Dietary l-tryptophan leaves a lasting impression on the brain and the stress response

Comparative models suggest that effects of dietary tryptophan (Trp) on brain serotonin (5-hydroxytryptamine; 5-HT) neurochemistry and stress responsiveness are present throughout the vertebrate lineage. Moreover, hypothalamic 5-HT seems to play a central role in control of the neuroendocrine stress axis in all vertebrates. Still, recent fish studies suggest long-term effects of dietary Trp on stress responsiveness, which are independent of hypothalamic 5-HT. Here, we investigated if dietary Trp treatment may result in long-lasting effects on stress responsiveness, including changes in plasma cortisol levels and 5-HT neurochemistry in the telencephalon and hypothalamus of Atlantic salmon. Fish were fed diets containing one, two or three times the Trp content in normal feed for 1 week. Subsequently, fish were reintroduced to control feed and were exposed to acute crowding stress for 1 h, 8 and 21 d post Trp treatment. Generally, acute crowding resulted in lower plasma cortisol levels in fish treated with 3×Trp compared with 1×Trp- and 2×Trp-treated fish. The same general pattern was reflected in telencephalic 5-HTergic turnover, for which 3×Trp-treated fish showed decreased values compared with 2×Trp-treated fish. These long-term effects on post-stress plasma cortisol levels and concomitant 5-HT turnover in the telencephalon lends further support to the fact that the extrahypothalamic control of the neuroendocrine stress response is conserved within the vertebrate lineage. Moreover, they indicate that trophic/structural effects in the brain underlie the effects of dietary Trp treatment on stress reactivity.
Effects of acute and chronic stress on telencephalic neurochemistry and gene expression in rainbow trout (Oncorhynchus mykiss)

By filtering relevant sensory inputs and initiating stress responses, the brain is an essential organ in stress coping and adaptation. However, exposure to chronic or repeated stress can lead to allostatic overload, where neuroendocrinial and behavioral reactions to stress become maladaptive. This work examines forebrain mechanisms involved in allostatic
processes in teleost fishes. Plasma cortisol, forebrain serotonergic (5-HTergic) neurochemistry, and mRNA levels of
corticotropin-releasing factor (CRF), CRF-binding protein (CRFBP), CRF receptors (CRFR1 and CRFR2),
mineralocorticoid receptor (MR), glucocorticoid receptors (GR1 and GR2) and serotonin type 1A (5-HT1A) receptors (5-
HT1Aα and 5-HT1Aβ) were investigated at 1 h before and 0, 1 and 4 h after acute stress, in two groups of rainbow trout
held in densities of 25 and 140 kg m-3 for 28 days. Generally, being held at 140 kg m-3 resulted in a less pronounced
cortisol response. This effect was also reflected in lower forebrain 5-HTergic turnover, but not in mRNA levels in any of
the investigated genes. This lends further support to reports that allostatic load causes fish to be incapable of mounting a
proper cortisol response to an acute stressor, and suggests that changes in forebrain 5-HT metabolism are involved in
allostatic processes in fish. Independent of rearing densities, mRNA levels of 5-HT1Aα and MR were downregulated 4 h
post-stress compared with values 1 h post-stress, suggesting that these receptors are under feedback control and take
part in the downregulation of the hypothalamic-pituitary-interrenal (HPI) axis after exposure to an acute stressor.
Improved growth performance in rainbow trout Oncorhynchus mykiss reared at high densities is linked to increased energy retention

Behaviour has been suggested as an underlying factor influencing how rearing density affects growth performance in Salmonid fishes. At low densities there is an elevated intensity of aggressive interactions and the formation of dominance hierarchies. As density increases, it is commonly assumed that aggression decreases, as the cost and effort required to establish and maintain dominance hierarchies increase. The increased energy expenditure associated with aggressive interactions has been identified as one mechanism causing a reduced efficiency in feed utilisation and therefore decreased growth performance. Manipulating aggressive behaviour through density may have advantages from a practical perspective. In the present study the energetic expenditure of rainbow trout held at three densities, 25, 80 and 140 kg m(-3), were related to growth performance parameters. Measurements for growth performance and parameters of energetics were investigated at the three densities during a four week growth period. The results showed a significant increase in routine metabolism in fish reared at 25 kg m(-3) compared to groups reared at higher densities. The study concludes that in fish reared at density of 25 kg m(-3), a higher fraction of the dietary energy intake was used to fuel activity rather than growth, as evidenced by significantly higher routine metabolism, reduced feed utilisation efficiency and a tendency for lower growth performance compared to fish reared at the higher densities. These results indicate a bioenergetic advantage of crowding. (C) 2015 Published by Elsevier B.V.
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FISHERIES, MARINE, SALVELINUS-ALPINUS L, BRAIN SEROTONERGIC ACTIVITY, STOCKING DENSITY, ARCTIC CHARR, SALMO-GAIRDNERI, RESPIRATORY PHYSIOLOGY, OXYGEN-CONSUMPTION, FEEDING-BEHAVIOR, REARING DENSITY, EXERCISE, Energetics, Aggression, Growth, Density, Rainbow trout
High oxygen consumption rates and scale loss indicate elevated aggressive behaviour at low rearing density, while elevated brain serotonergic activity suggest chronic stress at high rearing densities in farmed rainbow trout *Oncorhynchus mykiss*.

