis

Multifactorial analysis of variance (ANOVA, Genstat 5) was employed to assess the significance of changes with time and differences between selection lines (HR, LR) and treatment (confined, unconfined). Where mean and variance did not vary independently, as indicated by a plot of residuals against fitted values, an appropriate transformation was applied (log or square root) to improve homogeneity of variance. Significant differences between times or treatment groups were determined using the estimated standard error of the differences between means.

3. RESULTS

3.1. Plasma cortisol

After the onset of confinement, plasma cortisol levels in both LR and HR fish increased significantly in relation to levels in the control unstressed groups (Fig. 1). Plasma cortisol levels in confined HR fish were significantly higher at 2 hours (116 ± 13.2 ng/ml) than those in the LR confined fish (43.2 ± 7.7 ng/ml; $P<0.001$). Maximum plasma cortisol levels occurred in the LR fish at 6 hours (97.4 ± 18.3 ng/ml). The initial elevation of plasma cortisol levels was followed by a progressive decrease in both LR and HR stressed groups approaching baseline levels at 168 hours (6.1 ± 0.8 and 9.3 ± 1.9 ng /ml respectively)

although these levels were still significantly higher than those in control fish at this time (1.2 ± 0.4 and 1.3 ± 0.6 ng/ ml respectively). At 336 hours levels in confined and control fish were similar in both LR and HR lines (LR stress and control: 2.5 ± 0.5; 2.6 ± 1.2; HR stress and control: 4.1 ± 1.1; 1.0 ± 0.2) although the difference between confined and control HR fish remained significant ($P<0.05$).

3.2. Plasma glucose

There were pronounced and significant differences between plasma glucose levels of the confined HR and LR fish. During confinement plasma glucose levels in both HR and LR fish increased significantly above those in unconfined fish of the same lines ($P<0.01$ at 24h; Fig. 2). However, in confined LR fish at 24 h plasma glucose levels were markedly higher (326 ± 44.5 mg/100 ml) than those in HR fish at this time (156 ± 13.2 mg/100 ml; $P<0.001$) and plasma glucose levels in the HR confined fish did not exceed approximately 150 ng/ml during the first 24 hours of confinement. In confined fish of both lines plasma glucose levels declined between 24 h and 168 h and were significantly lower in confined HR fish than control HR fish at 168 hours ($P<0.05$). After 336 hours of confinement, plasma glucose levels in the LR and HR groups were similar to each other (83.5 ± 1.4 and 94.1 ± 9.7 ng/ml respectively) and similar to those in the LR and HR control groups (84.5 ± 5.70 and 87.5 ± 7.16 ng/ml respectively).

3.3. Plasma lactate

Plasma lactate levels in both HR and LR confined groups rose rapidly within 2 hours relative to the controls, and significant differences ($P<0.001$; Fig. 3) were evident between the confined LR (119.3 ± 10.5 mg/100 ml) and HR (93.2 ± 5.4 mg/100 ml) fish at 2 h. Lactate in

the confined groups returned to pre-confinement levels within 6 hours of the onset of confinement. No further changes in plasma lactate levels occurred.

3.4. Plasma amino acids

Plasma amino acids showed, in both confined groups, a slight tendency to decline during the first 6 hours of confinement. This was most obvious in the HR fish resulting in significant differences ($P<0.01$; Fig 4.) between LR (4.8 ± 0.4 mg/100 ml) and HR (3.8 ± 0.1 mg/100 ml) fish after 6 h. At 24 hours after the onset of confinement there was a marked increase in plasma amino acids in both confined groups, but this was most evident in the LR fish with amino acid levels (7.7 ± 0.5 mg/100 ml) significantly higher than those in the HR fish (4.7 ± 0.4 mg/100 ml; $P<0.001$) and in the unconfined LR fish (5.2 ± 0.3 mg/100 ml; $P<0.001$). Plasma amino acid levels in the confined HR fish returned to pre-stress levels within 24 hours, whereas in confined LR fish 168 hours was required. From this time, no further significant differences between the stress and control groups were apparent.

3.5. Liver glycogen

At 24 hours after the onset of confinement there were significantly lower levels of glycogen in the livers of confined LR fish (25.9 ± 4.5 mg/g wet weight; Fig. 6) than in the LR control group (67.9 ± 6.4 mg/ g wet weight; $P<0.05$) or the HR confined group (52.0 ± 8.3 mg/ g wet weight ; $P<0.01$). After 168 hours confinement, liver glycogen levels in both LR and HR groups (19.2 ± 9.6 and 15.8 ± 11.8; LR and HR respectively) were significantly lower ($P< 0.001$) than controls (58.8 ± 6.1 and 56.3 ± 8.7; LR and HR respectively). Between 168 hours and 336 hours after the start of confinement there was a marked increase in liver glycogen levels in both groups of confined fish (88.0 ± 12.8 and 95.8 ± 13.0; LR and HR respectively)

to levels significantly (P<0.001; P<0.01) higher than controls at 336 hours (43.9 ± 4.5 and 39.1 ± 8.1; LR and HR respectively).

