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# Melanin-based skin spots reflect stress responsiveness in salmonid fish

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**Within animal populations, genetic, epigenetic and environmental factors interact to shape individual neuroendocrine and behavioural profiles, conferring variable vulnerability to stress and disease. It remains debated how alternative behavioural syndromes and stress coping styles evolve and are maintained by natural selection. Here we show that individual variation in stress responsiveness is reflected in the visual appearance of two species of teleost fish; rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). Salmon and trout skin vary from nearly immaculate to densely spotted, with black spots formed by eumelanin-producing chromatophores. In rainbow trout, selection for divergent hypothalamus-pituitary-interrenal responsiveness has led to a change in dermal pigmentation patterns, with low cortisol-responsive fish being consistently more spotted. In an aquaculture population of Atlantic salmon individuals with more spots showed a reduced physiological and behavioural response to stress. Taken together, these data demonstrate a heritable behavioural-physiological and morphological trait correlation that may be specific to alternative coping styles. This observation may illuminate the evolution of contrasting coping styles and behavioural syndromes, as occurrence of phenotypes in different environments and their response to selective pressures can be precisely and easily recorded.**

## Introduction

Within the last decade, several influential publications have promoted the recognition of individual differences within a population as biologically meaningful adaptive traits (Koolhaas et al. 1999; Wilson 1998), and the emerging conceptual framework for individuality has had important consequences to both evolutionary ecology, animal husbandry, and biomedicine (Sih et al. 2004; Cavigelli 2005; Huntingford and Adams 2005; Korte et al., 2005; Reale et al. 2007). Individuals differ in the way they react towards stressful situations and novelties, and selection studies in several species show that physiological and behavioural responses to stress are often correlated in a coherent and heritable pattern (Øverli et al. 2005; van Oers et al. 2005). Stress-related physiological-behavioural trait associations are commonly referred to as stress coping styles (Koolhaas et al. 1999), while consistent individual variation in behaviour across other situations have been denoted behavioural syndromes, profiles, axes, temperament, or a shyness-boldness continuum  (Gosling 2001; Sih et al. 2004; Wilson et al. 1994; Réale et al. 2007). Notwithstanding a certain lack of consensus in terminology, the evolution of these analogues of human personality has evoked considerable scientific interest. Among the questions raised are by which mechanisms behavioural syndromes are inherited, how the variation is maintained, which circumstances benefit different complex phenotypes, and what are the costs and benefits of limited plasticity imposed by more or less fixed trait associations (DeWitt et al. 1998; Koolhaas et al. 1999; Sih et al. 2004; Dingemanse and Réale 2005; McElreath et al. 2007; Réale et al. 2007; Wolf et al. 2007).

Salmonid fish of the genera *Oncorhynchus* and *Salmo* attract the attention of both researchers and the general public, on account of their biological characteristics and wide use in sport and recreational fishing, aquaculture, and research (see examples in Thorgaard et al. 2002).  Salmonids are particularly known for their extensive genetic and life-history variation, resulting in part from their homing ability during spawning, with localized sub-populations being adapted to different sites and environments within a river system (Dittman and Quinn 1996; Garcia de Leaniz et al. 2007). Previously, we have used strains of rainbow trout (*Oncorhynchus mykiss*) selected for divergent (high-responsive, HR, and low-responsive, LR) post-stress cortisol levels to study heritable variation in neuroendocrine and behavioural stress responses (Øverli et al. 2005; Schjolden and Winberg 2007). Low post-stress cortisol levels are typically associated with proactive behavioural traits such as social dominance and boldness in new environments (Pottinger and Carrick, 2001; Øverli et al. 2007). In contrast, high cortisol levels are associated with social subordinance and shyness (Pottinger and Carrick, 2001; Øverli et al. 2007). Furthermore, high cortisol levels may confer increased susceptibility to some infectious diseases (Fevolden et al. 1992).

