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1 **Behavioral and Neuroendocrine Correlates of Selection for Stress Responsiveness in**
2 **Rainbow Trout - A Review¹**

3

4 RUNNING TITLE: SELECTION FOR STRESS RESPONSIVENESS IN TROUT

5

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23 **SYNOPSIS.**

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

47

INTRODUCTION

48

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

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

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

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

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

94 severity of the challenge (Haller et al., 1998; Øverli et al., 2004a). Seminal studies in
95 mammals indicated that the threshold at which the shift from an active to a passive
96 behavioral response occurs is subject to great individual variation (Engel and Schmale,
97 1972; Henry and Stephens, 1977).

98 More recently, it has been established that individual differences in the
99 physiological stress response are associated with differences in behavior. For instance, a
100 pro-active stress coping style is behaviorally characterized by a high level of active
101 avoidance, aggression, and other behavioral patterns indicating an active attempt to
102 counteract the stressful stimulus (e.g. defensive burying in rodents) (Bohus et al., 1987;
103 Benus et al., 1989, 1991a; Sluyter et al., 1996). Reactive (passive) coping, on the other
104 hand, involves immobility and low levels of aggression. Physiologically, the pro-active
105 strategy is associated with low hypothalamus-pituitary-adrenal axis (HPA axis, the
106 mammalian equivalent of the teleost HPI axis) responsiveness, but high sympathetic
107 reactivity, while the opposite is true for reactive coping (de Boer et al., 1990; Korte et al.,
108 1992; Fokkema et al., 1995). A genetic basis for the expression of behavioral and
109 physiological components of individual coping styles has repeatedly been demonstrated
110 (e.g. Driscoll et al., 1998; Ellenbroek and Cools, 2002; de Boer et al., 2003; Veenema et
111 al., 2003). Limited information, however, is available on whether different behavioral-
112 physiological stress coping styles are present in teleost fish (but see Francis, 1990; Van
113 Raaij et al., 1996; Budaev et al., 1999).

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

117 norepinephrine (NE) play a vital role in this co-ordination (Winberg and Nilsson, 1993;
118 Winberg et al., 1997, 2001; Øverli et al., 1998, 1999; Höglund et al., 2001, 2002a,b;
119 Lepage et al., 2002, 2003; Clements et al., 2003; Larson et al., 2003; Perreault et al.,
120 2003). Genetically determined variation in behavior and stress responsiveness has been
121 associated with differences in brain monoaminergic function in both fish and mammals
122 (e.g. Benus et al., 1991b; Popova et al., 1991a,b; Nikulina et al., 1992; Rots et al.,
123 1996a,b,c; de Kloet et al., 1996; Sallinen et al., 1999; Lepage et al., 2000; Giorgi et al.,
124 2003). In this paper we review the effects of selection for post stress cortisol production
125 in rainbow trout, with particular reference to a series of studies investigating the
126 association between altered plasma cortisol dynamics, behavior, and brain monoamine
127 neurotransmitters. In other words, we examine the hypothesis that behavioral and
128 physiological traits are linked in such a way that altering one trait, post-stress plasma
129 cortisol concentrations, incurs differences in other putative components of individual
130 stress coping styles. It should be noted that several additional physiological differences
131 between HR and LR lines, such as metabolic changes and interrenal sensitivity to
132 adenocorticotrophic hormone (ACTH) also has been reported, but for details of this
133 experimental work we refer to the original papers (Pottinger and Carrick, 2001b,
134 Trenzado et al., 2003).

135

136

137

MATERIAL AND METHODS

138 *Generation of HR and LR trout lines*

139 The selection procedure and the effect of the breeding program on the cortisol response
140 has been thoroughly described elsewhere (Pottinger and Carrick, 1999, 2001a), and will
141 only be briefly reviewed here. The parental generation of the HR and LR trout lines was
142 established in 1996 by repeated stress testing (3h confinement in 50 L water in groups of
143 6-7 individuals once monthly) of passive integrated transponder (PIT) tagged 2-year-old
144 rainbow trout. Following confinement, blood samples (0.5 ml) and PIT-tag readings were
145 obtained from anaesthetized (2-phenoxyethanol, 1:2000) fish, and plasma was later
146 analyzed for cortisol content by a previously validated radioimmunoassay (RIA)
147 procedure (Pickering et al., 1987). The mean post-stress plasma cortisol content across
148 five episodes of confinement was then calculated for each fish, and individuals were
149 ranked accordingly. Between testing fish had been kept in groups of 25 in 1500 L holding
150 tanks, and the four most high-responding (HR) and the four most low-responding (LR)
151 fish in each tank were removed from their home tank, segregated by sex, and kept
152 separate in 4 tanks based on group and sex until maturation.