3.6. Liver AAT

Hepatic AAT activity did not show changes during confinement in any group other than a slight but significant difference (P<0.05; Fig. 6) between the HR confined fish (1069 ± 72 mU/mg protein) and the HR control group (898 ± 33 mU/mg protein) at 168 hours.

3.7. Liver cortisol binding

The abundance of specific cortisol binding sites in liver was significantly reduced in both confinement groups at 24 hours, with significant differences (P<0.01; Fig. 7) between LR confined and control fish (26.9 ± 5.6 and 66.5 ± 9.5 fmol/mg protein respectively) and HR confined and control fish (29.3 ± 10.9 and 72.7 ± 12.4 fmol/mg protein respectively) being observed. As confinement progressed an increase in the abundance of binding sites in both HR and LR confined groups occurred such that by 336 hours there was no significant difference between LR and HR confined groups (7.2 ± 1.0 and 5.1 ± 0.5 fmol/mg protein respectively) and their corresponding controls. The number of binding sites in confined LR fish was significantly greater than that in confined HR fish at 168 hours (P<0.001) but no differences between the lines were evident at 24 hours or 336 hours. There was no significant variation with time in the number of binding sites in control groups in either line.

4. DISCUSSION

Exposure of fish from the LR and HR lines to the combined stressor of tank transfer followed by confinement resulted in a marked increase of plasma cortisol in both groups. Levels in the confined fish remained significantly greater than those in unconfined controls for almost the entire duration of the study although there was overall a marked decline in levels with time.

There was a clear difference between cortisol levels in fish from the HR and LR lines at 2 hours after transfer to confinement tank, however, this was not sustained throughout the period of confinement as might have been expected from previous studies with the F1 generation of these lines (Pottinger and Carrick, 1999a). Instead, little difference was evident in cortisol levels between the lines from 6 hours onwards. The main difference in the cortisol response between the selected lines appeared to be the rate of change during the early stage of the stressor, immediately following capture and transfer. However, it is difficult to assess which element of the combined stressor is most important in evoking this response. Similar observations were made during a previous study with rainbow trout selected for stress responsiveness (Pottinger et al., 1994b) and has been confirmed during a recent study with the same F2 lines (T. G. Pottinger and K. G. T. Pulman, unpublished observations). Further investigations are being made to establish whether the dynamics of change in plasma cortisol levels during stress in the HR and LR F2 lines has altered radically from that observed in the F1 lines.

In terms of the metabolic response to the stressor in the two lines, a clear trend was apparent in the results of this study for alterations to be more pronounced in the LR fish than in the HR fish as might instead have been predicted. A hyperglycaemic response was observed during confinement in both lines. Elevation of blood glucose levels is a characteristic response of salmonid fish subjected to acute or chronic stressors (Vijayan and Moon, 1992; Vijayan et al., 1994; Biron and Benfey, 1994; Pottinger and Carrick, 1999b; Diouf et al., 2000). The glucose response pattern in confined LR and HR fish displayed similar trends, increasing to attain peak values within 24 h before declining to control values. However, plasma glucose concentrations in LR fish were more than two-fold greater than those in HR fish during the first 24 hours of confinement. While in the short-term at least this response may be

catecholamine-mediated (Weber and Shanghavi, 2000) there is evidence that cortisol plays both direct and permissive roles in glycogenolysis (Mommsen et al., 1999). During recovery (24 hours-336 hours), there was an apparent undershoot in glucose levels in confined fish which declined to concentrations below those of controls, possibly as a result of continued utilisation of glucose in certain tissues with elevated energy requirements (Soengas et al., 1993). The difference in stress-induced plasma glucose levels observed here between the HR and LR lines contrasts with previous observations suggesting that selection for cortisol responsiveness had no effect on the glucose response to a stressor (Pottinger and Carrick, 1999b). However, in the previous study, glucose levels were determined at a single point, after 3 hours of confinement, and not at intervals during a prolonged period of confinement, raising the possibility that differences may have been overlooked. It is also possible that the response characteristics of the F2 lines differ from those of the F0 or F1 fish.

Several authors have demonstrated the contribution made by hepatic glycogen reserves to stressor-induced hyperglycaemia (Bourne, 1986; De Boek et al., 2000) particularly during short-term responses. In the present study only in the LR fish were liver glycogen concentrations significantly lower than control groups after 24 hours of confinement, although it must be borne in mind that the absence of data for time 0 for confined fish does not allow us to say with certainty that glycogen declined at 24 hours. If a genuine change, this lower level may be functionally related to the considerably higher plasma glucose levels observed in the LR fish than HR fish at this time and therefore might reflect differences in glucose mobilization between the lines, perhaps linked to differential activation of the glycogenolytic pathways. In particular those associated with an increase in glycogen phosphatase (GPase) activity linked to a decrease in glycogen synthetase (GSase) activity (Soengas et al., 1992b, 1993). The return of plasma glucose levels to pre-stress baseline levels coincided with the

recovery of hepatic glycogen that could be explained by a stimulation of GSase activity linked to a decrease in GPase activity (Soengas et al., 1992a). These results agree with previous experiments that demonstrate the recovery of glycogen stores after a period of stress (Morales et al., 1990). In both confined HR and LR fish hepatic glycogen concentrations were higher than those in control fish at the end of the study. The replenishment of glycogen reserves by glyconeogenesis may occur at the expense of other substrates such as lipids and proteins (Vijayan and Moon, 1992; Vijayan et al., 1994) and this may have occurred here.

