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# Uncontrollable chronic stress reduces growth disparities in farmed Atlantic salmon

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ABSTRACT

Individual variation in behavior and physiological traits in a wide variety of animals has been the focus of numerous studies in recent years. In this context, early life experiences shape responses that individuals have to subsequent environments, *i.e.* developmental plasticity. In this experiment, we subjected 10-month old fish to an unpredictable chronic stress (UCS) regime or no stress (control) for 3 weeks. These individuals then underwent the parr-smolt transformation, when salmonids become adapted for the seawater environment, and were subsequently transferred into seawater before the final sampling. Biometric data was collected at the end of each period. Sampling on the final day was conducted in order to analyze basal monoaminergic activity in the brain stem and hypothalamus, as well as gene expression of target genes in the telencephalon. We found that post-hoc sorting of individuals by their serotonergic activity (high and low) resulted in the elucidation of growth and gene expression differences. UCS groups were found to have less growth disparities throughout the experiment, compared to control fish. Furthermore, we found brain serotonergic signaling and corticotropic releasing factor binding protein expression were positively associated with brain stem serotonergic activity, which is consistent with fish showing a stress reactivity neurophysiological profile. In conclusion, we here submit evidence that sorting individuals by their basal serotonergic activity levels may be a useful tool in the study of developmental plasticity. These results may thus apply directly to improving husbandry practices in aquaculture and elucidating neural mechanisms for coping behavior.

## 1. Introduction

Individual variation in behavior and physiological traits in a wide variety of animals has been the focus of numerous studies in recent years [1–4]. The term coping style characterizes a group of individuals that express consistent physiological and behavioral responses to stressful stimuli [3]. Much of the research leading to the characterization of the proactive and reactive coping styles in fish has been based upon the rainbow trout (*Oncorhynchus mykiss*) post-stress cortisol selected lines [5]. Notably, selection by post-stress cortisol levels is consistently proportional with the serotonergic system's reactivity. That is, while proactive (*i.e.* bold/aggressive) consistently exhibit low serotonergic activity, reactive (*i.e.* shy) have enhanced serotonergic responsiveness [6,7]. The serotonergic system is phylogenetically ancient and anatomically well conserved across species [7]. The neurotransmitter serotonin (5-hydroxytryptamine; 5-HT) has been associated with energy regulation, neural plasticity, stress regulation and behavioral/emotional control [8–11]. Therefore, serotonergic reactivity has been crucial in the study of individual differences in stress responses. Interestingly, even though there is compelling evidence that early life experiences, particularly stress, shape how individuals cope with their present and future environments [12–14], this is not often taken into account when studying individual variation in animals. Even though there are consistent differences reported within individuals comprising

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a population, there is still a lack of consensus regarding the consistency of a given individual response throughout different contexts and across time [2]. For example, proactive individuals are often characterized as responding in an active and aggressive way, even when it appears to not be adaptive [15]. However, behavioral traits are not as fixed as once proposed, but highly dynamic and animals exhibit a series of plastic responses to stimuli that are based upon external and internal cues. For example, Ruiz-Gomez et al. [16] reported that reactive trout adopted a more proactive style for up to one year when they experienced a decrease in body fat after transport. This plasticity in behavioral outputs to exogenous stimuli may involve immediate responses (contextual plasticity) or it may involve responses shaped by past events (developmental plasticity) [2,17]. Therefore, it is not always possible to find consistent responses across different situations. Importantly, since behavioral responses are regulated by physiological systems, it is fundamental to study differences in physiological traits. In this context, it has been particularly useful to apply strong artificial selection of extreme values of a given physiological trait in order to elucidate relationships between individual behavioral responses and physiological regulation [6,18–22].

Here we explore the effect of an unpredictable chronic stress regime (UCS) on Atlantic salmon performance and physiology through different early life stages compared to control fish. Furthermore, *via* the *post hoc* sorting of individuals by their high (H) and low (L) serotonergic activity, we explore the regulation of gene expression of target serotonergic, neural plasticity and corticotropic genes. We hypothesize that long-term developmental plasticity of growth and neural responses to an early life stress regime will be elucidated in terms of the fish's basal brain serotonergic activity. We collected data at several timepoints during development and analyzed the final monoamine neurochemistry in the hypothalamus and brain stem (which contain the main serotonergic nuclei innervating the brain [23]). In addition, we studied the telencephalic gene expression of the aforementioned genes, since this area has been associated with the top-down regulation of the serotonergic stress response [24,25].

