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Behavioral and Neuroendocrine Correlates of Selection for Stress Responsiveness in Rainbow Trout - A Review[1]

RUNNING TITLE: SELECTION FOR STRESS RESPONSIVENESS IN TROUT

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SYNOPSIS.

In rainbow trout the magnitude of the cortisol response to stress shows both consistency over time and a moderate to high degree of heritability, and high responding (HR) and low responding (LR) lines of rainbow trout have been generated by individual selection for consistently high or low post-stress cortisol values. Using 2nd and 3rd generation fish, we tested the hypothesis that differential stress responsiveness is associated with behavioral alterations in the HR-LR trout model. LR fish showed a tendency to become socially dominant, a rapid recovery of food intake after transfer to a novel environment, and a reduced locomotor response in a territorial intrusion test. Furthermore, stress induced elevation of brain stem and optic tectum concentrations of the monoamine neurotransmitters serotonin, dopamine, and norepinephrine and their metabolites suggests that both synthesis and metabolism of these transmitters were elevated after stress to a larger degree in HR than in LR trout. A divergent pattern was seen in the hypothalamus, where LR fish displayed elevated levels of 5-hydroxyindoleacetic acid (a serotonin metabolite) and 3-methoxy-4-hydroxyphenylglycol (a norepinephrine metabolite). Thus, selection for a single trait, cortisol responsiveness, in rainbow trout is associated with concurrent changes in both behavior and central signaling systems. The apparent parallel to genetically determined stress coping styles in mammals, and the existence of similar trait associations in unselected populations of rainbow trout, suggests an evolutionarily conserved correlation between multiple traits. Continuing studies on the HR and LR trout lines are aimed at providing the physiological and genetic basis for new marker-assisted selection strategies in the rapidly developing finfish aquaculture industry, as well as increased knowledge of the function and evolution of central neuroendocrine signaling systems.

INTRODUCTION

The rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) is native to the Pacific coast of North America and Russia, but has been widely cultivated and introduced as a food and game fish in temperate regions around the world. Wide occurrence, availability and ease of culture have made the rainbow trout one of the most intensively studied fish species in biological research - this species has been used in fields ranging from evolutionary ecology to behavior, physiology, genetics, toxicology and cancer research (see Thorgaard et al., 2002). Rainbow trout also attracts interest as a model species for the commercially important family salmonidae, with focus on nutrition, reproduction, and stress physiology.

Intensive aquaculture of rainbow trout and other salmonids is a relatively new innovation, compared to the cultivation of most other animals. The process of domestication has only just begun in salmonids, and considerable effort is devoted to selecting for and defining the heritability of economically important traits in these fish, such as growth rate, age at maturity, fillet composition, and disease resistance (see e.g. Gall and Huang 1988a,b; Gjerde, 1993; Gjøen and Bentsen, 1997; Gjedrem, 2000; Midtlyng et al., 2002). Natural populations of salmonids are characteristic in displaying an immense variability in genetic composition, life history, and a range of phenotypic traits (Ryman, 1983; Taylor, 1991; Hershberger, 1992; Carlsson et al., 1999; Waples et al., 2001; Hansen et al., 2002), which makes these fish make excellent raw material for genetic studies and selection experiments.

Fish under intensive culture conditions are exposed to a regime of acute and chronic stressors, which have adverse effects on growth, reproduction,

immunocompetence, and flesh quality, among other things (Barton et al., 1987; Maule et al., 1989; Barton and Iwama, 1991; Lowe et al., 1993; Pickering, 1993; Balm, 1997; Pankhurst and Van der Kraak, 1997; Sigholt et al., 1997; Schreck et al., 2001). As in other vertebrates, the blood concentration of corticosteroid hormones is a major index of stress in fish, and elevated levels of these hormones arise from activation of the hypothalamus-pituitary-interrenal (HPI) axis (Wendelaar-Bonga, 1997). The main corticosteroid in teleost fish is cortisol (Kime, 1987), and this steroid is a causal factor in many of the deleterious effects of stress (Barton et al., 1987; Barton and Iwama, 1991; Harris and Bird, 2000; Pankhurst and Van der Kraak, 2000; Schreck et al., 2001; Consten et al., 2002; Bernier et al., 2004). In rainbow trout the magnitude of the cortisol response to stress is an individual characteristic which is stable over time, with a moderate to high degree of heritability (Pottinger et al., 1992, 1994; Fevolden et al., 1999). Thus, post-stress cortisol levels provide a trait of functional and economical significance upon which selection pressure can be directed, and lines of high- (HR) and low-responsive (LR) trout have been established at the Windermere laboratory of the UK Centre for Ecology and Hydrology (Pottinger and Carrick, 1999, 2001a).

In association with the physiological response, stress may lead to drastic changes in behavior, which has been classified and interpreted in various ways (see e.g. Koolhaas et al., 1999; Wingfield, 2003). A distinction is often made between proactive (active coping, or ‘fight or flight’) and reactive (passive coping, or ‘conservation-withdrawal’) responses (Engel and Schmale, 1972; Henry and Stephens, 1977; Benus et al., 1991; Henry, 1993; Koolhaas et al., 1999). In both mammals and fish, a transition from behavioral activation to an inhibiting effect is often seen with increasing duration or

severity of the challenge (Haller et al., 1998; Øverli et al., 2004a). Seminal studies in mammals indicated that the threshold at which the shift from an active to a passive behavioral response occurs is subject to great individual variation (Engel and Schmale, 1972; Henry and Stephens, 1977).