The glycogenolytic action of catecholamines during stress is well established (Janssens and Waterman, 1988; Ottolenghi et al., 1989; Fabri et al., 1998) but there is also evidence that cortisol makes an important contribution to glycogen mobilization (Paxton et. al., 1984; Morales et al., 1990; Soengas et al., 1992b, 1993; Reubush and Heath, 1996). However, in this case the most pronounced elevation of plasma glucose was observed in the LR group and the hyperglycaemic response in the HR fish was muted, suggesting, on the basis of blood cortisol levels in the two lines, little direct involvement of cortisol in glucose mobilisation. Furthermore, at 168 hours after the onset of confinement the HR fish showed a decrease in hepatic glycogen concentration that was not clearly correlated to an increase in either cortisol or glucose. Of course, hormones other than the catecholamines and cortisol, such as thyroxine and glucagon, are implicated in glycogen breakdown (Janssens and Waterman, 1988; Soengas et al., 1992b). Enhanced glycogen breakdown in the absence of increased total glucose production has previously been interpreted to indicate a mobilization of carbohydrate reserves for endogenous use by the liver (Vijayan et al., 1993).

An increase in plasma lactate levels is one of the earliest responses associated with "urgent" fuel consumption in tissues. It is manifested as a result of anaerobic metabolism in white

muscle. Both acute and chronic stressors elicit a rise in plasma lactate levels, presumably primarily due to physical disturbance and hypoxia arising from the onset of stressful conditions, and this response has been widely used as an indicator of stress in fish (Huber et al., 1989; Rotllant et al., 2001). Results in the present study are consistent with those from others (Schoonbee et al., 1989; Vijayan and Moon, 1992; Vijayan et al., 1994; Barton et al., 1998; Iversen et al., 1998; Barton et al., 2000) with confined groups displaying a rapid increase in plasma lactate levels within 2 hours of the onset of confinement. Interestingly, this elevation of lactate was significantly greater in the LR than HR fish. Baseline levels of lactate were re-established in the confined fish of both lines within 6 hours. The rapid removal of circulating lactate may be in part the result of a gluconeogenic conversion to glucose (Reubush and Heath, 1996) but it is possible that some of the lactate used by the liver is channelled for the replenishment of glycogen in addition to glucose production (Vijayan et al., 1994).

Plasma amino acid levels in both groups of confined fish displayed a slight decrease relative to control groups during the first 6 hours of confinement. It has previously been suggested that a decline in plasma amino acids observed in hypoxic conditions in rainbow trout was due to an increased utilization by tissues for energy supply, for example via participation in hepatic gluconeogensis (Medale et al. 1987). After 6 hours of confinement the decrease was followed by an elevation in plasma total amino acid concentration that in LR fish reached significantly higher values than HR, which only returned to pre-stress basal levels. A number of authors have reported an increase in plasma amino acids after exhaustive exercise, handling, or confinement (Morales et al., 1990; Braley and Anderson, 1992; Milligan, 1997; Vijayan et al., 1997). It has been shown that elevated cortisol levels can provoke a net catabolism of peripheral proteins (synthesis is also inhibited) and an elevation of plasma free

amino acid levels would be followed by a gluconeogenic removal (van der Boon et al., 1991; Vijayan et al., 1997). High plasma amino acid levels lead to induction of liver aminotransferases and consequently the participation of these metabolites in hepatic gluconeogenesis (Morales et al., 1990; van der Boon et al., 1991; Milligan, 1997). Several studies have provided evidence that alanine constitutes a good hepatic gluconeogenic precursor in fish (Medale et al., 1987; Braley and Anderson, 1992; Vijayan et al., 1994). However, in this study there was no evidence of a systematic induction of AAT activity in confined groups. This is consistent with the findings of Foster and Moon (1986) who showed that AAT activity was not affected by cortisol administration. Other transaminases (TAT, GOT) may have been affected (van Der Boon et al., 1991), but increased activity in these enzymes has not been reported to occur in response to cortisol administration (Leach and Taylor, 1982). The disparity in the extent to which plasma amino acid levels were elevated in the two lines during confinement contrasts with the similarity in hepatic glycogen concentrations that was evident by the end of the confinement period and counters the likelihood that transamination contributed significantly to the re-establishment of liver glycogen levels.