Anybody who has studied, fished for, handled, or consumed salmon or rainbow trout may have noticed the black spots dappling the skin of these fishes. The spots are formed by specialised chromatophores – melanophores (or melanocytes) - which produce the dark pigment eumelanin (Bagnara 1998). During work with the fourth generation of HR-LR rainbow trout, it became evident that the two strains differed in pigment patterns. Specifically, LR fish exhibited far more eumelanin spots than HR fish. Melanin-based coloration in vertebrates and its association with behavioural syndromes was recently reviewed by Ducrest et al. (2008). This literature review showed that darker wild vertebrates are generally more aggressive, sexually active and resistant to stress than lighter individuals (Ducrest et al. 2008). Furthermore, in the barn owl (*Tyto Alba*) melanin-based colouration is associated with several behavioural, morphological and physiological characteristics that have been linked to stress coping ability (Almasi et al. 2008).

Based on observations that HR and LR trout differed in melanin pigmentation our hypothesis was that spotted fish, in the closely related specie Atlantic salmon (*Salmo Salar*), would show a lower cortisol reponse to stress when compared to a non-spotted group of individuals. Furthermore, several documented changes in the behaviour of the two trout lines made us predict that also behaviour would differ between groups of spotted and non-spotted fish. The main focus of this study was therefore to test the hypothesis that there exist an association between melanin-based pigmentation and hypothalamus-pituitary-interrenal (HPI-axis) reactivity in salmonid fish.  Furthermore, we tested the prediction that behavioural differences are present in two groups of fish chosen divergently on melanin pigmentation.

## Materials and methods

### Quantification of melanin-based coloration

In the studies described below, photographs of experimental fish were captured with a Canon EOS 450D digital camera and transferred to a computer for further analyses of melanin-based coloration. Prior to photography, each fish were sedated in 200 mg/l MS-222 and placed adjacent to a measuring tape with millimetre resolution, allowing for an accurate estimation of the area where spots were counted. The degree of melanin-based coloration is presented as number of spots per $cm^2$ of body surface in a defined area above the sideline, reaching from the gill cover to the base of the dorsal fin. These data are intended only to document that melanin- based coloration patterns differed between the HR-LR lines and between experimental groups of Atlantic salmon, and more accurate calculations of pigmented areas of curved skin surfaces were omitted at present.

### HR and LR rainbow trout

The HR and LR rainbow trout lines were initiated in 1996 by repeated stress testing (3 h confinement in 50 l water once monthly) of passive integrated transponder (PIT) tagged 2-year-old rainbow trout (for details see Pottinger and Carrick 1999). The mean post-stress plasma cortisol content across five episodes of confinement was used as a basis to create an

F1 generation consisting of 15 HR and 14 LR families, each resulting from a unique male–female crossing. Stress testing of the F1 generation was carried out on five different occasions between September 1997 and September 1998, and on five occasions in 1999. A highly significant parent-progeny regression in confinement-induced cortisol response was seen, 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 in further breeding (Pottinger and Carrick 1999).

A series of behavioural studies, starting with adult F1 fish, have revealed that selection for divergent cortisol responses is associated with multiple behavioural alterations in the HR-LR trout model. In summary, LR fish are characterized by a low locomotor response to acute stress, social dominance, and proactive behaviour in novel environments. HR individuals most often become socially subordinate, they exhibit more shy behaviour in novel environments and are characterised by a high locomotor response to stress. These differences in behavioural and neuroendocrine responses to stress are well documented and reviewed elsewhere (Øverli et al. 2002a; 2005, 2007; Schjolden and Winberg 2007). Therefore, no further behavioural studies using the HR-LR trout lines were included in this report. Melanin-based colouration, i.e. spot density, was quantified in juvenile (age 6 months, weight 25.2 ± 1.4 g, mean ± SEM, n=16) and adult (age 18 months, weight 434.3 ± 18.3 g, mean ± SEM, n=20) fish from the F4 generation. Photographs of one adult HR and one LR fish are shown in Fig. 1A.

### Behaviour and physiology during stress in Atlantic salmon

A group of 60 juvenile salmon from a commercial breeding programme (Aquagen AS, Trondheim, Norway) was raised from a size of approximately 20 g in freshwater in a 250-litre glass aquarium. When the fish had reached a size suitable for behavioural studies and blood sampling (population mean weight 74.7 g), we visually identified individuals that appeared as "spotted" or "non-spotted" (see example in Fig.1A), confirmed the difference by counting eumelanin spots as described above, and subjected 8 individuals from each group to behavioural tests previously used to describe alternative coping styles in rainbow trout (Øverli et al. 2006a; 2007).

