Article (refereed) - postprint

Pottinger, T. G.; Carrick, T.R.. 1999 A comparison of plasma glucose and plasma cortisol as selection markers for high and low stress-responsiveness in female rainbow trout. *Aquaculture*, 175 (3-4). 351-363. 10.1016/S0044-8486(99)00107-6

A comparison of plasma glucose and plasma cortisol as selection markers for high and low stress-responsiveness in female rainbow trout

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Abstract

Two groups of female rainbow trout displaying consistently divergent plasma cortisol responses to a 3 h period of confinement were identified following five separate confinement episodes at monthly intervals. High-responding (HRC) and low-responding individuals (LRC) continued to display divergent cortisol responses to confinement up to 21 months after the start of the study (342 ± 34 ng ml^{-1} and 208 ± 21 ng ml^{-1} respectively at the final sample; $p<0.01$). HRC fish were significantly larger than LRC fish throughout the study period (533 ± 13 g and 422 ± 10 g respectively overall; $p<0.001$), although significant differences in specific growth rate (SGR) were apparent only at the start of the study. Individual fish were also selected from the same population on the basis of their plasma glucose levels following confinement (HRG: 189 ± 6; LRG 121 ± 3 mg dl^{-1}; $p<0.001$). However, the two selection traits (cortisol and glucose) identified separate subsets of the experimental population. HRG fish were also significantly larger than LRG fish although this difference was not so pronounced as for the cortisol-selected fish. There was no reciprocal relationship between body weight and stress responsiveness; fish selected from the population on the basis of high or low body weight displayed no divergence in either cortisol or glucose responses to confinement. Differences in size and SGR may indicate that HR fish adapted more rapidly to changes in environmental and social factors at the start of the study than LR fish did.

Key words: cortisol, glucose, rainbow trout, stress, selection

1. Introduction

Repeated or prolonged exposure of salmonid fish to stressful stimuli can result in reduced growth, impaired reproductive performance and immunosuppression (Barton, 1997). A wide range of responsiveness to stressors has been reported both for strains of salmonid fish (Pickering and Pottinger, 1989; McGeer et al., 1991) and for individual fish within strains (Pottinger et al., 1992). Because stress is an unavoidable component of the finfish aquaculture environment, and because of the adverse effects of chronic stress on performance characteristics, it has been suggested that fish which display a lower degree of responsiveness to stressors may perform better under intensive culture conditions than those which display a higher level of responsiveness (Pottinger and Pickering, 1997). It is also acknowledged that the converse may be true; given the adaptive significance of the stress response, high-responding fish may prove to be at a greater advantage, or low-responding fish at a relative disadvantage, overall. The prospect of selectively breeding fish for reduced responsiveness to stress has been addressed with respect to disease resistance (Fevolden et al., 1993) and overall performance (Pottinger et al., 1994). It is clear from these studies and similar work in poultry (Satterlee and Johnson, 1988), that selection of fish on the basis of stress responsiveness is practicable. However, as yet no thorough evaluation of the performance of fish selected for divergent stress responsiveness exists. The present study was carried out as one of a number of preliminary elements of a selective breeding programme, the aim of which is to generate lines of rainbow trout (*Oncorhynchus mykiss*) which are divergent for stress-responsiveness. If successful, this will allow the relative performance of fish with high- and low-responsiveness to stressors to be compared and permit an assessment to be made of which trait, if either, is desirable in fish under aquaculture conditions.

The changes in blood cortisol levels which occur in fish following the perception of a stressful stimuli are widely employed as a reliable index of activation of the neuroendocrine stress response (Barton and Iwama, 1991). Furthermore, cortisol itself is closely linked to many of the adverse consequences of chronic stress including effects on growth (Pickering, 1993), reproduction (Pickering et al., 1987), and the immune system (Pickering and Pottinger, 1989). Consequently, the extent of cortisol elevation following exposure to a standardised stressor has been adopted as a trait of physiological significance on which a selection procedure may be based (Fevolden et al., 1991; Pottinger et al., 1994). Blood glucose levels are also increased in fish following exposure to stressful stimuli. In the short-term hyperglycaemia is mediated primarily as a consequence of the rapid increase in blood catecholamines which occurs during the first phase of the stress response (Wendelaar Bonga, 1997) although in the longer-term, cortisol-dependent gluconeogensis may be responsible (van Raaij et al., 1996). The measurement of post-stress blood glucose levels therefore offers an indirect alternative marker of the magnitude of the neuroendocrine stress response. The measurement of blood glucose can be carried out rapidly using portable equipment (Morgan and Iwama, 1997), in contrast to the more sophisticated facilities required for the determination of cortisol. This is an important consideration when assessing the practicality of employing either selection trait within a commercial environment.

The aim of this study was to compare the utility of both plasma cortisol and plasma glucose levels as selection markers for stress responsiveness and to assess the reliability of each as identifiers of consistency in stress-responsiveness with time. Because uncertainty exists regarding the relative benefits of a high or low responsiveness to stressors, individuals with divergent responses were sought to permit comparison.