Cortisol-dependent effects are mediated at the target tissue by specific receptors. Persistent stimulation of cortisol-sensitive pathways may be detrimental and consequently target tissue receptor abundance may be down-regulated. In this study, re-establishment of hepatic cytosolic cortisol receptor numbers in confined fish appears to proceed more rapidly in LR than HR fish, despite there being no substantial difference in the blood cortisol profiles for the two groups of confined fish from 24 h onwards. Most of the pronounced differences in plasma and hepatic metabolite levels between HR and LR fish that occurred during confinement were

evident within the first 24 h of the onset of confinement. At this time, hepatic cortisol receptor numbers were similar in both HR and LR fish.

Overall, these observations suggest that the divergent alterations in intermediary metabolism observed during confinement in fish from the HR and LR lines were not dependent on either plasma cortisol levels or on target tissue sensitivity to cortisol. We interpret these data to indicate that the response of the HR and LR fish to stressors may be characterised by divergence across a range of responses that share a commonality other than the hypothalamic-pituitary-interrenal axis. This is not entirely consistent with emerging data on differences between the lines in behaviour (Pottinger and Carrick, 2001a; Øverli et al., 2002) and in brain monoaminergic profiles (Øverli et al., 2001). A recent study suggests that the interrenal is the site at which HPI divergence is mediated in these lines (Pottinger and Carrick, 2001b) and that therefore differences in behaviour and brain neuroendocrinology can be explained in terms of direct effects of cortisol. The present data do not readily fit this hypothesis and the possibility that selection may have altered, for example, the chromaffin catecholaminergic response to stress must also be considered. Further work will be required to clarify the causality of the differences in metabolic responsiveness between the two lines. In practical terms, from an aquaculture perspective these data clearly indicate that selection of one specific trait may inadvertently co-select for other alterations in ways that are unpredictable and may even be detrimental to the performance of the fish in culture conditions.

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FIGURE LEGENDS

Fig. 1. Plasma cortisol levels in LR and HR unconfined control and confined fish at intervals following the onset of confinement. Each point is the mean ± SEM, n = 6. Significant differences between confined LR and HR groups are denoted by: ***** *P*<0.05**, **** *P*<0.02, ******* *P*<0.01, ******** *P*<0.001. Significant differences between confined and unconfined control groups from the same line (LR or HR) are denoted by: **a** p < 0.05, **b** p < 0.02, **c** p < 0.01, **d** p < 0.001.

Fig. 2. Plasma glucose levels in LR and HR unconfined control and confined fish at intervals following the onset of confinement. Each point is the mean ± SEM, n = 6. Significant differences between confined LR and HR groups are denoted by: ***** *P*<0.05**, **** *P*<0.02, ******* *P*<0.01, ******** *P*<0.001. Significant differences between confined and unconfined control groups from the same selection (LR or HR) are denoted by: **a** p < 0.05, **b** p < 0.02, **c** p < 0.01, **d** p < 0.001.

Fig. 3. Plasma lactate levels in LR and HR unconfined control and confined fish at intervals following the onset of confinement. Each point is mean ± SEM, n = 6. Significant differences between LR and HR confined groups are denoted by: ***** *P*<0.05**, **** *P*<0.02, ******* *P*<0.01, ******** *P*<0.001.

Fig. 4. Plasma total amino acid levels in LR and HR unconfined control and confined fish at intervals following the onset of confinement. Each point is the mean ± SEM, n = 6. Significant differences between LR and HR confined groups are denoted by: ***** *P*<0.05**, **** *P*<0.02, ******* *P*<0.01, ******** *P*<0.001. Significant differences between confined and unconfined

control groups from the same line (LR or HR) are denoted by: **a** *P*<0.05, **b** *P*<0.02, **c** *P*<0.01, **d** *P*<0.001.

Fig. 5. Liver glycogen levels in LR and HR unconfined control and confined fish at 24, 168, and 336 hours of confinement. Each point is the mean ± SEM, n = 6. Significant differences between LR and HR confined groups are denoted by: ***** *P*<0.05**, **** *P*<0.02, ******* *P*<0.01, ******** *P*<0.001. Significant differences between confined and unconfined control groups from the same line (LR or HR) are denoted by: **a** *P*<0.05, **b** *P*<0.02, **c** *P*<0.01, **d** *P*<0.001.

Fig. 6. Liver AAT activity in LR and HR unconfined control and confined fish at 24, 168, and 336 hours of confinement. Each point is the mean ± SEM, n = 6. Significant differences between LR and HR confined groups are denoted by: ***** *P*<0.05**, **** *P*<0.02, ******* *P*<0.01, ******** *P*<0.001. Significant differences between confined and unconfined control groups from the same selection (LR or HR) are denoted by: **a** *P*<0.05, **b** *P*<0.02, **c** *P*<0.01, **d** *P*<0.001.

