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Behavioural and neuroendocrine effects of environmental background colour and social interaction in Arctic charr (*Salvelinus alpinus*)

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Summary

In salmonid fish, a darker skin colour has been suggested to signal social subordination. Substratum colour is another factor affecting skin pigmentation in fish; in the present experiment, juvenile Arctic charr (*Salvelinus alpinus*) were acclimated and allowed to interact in pairs for 5 days on a pale or dark background colour. Skin darkness was quantified prior to and following social interaction. Furthermore, agonistic behaviour and skin darkness were quantified, together with plasma levels of cortisol, adrenocorticotropin (ACTH) and α-melanocyte-stimulating hormone (α-MSH), and brain levels of monoamines and monoamine metabolites. The results show that fish interacting on a white background were more aggressive than those interacting on a black background. Social subordination resulted in skin darkening in fish kept on a white background, but not in fish kept on a black background. Furthermore, subordinate fish on a white background showed an elevation of brain norepinephric activity, an effect not seen in subordinate fish on a black background. Subordinate fish on both white and black backgrounds showed a similar activation of the brain serotonergic system and the hypothalamic–pituitary–interrenal axis. These results support the suggestion that skin darkening in subordinates acts as a social signal announcing social submission.

Key words: Salmonidae, serotonin, dopamine, norepinephrine, pro-opiomelanocortin-derived peptide, skin darkening, brain, social signal, Arctic charr, *Salvelinus alpinus.*

Introduction

Visual signals mediated through differential colour patterns often play an important role in the control of agonistic behaviour in fish (Huntingford and Turner, 1987). In salmonids, social subordination results in darkening of the skin, and skin darkening of subordinate fish may act as a social signal, reducing aggressive interactions in established dominance hierarchies (Abbott et al., 1985; O'Connor et al., 1999; Höglund et al., 2000).

In socially organised teleosts, as in many other vertebrates, subordinate individuals are subjected to chronic stress induced by a general lack of control, as well as by direct aggressive acts from individuals of higher social rank (Winberg and Lepage, 1998). Sustained social stress leads to chronic activation of the hypothalamic–pituitary–interrenal (HPI) axis, the teleost homologue of the mammalian hypothalamic–pituitary–adrenal (HPA) axis (Winberg and Lepage, 1998; Øverli et al., 1999; Höglund et al., 2000). Alpha-melanocyte-stimulating hormone (α-MSH) and adrenocorticotrophic hormone (ACTH), two hormones involved in the control of interrenal cortisol release (Balm et al., 1995), also have the ability to induce skin darkening (Fujii and Oshima, 1986).

Brain monoamines are important in the control of pituitary release of α-MSH and ACTH (Höglund et al., 2000; Bentley, 1998). Serotonin (5-hydroxytrypamine, 5-HT) stimulates the release of α-MSH in mammals (Carr et al., 1991), and Olivereau et al. (1980) obtained results suggesting that 5-HT might serve a similar function in teleosts. Moreover, 5-HT seems to stimulate the HPA axis in mammals (Dinan, 1996) and 5-HT has also been reported to stimulate HPI axis activity in rainbow trout (*Oncorhynchus mykiss*) (Winberg et al., 1997). In addition, the behavioural suppression observed in socially subordinate fish is an effect that, at least in part, appears to be mediated by a stress-induced activation of the central 5-HT system (Winberg and Nilsson, 1993; Øverli et al., 1998). By contrast, the brain catecholamines dopamine and norepinephrine exert inhibitory effects on the release of α-MSH from the pituitary (Bentley, 1998), and these neurotransmitter systems also appear to facilitate intraspecific

aggressive behaviour, thus having effects opposing those of 5-HT on both behaviour and skin colour (reviewed by Winberg and Nilsson, 1993).

Social subordination results in an elevation of plasma α-MSH levels and skin darkening in juvenile Arctic charr (*Salvelinus alpinus*) interacting in small groups on a pale background (Höglund et al., 2000). If skin darkening in subordinate Arctic charr acts as a signal announcing submissive behaviour, it is possible that environmental background colour, through its effect on body pigmentation, might affect agonistic behaviour in socially interacting fish.

