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Research report

Social stress effects on pigmentation and monoamines in Arctic charr

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HIGHLIGHTS

- Social stress induces differences in carotenoid spots of Arctic charr.
- Carotenoid spots are associated with behavioural stress responses in Arctic charr.
- Monoaminergic activity is to some extent associated with carotenoid spots in Arctic charr.

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ABSTRACT

Pigmentation often signals status and in general melanin-based pigmentation is indicative of aggression and stress resilience in vertebrates. This is evident in the salmonids Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) where more melanin spotted individuals are more stress resilient. However, in the salmonid Arctic charr (*Salvelinus alpinus*) it seems as if it is carotenoid-based pigmentation that signals aggression and stress resilience. In our study, social stress effects on carotenoid-based spots, and behavioural and physiological stress responses were investigated. Socially stressed individuals have more spots, and behavioural stress responses were associated with spots. Some of the results concerning physiological stress responses, such as plasma cortisol levels and monoaminergic activity, are associated with spottiness. Further, the earlier proposed lateralization of spots, with left side connected to stress responsiveness and right side to aggression, is to some extent validated although not conclusively. In conclusion, this study provides further evidence that more stressed charr have more carotenoid spots, and for the first time monoaminergic activity is shown to be connected with carotenoid pigmentation.

1. Introduction

Animal's visual appearance often signals status or condition [1], and in vertebrates the two pigment groups of carotenoids and melanins cause this colour variation. This is often evident in agonistic interactions where aggression, dominance, and/or subordinance can be signalled with colours. For instance, eye darkening signals dominance in lizards [2,3], whereas eye darkening signals subordinance in several teleost fish [4–8]. Further, melanin-based pigmentation correlates positively with aggression in birds [9–11] and mammals [12]. Similarly, some evidence for correlations between carotenoid pigmentation and aggression is also reported. Male firemouth cichlids (*Cichlasoma meeki*) that obtained more carotenoid pigmentation via feed enrichment won more dyadic fights compared to controls [13]. Fights lasted longer in male three-spined stickleback (*Gasterosteus aculeatus*) if losers had higher carotenoid levels than winners [14].

Interestingly, the more melanised animals that typically are more aggressive [15,16] also seem to be more stress resilient [15]. Similar results have also been reported in teleost fish. In the salmonids Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), fish with more permanent melanin-based skin spots have a lower physiological stress response than the fish with few spots [17]. However, in the Arctic charr (*Salvelinus alpinus*) pigmentation is different compared to most salmonids [18] and social subordination induces a transient skin darkening [19,20] similar to other salmonids [21,22]. Thus, in Arctic charr melanisation does not seem to be indicative of stress resilience. Instead, a recent study reported that the carotenoid pigmentation in Arctic

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charr could be used as an indicator of physiological stress response [23]. Additionally, social subordinate charr have more carotenoid spots compared to dominant individuals [24]. Therefore, carotenoid pigmentation, rather than melanin-based pigmentation, seems to be associated with both aggression and stress in Arctic charr.

There are both behavioural and physiological responses following social stress. The behavioural responses following defeat include inhibited aggression [25]. For the physiological responses, there is an increase of glucocorticoids as well as monoaminergic activity changes [25]. Therefore, the monoaminergic activity can also be used as a stress indicator [26,27]. Monoaminergic activity also regulates aggression [26] and there are interactions between the regulation of stress and aggression [27]. Thus it seems likely that pigmentation will be associated with monoaminergic activity as well.

Further, both aggression and stress have been suggested to be lateralized, that is, one side being different from the other side in function or structure [28,29]. For instance, aggression has been shown to be lateralized in teleost fish [30–33] and mammals [34–38]. In addition, some behaviour during stress seems to be lateralized [29,39]. Recently it was suggested that the carotenoid spots in Arctic charr are lateralized, with the right side connected with aggression and the left side connected with stress responsiveness [24].

Based on these earlier results, we investigated how social stress in Arctic charr affected behavioural and physiological stress responses, including brain monoamines, and carotenoid-based pigmentation, and if there was any lateralization effects. Specifically, three different hypotheses were tested using social stress: (1) stressor affects number of carotenoid spots, (2) behavioural and physiological responses during stress can be coupled to number of spots before and/or after stressor, and (3) there is a lateralized effect of spots associated with behaviour and/or physiology.