2. Materials and methods

2.1. Fish

During February 1996, 125 2-year old female rainbow trout (Stirling strain) were divided evenly between five 1500-l holding tanks (25 fish/tank), each supplied with a constant flow of lake water (25 l min^{-1}). Each fish was weighed, measured, and individually tagged by two methods. A passive integrated transponder (PIT) tag (Fish Eagle Co.) was inserted into the peritoneal cavity of each fish and a visible implant (VI) tag (Northwest Marine Technology Inc.) was inserted under the clear tissue posterior to the eye. Because of tag loss associated with the ejection of eggs from the body cavity in ripe females, PIT tags were re-implanted during subsequent samples into the dorsal musculature. Overall, PIT tags were found to be more reliable during the course of the study than VI tags whose retention was variable and operator-dependent. The fish were fed five times per week at the manufacturers recommended rate (1-2% body weight) with Trouw Standard Expanded 60 trout feed.

2.2. Stress testing

One month after the fish were distributed and tagged, and at monthly intervals for four months afterwards, the fish from each holding tank were transferred, in turn, to four 50-l confinement tanks, 6-7 fish per tank. Each confinement tank was supplied with a constant flow of lake water (15 l min^{-1}). After 3h confinement, the contents of the first confinement tank were transferred to anaesthetic (2-phenoxyethanol, 1:2000) and a 1.0-ml blood sample

was removed from the cuverian sinus of each fish into a heparinized syringe. After blood sampling, the fish was identified by reading both PIT and VI tags, and weight and length were recorded before the fish was returned to its original holding tank to recover. The blood samples were kept on ice until being centrifuged to separate plasma. Plasma was stored frozen (-20^{o}C) in aliquots until required for assay. It was not possible to obtain resting cortisol levels for unstressed fish because of the difficulty in catching, anaesthetizing and blood sampling all the fish from a single holding tank within the few minutes required to avoid effects of the associated disturbance on plasma cortisol levels.

At 5 and 11 months following spawning (15 and 21 months after the start of the study) a group of surviving fish (some mortality due to fungal infection, particularly among male fish, occurred immediately prior to, and following, the spawning period) were further tested to assess the stability of responsiveness to confinement with time.

For each tank in the experiment, weight, length, coefficient of condition (100.weight/length^3), specific growth rate (SGR, where SGR=[(lnW_2-lnW_1)/(t_2-t_1)].100; W_1 and W_2 are body weight at the start and end of the growth period, and t_2-t_1 is the length of the period in days), and post-stress plasma glucose or plasma cortisol levels (where appropriate) were determined each fish.

2.3 Assay procedures

Plasma cortisol levels were determined by a validated radioimmunoassay procedure (Pickering et al., 1987) and plasma glucose concentrations were measured by the glucose

oxidase method (Sigma Diagnostics, Kit no. 510A).

2.4. Identification and segregation of high- and low-responding fish

When all five monthly samples were completed and assayed, the mean post-stress plasma cortisol level for each fish was calculated and the fish within each tank were ranked on the basis of this figure. The four fish showing the consistently greatest response and the four fish showing the consistently lowest response were designated high-responders-cortisol (HRC) or low-responders-cortisol (LRC) respectively. The same procedure was carried out using post-stress plasma glucose levels to rank the fish as HRG and LRG. The use of only the four most divergent fish per tank for each group was dictated by consideration of the number of progeny groups (families) required for the next phase of the study and uncertainty regarding the possible loss, through disease or accident, of selected fish between the end of the selection period and the onset of spawning.

2.5. Statistical analysis

Multifactorial analysis of variance (ANOVA, Genstat) was employed to assess the significance of changes with time and differences within and between groups in length, weight, coefficient of condition, specific growth rate, plasma glucose, and plasma cortisol levels. Where appropriate, the data were transformed (log, square root) prior to analysis to ensure homogeneity of variances.

3. Results

3.1. Selection by post-stress cortisol level

The procedure by which HRC and LRC fish were selected within a single experimental tank, on the basis of their plasma cortisol response to a 3h confinement stress, is illustrated by Table 1. The mean post-stress plasma cortisol level was determined for each fish from the plasma cortisol values obtained on each of the sample dates (March - July). The mean plasma cortisol values were then ranked and the four fish with lowest mean post-stress cortisol levels and the four fish with highest mean post-stress cortisol levels were designated LRC (low-responding-cortisol) and HRC (high-responding-cortisol) respectively. This procedure was repeated for all the experimental tanks.

The mean post-stress plasma cortisol levels on all five sample dates in HRC and LRC fish are depicted in Fig. 1e. Throughout the test period a highly significant difference between HRC and LRC fish was maintained ($p<0.001$), the overall mean levels for the two groups over the five samples being 118 ± 5 and 53 ± 3 ng ml^{-1} (n = 100) respectively. Significant differences between HRC and LRC fish were also observed elsewhere. Body weight was significantly greater overall in HRC (533 ± 13 g) than LRC fish (422 ± 10 g) ($p<0.001$; Fig. 1a) as were length (34.0 ± 0.2 cm c.f. 31.8 ± 0.2 cm; $p<0.001$; Fig. 1b) and coefficient of condition (1.333 ± 0.014 c.f. 1.258 ± 0.012; $p<0.05$; Fig. 1c). There was no overall significant difference in SGR between the two groups although HRC fish displayed a significantly greater SGR than LRC fish at time 2 (0.38 ± 0.07 c.f. 0.18 ± 0.07 %body weight day^{-1}; $p<0.05$; Fig. 1d).

There was no significant difference in post-stress plasma glucose levels in HRC and LRC fish

either at individual samples (Fig. 3a) or overall (159 ± 5 c.f. 150 ± 5 mg dl^{-1}).