153 Confinement stress testing of the 1st generation offspring (F1), consisting of 15
154 HR and 14 LR families each resulting from a unique male-female crossing, were carried
155 out on five different occasions between September 1997 and September 1998, and on five
156 occasions in 1999. A highly significant regression of mid-parent cortisol response on
157 progeny response was seen (estimated r^2 [h2] value = 0.41) and the six LR families with
158 the lowest mean cortisol response and the six HR families with the highest mean cortisol
159 response were identified and used for further work (Pottinger and Carrick, 1999). An
160 unselected (US) population resulting from random breeding of fish not designated as HR
161 or LR in the parental group (6 families) was tested on one occasion, and showed a

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

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

171

172 *Behavioral experiments*

173 Locomotor activity, feed intake, and brain monoaminergic activity in HR and LR trout:

174 These experiments are described in detail in Øverli et al. (2001, 2002a). Recovery of feed
175 intake after transfer to a new and potentially stressful environment was used as an index
176 of adaptiveness. Only adult female F1 fish were available for these studies. Locomotor
177 activity when in isolation and in response to a territorial intruder was also analyzed, along
178 with basal and stress-induced brain 5-HT, DA and NE activity. During April 2000 adult
179 F1 female HR (n=18) and LR (n=18) rainbow trout weighing 987.5 ± 39.5 g (mean \pm SE)
180 were transferred individually from communal holding tanks to rearing in isolation in
181 white 250 L polypropylene observation tanks. From day 1 after transfer to rearing in
182 isolation, fish were fed daily by hand (1.5% of body weight), and fish were observed for
183 3 min after distribution of food to register food intake. Behavioral observations and blood
184 sampling (see below) were carried out between 10.00 and 14.00.

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

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

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

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

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

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

232

233 RESULTS AND DISCUSSION

234 *Cortisol responsiveness in HR and LR rainbow trout*

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

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

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

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

266

267 *Behavioral effects of selection for stress responsiveness*

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

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

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

286

287 *Brain monoaminergic activity in HR and LR trout*

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

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

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

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

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

327

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

329 The creation of HR and LR lines of rainbow trout confirm that the magnitude of the
330 cortisol response to a standardized stressor is an individual, heritable characteristic in this
331 species (Pottinger et al., 1992, 1994; Fevolden et al., 1999). Furthermore, it appears that
332 the magnitude of stress-induced elevation of blood cortisol is part of a complex trait
333 incorporating several correlated physiological and behavioral responses (Pottinger and
334 Carrick 2001a,b; Øverli et al., 2001, 2002; Trenzado et al., 2003). Taken together, these
335 observations suggest that the HR and LR rainbow trout may represent selection for
336 different physiological/behavioral stress-coping styles, as defined by Koolhaas et al.
337 (1999). These authors stated: “*A coping style can be defined as a coherent set of*
338 *behavioral and physiological stress responses which is consistent over time and which is*
339 *characteristic to a certain group of individuals.*”

340 As noted previously, the plasma cortisol response is an individual trait which is
341 consistent over time in rainbow trout (Pottinger et al., 1992; Pottinger and Carrick, 1999).
342 The degree to which behavioral traits are consistent in individual HR and LR fish has not
343 been determined, but comparisons of behavior between the two lines have revealed
344 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-

368 related behavior lines (Liebsch et al., 1998; Yilmazer-Hanke et al., 2004), the Maudsley
369 reactive and nonreactive strains (Blizard and Adams, 2002), the short (SAL) and long
370 attack latency (LAL) house mice (Veenema et al., 2003), apomorphine susceptible and
371 unsusceptible rats (Ellenbroek and Cools, 2002), and several others (e.g. Klomberg et al.,
372 2002; Viggiano et al., 2002, 2003; Brush, 2003; Ramos et al., 2003).