Behavioural and physiological responses to acute stress were investigated by a confinement test. Each individual was subjected to 30 minutes of confinement in a transparent plastic chamber with 1 l aerated water. During acute challenges, high cortisol production is typically associated with high locomotor activity, while low-responsive individuals settle down quickly (Øverli et al. 2002a; 2006a). The difference between alternative stress-coping styles is typically not apparent until 10-15 min after the initiation of stress. Therefore, 10 minutes after initiation of acute stress, behaviour was videotaped for a duration of 15 minutes (Canon Digital Videocamera MD101) for analysis of locomotor activity (% of observation time spent moving). At 30 min following the onset of stress, the fish were anaesthetized by a high dose of MS-222 (500 mg/l). Within two minutes after capture blood samples were drawn from the caudal vein using heparinized 1 ml syringes. The blood samples were kept on ice until centrifuged for 7 minutes at 10 000 rpm. Plasma were decanted off and frozen at -80 ºC until analyses of plasma cortisol content. Plasma cortisol concentrations were quantified using a radioimmuno assay (RIA) after the protocol of Pickering et al. (1987). All samples were analysed in a single assay, and the intra-assay coefficient of variation was 5.1% (n=8). The antibody used was Abcam@1:1500 and the lower detection limit for the assay was 0.3 ng/ml. Cross-reactivity of the antibody with cortisone, the most abundant potential competitor in rainbow trout and salmon plasma, was 2.6%.

One week prior to the confinement test experimental fish were weighed and isolated in 250-litre aquaria divided into four compartments by removable, opaque PVC walls. There was no significant difference in weight between spotted and non-spotted fish prior to the behavioural tests (mean ± SEM: 76.9±4.5 g and 65.9±5.3 g, respectively, $t_{[14]}$=1.60, p=0.33). Test aquaria were supplied with a continuous flow of aerated tap water (1.0 l/min) at 8 °C. Light was provided by fluorescent tubes (30 W, natural light) 25 cm above the aquaria, and the diurnal cycle was 12 hours of light and 12 hours of darkness. The fish were fed once a day for 8 days beginning at the day of transfer from group rearing. During feeding trials fish were handfed with commercial pelleted fish feed, until the fish had rejected to eat three consecutive pellets. The total amount of food consumed daily during the test period was registered and expressed relative to the body mass for each individual. Behavioural observations, blood sampling and feeding took place between 12.00 and 14.00. All animal procedures were approved by the local Laboratory animal's sciences committee. In general, unnecessary distress was as far as possible avoided.

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**Statistical analysis**

All data were checked for homogeneity of variance (Levene's test) and normality (Kolmogorov-Smirnov method). Time spent moving was analysed by non-parametric median test. All other data are presented as mean ± S.E.M., and group differences were analysed using parametric t-tests (Statistica for Windows software package and JMP version 7.0). The amount of food ingested (a continuous, normally distributed variable) was expressed as group mean ± S.E.M. on each day, and analysed by two-way repeated measures ANOVA followed by the Tukey post-hoc test.

## Results

Melanin-based colouration in trout and salmon

Melanin-based colouration differed significantly in both juvenile ($t_{[14]}$=3.3, p=0.005) and adult ($t_{[18]}$ = 5.4, $p<0.001$) HR and LR trout, with individuals from the LR selection line consistently exhibiting more melanin spots per $cm^2$ than HR individuals (Fig.2A-B). Juvenile salmon were on average less spotted than juvenile rainbow trout, while variation between groups based on visual identification was highly significant ($t_{[14]}$=9.7, $p<0.001$, Fig. 2C).

## Behaviour and physiology of spotted and non-spotted Atlantic salmon

### Post-stress cortisol response

Analysis of cortisol levels after a 30 minute confinement challenge revealed differences between the spotted and non-spotted group. The spotted group showed significantly lower post-stress plasma cortisol levels than their non-spotted conspecifics ($t_{[14]}$=4.1, $p=0.001$) (Fig.3A).

### Locomcotor behaviour under acute stress

Locomotor activity during the acute confinement challenge was characterized by active swimming attempts against the walls or other locomotor escape behaviour. The amount of locomotor behaviour differed significantly between the two groups. Spotted fish spent less time moving when compared to non-spotted individuals ($\chi^2$= 4.0, $p=0.046$) (Fig 3B).