Fig. 7. The abundance of specific hepatic cortisol binding sites (Bs) in LR and HR unconfined control and confined fish at 24, 168, and 336 hours of confinement. Each point is the mean ± SEM, n = 6. Significant differences between LR and HR confined groups are denoted by: ***** *P*<0.05**, **** *P*<0.02, ******* *P*<0.01, ******** *P*<0.001. Significant differences between stress and control groups from the same selection (LR or HR) are denoted by: **a** *P*<0.05, **b** *P*<0.02, **c** *P*<0.01, **d** *P*<0.001.

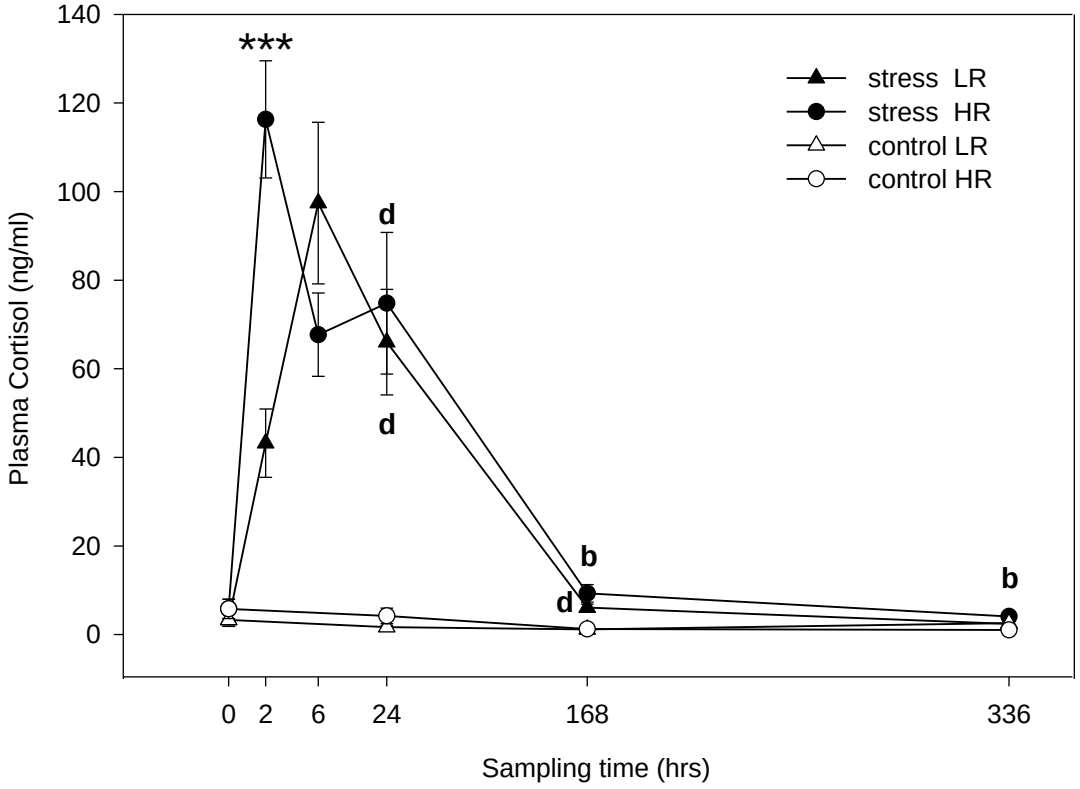

140
120
100
80
60
40
20
0
Plasma Cortisol (ng/ml)
***
d
d
b
d
b
stress LR
stress HR
control LR
control HR
0
2
6
24
168
336
Sampling time (hrs)

Fig 1.