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

385

386 *Neuroendocrine mechanisms integrating physiological and behavioral stress responses*

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

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

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

403 Interestingly, CRH administration also increases DA concentrations in dorsal
404 medial hypothalamus of newts (*Taricha granulosa*) (Lowry et al., 2001), and one of the
405 main neurochemical differences between HR and LR rainbow trout was that HR fish
406 responded to stress by increased DA concentrations and turnover in several brain areas,
407 while LR fish did not (Table I). Genetically determined differences in DA systems has
408 been reported in several other models (Rots et al., 1996a,b,c; Lecca et al., 2004).
409 However, increased DA synthesis and release may also be an effect of acute elevations in
410 glucocorticoid concentrations (Dunn et al., 1978; Piazza et al., 1996a,b; Barrot et al.,
411 2000, 2001). Effects of glucocorticoids on dopaminergic activity are also strongly context
412 (Piazza et al., 1996b) and regionally dependent (Lucas et al., 1998; Barrot et al., 2000,
413 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.,

437 2002,2003). Cortisol also affects behavior (Øverli et al., 2002), while both stressful and
438 rewarding behavior influence 5-HT as well as stress hormones (Winberg and Lepage
439 1997; Øverli et al., 1999, 2004a). Finally, there is preliminary evidence that circulating
440 cortisol act on brain 5-HT neurons and can modify neurotransmission also in fish (Øverli
441 et al., 2003).

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

457

458 *Further experiments with HR and LR trout lines*

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

468 Continuing studies on the 3rd generation HR and LR trout lines are providing
469 evidence that the range of behavioral traits in which there are pronounced differences
470 between the two lines far exceeds what has so far been published. For instance, a recent
471 study has demonstrated that the extinction of a conditioned response occurs more rapidly
472 among fish from the HR line than fish from the LR line, suggesting that the two lines
473 differ in cognitive function as well as in behavioral characteristics (Moreira et al., 2004).
474 It is, however, not known whether this result depends on differences in memory
475 formation, consolidation, or retrieval. This latter point is of particular interest, since some
476 studies suggest dual effects of glucocorticoids on specific memory phases (Rooszendaal,
477 2002). Furthermore, the results of Moreira et al. (2004) suggest that densities and
478 composition of *N*-methyl-D-aspartate (NMDA) are also likely to differ between the lines,
479 in view of the important role of these receptors in excitatory neurotransmission, synaptic
480 plasticity, and cognition in other vertebrates.

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875 Figure legends

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

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

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

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

891 Table I. Tissue concentrations of monoamines and monoamine metabolites (ng/g), and
892 corresponding metabolite/monoamine ratios (mean \pm SE) in different brain regions of LR
893 and HR rainbow trout when reared in isolation (controls) or following 1 h confinement
894 stress. Ratios are in italics. Values that are significantly higher in one selection line (LR
895 vs HR) or in stressed than control fish are in bold font. Asterisks indicate an effect of
896 stress, asterisks in brackets [*] indicates a difference between HR and LR fish, * =
897 $p < 0.01$, ** = $p < 0.001$, *** = $p < 0.0001$, analysis of variance followed Tukey HSD post-
898 hoc test (data from Øverli et al., 2001).