More recently, it has been established that individual differences in the physiological stress response are associated with differences in behavior. For instance, a pro-active stress coping style is behaviorally characterized by a high level of active avoidance, aggression, and other behavioral patterns indicating an active attempt to counteract the stressful stimulus (e.g. defensive burying in rodents) (Bohus et al., 1987; Benus et al., 1989, 1991a; Sluyter et al., 1996). Reactive (passive) coping, on the other hand, involves immobility and low levels of aggression. Physiologically, the pro-active strategy is associated with low hypothalamus-pituitary-adrenal axis (HPA axis, the mammalian equivalent of the teleost HPI axis) responsiveness, but high sympathetic reactivity, while the opposite is true for reactive coping (de Boer et al., 1990; Korte et al., 1992; Fokkema et al., 1995). A genetic basis for the expression of behavioral and physiological components of individual coping styles has repeatedly been demonstrated (e.g. Driscoll et al., 1998; Ellenbroek and Cools, 2002; de Boer et al., 2003; Veenema et al., 2003). Limited information, however, is available on whether different behavioral-physiological stress coping styles are present in teleost fish (but see Francis, 1990; Van Raaij et al., 1996; Budaev et al., 1999).

In fish, like in other vertebrates, behavioral and physiological stress responses are to a large degree linked by common control mechanisms in the brain, and the monoamine neurotransmitters serotonin (5-hydroxytryptamine, 5-HT), dopamine (DA), and

norepinephrine (NE) play a vital role in this co-ordination (Winberg and Nilsson, 1993; Winberg et al., 1997, 2001; Øverli et al., 1998, 1999; Höglund et al., 2001, 2002a,b; Lepage et al., 2002, 2003; Clements et al., 2003; Larson et al., 2003; Perreault et al., 2003). Genetically determined variation in behavior and stress responsiveness has been associated with differences in brain monoaminergic function in both fish and mammals (e.g. Benus et al., 1991b; Popova et al., 1991a,b; Nikulina et al., 1992; Rots et al., 1996a,b,c; de Kloet et al., 1996; Sallinen et al., 1999; Lepage et al., 2000; Giorgi et al., 2003). In this paper we review the effects of selection for post stress cortisol production in rainbow trout, with particular reference to a series of studies investigating the association between altered plasma cortisol dynamics, behavior, and brain monoamine neurotransmitters. In other words, we examine the hypothesis that behavioral and physiological traits are linked in such a way that altering one trait, post-stress plasma cortisol concentrations, incurs differences in other putative components of individual stress coping styles. It should be noted that several additional physiological differences between HR and LR lines, such as metabolic changes and interrenal sensitivity to adenocorticotrophic hormone (ACTH) also has been reported, but for details of this experimental work we refer to the original papers (Pottinger and Carrick, 2001b, Trenzado et al., 2003).

MATERIAL AND METHODS

Generation of HR and LR trout lines

The selection procedure and the effect of the breeding program on the cortisol response has been thoroughly described elsewhere (Pottinger and Carrick, 1999, 2001a), and will only be briefly reviewed here. The parental generation of the HR and LR trout lines was established in 1996 by repeated stress testing (3h confinement in 50 L water in groups of 6-7 individuals once monthly) of passive integrated transponder (PIT) tagged 2-year-old rainbow trout. Following confinement, blood samples (0.5 ml) and PIT-tag readings were obtained from anaesthetized (2-phenoxyethanol, 1:2000) fish, and plasma was later analyzed for cortisol content by a previously validated radioimmunoassay (RIA) procedure (Pickering et al., 1987). The mean post-stress plasma cortisol content across five episodes of confinement was then calculated for each fish, and individuals were ranked accordingly. Between testing fish had been kept in groups of 25 in 1500 L holding tanks, and the four most high-responding (HR) and the four most low-responding (LR) fish in each tank were removed from their home tank, segregated by sex, and kept separate in 4 tanks based on group and sex until maturation.

Confinement stress testing of the 1st generation offspring (F1), consisting of 15 HR and 14 LR families each resulting from a unique male-female crossing, were carried out on five different occasions between September 1997 and September 1998, and on five occasions in 1999. A highly significant regression of mid-parent cortisol response on progeny response was seen (estimated r2 [h2] value = 0.41) and the six LR families with the lowest mean cortisol response and the six HR families with the highest mean cortisol response were identified and used for further work (Pottinger and Carrick, 1999). An unselected (US) population resulting from random breeding of fish not designated as HR or LR in the parental group (6 families) was tested on one occasion, and showed a

cortisol response intermediate to the mean of these most divergent HR and LR groups. Adult female F1 generation HR and LR fish were later used in a study investigating the effect of selection for stress responsiveness on behavior and brain monoamine neurotransmitters (Øverli et al., 2001, 2002a).

The 2nd generation of offspring consisted of 11 HR, 11 LR and 3 US families. The regression of midparent [(male + female)/2] cortisol response on progeny cortisol response provided an estimated h2 of 0.6 in this generation, while male and female parent–progeny regressions provided estimates for h2 of 0.73 and 0.44, respectively (Pottinger and Carrick, 2001a).

Behavioral experiments

Locomotor activity, feed intake, and brain monoaminergic activity in HR and LR trout: These experiments are described in detail in Øverli et al. (2001, 2002a). Recovery of feed intake after transfer to a new and potentially stressful environment was used as an index of adaptiveness. Only adult female F1 fish were available for these studies. Locomotor activity when in isolation and in response to a territorial intruder was also analyzed, along with basal and stress-induced brain 5-HT, DA and NE activity. During April 2000 adult F1 female HR (n=18) and LR (n=18) rainbow trout weighing 987.5 ± 39.5 g (mean ± SE) were transferred individually from communal holding tanks to rearing in isolation in white 250 L polypropylene observation tanks. From day 1 after transfer to rearing in isolation, fish were fed daily by hand (1.5% of body weight), and fish were observed for 3 min after distribution of food to register food intake. Behavioral observations and blood sampling (see below) were carried out between 10.00 and 14.00.