2. Material and methods

2.1. Experimental animals and location

This study was carried out on 1 year old juvenile Arctic charr from the 7th generation of the Swedish Arctic charr breeding programme (Arctic superior, for details on the programme see Nilsson et al. [40]). Several months before the experiment, the fish were transported to Umeå Marine Research Station (UMF). At UMF, the fish were kept in tanks supplied with running brackish water (3–4‰) from the Bothnian Bay with a temperature ranging between 5 and 10 °C and a photoperiod set to 12 h light/12 h dark. The experiments were performed in May–June 2012 and the methodology was approved by the Umeå Animal Research Ethical Committee.

2.2. Social stress experiment

At the start of the experiment (day 1) fish (body mass: 149.8 ± 28.5 g, fork length (L_F): 24.3 ± 1.5 cm, mean ± SD, N = 32), randomly selected from stock, were photographed on both sides using a Canon EOS 500D digital camera in a setup providing constant bright light. During the photographing, fish were restrained in a box with a transparent lid (see Backström et al. [24]), and after the photographs were taken the fish were anaesthetized with Tricaine methanesulfonate (MS-222, 0.15 g/L), weighed and measured. This procedure with anaesthetics following photographs instead of before, which would be easier and maybe less stressful for the fish, was based on that anaesthetics induce spots in Arctic charr [41]. The fish were marked by a small cut in the caudal fin, either dorsally or ventrally, and put into social isolation in individual compartments. Individual compartments were created by separating experimental aquaria (170 L, 95 × 41 × 44 cm) into four equally-sized 42.5 L compartments using removable dark PVC walls. Two neighbouring fish were matched for mass (asymmetries in mass within pairs was less than 5%) thus creating two pairs per aquarium. The fish were allowed to acclimate for one week during which they were hand-fed commercial pellets. Each aquarium was continuously supplied with running water from the Bothnian Bay (5–10 °C) and the light/dark regimen was set to 12 h light/12 h dark (light on at 06.00 and light off at 18.00 h).

The dominance experiments were conducted on day 8, and started by removing the PVC walls separating each pair of fish. The fish were allowed to interact in their size-matched pairs, and the interaction lasted for 1 h and was recorded by a camcorder for later behavioural analysis. After 1 h a clear dominant-subordinate relationship was established in all pairs, and the fish were immediately taken from the aquaria and photographed (as described above). After the photographs were taken, fish were sacrificed in a high dose of MS-222 (0.30 g/L). Blood was immediately sampled via a heparinized syringe from the caudal vein, then the fish was decapitated and the brain dissected out. Sex was determined by visual inspection of gonads. The blood was subsequently centrifuged, the plasma collected and stored at −20 °C until further analysis. The brains were divided into the easily identified telencephalon, cerebellum, optic tectum and brainstem (including hypothalamus) and were stored at −80 °C until further analysis.

2.3. Image analysis

Photographs were analysed as described and validated earlier [24]. Briefly, carotenoid-based spots were counted systematically in a rectangle (2 cm × 10 cm) on each side of the fish using ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997–2012.).

2.4. Assays

Plasma was analysed for cortisol using a commercial enzyme linked immunosorbent assay (ELISA) kit (product no. 402710, Neogen corporation, Lexington, USA). Each sample was run in duplicates during a single assay with an intra-assay coefficient of variation of 1.82%.

Tissue levels of serotonin (5-hydroxytryptamine; 5-HT) and the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA) and the DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC), and norepinephrine (NE) were analysed in telencephalon, cerebellum, optic tectum and brainstem samples using high performance liquid chromatography with electrochemical detection (HPLC–EC) as described by Höglund et al. [19]. The concentrations were standardized against the weight of the brain tissues. The ratios of 5-HIAA to 5-HT concentration and DOPAC to DA concentration were calculated and used as an index of serotonergic and dopaminergic activity, respectively.

2.5. Behavioural observations

In our analysis of the 1 h dyadic agonistic interactions the following was recorded per pair by one observer (TB): (1) latency to first attack within the pair, (2) time until the dominant-subordinate relationship was settled after first attack, (3) time remaining of the hour after the dominant-subordinate relationship was settled, (4) the number of aggressive acts per minute performed prior to the dominant-subordinate relationship was settled (attack rate prior dominance), (5) the number of aggressive acts per minute performed by dominant individual after the dominant-subordinate relationship was settled (attack rate post dominance), and finally