The effect of stocking density on indicators of welfare has been investigated by several studies on farmed rainbow trout *Oncorhynchus mykiss*. However, the densities at which welfare are compromised remain ambiguous. Here three different stocking density treatments were selected based on the results of a previous study, where levels of crowding where determined using the spatial distribution of fish in two-tank systems. An un-crowded low density of 25 kg m$^{-3}$, the highest density accepted by the fish without showing indications of crowding stress of 80 kg m$^{-3}$ as the intermediate density, and the highest density accepted by the fish showing indications of crowding stress of 140 kg m$^{-3}$ as the high density were investigated. The aim of the present study was to examine the effect of being held at these densities on indicators of welfare. This was achieved through oxygen consumption measurements using automated respirometry, recording fin erosion, determining scale loss and analysing plasma cortisol and brain serotonergic activity levels. The results obtained in the present study indicated that at the lowest density the fish had the space and opportunity to display their natural aggressive behaviour and that the fish held at the highest density were exposed to a situation of confinement.
The relationship between emergence from spawning gravel and growth in farmed rainbow trout Oncorhynchus mykiss

The relationship between the timing of emergence from spawning gravel and growth after emergence was investigated in farmed Oncorhynchus mykiss. A relationship between the time of emergence and growth became evident after 6 months of rearing, where individuals with an intermediate emergence time had grown larger compared with early and late emerging individuals.

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Utilising spatial distribution in two-tank systems to investigate the level of aversiveness to crowding in farmed rainbow trout Oncorhynchus mykiss

In aquaculture, fish are exposed to a range of unfavourable environmental conditions. Amongst these, stocking density has attracted considerable attention as inappropriate densities may compromise welfare and negatively impact production. However, the recommendations for stocking remain elusive. The aim of the present study was to apply a novel method to investigate a level of crowding that indicated aversiveness in rainbow trout (Oncorhynchus mykiss). In a two-tank system, where two identical tanks were connected via a doorway, it was observed that social behaviour controlled the distribution of the fish between the tanks. Fish were stocked at equal quantities in each tank of the system. The doorway was opened and the fish moved between the two tanks. Typically, this resulted in one tank being occupied by a few highly aggressive dominant individuals (“dominant” tank) and the majority of the fish occupying the second tank (“crowded” tank). Here, the potential of this unequal spatial distribution for quantifying aversion to crowding was explored. Fish were stocked in three two-tank systems at a total density of 20, 40 and 80 kg m⁻³ respectively. The number of fish in each tank was determined every three days throughout the duration of the experiment and the percentage of fish in the “crowded” tank was used as an indicator of the distribution pattern in the two-tank systems. The results indicated a negative relationship between the total density stocked (20, 40 and 80 kg m⁻³) and the percentage of fish in the “crowded” tank. A subsample of individuals was sacrificed for blood and brain samples every three days from the “crowded” tank, prior to the fish count. The neuroendocrine indicators of stress, elevated serotonergic activity levels which were not associated with high plasma levels of cortisol, suggested chronic stress in the fish at the highest total density stocked (80 kg m⁻³). Taken together, these results indicated that a level of aversiveness to crowding had been reached at the highest density stocked, where the mean absolute density, irrespective of time of day, observed in the “crowded” tank was 126.5±3.7 kg m⁻³.
Welfare aspects of stocking density in farmed rainbow trout, assessed by behavioural and physiological methods