After being held for 6 days in isolation, locomotor activity was quantified by observing time spent moving during 20 min for each fish, starting 1 h after feeding. Locomotor activity was quantified again the next day, this time for 20 min immediately following the introduction of an intruder fish in the observation tank. Intruder fish were smaller (< 50% body weight of the resident fish) group reared rainbow trout from a hatchery population, and previously unfamiliar to the test fish.

On the day after the intruder test, 50% of the fish from each line (HR, LR) were randomly selected for individual stress testing in 50 L confinement tanks. After 1h in the confinement tanks, fish were netted, anaesthetized in 0.5 ml/L 2-phenoxyethanol, and a blood sample was obtained from the caudal sinus into a heparinized syringe. The remaining 50% of the fish were sampled directly from observation tanks to serve as undisturbed controls. Immediately following blood sampling fish were killed by decapitation and dissected, and the presence or absence of food in the stomach and / or intestines was registered. Brains were removed and dissected into telencephalon (excluding the olfactory bulbs), hypothalamus (excluding the pituitary), optic tectum, and brain stem (excluding the cerebellum). Brain samples were immediately wrapped in aluminum foil and snap frozen in liquid nitrogen, where after concentrations of monoamines and monoamine metabolites in brain samples were analyzed by HPLC with electrochemical detection (Øverli et al., 1999). Plasma cortisol levels were determined using the RIA procedure described by Pickering et al. (1987).

Tests for social dominance in HR and LR trout: Tests to investigate the relative tendency toward dominant or subordinate social status of the HR and LR lines were carried out in September and October 2000 using juvenile F2 progeny (Pottinger and

Carrick, 2001a). Mixed-sex juvenile fish were used in these tests since they are generally more territorial than adults, and there is no effect of sex on the ability to gain dominance at this life stage (Ø. Øverli and S. Winberg, unpublished results). Dyadic contests were performed following broadly the protocol of Øverli et al. (1999). In all, 47 size matched pairs consisting of 1 HR and 1 LR individual were transferred from holding tanks to rearing in glass observation aquaria (63 L volume) covered with black plastic on three sides. In each aquarium two fish were kept separate by a removable opaque plastic barrier.

Fish were offered food (five crumbs, Trouw Fry 18) twice each day after being transferred to the aquaria. Previous experiments have shown that holding juvenile salmonid fish in isolation for approximately one week with sufficient access to food is highly effective in inducing territoriality and motivation to express aggressive behavior (Winberg et al., 1991; Winberg and Lepage, 1998; Øverli et al., 1999). Barriers separating HR/LR pairs were removed after 5 days of acclimation, resulting in escalated contests for social dominance within each pair. Behavioral observations started at 3 h after the onset of interaction, by which time the conflict was resolved and the identity of the dominant and subordinate individuals within each pair was clear. At this time dominant fish were characterized by holding a midwater position, displaying extensive movement around the tank, nipping and/or chasing the subordinate, and intercepting food. Subordinate fish were typically located on the base of the aquarium, usually immediately adjacent to the aquarium wall, exhibited little swimming activity, and directed no aggressive acts toward the dominant individual. After 5 h of social interaction

fish were killed and, a blood sample was obtained, and blood plasma was analyzed for cortisol content using a RIA with ethyl acetate extraction (Pottinger and Carrick, 2001a).

RESULTS AND DISCUSSION

Cortisol responsiveness in HR and LR rainbow trout

Post-stress blood plasma cortisol levels of HR and LR trout for every occasion on which they were tested, from the establishment of the parental generation (F0) up to present (F3), is shown in Figure 1. There is considerable variation in the magnitude of the cortisol response over time, and between generations, but a difference between the lines (HR>LR) is always evident. The exception to this is the final sample for the F0 fish in which reproductive status may have confounded the normal previously observed divergence. Some of the variation in overall magnitude of the stress response can no doubt be accounted for by seasonal changes in water temperature (Sumpter et al., 1985; Barton and Schreck, 1987; Pickering and Pottinger, 1987) and in reproductive status of the fish (Pottinger et al., 1995; Pottinger and Carrick, 2000). However, it must be borne in mind that these tests were carried out for a variety of purposes and did not employ exactly the same protocol on each occasion. Therefore, while it is appropriate to conclude that divergence in stress responsiveness has been sustained across three generations, it is not possible to directly compare successive time points and evaluate temporal trends.

Notwithstanding this caveat, the type of test that were employed for the first occasion on which each generation was assessed were similar and the results of these tests may be directly compared. The magnitude of the difference in cortisol responsiveness between lines has remained similar over 3 successive generations for

juvenile fish subjected to their first stress test (age 6-7 months, tested in September 1997, September 2000 and October 2003). The post-stress plasma cortisol levels in HR juveniles represents 135%, 224%, and 220% that of the LR fish for the F1-F3 generations respectively. The similarity in the magnitude of divergence of these generations might indicate that there are physiological constraints on the range of plasma cortisol responsiveness to stress in trout. Such constraints may be imposed upon low-responders by the need to retain a functional cortisol response and upon high-responders by problems associated with hypercortisolism.

Finally, it should be noted that strain differences in plasma cortisol levels was never observed in unstressed fish. Thus, it seems likely that the effect of the selection program on post-stress levels of cortisol represents divergent responses to stressors, rather than differences in basal HPI-axis activity, which in turn could affect responsiveness.