## 2. Methods

### 2.1. Ethics statement

This work was conducted in accordance with the laws and regulations controlling experiments and procedures on live animals in Norway and was approved by the Norwegian Animal Research Authority (NARA), following the Norwegian Regulation on Animal Experimentation of 1996.

### 2.2. Experimental animals and facilities

Atlantic salmon eggs (Aqua Gen strain, Aqua Gen AS, Trondheim, Norway) were hatched and reared at the Institute of Marine Research (IMR), Matre, Norway. Experimental fish were kept in 10,000 L outdoor tanks under natural conditions (9 °C). A month before the start of the experiment, 1110 fish (approx. 10 months old) were transferred into 9 indoor tanks (400 L; density: 7 kg fish/tank) supplied with flow-through freshwater. Fish were kept at 12 °C on a 12:12 photoperiod with a water flow of 15 L/min which provided an approximately a 92% oxygen saturation. Fish were fed with dry pellets (2 mm Skretting Nutra Olimpic, Stavanger, Norway) distributed *ad libitum* three times a day with automatic feeders (Arvo-tec feeding units: Arvo-Tec T drum 2000, Huutokoski, Finland). Tank conditions were monitored and regulated by a fully automated system (SD Matre, Normatic AS, Nordfjordeid, Norway).

### 2.3. Experimental procedure

At the beginning of the experiment, tank groups were randomly assigned to one of 2 treatments (3 replicates/treatment, 124 fish per tank), unpredictable chronic stress (UCS) or no stress (control). The UCS treatment consisted of stressing fish three times per day (at 8:30, 13:00, and 17:00) using 8 different stressors in a random and unpredictable order throughout the week for a total of 3 weeks, following the protocol previously described in Madaro et al. [26] and Vindas et al. [27]. Control fish were only subjected to routine practices of tank maintenance, but otherwise left undisturbed. The 3 feedings/day were maintained throughout the experiment starting approximately 1 h after the stressors. Importantly, throughout this period fish were sequentially sampled terminally ($n = 50$) in order to quantify their stress response through this period. These data were previously reported by Madaro et al. [26]. At the end of the stress regime, all fish were mildly sedated in metacaine (25 mg/L, Finquel®vet, ScanAqua AS, Årnes, Norway, buffered with 25 mg/L sodium bicarbonate) and fork length and body weight recorded (Sampling 1). The remaining fish were individually tagged with a PIT-tag inserted into the abdominal cavity for individual recognition and distributed into two tanks per treatment (111 fish; 7 kg/tank). The fish then underwent light controlled parr-smolt transformation (6 weeks L:D 24:0) At the end of this period all fish were mildly sedated, measured and weighed (Sampling 2). To maintain a density of 7 kg/tank, the groups were reduced to 74 fish per tank. At this point, the water flow was switched into full strength seawater (35 ppt.) for a period of 4 weeks before the final sampling (Sampling 3).

### 2.4. Final sampling protocol

During the final sampling (Sample 3) a total of 60 fish were sampled directly from holding tanks and immediately killed with an overdose of MS-222 (1 g/L) which rendered them completely motionless (no opercular movement) within 10 s of immersion. Fish were rapidly weighed, fork length measured and decapitated for brain dissection. The brain stem, hypothalamus and telencephalon were quickly excised within 2 min, snap-frozen in liquid nitrogen and stored at − 80 °C for later analysis.

### 2.5. The specific growth rate (SGR)

The percent of body weight gain per day (standardized growth into % body mass per time unit) may be studied by calculating the SGR which allows for comparison of growth rate and fish weight in a linear manner by correcting for fish size effects (although it needs to be considered that small fish grow faster in % of body mass). This is done by using the formula (1):

$$\mathrm{SGR} = \left[\frac{(\ln W_2 - \ln W_1)}{(t_2 - t_1)}\right] \times 100 \quad (1)$$

where $W_1$ and $W_2$ are the Mass (g) at the start ($t_1$) and end ($t_2$) of the specific growth period of interest [28].

### 2.6. Serotonergic neurochemistry

Frozen brain stems and hypothalamus were analyzed by means of high-performance liquid chromatography (HPLC) as described by Vindas et al. [27].

### 2.7. Gene expression analysis

Total RNA was extracted from the telencephalon using TRIzol® reagent. All RNA concentrations were assessed using a NanoDrop® ND-1000 UV–Vis Spectrophotometer (NanoDrop Technologies, Rockland, DE, USA). The RNA quality was determined from RNA integrity numbers (RINs) calculated by a 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). A RIN equal or above eight confirmed excellent RNA quality. First strand cDNA was synthesized from 1260 ng/μL