### Feed intake

Neither spotted nor non-spotted fish showed any feed intake on the day after transfer to isolation. On the second day, one individual in each group took a few pellets falling directly in front of its mouth, while all other fish still showed complete anorexia. Thereafter, feed intake increased gradually in both groups, but with a generally quicker resumption of normal feeding behaviour in the spotted group. Feed intake throughout the experiment expressed as consumed food relative to body weight is shown in Fig. 4. For this variable, repeated measures ANOVA indicated a significant effect of time ($F_{[7,98]}$=17.3, p<0.001) and a significant interaction between time and group ($F_{[7,98]}$=3.8, p=0.001), with post-hoc tests indicating significant group differences on single days only on day 7, at which spotted fish consumed more food than non-spotted conspecifics.

In addition to group means, we investigated whether there were significant correlations between different parameters within each group. There were no significant correlations between behaviours, cortisol response and spots within each group. This result does not imply that behavioural syndromes, behavioural-physiological or behavioural-morphological correlations were not present in the population as such, since 1) The number of individuals analysed here was tailored to reveal differences in group means, and 2) There was restricted variation within in each group, as groups were selected to represent the opposite ends of a continuum within a larger population.

## Discussion

Aquatic animals are known for physiological and environmentally induced colour changes which involve melanophore dispersal and contraction (Baker 1993; Nery and de Lauro Castrucci 1997; Höglund et al. 2000). However, there is little evidence that persistent melanin-based pigment patterns arising from melanophore aggregations in fishes are subject to environmental influence. In teleosts, pigment patterns have been addressed mainly in the *Danio* fish model to study genes underlying evolutionary change in cell development, and their effects on adult form (see e.g. Kelsh et al. 2000; Mills et al. 2007). One of the few studies dealing with this topic in salmonid fish reports strong heritability in the number of black skin spots, with weak genotype-by-environment interactions across environments (Kause et al. 2004). The current study demonstrates that eumelanin pigmentation patterns are associated with, and can be co-selected with, responsiveness of the hypothalamus-pituitary-interrenal axis (HPI-axis, the teleost equivalent of the mammalian HPA-axis). Furthermore, behavioural components of genetically determined stress coping styles also differ between more or less pigmented individuals.

Spotted salmon had significantly lower post-stress cortisol levels than those of their non-spotted conspecifics, furthermore spotted fish spent less time moving during acute stress when compared to non-spotted individuals. These aspects of the behaviour and physiology of spotted and non-spotted Atlantic salmon are entirely consistent with the behavioural patterns previously reported in LR and HR trout, respectively (Øverli et al. 2002a; 2005; 2007; Schjolden and Winberg 2007). In ethological studies, care must be taken to interpret the functional significance of behaviour shown by animals in a laboratory setting. The interpretation of time spent moving during confinement may have alternative explanations. It is possible that the fish that moved less were indeed stressed, but displayed fear-related freezing behaviour. Nevertheless, taken together with the data on plasma cortisol we argue that time spent moving is more likely related to stress response, in that individuals who move more show an elevated or prolonged physiological stress response compared to individuals that move less. This interpretation is in accordance with reported short-term stimulatory effects of cortisol and corticotrophin-releasing factor on locomotion (Øverli et al. 2002b; Lowry and Moore 2006; Carpenter et al. 2007). Almasi et al. (2008) found that the amount of eumelanin predicted parental provisioning in the barn owl and that individuals with large spots had a less pronounced behavioural change when exposed to exogenous corticosterone (a simulated stressful situation). Also in the present study individuals with higher amount of eumelanin (spotted group) showed a reduced behavioural response to a stressful situation compared to the non-spotted group.