Behavioral effects of selection for stress responsiveness

Pottinger and Carrick (2001a) reported a tendency of LR fish to establish social dominance over HR fish when held in pairs (Figure 2), and Øverli et al. (2001) observed that only LR fish had regained feed intake within one week after transfer to a new environment (Figure 3). Interestingly, an association between rapidly regaining feed intake after environmental change and the ability to win fights for social dominance was also observed in an unselected population of rainbow trout, using juvenile fish of both sexes (Øverli et al., 2004b). These observations indicate that the HR and LR lines are based on multi-trait phenotypes that display a significant amount of variation also in

unselected populations, but it has not yet been studied whether the divergence in behavior has increased with successive generations.

Locomotor activity in isolated HR and LR female rainbow trout, when held alone and when challenged with a conspecific intruder, is depicted in Figure 4. HR rainbow trout displayed higher locomotor activity than LR trout in the presence of an intruder, but there was no significant difference between the two groups in the isolated condition. However, both HR and LR rainbow trout increased their activity level when the intruder was present. Thus, it appears that some behavioral differences between HR and LR lines occur only under an acute challenge, which may indicate that they depend on control mechanisms activated in synchronization with the physiological stress response.

Brain monoaminergic activity in HR and LR trout

Concentrations of the three monoamine neurotransmitters 5-HT, DA, and NE and their respective metabolites 5-hydroxyindoleactetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC), and 3-methoxy-4-hydroxyphenylglycol (MHPG), and corresponding metabolite/monoamine ratios in four different brain regions of stressed and control HR and LR trout are shown in Table I. Since monoamine neurotransmitters are not exposed to monoamine oxidase (MAO) while stored in vesicles, increased concentrations of their deaminated metabolites are thought to indicate increased release and turnover of the neurotransmitter (Fillenz, 1993).

In particular, metabolite/monoamine ratios are frequently used as an index of neural activity. However, attention should be paid to the fact that altered metabolite / monoamine ratios may be caused by changes in the concentrations of monoamine

neurotransmitters as well as metabolites, or by combinations of such changes. Thus, differential patterns of activation may be assumed depending on whether concentrations or ratios are studied. For instance, brain stem 5-HIAA concentrations were significantly affected by confinement stress only in HR fish (Table I). Therefore, it could be concluded that the brain 5-HT system was activated by confinement stress to a larger extent in HR than in LR fish. On the other hand, brain stem 5-HIAA/5-HT ratios were higher in LR than HR fish both in the control condition and after stress. This apparent contradiction is probably caused by the fact that HR fish, but not LR fish, responded to stress by an increase in 5-HT concentrations in the brain stem. Apart from that, three possible explanations can be given to the observation that 5-HIAA/5-HT ratios were elevated in LR fish relative to HR fish: 1. The proportion of 5-HT that was actually released, and thereby exposed to the action of MAO after re-uptake from the intercellular space, was greater in LR fish. 2. MAO enzyme activity was decreased in HR fish. 3. Re-uptake of 5-HT was more effective in LR fish.

Nonetheless, one of the most evident findings of this study was that HR trout reacted to stress by an increase in the tissue concentrations of serotonin (brain stem), dopamine (brain stem), and norepinephrine (optic tectum, telencephalon), whereas low-responsive fish did not (Table I). Brain stem and optic tectum concentrations of monoamine metabolites were also elevated after stress in HR, but not in LR fish. A divergent pattern was seen in the hypothalamus, were LR fish displayed elevated levels of 5-HIAA and MHPG. Both populations had elevated telencephalic concentrations of these metabolites after stress. Some differences were also seen in fish sampled directly from rearing in isolation, suggesting that the rearing environment was not entirely

optimal, and the experimental fish may have experienced a mild stress even in the undisturbed condition. This notion is also in line with the lack of aggressive behavior and low level of feed intake in these fish (Øverli et al., 2002a). Alternatively, differential stress responsiveness in HR and LR fish is associated with permanent differences in brain monaminergic systems that are expressed even in unstressed individuals.

Correlated physiological and behavioral stress responses in HR and LR trout lines

The creation of HR and LR lines of rainbow trout confirm that the magnitude of the cortisol response to a standardized stressor is an individual, heritable characteristic in this species (Pottinger et al., 1992, 1994; Fevolden et al., 1999). Furthermore, it appears that the magnitude of stress-induced elevation of blood cortisol is part of a complex trait incorporating several correlated physiological and behavioral responses (Pottinger and Carrick 2001a,b; Øverli et al., 2001, 2002; Trenzado et al., 2003). Taken together, these observations suggest that the HR and LR rainbow trout may represent selection for different physiological/behavioral stress-coping styles, as defined by Koolhaas et al. (1999). These authors stated: "*A coping style can be defined as a coherent set of behavioral and physiological stress responses which is consistent over time and which is characteristic to a certain group of individuals."*

As noted previously, the plasma cortisol response is an individual trait which is consistent over time in rainbow trout (Pottinger et al., 1992; Pottinger and Carrick, 1999). The degree to which behavioral traits are consistent in individual HR and LR fish has not been determined, but comparisons of behavior between the two lines have revealed several striking differences (*c.f.* figure 2-4). Behavioral and physiological stress responses

345 are to a large degree controlled by common neuroendocrine signaling systems. Thus, if
346 the cortisol response is a consistent individual trait, it seems likely permanent differences
347 exist with respect to the behavioral components of different stress coping styles.