There is an increasing amount of interest in the welfare of fish from aquaculture. There are several aquaculture practices that may act as chronic stressors and therefore have the potential to negatively impact welfare. Stocking density has been highlighted as a particular welfare concern, from both an ethical and practical point of view. A quantity of research has been conducted on the relationship between stocking density and indicators of welfare in farmed rainbow trout Oncorhynchus mykiss. The studies to date have revealed that both low and high densities have the potential to detrimentally affect welfare in rainbow trout. Several studies have endeavoured to make specific recommendations for maximum stocking density limits for rainbow trout. However, wide discrepancies exist, highlighting the fact that it has been a challenge to identify density limits that promote optimal welfare and production in rainbow trout. This emphasises the significance of developing alternative methods that provide insight into the potential density limits that are optimal for welfare and performance in rainbow trout. Here, a behavioural method using two-tank systems was developed and applied. The twotank systems consisted of two identical tanks which were attached to each other with a doorway allowing the fish to move freely between the two tanks. By studying the spatial distribution of fish in two-tank systems stocked with different densities and the neuroendocrine stress levels of the fish, a density level was established that showed indications of crowding. The results revealed that a level of aversion to crowding had been reached at an absolute density of approximately 140 kg m\(^{-3}\). Additionally, the influence of the established density limit on physiological indicators of welfare and performance were investigated. At this density of 140 kg m\(^{-3}\), the lower oxygen consumption rates and lower quantity of scale loss collected from the tanks suggested reduced levels of social hierarchy related aggressive encounters. Higher brain serotonergic activity in the brain stem of individuals held at this density indicated elevated stress levels, despite low concentrations of plasma cortisol. The reduced energetic expenditure at 140 kg m\(^{-3}\) resulted in a better utilisation of ingested feed and hence growth performance. Taken together, despite the chronic stress levels at this density, the results showed that at this density the reduced energy expenditure, attributed to reduced aggressive social interactions, resulted in a better growth performance. Therefore, it may be concluded that application of
the method using the two-tank systems provided new insight into an optimal stocking density limit for rainbow trout. Furthermore, the method presented here provides a promising tool for investigating stocking density levels in rainbow trout. Further development of the current method would consider it applicable for determining limits for a range of culture situations.

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Behavioural responses to hypoxia provide a non-invasive method for distinguishing between stress coping styles in fish
Two divergent behavioural and physiological response patterns to challenges have been identified in mammals and birds, frequently termed the proactive and reactive coping styles. In recent years, individually distinct coping styles have also been observed in several species of fish. These individual differences may result in suboptimal production and compromised welfare in aquaculture. An approach to overcome these problems could be to sort fish and optimise rearing conditions according to coping style. It has been previously demonstrated that the proactive and reactive coping styles in fish can be characterised by contrasting behavioural responses to hypoxia. Two rainbow trout (Oncorhynchus mykiss) strains, bred for a low- (LR) and high- (HR) cortisol response to a standardized stressor, are suggested to resemble the proactive and reactive coping styles respectively. Therefore, these fish provided an opportunity for verifying a method for sorting fish with respect to coping style by exposure to hypoxia. Groups consisting of 24 individually tagged fish, 12 HR and 12 LR were exposed to hypoxia in a two choice system. The system consisted of a “home” tank provided with cover connected to a second brightly “illuminated” tank via a closable doorway. During the experiment, the doorway between the two tanks was opened and hypoxic conditions were gradually induced in the “home” tank by bubbling with nitrogen. The latency time to move away from hypoxic conditions to normoxic conditions in the second tank was recorded for each individual. The oxygen saturation in each tank was measured every 30 min. The experiment consisted of two trials. Each trial was carried out in two sessions, switching the “home” tank and “illuminated” tank between Sessions 1 and 2. The results indicated that the response to hypoxia differed significantly between LR and HR individuals in both Session 1 (P < 0.05) and Session 2 (P ≤ 0.001). Furthermore, a higher number of HR individuals left hypoxic conditions compared to LR individuals in both Session 1 (P = 0.001) and Session 2 (P ≤ 0.001). Taken together, the findings of the present study demonstrate a repeatable difference in behavioural response to hypoxia between the two strains. The method presented could be utilized as a non-invasive method for sorting fish according to stress coping style.

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The two-choice system is a non-invasive method for identifying socially dominant individuals from a group of fish

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Welfare of farmed rainbow trout (Oncorhynchus mykiss), preferences for stocking density

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Welfare of farmed rainbow trout (Oncorhynchus mykiss), preferences for stocking density

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Avoidance behaviour of rainbow trout (Oncorhynchus mykiss) to hypoxia: a non-invasive method for sorting fish according to stress coping style

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Welfare in farmed rainbow trout, social and environmental preferences

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Phd Student: Laursen, Danielle Caroline (Intern)
Supervisor: Skov, Peter Vilhelm (Intern)
Main Supervisor: Höglund, Erik (Intern)
Examiner: Jokumsen, Alfred (Intern)
Kristiansen, Tore S. (Ekstern)
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Animal welfare: social and environmental preferences of reared rainbow trout (38697)
The principle objective of this project is to evaluate the effect of rearing densities, current and cover on animal welfare. We will use preference test to investigate behavioral and environmental needs of farmed rainbow trout. Furthermore, for investigating the effects of not fulfilling these needs we will use neurophysiological and endocrine responses involved in the stress reaction as biomarkers for compromised welfare. The obtained knowledge is expected to contribute to a scientific based governmental guideline for welfare based intensive fish rearing.

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Project participant: Laursen, Danielle Caroline (Intern)
Project Manager, academic: Höglund, Erik (Intern)