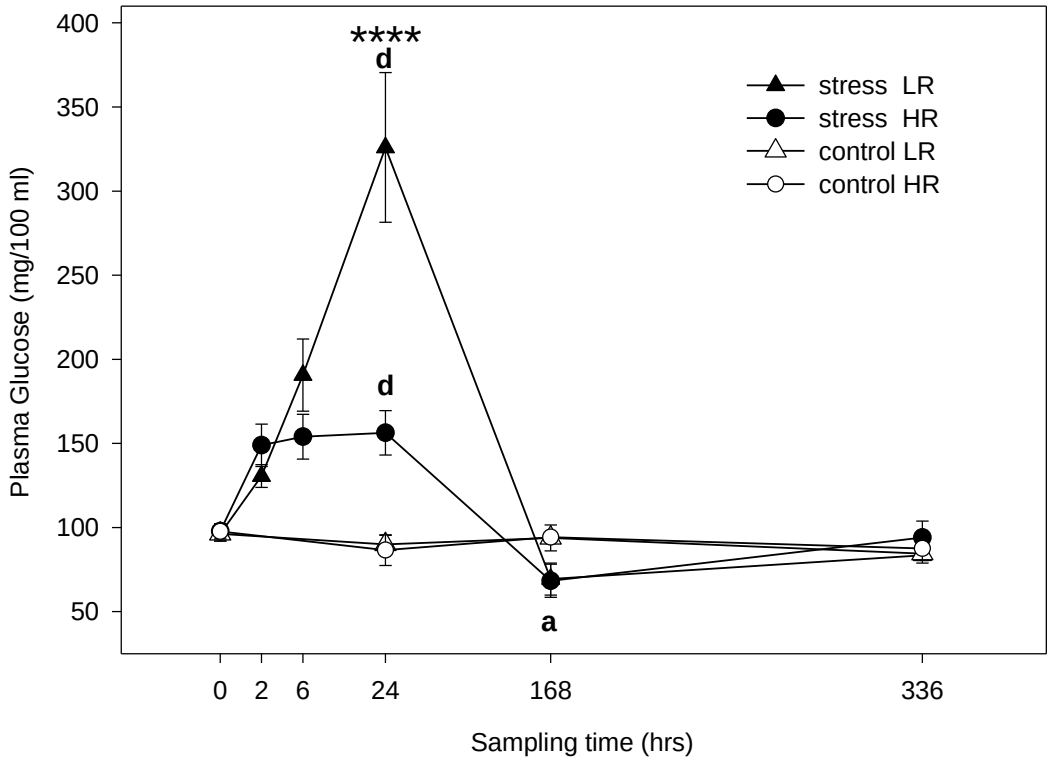

400
350
300
250
200
150
100
50
Plasma Glucose (mg/100 ml)
****
d
d
a
stress LR
stress HR
control LR
control HR
0
2
6
24
168
336
Sampling time (hrs)

Fig 2.

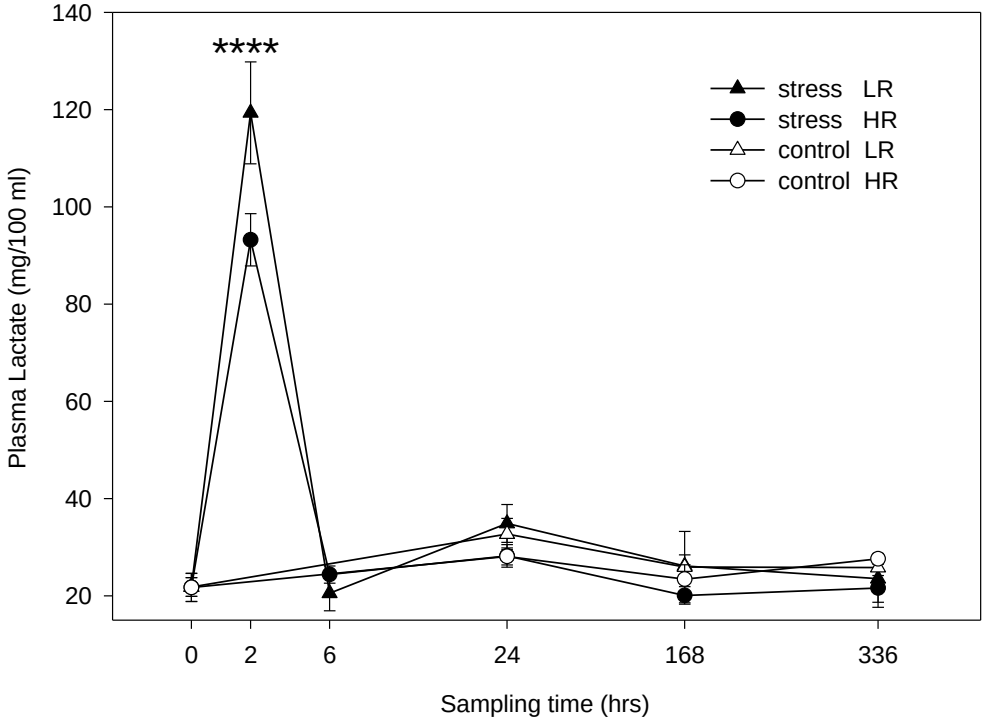
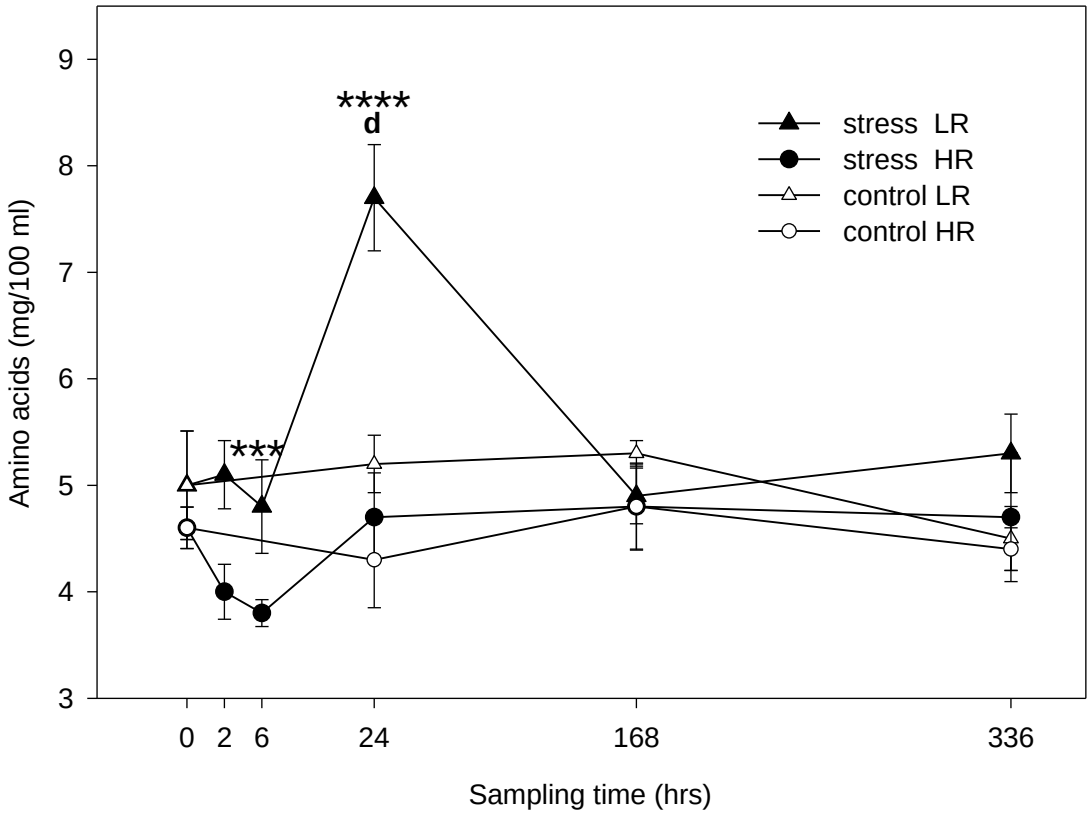