348 Most likely, some degree of trait associations exist in all animals with respect to a
349 fundamental response such as stress coping, but the evolutionary success of different
350 profiles may vary between species. It is not clear whether the behavioral and endocrine
351 traits of LR and HR rainbow correspond exactly to the proactive (active) and reactive
352 (passive) coping styles described in mammals. The proactive stress coping style in
353 rodents involves low glucocorticoid production (de Boer et al., 1990; Korte et al., 1992;
354 Fokkema et al., 1995), while in HR trout high cortisol responsiveness was associated with
355 increased swimming activity during an intruder test (figure 4). The impression of the
356 observers was that the HR trout showed anxiety-like erratic behavior, rather than targeted
357 attempts to actively cope with the experimental situation. If that is the case, the
358 behavioral strategy of the LR fish (to remain passive and ignore the presence of an
359 intruder in a low-quality territory) may be more equivalent to the active coping style that
360 is normally associated with low HPA axis responsiveness in rats (Koolhaas et al., 1999).
361 Furthermore, the observation that LR trout tend to win encounters for social dominance
362 (figure 2) is in accordance with an active coping style (see e.g. Verbeek et al., 1996;
363 Klomberg et al., 2002; Zhukov and Vinogradova, 2002).

364 Several rodent models consisting of two contrasting lines/strains that respond
365 differently to stressful environments have also been developed through bidirectional
366 selection. Examples are the Roman high (RHA) and low (RLA) avoidance rats (Driscoll
367 et al., 1998; Steimer and Driscoll, 2003), the Wistar high (HAB) and low (LAB) anxiety-

related behavior lines (Liebsch et al., 1998; Yilmazer-Hanke et al., 2004), the Maudsley reactive and nonreactive strains (Blizard and Adams, 2002), the short (SAL) and long attack latency (LAL) house mice (Veenema et al., 2003), apomorphine susceptible and unsusceptible rats (Ellenbroek and Cools, 2002), and several others (e.g. Klomberg et al., 2002; Viggiano et al., 2002, 2003; Brush, 2003; Ramos et al., 2003).

Like the HR and LR rainbow trout, these models were created by selective mating of animals with the highest and lowest scores for a given behavioral or physiological trait over several generations. In general, it is assumed that maximizing differences in this way produces one line with more genes that affect the selected trait positively, and one line carrying more genes with negative effects on the same trait (Ramos and Mormède, 1998; Crabbe, 1999). Ideally, correlated responses should be consistent across several replicate lines to indicate the presence of genetic correlations (Henderson, 1997). Thus, in the case of the HR and LR trout lines, founder effects, unique mutations and random genetic drift can not be ruled out as contributing factors in the simultaneous divergence of multiple traits. However, the apparent parallel to genetically determined stress coping styles in mammals, and the existence of similar trait associations in unselected populations (Øverli et al., 2004b), suggest an evolutionarily conserved correlation between multiple traits.

Neuroendocrine mechanisms integrating physiological and behavioral stress responses

The mechanisms integrating the behavioral and physiological characteristics of HR and LR trout lines remain largely unknown. The behavioral differences between HR and LR trout are consistent with some reported effects of corticosteroid hormones in poikilotherms (decreased appetite: Gregory and Wood, 1999; increased locomotor

activity: Cash and Holberton, 1999; Øverli et al., 2002b). In fish, like in mammals, these steroids typically have time-, context-, and dose-dependent effects (Øverli et al., 2002b; Bernier et al., 2004). Thus, altered competitive ability in a stressful situation such as a fight for social dominance (Pottinger and Carrick, 2001a) may also be directly caused by differences in circulating hormone levels.

The behavioral and physiological characteristics of HR and LR rainbow trout may also be functionally linked through a number of factors which influence both endocrine and behavioral responses. For instance, it seems likely that corticotrophin releasing hormone (CRH) is involved in the increase in locomotor activity observed in HR trout. In juvenile chinook salmon (*Oncorhynchus tshawytscha*), intracerebroventricular injections of CRH induced hyperactivity, an effect that was shown to depend on concurrent (i.e. CRH induced) 5-HT activation (Clements et al., 2003).

Interestingly, CRH administration also increases DA concentrations in dorsal medial hypothalamus of newts (*Taricha granulosa*) (Lowry et al., 2001), and one of the main neurochemical differences between HR and LR rainbow trout was that HR fish responded to stress by increased DA concentrations and turnover in several brain areas, while LR fish did not (Table I). Genetically determined differences in DA systems has been reported in several other models (Rots et al., 1996a,b,c; Lecca et al., 2004). However, increased DA synthesis and release may also be an effect of acute elevations in glucocorticoid concentrations (Dunn et al., 1978; Piazza et al., 1996a,b; Barrot et al., 2000, 2001). Effects of glucocorticoids on dopaminergic activity are also strongly context (Piazza et al., 1996b) and regionally dependent (Lucas et al., 1998; Barrot et al., 2000, 2001). Thus, at present it is not known whether differences in DA systems between HR