899

901 Table I.

	LR control	HR control	LR stressed	HR stressed
<i>Telencephalon</i>				
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 **
<i>Hypothalamus</i>				
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
<i>Optic tectum</i>				
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
<i>Brain stem</i>				
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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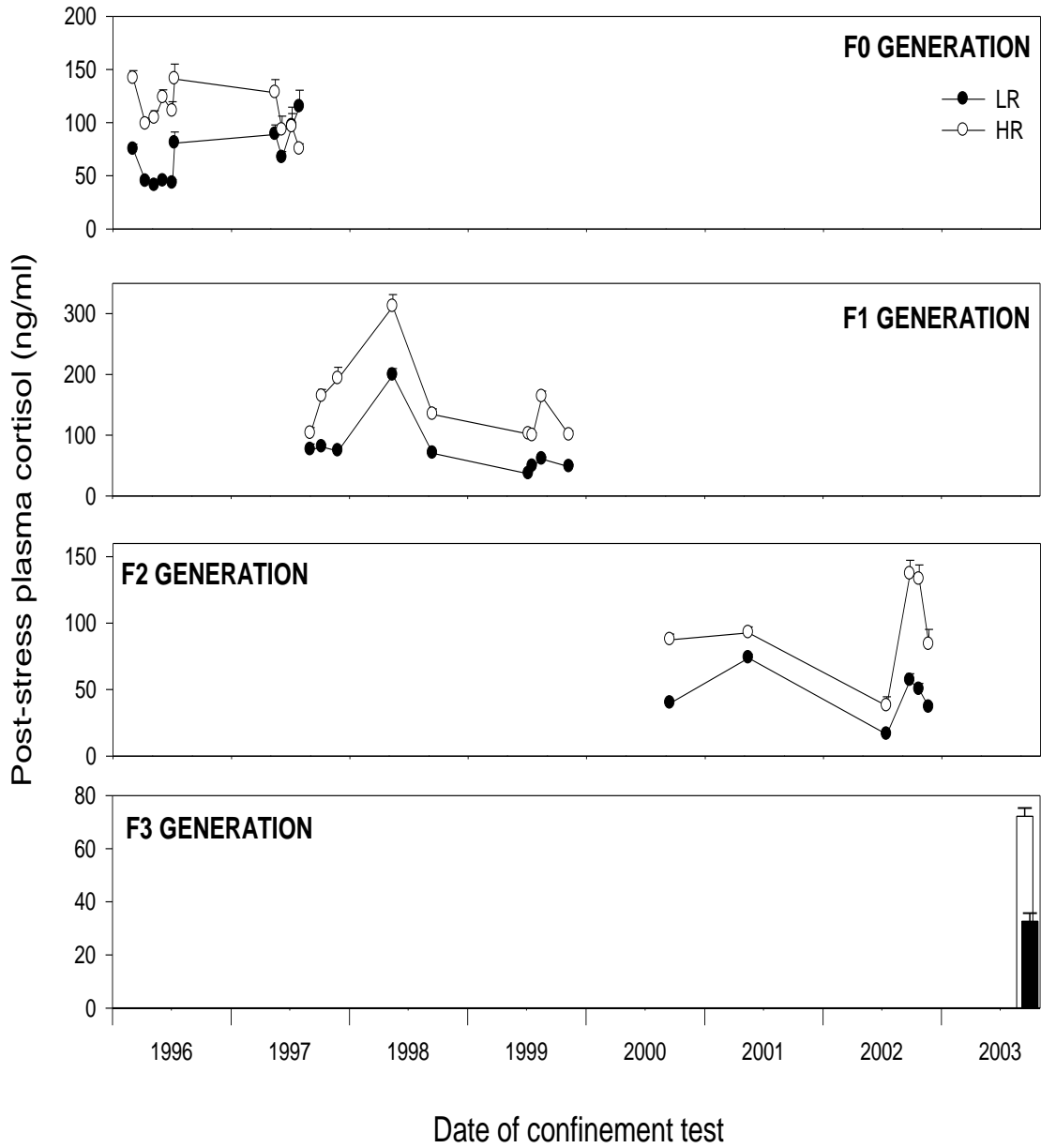
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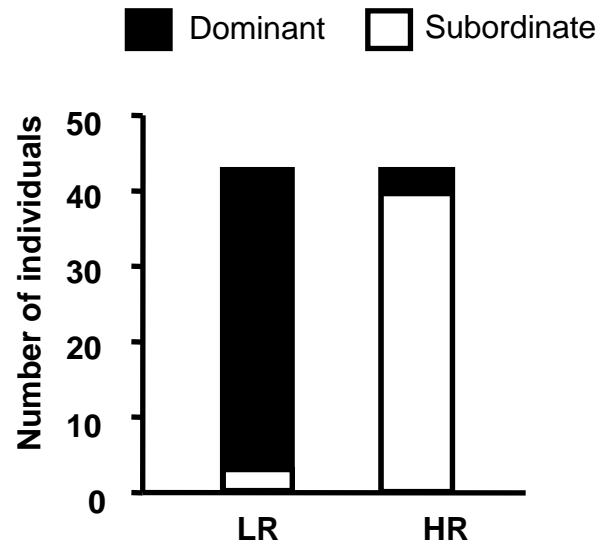
904 Figure 1

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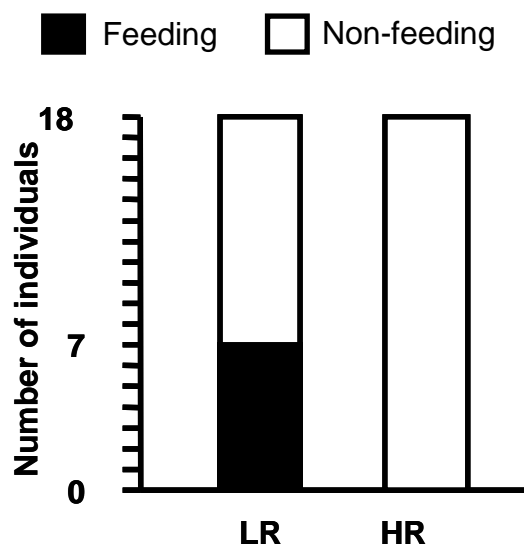


909 Figure 3

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913 Figure 4

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