3.2. Selection by post-stress plasma glucose level

The same selection procedure which was employed to identify HRC and LRC fish on the basis of cortisol response to confinement was used to identify HRG (high-responding-glucose) and LRG (low-responding-glucose) fish within the same population based on the hyperglycaemic response to confinement. A significant difference in plasma glucose levels between the HRG and LRG fish following confinement was sustained throughout the test period ($p<0.001$; Fig. 2e) with overall mean levels of 189 ± 6 and 121 ± 3 mg dl^{-1} respectively. Differences were also apparent in size and growth of the HRG and LRG fish but less so than for the cortisol-selected individuals. HRG fish were consistently heavier than LRG fish overall (508 ± 13 c.f. 448 ± 12 g; $p = 0.079$) and significant differences were apparent on three occasions ($p<0.05$; Fig 2a). There was no overall significant difference in length between the two groups (33.6 ± 0.2 c.f. 32.5 ± 0.3 cm) although HRG fish were found to be significantly longer than LRG fish at the final sample ($p<0.05$; Fig. 2b). The coefficient of condition did not differ significantly between HRG and LRG fish (Fig. 2c) and SGR was significantly different on only the second sample point (0.348 ± 0.07 c.f. 0.115 ± 0.07 %body weight day^{-1}; $p<0.05$; Fig. 2d).

There was no significant difference in post-stress plasma cortisol levels in HRG and LRG fish either at individual samples (Fig. 3b) or overall (82 ± 4 c.f. 75 ± 5 ng ml^{-1}).

3.3. The effect of fish size on the cortisol and glucose response to confinement

When fish from a single experimental tank were ranked on the basis of body weight (Fig 4a) no significant differences were apparent in either the glucose or cortisol responses to confinement in these fish (Fig. 4b,c). Regressions of body weight against post-stress cortisol levels for all fish within each of the experimental tanks at a single sample point confirmed that there was no significant relationship between body weight and stress responsiveness overall.

3.4. Persistence of differences in the response to confinement and body weight with time

Twelve of the surviving HRC and LRC fish were subjected to a 3-h period of confinement at 5 and 11 months following spawning. On each occasion the significant difference in cortisol response between HRC and LRC fish was retained (Fig. 5b), values at the final sample being 342 ± 34 ng ml^{-1} and 208 ± 21 ng ml^{-1} respectively ($p<0.01$; $n = 12$). In addition, the HRC fish remained significantly heavier than the LRC fish throughout this period (Fig. 5a) body weights at the final sample being 3091 ± 106 g and 2433 ± 98 g respectively ($p<0.01$). The proportional difference between the body weight of HRC and LRC fish and the plasma cortisol response of HRC and LRC fish remained at approximately 25% and 40% respectively at each sample point. Limitations on available space and resources meant that HRG and LRG fish were not maintained beyond the initial 5-month evaluation period.

4. Discussion

These results confirm and extend earlier observations (Pottinger et al., 1992) that the cortisol response to confinement stress in rainbow trout is an individual characteristic that is stable

with time, allowing individual fish with divergent responses to be selected. These data also demonstrate that fish displaying a hyperglycaemic response to confinement, which is consistently higher or lower than the population mean, may be identified. However, selection of individuals on the basis of their cortisol response to confinement did not co-select for glucose responsiveness and the converse was also true; selection on the basis of glucose responsiveness did not co-select for cortisol responsiveness. Each selection trait identified a different sub-set of the population. Furthermore, body weight was positively related to stress responsiveness in both glucose- and cortisol-selected fish, with HR fish selected for either trait being significantly heavier than LR fish.

Changes in blood cortisol levels following stress are considered to be a more direct indicator of the activity of the hypothalamic-pituitary-interrenal (HPI) axis than changes in glucose levels following stress. The stress-induced increase in circulating levels of cortisol is believed to be wholly dependent on *de novo* biosynthetic activity within the interrenal tissue. This in turn is dependent on stimulation by adrenocorticotropin (ACTH) secreted by the pituitary, following stimulation of the pituitary corticotropes by corticotropin-releasing hormone (CRH) of hypothalamic origin (Sumpter, 1997). "Resting" cortisol levels are low (<5 ng ml^{-1}) in unstressed immature rainbow trout (Pickering and Pottinger, 1989), and can increase more than 100-fold following exposure of the fish to a stressful stimulus (Barton and Iwama, 1991). In contrast, blood glucose levels in rainbow trout may only increase 2-fold following exposure to stressful stimuli (T. G. Pottinger and L. Machin, unpublished) and, in addition, resting and stress-induced blood glucose levels are subject to influence of diet and nutritional status (Barton et al., 1988; Holloway et al., 1994; Jayaram & Beamish, 1992; Vijayan & Moon, 1992). Nonetheless, some agreement between the cortisol and glucose response might

have been expected in terms of the individual fish selected as HR and LR, given that the magnitude of the blood cortisol response to a stressor is widely taken to be proportional to the stress response in its entirety. These data suggest that the elements within the neuroendocrine stress response that control rapid glycogenolytic mobilisation of glucose may not be closely tied to the neuroendocrine factors controlling the release of cortisol into the bloodstream. This lack of intraspecific consistency in the magnitude of the cortisol and glucose responses to stress has also been reported to occur at an interspecific level (Barton and Iwama, 1991). If the rapid mobilisation of glucose in fish during stress is primarily related to the catecholamine pulse then it is possible that the two axes (pituitary/interrenal tissue and sympathetic nervous system/chromaffin tissue) of the stress response in rainbow trout are not closely coupled. Therefore, a selection process based on the corticosteroidogenic response alone may fail to account for other elements of the response. Whether in practical terms this is disadvantageous or beneficial depends on whether there is any improvement in performance to be gained from modifying the catecholamine component of the stress response.