414 and LR trout are a cause or a consequence of hormone dynamics, but there is an
415 interesting parallel to mammalian models which suggest the presence of evolutionary
416 conserved trait correlations. Notably, a similar negative relationship between DA
417 reactivity, stressor or novelty-induced locomotor activity, and social competitive ability
418 has also been demonstrated in cynomolgus monkeys (*Macaca fascicularis*) (Morgan et
419 al., 2000). Individually housed monkeys with high levels of locomotion in an open-field
420 test after a low dose of cocaine (which increases CNS dopamine) were more likely to
421 become subordinate in subsequent group housing (Morgan et al., 2000).
422 Like with DA, corticosteroids may affect brain 5-HT signaling directly and
423 through interaction with other neurotransmitter systems (Chaouloff, 2000). Inheritable
424 properties of the 5-HT system have also been associated with HPA-axis activity,
425 personality and mood alterations in human and other animals. Examples are differences
426 in MAO and polymorphisms in the 5-HT transporter gene or promoter region (Lesch et
427 al., 1996; Shih et al., 1999; Fernandez et al., 2003).
428 The interaction between different signaling systems involved in the stress
429 response is, in fact, so complex that on occasions it appears futile to disentangle causes
430 and consequences. Most research on neuroendocrine control of behavioral and
431 physiological stress responses has been carried out on mammals. However, the
432 complexity of the interaction between a single neurotransmitter, 5-HT, and stress is well
433 illustrated by a series of studies with salmonid fish: Stress affects 5-HT metabolism and
434 most likely also functional release (Winberg and Nilsson, 1993; Øverli et al., 1998, 1999,
435 2001; Lepage et al., 2002) and 5-HT in turn affects behavior (Winberg et al., 1993, 2001)
436 and cortisol release (Winberg et al., 1997; Höglund et al., 2002b; Lepage et al.,

2002,2003). Cortisol also affects behavior (Øverli et al., 2002), while both stressful and rewarding behavior influence 5-HT as well as stress hormones (Winberg and Lepage 1997; Øverli et al., 1999, 2004a). Finally, there is preliminary evidence that circulating cortisol act on brain 5-HT neurons and can modify neurotransmission also in fish (Øverli et al., 2003).

Thus, it is not surprising that selection for stress responsiveness is also associated with alterations in brain 5-HT activity (Øverli et al., 2001, Table I). However, at present it is not known to what degree the physiological and behavioral profiles of HR and LR trout are a result of innate differences in central signaling systems, of glucocorticoid influence on brain function, or a combination of these factors. Finally, it should be kept in mind that the differences in stress induced cortisol concentrations between HR and LR fish might arise from differences in interrenal function, rather than in central HPI-axis control (Pottinger and Carrick 2001b). Similarly, seasonal variations in stress-induced plasma corticosteroid levels was correlated to alterations in adrenocortical cell steroidogenic function in lizards (*Sceloporus undulatus*) (Carsia and John-Alder, 2003). Thus, the possibility should be considered that differences in neurochemistry and behavior between HR and LR trout are a result of variation in interrenal influence on brain functions, rather than the opposite. However, differences between unstressed HR and LR lines also have been observed, and in these cases interrenal function are unlikely to be directly responsible.

Further experiments with HR and LR trout lines

In summary, experiments carried out on the HR and LR trout lines demonstrate a tight coupling of physiological and behavioral stress responses. The neuroendocrine control mechanisms behind this connection appear to be evolutionarily conserved, and are well illustrated by genetically selected strains of animals that display simultaneous differences in physiology and behavior. In many ways, selection models are superior to targeted gene knock-outs, since an entire suite of neuroendocrine mechanisms are selected for, instead of altering just one specific gene product. The systems that produce behavioral and neuroendocrine stress responses work in an integrated fashion, and selection models keep that integration intact.

Continuing studies on the 3rd generation HR and LR trout lines are providing evidence that the range of behavioral traits in which there are pronounced differences between the two lines far exceeds what has so far been published. For instance, a recent study has demonstrated that the extinction of a conditioned response occurs more rapidly among fish from the HR line than fish from the LR line, suggesting that the two lines differ in cognitive function as well as in behavioral characteristics (Moreira et al., 2004). It is, however, not known whether this result depends on differences in memory formation, consolidation, or retrieval. This latter point is of particular interest, since some studies suggest dual effects of glucocorticoids on specific memory phases (Roozendaal, 2002). Furthermore, the results of Moreira et al. (2004) suggest that densities and composition of *N*-methyl-$_{D}$-aspartate (NMDA) are also likely to differ between the lines, in view of the important role of these receptors in excitatory neurotransmission, synaptic plasticity, and cognition in other vertebrates.

One neurotransmitter system that is also likely to play a central role in shaping behavioral profiles, but that has not been studied in the HR/LR trout model, is CRH. It is, for instance, not known whether basal or stress-induced CRH activity differs between the lines, although their behavioral and physiological profile strongly suggests such a difference. As suggested by Clements et al. (2003), it would also be interesting to compare the responses of these two lines to CRH treatment.

In a different venue of research, recently developed genomic tools, such as microarray technology, are being utilized in an EU program (STRESSGENES) to identify candidate genes associated with resistance to stress. Knowledge of the genes responsible for trait variability will further point out the pathways responsible for the phenotypical differences between HR and LR lines. This should provide the physiological and genetic basis for new marker-assisted selection strategies in the rapidly developing finfish aquaculture industry, as well as increased knowledge of the function and evolution of central neuroendocrine signaling systems.

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Figure legends

Figure 1: Post-stress blood plasma cortisol levels (mean ± SE) of LR and HR rainbow trout (mixed sex samples) for all test occasions from the establishment of the parental generation (F0) up to present (F3).

Figure 2: The number of LR and HR rainbow trout identified as either dominant or subordinate during paired contests. HR subordinates>LR subordinates, $p < 0.001$, χ^2 test (data from Pottinger and Carrick, 2001a.

Figure 3. The number of LR and HR rainbow trout regaining feed intake within 1 week of transfer to a new environment. LR feeding>HR feeding, $p = 0.003$, χ^2 test (data from Øverli et al., 2002a).