Speed of release from stress-induced anorexia has previously been found to correlate positively with social dominance, and negatively with locomotor behaviour during acute stress (Øverli et al. 2004; 2006a). It could be argued that feeding in isolation reflects motivation to feed and differences in hunger at the time of testing, rather than stress responsiveness. A recent study has provided evidence that melanocortins play an important role in the regulation of energy homeostasis in salmonid fish (Schjolden et al. 2009). Furthermore, Ducrest et al. (2008) report associations between eumelanin pigmentation and regulation of feed intake in vertebrates in general. It appears that darker eumelanic individuals have higher resting metabolic rate, yielding increased feed intake to maintain energy homeostasis. At the same time stress has an appetite inhibiting effect (Bernier and Peter 2001), and when feeding behaviour is resumed after a stressful experience (such as introduction to a novel environment), this might reflect a downregulation of the physiological stress response, including the melanocortins. A high feed intake in a novel environment may therefore to some extent indicate quick acclimatization to the new environment, however it should be noted that this test is easily influenced by individual differences in previous growth rate (Ruiz-Gomez et al. 2008). Taken together these two aspects probably explain the higher feed intake in the spotted group. In the current experiment, the difference in feeding behaviour between spotted and non-spotted salmon was most evident after approximately one week of rearing in isolation but not immediately after transfer. As seen in Fig 4, it would appear that spotted and non-spotted fish showed a rather similar initial response to isolation, but after a few days spotted fish had a higher feed intake.

From these data it is not possible to conclude whether feed intake had reached a plateau in the non-spotted group, or would have continued to increase to eventually equal that of spotted fish. Further tests are therefore required to determine whether different feeding behaviour reflects a permanent difference in the behavioural strategy and possibly also resting metabolism of spotted vs. non-spotted Atlantic salmon. There was no initial size difference in fish sampled from group rearing. The most likely explanation for the difference in feed intake is that spotted and non-spotted fish reacted differently to novelty and social isolation, and that different feed intake in this case reflects a behavioural and/or metabolic component of contrasting stress coping styles akin to those seen in mammals (Koolhaas et al. 1999).

In summary, our results indicate that the association between melanin-based colouration and stress coping style is generally present in salmonid fish, and is not merely an artefact of the HR-LR selection regime. This observation is consistent with findings reviewed by Ducrest et al. (2008). These authors report co-variation between melanin-based colouration and other phenotypic traits. For example in white-throated sparrow, males of two different colour morphs (white and tan) showed different stress responses. White-morph males had a longer and more pronounced secretion of corticosterone in response to handling than tan-morph males (Schwabl 1995).

Eumelanin pigmentation may be linked to social status, for example in passerine birds (Järvi and Bakken 1984), thus it is important to control for immediate effects of social interactions on glucocorticoid production. We attempted to exclude the social factor by studying isolated fish.  Our results support the idea that differing dynamics of the stress response may be a consistent and partly inherited trait. Other studies have indicated that this trait may be a cause, as much as a consequence, of social status (Pottinger and Pickering, 2001; Øverli et al. 2004; Summers et al. 2005).  Salmonid fish can exhibit physiological colour change during stress; subordinate individuals e.g. often become darker. This is however not due to increased number of spots but rather a darkening of the entire dorsal body surface (see for example Höglund et al. 2000). Whether stress, e.g. caused by social subordination, in other cases also can alter eumelanic spots are a topic for future experiments.

A possibly important aspect which remains unresolved is whether melanin-based skin-spots in teleost fish serve a signalling function targeted by sexual selection. Colour pigments are used in the animal kingdom to advertise quality and attract mates. A recent study shows that embryo viability in the brown trout was revealed by the sires' dark pigmentation with darker males siring more viable offspring (Wedekind et al. 2008). In the barn owl males are on average 1.9 times less spotted and female spottiness is considered to be a criterion in mate choice (Roulin 1999). For example; the extent to which female barn owls display black eumelanin spots signal offspring parasite resistance and developmental homoeostasis (Roulin et al. 2003). Furthermore, the degree of eumelanism in the barn owl has been linked to the ability to cope with different environments and thus stressful factors (Roulin et al. 2008; Almasi et al. 2008). Determination of gender was not done in the present study.  Possible gender differences and a signalling function of melanin-based skin-spots in salmonid fish should be investigated.