Somatic data were collected for all fish in the five experimental tanks with the intention of identifying any trends in performance linked to stress responsiveness. Unexpectedly, fish with a consistently high plasma cortisol response to stress were significantly larger with a significantly higher condition factor (heavier for a given length) than fish selected as low plasma cortisol responders. To a lesser extent, the same trend in weight difference was apparent in fish selected on the basis of their blood glucose response to confinement although differences in condition factor were not detected in these fish. Intuitively, a negative rather than positive correlation between stress responsiveness and weight might be expected given the overall negative (catabolic) effects of stress and cortisol on growth and metabolism

(Pickering, 1993). However, these fish were maintained at a low density (initially 7 g l^{-1}) and loading (initially 500 g l^{-1} inflow) and were subject to no disturbance other than the monthly confinement stressor. It is therefore unlikely that the fish had to contend with the effects of prolonged elevation of blood cortisol levels and stress responsiveness *per se* is unlikely to be a relevant factor..

The SGR derived from incremental weight gain was significantly greater in both HRC and HRG fish than in the corresponding LR groups at the first sample point for which SGR was calculable, but did not differ between groups thereafter. Neither was there a statistically significant difference in weight between the HR and LR fish one month after the start of the experimental period for either cortisol or glucose-selected groups. The statistically significant differences in weight and SGR only became manifest during the second month of the study. Therefore, these data do not appear to suggest that fish identified as HR are intrinsically faster growing individuals than LR fish. If there were an intrinsic and consistent difference between LR and HR fish in food conversion efficiency or in food intake, either because of inherently greater appetite or success in competing for food on the part of HR fish, it is arguable that this would have been apparent as significant differences in weight at the start of the study and a continuing difference in SGR. Instead, the data suggest that prior to the start of the study growth in the two groups was similar and the divergence in SGR was limited to the first two months of the experimental period, an interpretation supported by inspection of the LRG and HRG data. The convergence of SGR for the HR and LR fish during months 3 - 5 of the study indicates that, after the initial divergence in performance, both groups grew at a similar rate, sustaining the difference in weight achieved during the first two months of the study. Thus, the data are suggestive of alterations in the relative

performance of the two groups associated with the change in the holding environment which occurred at the start of the experimental period. This may be related to the decline in stocking density from 25 gl^{-1} to 7 g l^{-1}.

If the observed differences in growth arose because the HR fish adapted more quickly than LR fish to the change in environmental and social conditions associated with the onset of experimental conditions, then the differences in size may have a behavioural rather than physiological basis. It is possible that the physiological differences between HR and LR fish are accompanied by differences in behavioural characteristics between the two groups. Strong links between behavioural traits and physiological responsiveness to stressors have been demonstrated in a number of strains or lines of rodents (Ramos and Mormède, 1998) and have also been established for poultry (Jones, 1996). Behavioural traits associated with territoriality, aggression and competitiveness have been strongly linked with food acquisition in salmonid fish, even in holding tanks under relatively high densities (see references in Kadri et al., 1996). Stressful stimuli are known to alter certain behaviours in fish (Schreck et al., 1997) so it is conceivable that the HR and LR fish responded differently to the manipulations associated with the start of the experiment.

The possibility that the size or weight of the fish is itself a determinant of stress responsiveness was also considered. However, when fish within a tank were ranked by weight, and mean post stress plasma cortisol levels were plotted for the four fish with highest body weights and the four fish with the lowest body weights, there was no evidence of a link between stress-induced cortisol elevation and body weight. This conclusion was supported by the observation that the regression of body weight and post-stress plasma cortisol for all fish

on a single sample was not statistically significant. Furthermore, the difference in stress responsiveness between the HR and LR fish was clearly detectable at the time of the first sample whereas the significant differences in weight associated with responsiveness did not emerge until the second or third months of the experimental period. The results therefore appear to indicate that it is factors associated with the HR and LR trait which have influenced growth, not growth rate or size which influence stress responsiveness.

In conclusion, the cortisol and glucose response to stress in individual fish may be regulated independently and do not provide an equivalent index of stress responsiveness. It remains to be determined whether selection for stress responsiveness for production enhancement purposes should take into account responsiveness as measured by both traits or only one.

Acknowledgements

This study forms part of a project co-funded by the European Commission (contract FAIR-CT95-0152) and the Natural Environment Research Council, U.K.

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Table 1. Post-stress plasma cortisol levels in all fish in a single representative tank on each sampling date. The fish have been ranked on the basis of the overall mean post-stress plasma cortisol value for each individual, across the five samples. Those individuals selected as HR and LR are indicated.