Figure 4. Locomotor activity in HR and LR rainbow trout quantified as time spent moving during a 20 min observation period, with or without the presence of a conspecific intruder. Asterisks indicate an effect of the intruder, asterisks in brackets [∗] indicates a difference between HR and LR fish, ∗ = $p<0.01$, ∗∗ = $p<0.001$, ∗∗∗ = $p<0.0001$, Kruskal–Wallis analysis of variance followed by groupwise comparisons by the Mann–Whitney U-test (data from Øverli et al., 2002a).

Table I. Tissue concentrations of monoamines and monoamine metabolites (ng/g), and corresponding metabolite/monoamine ratios (mean ± SE) in different brain regions of LR and HR rainbow trout when reared in isolation (controls) or following 1 h confinement stress. Ratios are in italics. Values that are significantly higher in one selection line (LR vs HR) or in stressed than control fish are in bold font. Asterisks indicate an effect of stress, asterisks in brackets [∗] indicates a difference between HR and LR fish, ∗ = $p<0.01$, ∗∗ = $p<0.001$, ∗∗∗ = $p<0.0001$, analysis of variance followed Tukey HSD post-hoc test (data from Øverli et al., 2001).

900

901 Table I.

	LR control	HR control	LR stressed	HR stressed
Telencephalon				
DOPAC	17.1±4.2	15.0±3.9	23.6±5.9	22.4±2.8
DA	218.3±30.7	188.8±39.2	268.6±57.5	229.9±14.5
DOPAC/DA	*0.074±0.009*	*0.076±0.005*	*0.086±0.008*	*0.095±0.008*
MHPG	13.5±1.0	11.6±0.6	**19.8±1.2** ***	**21.0±1.2** ***
NE	1264±78	1282±50	1347±93	**1674±76** *
MHPG/NE	*0.010±0.001*	*0.009±0.001*	***0.015±0.001*** ***	*0.012±0.001*
5-HIAA	752.8±39.3	595.1±31.6	**914.7±51.3** *	**838.6±48.0** ***
5-HT	1516±75	1490±34	1528±64	1588±77
5-HIAA/5-HT	***0.50±0.02*** [*]	*0.40±0.02*	***0.60±0.03*** *	***0.53±0.03*** **
Hypothalamus				
DOPAC	3.62±0.41	4.80±0.89	4.87±0.66	4.93±0.86
DA	754.8±15.6	734.8±37.8	696.2±23.6	758.6±36.2
DOPAC/DA	*0.0049±0.0006*	*0.0065±0.0012*	*0.0072±0.0011*	*0.0065±0.0012*
MHPG	8.08±0.84	7.27±0.88	**11.3±0.89** *	10.2±0.58
NE	403.5±29.7	379.2±23.7	418.9±31.4	418.4±34.3
MHPG/NE	*0.020±0.002*	*0.020±0.003*	*0.028±0.003*	*0.025±0.002*
5-HIAA	**362.6±19.3** [*]	283.0±21.6	**420.0±18.9** [*]	345.2±14.5
5-HT	1883±243	2080±306	1923±263	2058±303
5-HIAA/5-HT	*0.21±0.03*	*0.16±0.03*	*0.23±0.03*	*0.20±0.03*
Optic tectum				
DOPAC	6.00±0.25	6.90±0.49	6.31±0.25	**8.46±0.41** *[**]
DA	61.0±3.6	59.3±3.2	75.2±8.5	73.7±5.8
DOPAC/DA	*0.099±0.003*	*0.117±0.007*	*0.090±0.007*	***0.117±0.004*** [*]
MHPG	6.13±0.38	6.21±0.67	7.43±0.26	**8.54±0.31** **
NE	189.9±11.7	205.2±5.9	200.4±7.5	**262.8±15.6** **[**]
MHPG/NE	*0.032±0.001*	*0.030±0.003*	*0.037±0.001*	*0.033±0.002*
5-HIAA	114.7±7.4	121.8±11.8	145.1±8.0	154.1±9.8
5-HT	496.2±19.9	534.7±44.1	557.1±39.8	588.8±49.3
5-HIAA/5-HT	*0.23±0.01*	*0.23±0.01*	*0.26±0.01*	*0.27±0.01*
Brain stem				
DOPAC	4.03 ± 0.13	4.51±0.19	4.26±0.17	**5.40±0.21** **[***]
DA	140.3±6.4	139.3±5.3	127.0±5.2	**149.9±5.7** [*]
DOPAC/DA	*0.029±0.002*	*0.033±0.002*	*0.034±0.002*	*0.036±0.001*
MHPG	5.74±0.7	5.8±0.4	7.51±0.5	**7.95±0.48** *
NE	248.6±15.1	240.7±7.8	234.6±8	253.6±10.6
MHPG/NE	*0.023±0.003*	*0.024±0.002*	***0.034±0.003*** *	*0.031±0.001*
5-HIAA	125.6±6.3	103.6±4.9	144.2±6.9	**140.6±5.7** ***
5-HT	595.8±31.1	610.6±31.6	532.8±24.3	**646.1±28.8** [*]
5-HIAA/5-HT	***0.21±0.006*** [*]	*0.17±0.005*	***0.27±0.016*** ***	***0.21±0.005*** **[**]