140
120
100
80
60
40
20
****
stress LR
stress HR
control LR
control HR
Plasma Lactate (mg/100 ml)
0
2
6
24
168
336
Sampling time (hrs)

Fig 3.

Fig 4.


****
d
***
stress LR
stress HR
control LR
control HR
Amino acids (mg/100 ml)
9
8
7
6
5
4
3
0
2
6
24
168
336
Sampling time (hrs)

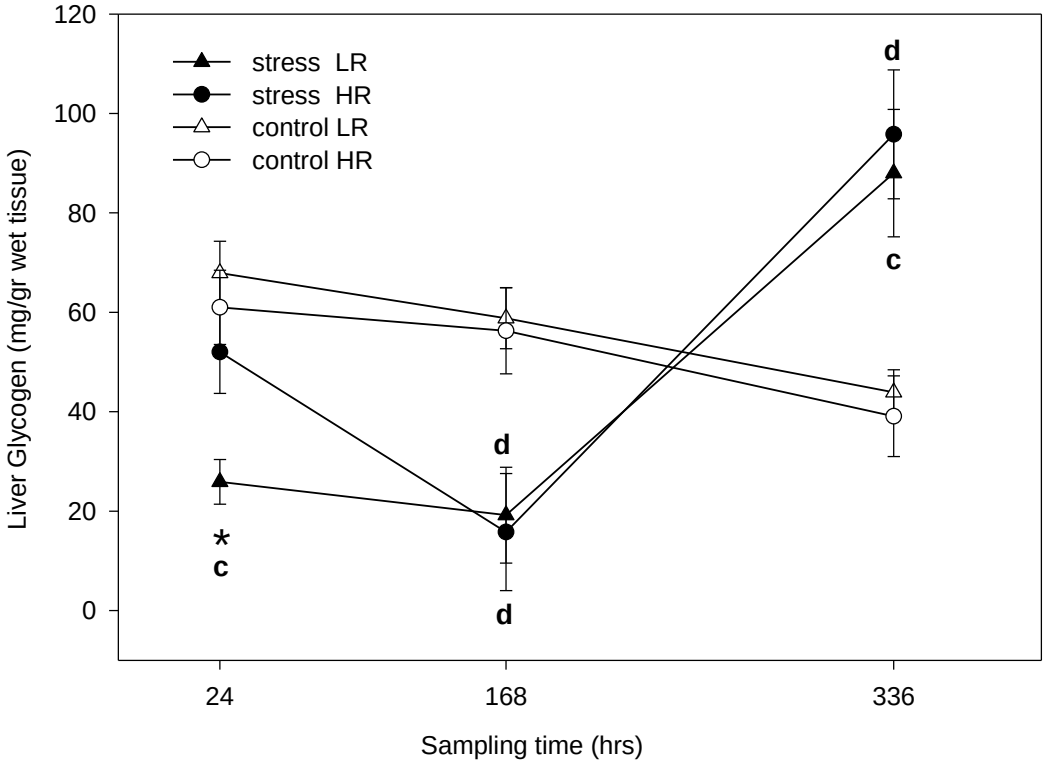

120
100
80
60
40
20
0
Liver Glycogen (mg/gr wet tissue)
stress LR
stress HR
control LR
control HR
d
c
d
*
c
d
24
168
336
Sampling time (hrs)

Fig 5.