Date of sample

VI tag no.	Mar 96	Apr 96	May 96	Jun 96	Jul 96	mean	
ME6	58	37.2	12.9	21.7	18.8	29.72	LR
MD9	73	15.7	17.2	41.7	10.2	31.56	LR
MISSING	48	22.9	25	43	32.1	34.2	LR
MM3	122	40.8	28.4	33.2	8.7	46.62	LR
MM0	118	23	40.2	64.2	27.3	54.54	
MM2	87	18.5	42.9	93.5	41.9	56.76	
MD0	119	36.9	36.3	75.2	23.8	58.24	
MM1	118	87.1	58.8	30.5	41	67.08	
ME1	132	47.4	26.1	64.5	66.3	67.26	
MD8	148	79.8	31.7	69.6	40	73.82	
MD7	128	58.6	89.7	85.1	24.8	77.24	
ME3	107	61.3	75.8	131.3	18.5	78.78	
ME4	161	112.8	44.5	43.3	35.5	79.42	
ME5	203	76.7	28.6	74.5	40.4	84.64	
ME8	142	53.9	85	109.5	42.4	86.56	
MD3	143	79.4	89.9	78.9	58.6	89.96	HR
ME2	154	144.7	33.7	81.5	90.9	100.96	HR
MD5	203	107.5	114.6	121.8	68.3	123.04	HR
MD2	155	159.8	192.7	256.7	31.9	159.22	HR

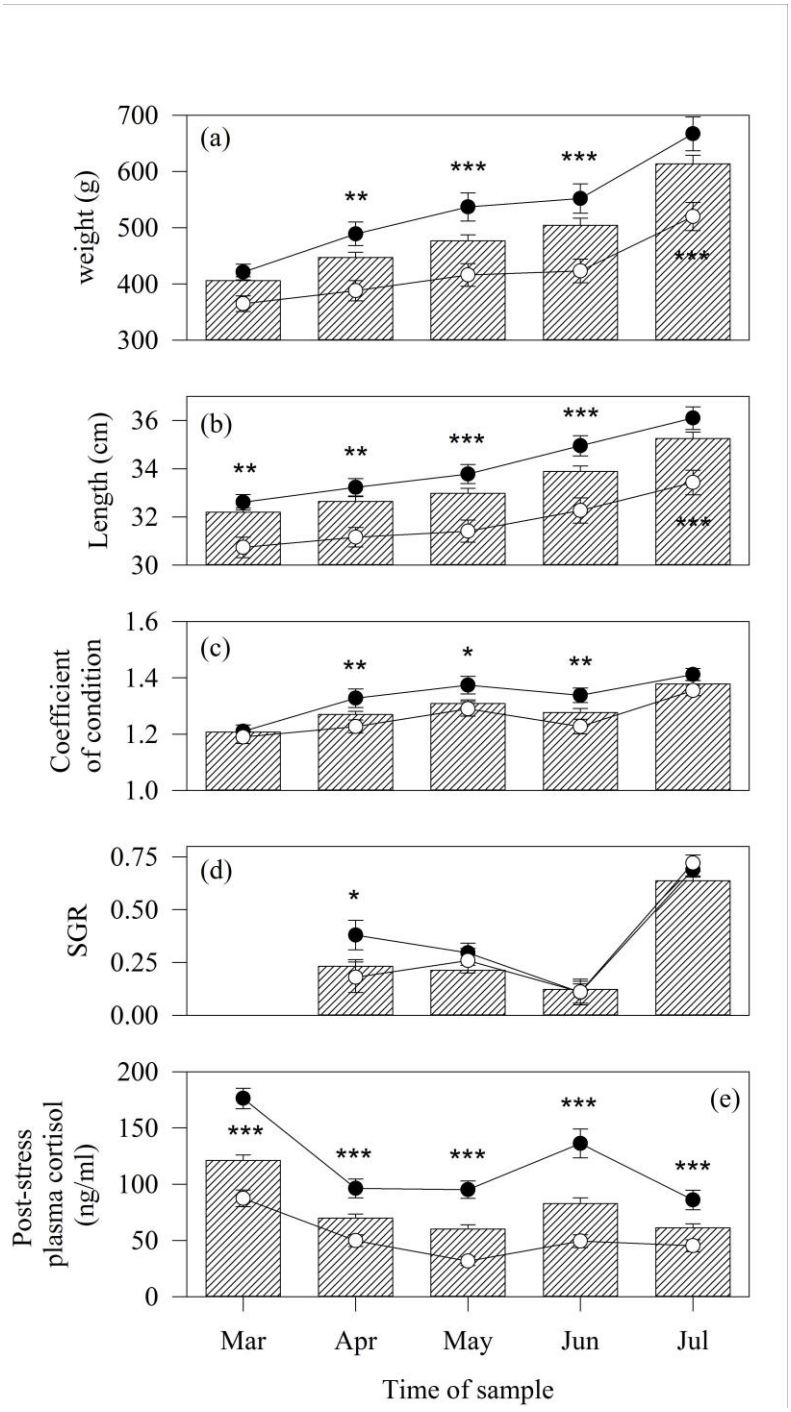

Figure 1. (a) Body weight, (b) fork length, (c) coefficient of condition, (d) specific growth rate, and (e) post-stress plasma cortisol levels, for all fish in the experimental tanks (shaded bars; n = 125), cortisol-selected HRC fish (•; n = 20) and cortisol-selected LRC fish (❍; n = 20) at each of 5 monthly samples. Each point is the mean ± SEM. Significant differences between HR and LR fish are denoted by * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