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904 Figure 1

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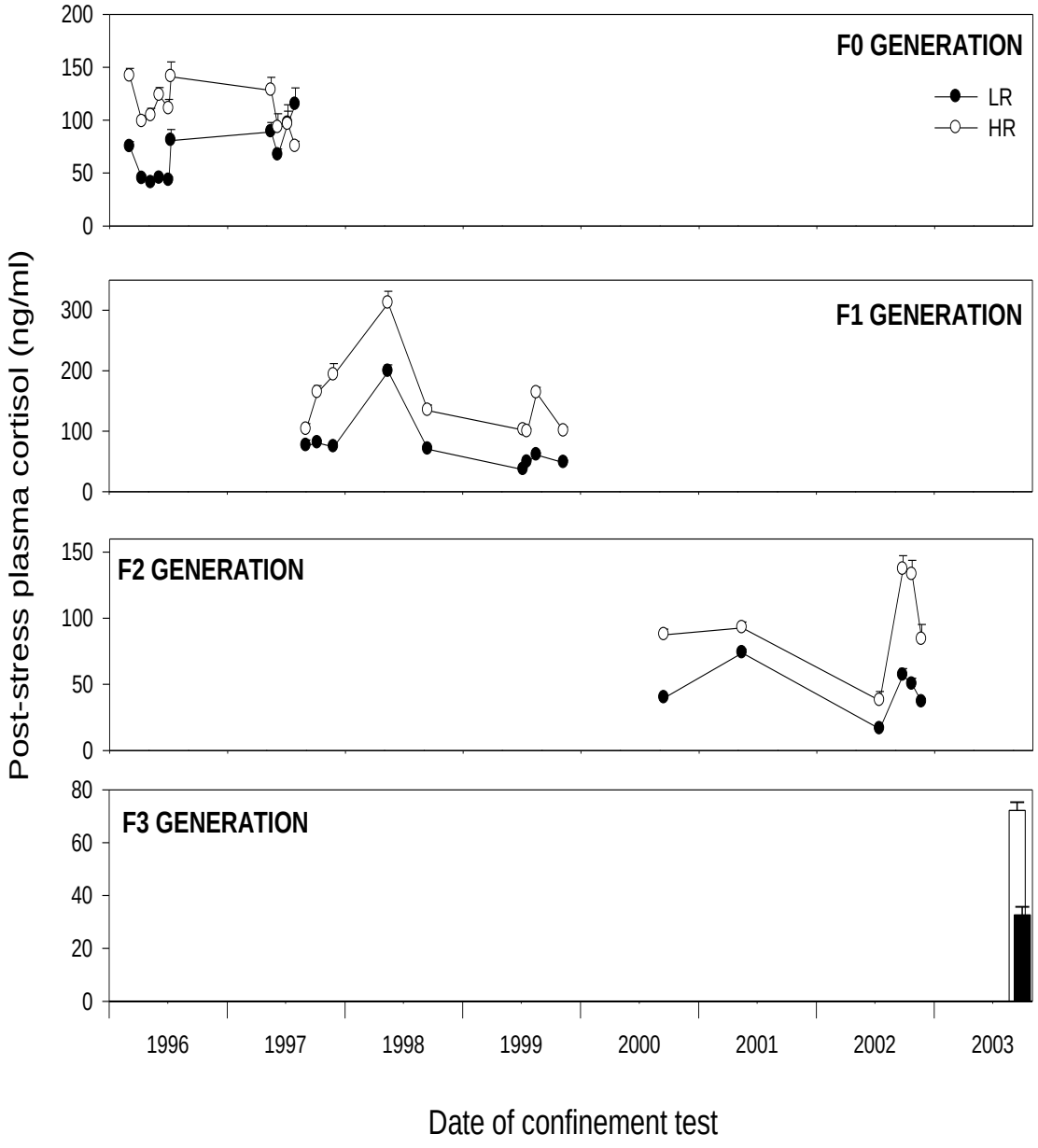
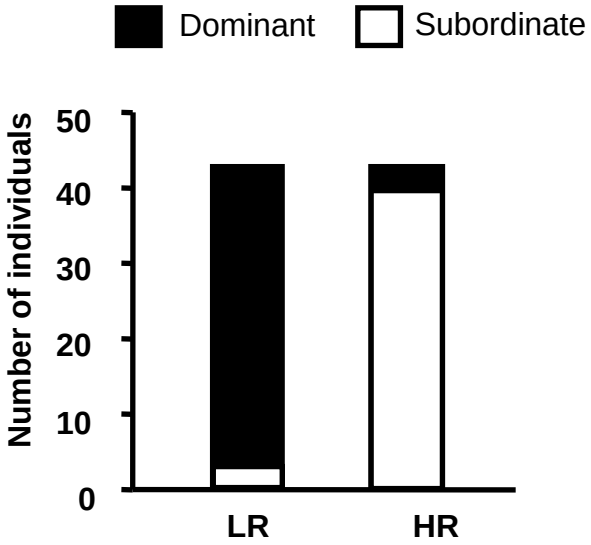


908 Figure 2


Dominant
Subordinate
Number of individuals
50
40
30
20
10
0
LR
HR

909 Figure 3

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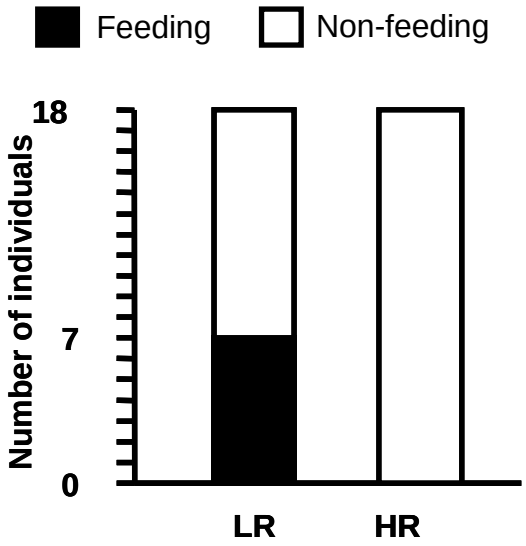

913 Figure 4

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