Melanin-based colouration is also involved in ornamentation, camouflage and protection against physical damage, infectious agents, UV-light, and toxicants (Cesarini 1996; Majerus 1998; Mackintosh 2001; Roulin 2004a; Hoekstra 2006). The proximate and ultimate causes for the association between eumelanin-based ornamentation and other fitness-related traits such as immunocompetence remains debated (see e.g. Roulin 2004b; Griffith et al. 2006). The fact that glucocorticoid suppression of immune function has been observed throughout various vertebrate taxa (McEwen et al. 1997; Weyts et al. 1999) provide a likely proximate link between eumelanin colouration and disease resistance which so far has received little attention. On an applied level, there are some potentially important implications of this trait correlation in fishes. For instance, concerns are frequently raised about the impact of salmon rearing on wild fish populations and the surrounding ecosystems. The ectoparasitic copepods commonly referred to as salmon lice (or sea lice) have been identified as an immediate aquaculture-related threat to wild stocks (Krkošek et al. 2007). It is therefore of interest to investigate whether increased melanism in salmon is associated with reduced ectoparasite prevalence and fecundity, as has already been reported in birds (Roulin et al. 2003, 2001). Identification and concomitant selection for more stress-resistant individuals in aquaculture could also potentially alleviate other critical industry issues related to disease, welfare and sustainability of feed resources (Fevolden, 1992; Huntingford et al., 2006; Small et al., 2008). For instance, one study reported reduced feed waste from LR as compared to HR rainbow trout rearing units, along with improved feed conversion ratios in LR fish (Øverli et al. 2006b). There is however some concern that selection for fast growth and low stress-responsiveness may result in bolder individuals with more aggressive behavioural traits (Huntingford and Adams, 2005). Ducrest et al. 2008 reports that in 18 out of 20 species darker eumelanic individuals are more aggressive. Agonistic interactions between farmed fish are undesirable. In addition these individuals are prone to display more risk-taking behaviour making them more susceptible to predators (Huntingford and Adams 2005).

A more fundamental aspect of our results is that dermal eumelanin pigment patterns provide a novel and convenient way to identify stress-sensitive and stress-resistant individuals. Correlated behavioural and physiological traits are thought to reflect adaptation to different population densities, the relative frequency of occurrence of similarly or differently behaving individuals, and/or other fluctuating aspects of the environment, including stability and predictability of environmental change (Koolhaas et al. 1999; Dingemanse et al. 2004; Höjesjö et al. 2004; Wolf et al. 2008). One important consequence of eumelanin pigment as a visual marker of stress coping style is that it may ease the work of understanding the evolution of correlated traits by providing a convenient and reliable identification of the occurrence of different phenotypes in different environments.

The suggested proximate explanations for covariance between melanin-based colouration and other phenotypic traits such as behaviour and HPA/HPI reactivity are pleiotropic effects of melanocortins and their receptors (MCRs) (Ducrest et al. 2008). In particular the MC1R (regulating synthesis of the dark pigment eumelanin) and MC2R (controlling corticosteroid production) might play important roles. These receptors share a substantial amount of homology, but have no physical linkage in the genome of any vertebrate species examined so far (Logan et al. 2003; Schiöth et al. 2003). Thus, the causal mechanisms for correlated activity between the MCR receptors remain unknown. It appears that melanin-based pigment patterns and stress-coping style are linked by largely unmapped, evolutionary processes. Hopefully, a combination of molecular genetics studies and experiments addressing the specific gene-environment interactions which maintain, or disrupt, the correlation between pigment patterns, stress-coping style, and other phenotypic traits will provide more knowledge in this area.