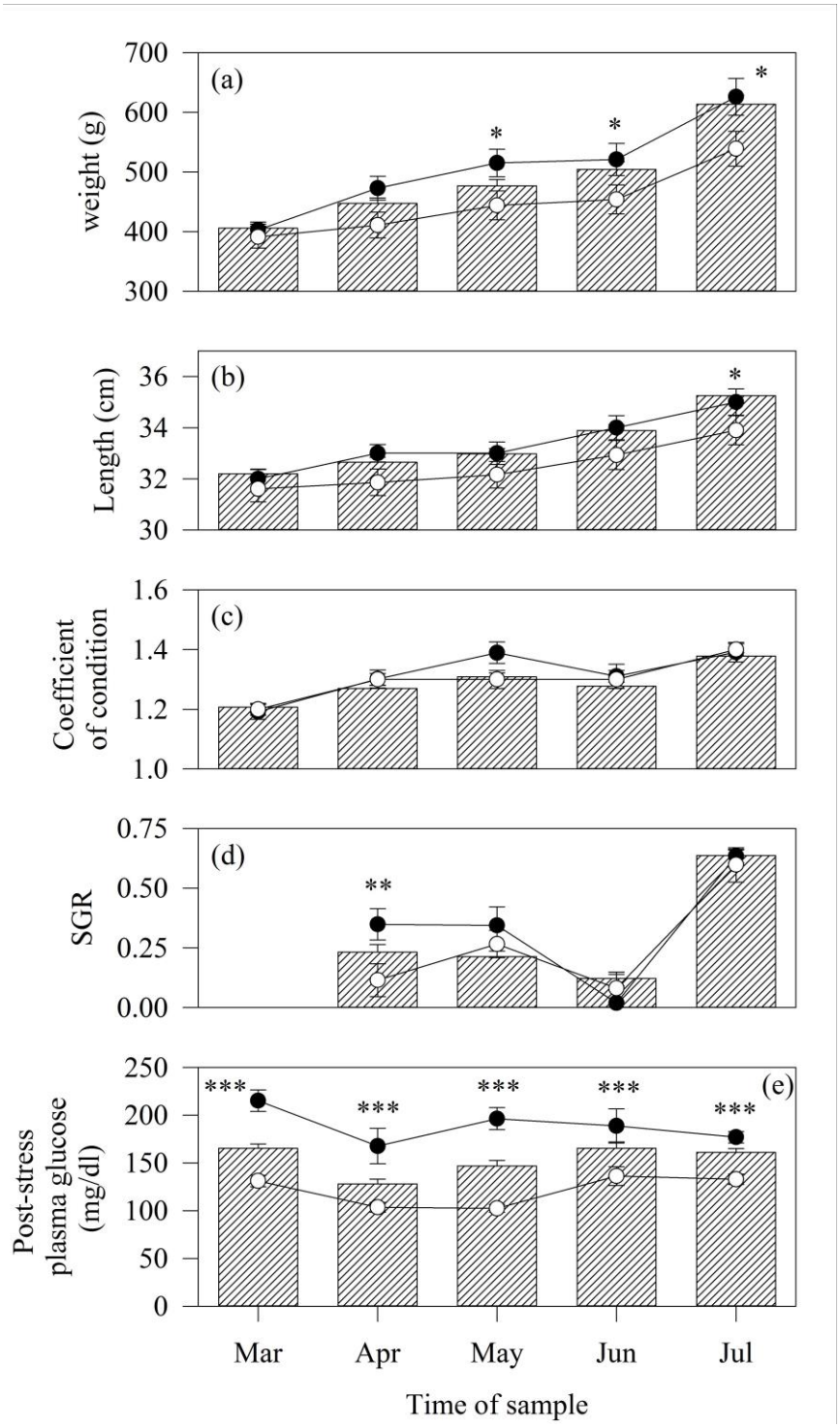

Figure 2. (a) Body weight, (b) fork length, (c) coefficient of condition, (d) specific growth rate, and (e) post-stress plasma glucose levels, for all fish in the experimental tanks (bars; n = 125), glucose-selected HRG fish (•; n = 20) and glucose-selected LRG fish (❍; n = 20) at each of 5 monthly samples. Each point is the mean ± SEM. Significant differences between HR and LR fish are denoted by * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