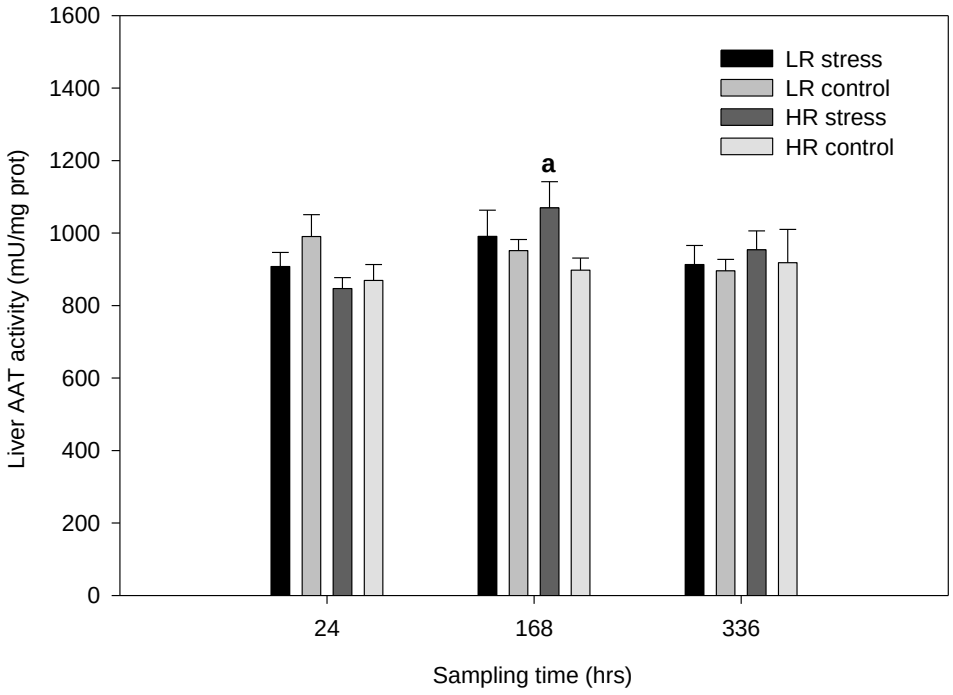

1600
1400
1200
1000
800
600
400
200
0
Liver AAT activity (mU/mg prot)
LR stress
LR control
HR stress
HR control
a
24
168
336
Sampling time (hrs)

Fig 6.

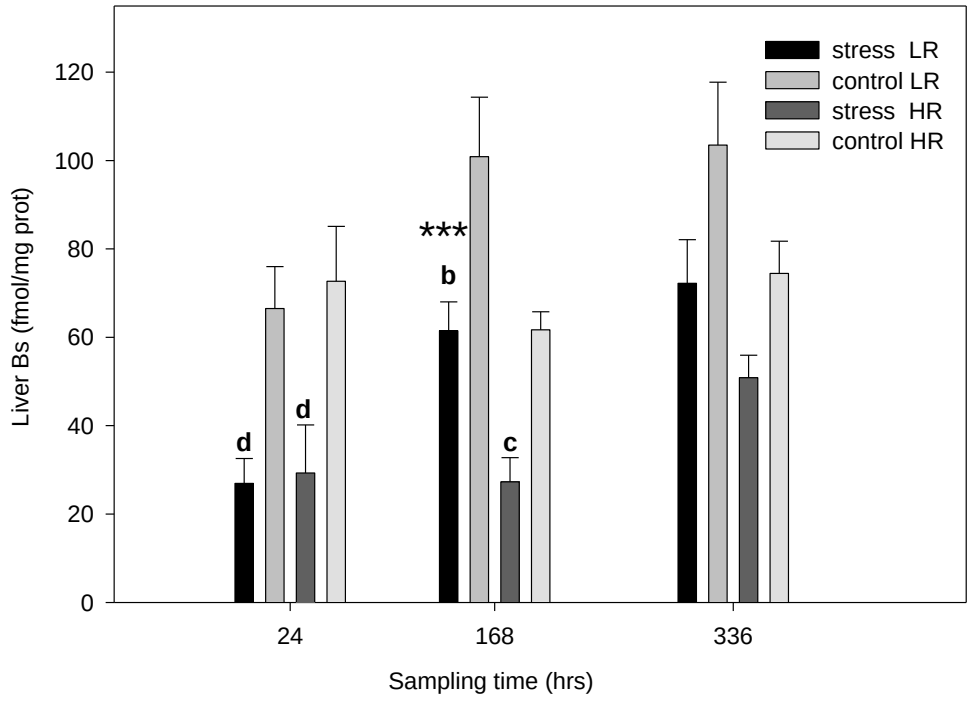

stress LR
control LR
stress HR
control HR
Liver Bs (fmol/mg prot)
120
100
80
60
40
20
0
d
d
***
b
c
24
168
336
Sampling time (hrs)

Fig 7.