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## Figures

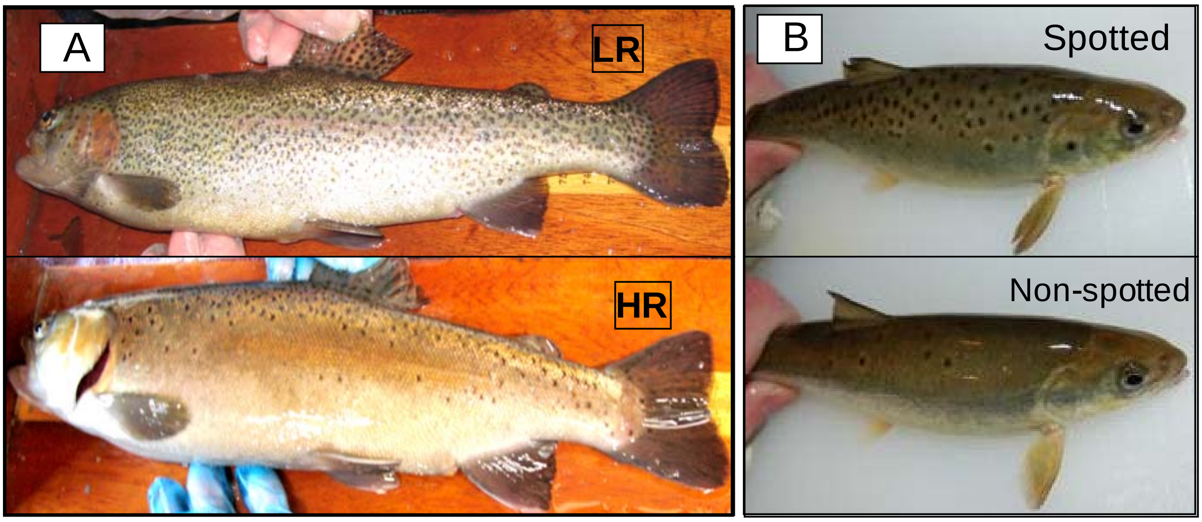

**Figure. 1.** Phenotypic variation in dermal melanin pigmentation in salmonid fish. A) Rainbow trout selected for low (LR, top) and high (HR, bottom) post-stress cortisol levels. B) Atlantic salmon defined as 'spotted' (top) and 'non-spotted' (bottom).

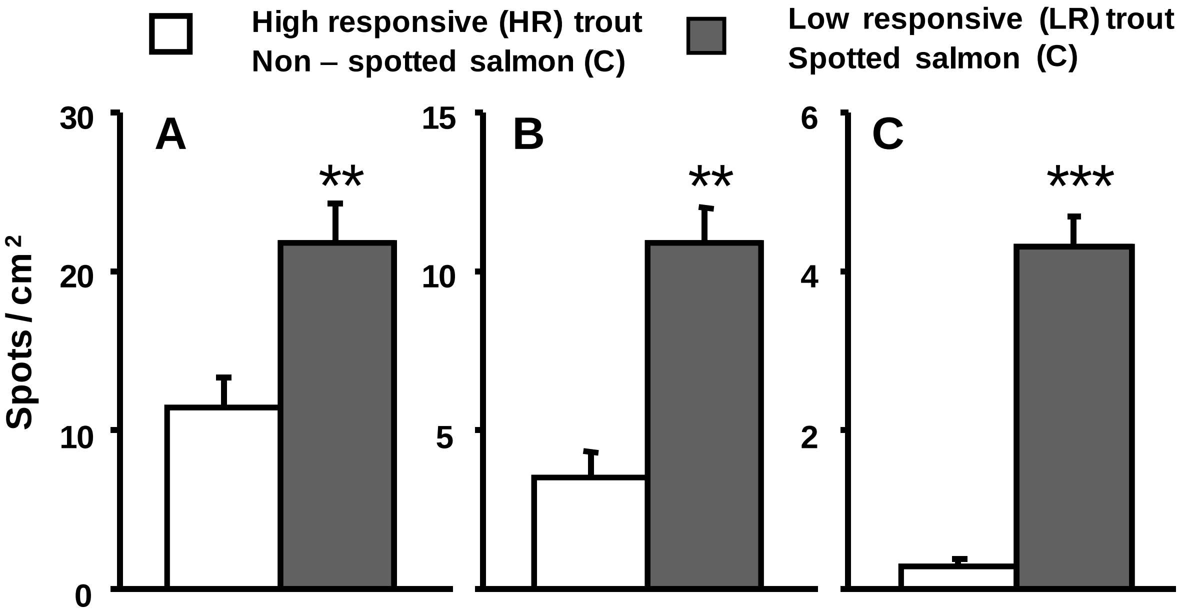

**Figure 2.** Eumelanin pigmentation in juvenile (A) and adult (B) rainbow trout of the HR and LR selection lines, and in spotted vs. non-spotted salmon (C). Bar graphs depict average number of black spots + S.E.M. pr cm$^2$ of skin surface in a defined area, above the sideline from the opercular lid to the dorsal fin. Asterisks indicate statistical differences ($p<0.01$**, $p<0.001$***, t-test).