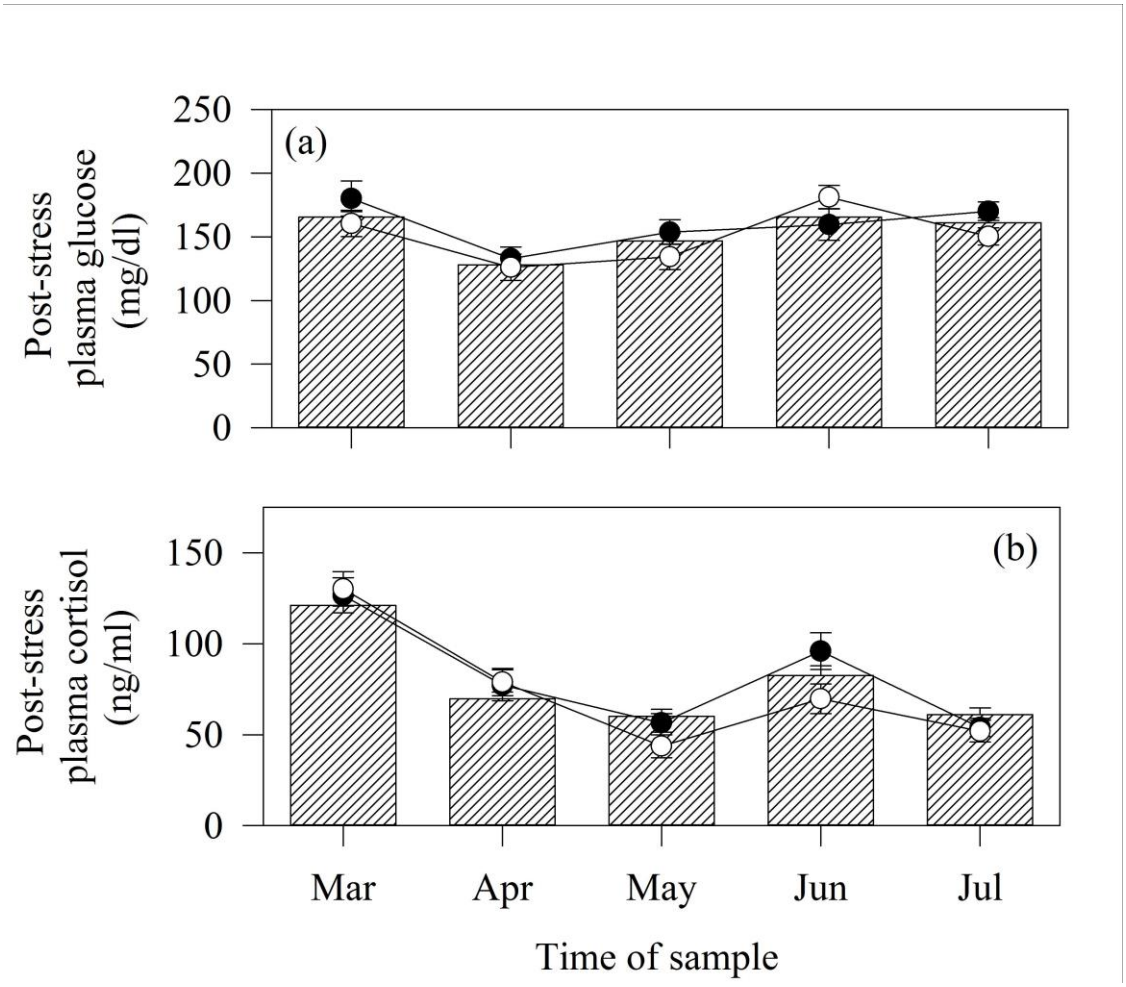

Figure 3. (a) Post-stress plasma glucose levels in cortisol-selected HRC and LRC fish and (b) post-stress plasma cortisol levels in glucose-selected HRG and LRG rainbow trout after 3 h confinement at each of 5 monthly samples. Mean of all fish in the experimental tanks (shaded bars; n = 125), HR fish (•; n = 20) and LR fish (❍; n = 20). Each point is the mean ± SEM.

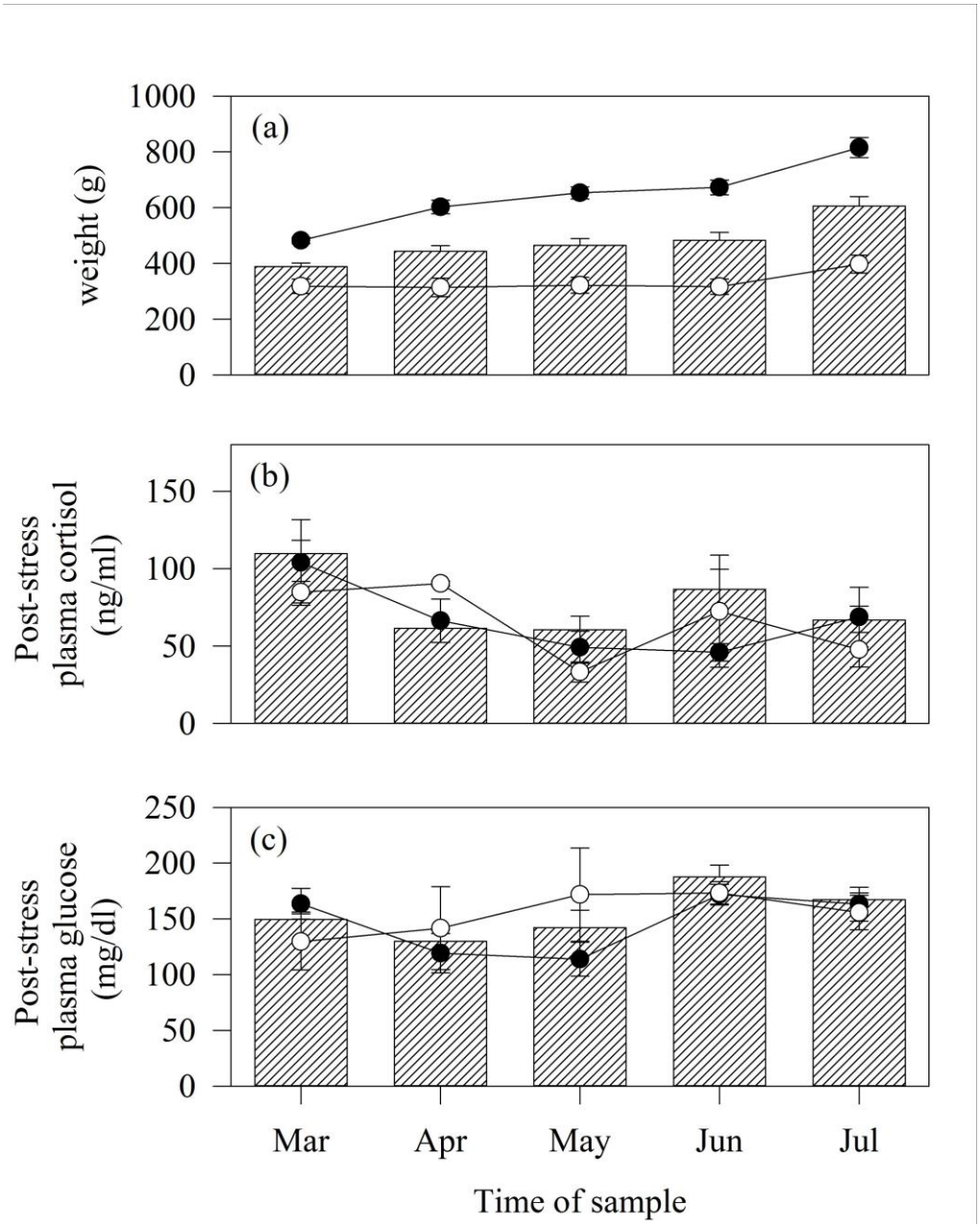

Figure 4. (a) Body weight, (b) post-stress plasma cortisol, and (c) post-stress plasma glucose in fish from a single tank at each of 5 monthly samples. Each point is the mean ± SEM. All fish (shaded bars; n = 25), selected for high body weight (•; n = 4), selected for low body weight (❍; n = 4).

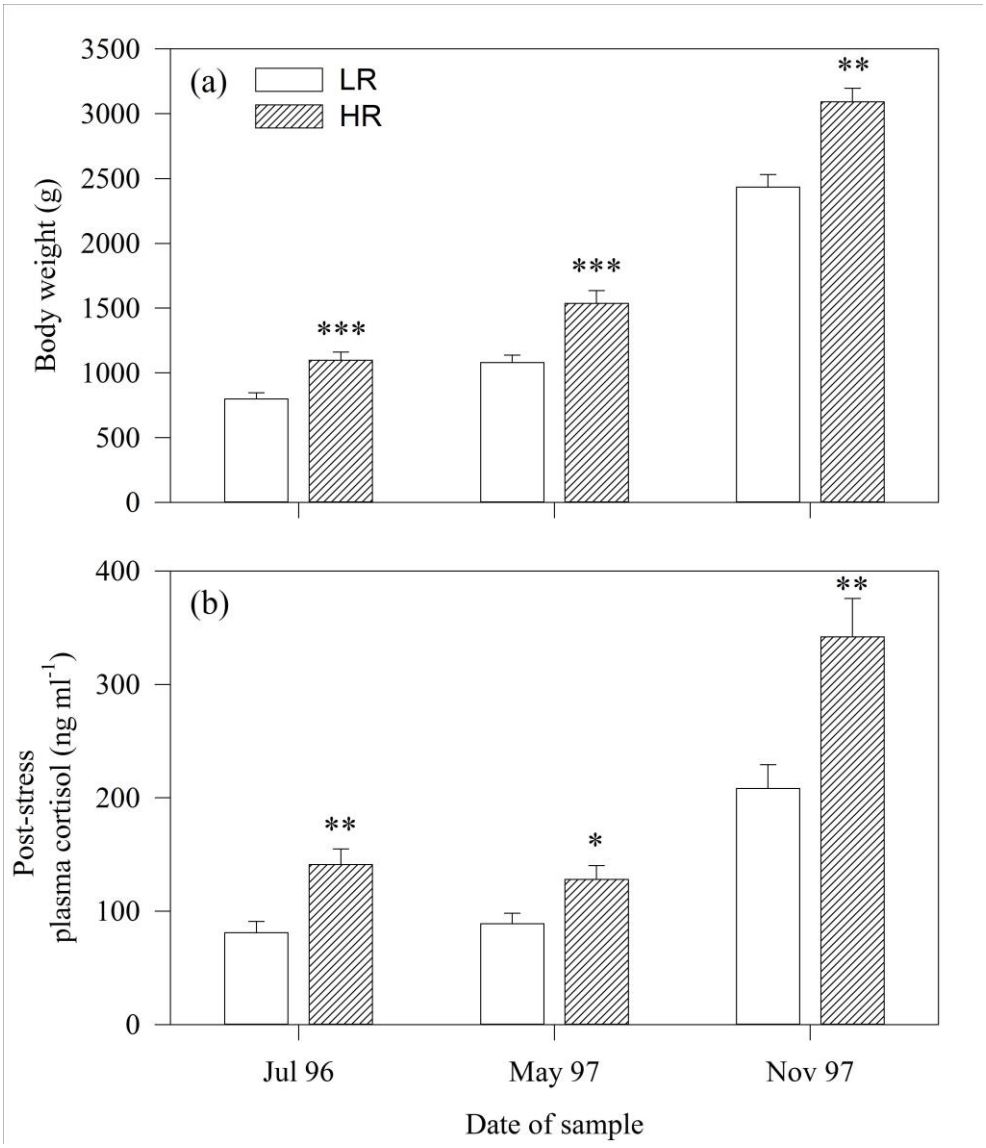

Figure 5. (a) Body weight and (b) post-stress plasma cortisol in cortisol-selected HRC fish (dark bars) and cortisol-selected LRC fish (light bars) on the final sample of the selection test series and on two subsequent occasions. Each point is the mean ± SEM (n = 12). Significant differences between HRC and LRC fish are denoted by * $p<0.05$; ** $p<0.01$; *** $p<0.001$.