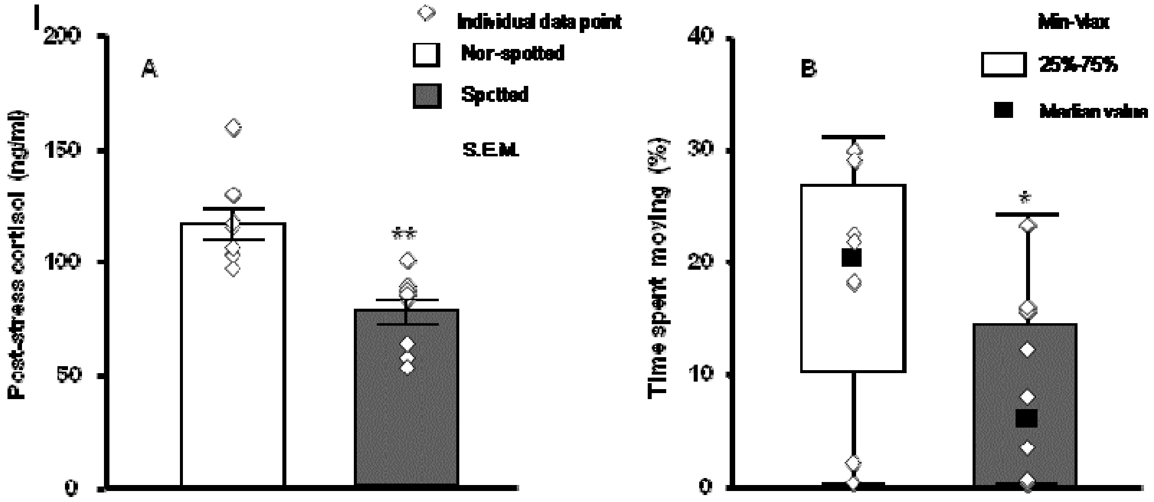

**Figure 3.** Behavioural and physiological responses to acute stress in spotted and non-spotted juvenile Atlantic salmon. A) Post-stress plasma cortisol (mean + S.E.M., t-test). B) Time spent moving in the acute stress test (box plot, median test). Asterisks indicate statistically significant differences (p<0.05*, p<0.01**).

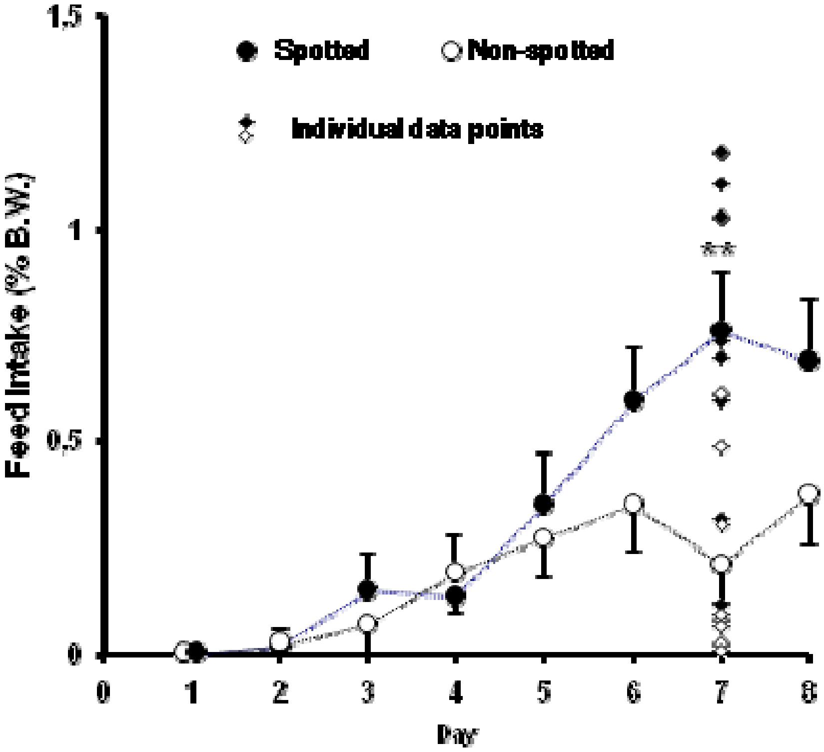

**Figure 4.** Feeding behaviour of juvenile Atlantic salmon defined as “spotted” or “non-spotted” after transfer from group rearing to isolation in a novel environment. Daily feed intake is expressed as % of initial body mass (mean ± S.E.M.). Asterisks indicate statistically significant differences ($p<0.01$**, repeated measures ANOVA with Tukey post-hoc test).