The aim of the present study was to investigate the effect of environmental background colour on socially induced skin darkening and aggressive behaviour in juvenile Arctic charr.

Materials and methods

Animals

The fish were 1-year-old offspring of Arctic charr (*Salvelinus alpinus*, L.) caught in lake Hornavan, Lapland, Sweden, and weighed 38.2±8.53 g (mean ± S.D., N=48). Fish were kept indoors at the Evolutionary Biology Centre, Uppsala University, at a density of 400 fish m^{-3}, in a dark green holding tank supplied continuously with Uppsala tap water (8–10 °C, 1.5 l min^{-1}), for more than 6 months before the experiment. The photoperiod was continuously and automatically adjusted to conditions at latitude 51°N. The fish were hand-fed with commercial trout pellets (Ewos ST40) at 1–2 % of body mass per day.

Behavioural observations

Behavioural observations were performed in six glass aquaria (1000 mm×300 mm×500 mm) continuously supplied with aerated tap water (1 l min^{-1}, 10–12 °C). Each aquarium was divided into four 25 l chambers by removable black plastic walls. Half of these chambers had a white base and back, whereas the other half had a black base and back. Since the removable plastic walls, and tank ends, were black in all cases, the fish on white backgrounds still experienced black lateral sides. Light was provided by two 20 W fluorescent tubes (warm white) placed 250 mm above the water surface. At the start of the experiment, fish were transferred from the holding tank and tagged by small clips in the caudal fin, before being isolated in individual chambers within the observation aquaria. The fish were kept visually isolated for 3 weeks before the experiment to reduce the effects of previous tank colour and social experience. After the isolation period, size-matched pairs (within-pair body mass deviation <5 %, based on mass determined prior to acclimation), consisting of fish that had been isolated on the same background colour, were formed by gently removing the plastic walls that had kept them separated. Experimental fish were allowed to interact in pairs for 5 days. Eight fish on white and eight fish on black background colour were kept visually isolated throughout the experiment and served as controls.

The fish were hand-fed commercial trout pellets (Ewos, ST40) daily at 15:00 h to satiation during the isolation period as well as while interacting in pairs, except on the day of sampling when the fish were not fed.

Aggressive acts performed and received by individual fish were counted during two daily observation sessions of 5 min each, at 10:00 h and 16:00 h. Three types of aggressive acts were registered: attack, charge and bite. The fish were observed through the front of the aquaria by an observer who was not screened from the fish but remained motionless during the observation session. To reduce further the disturbance of the fish during behavioural observations, light was kept low in the room. The first observation was performed 30 min after pairing of the fish and the last on day 4, the day before terminating the experiment. Since the number of experimental aquaria was limited, the experiment was performed in two consecutive rounds, the second following immediately after the first. The first round consisted of four controls (two on black and two on white backgrounds) and 10 pairs of socially interacting fish (five pairs on black backgrounds and five on white backgrounds) and the second round of 12 controls (six on black and six on white backgrounds) and six pairs of socially interacting pairs (three on black and three on white backgrounds).

In one pair of fish kept on a black background, no aggressive acts were observed and in one pair on a white background the subordinate died. These two pairs were excluded from analysis of skin colour, hormones and brain monoamine levels.

Skin pigmentation measurements, and blood and brain tissue sampling

Skin pigmentation, quantified as the darkness of the skin, was measured by an image-analysing system described by Höglund et al. (2000), 24 h prior to and on day 5 of social interaction. In short, skin pigmentation was measured by placing the fish in a plastic box with a transparent cover. The box was stuffed with foam rubber, which immobilised the fish against the transparent cover. The fish was filmed with a ccd-video camera through the plastic cover under constant light conditions. Thereafter, the filmed fish was analysed by an image analysis program (Scion Image, based on NIH image for Macintosh modified for windows, by Wayne Rasband, NIH, Betheseda, MD, USA). Skin darkness was measured on a linear black-white scale where 0 corresponded to white and 255 to black. A grey-scale with eleven standard measure points ranging from 0 to 250 and a step value of 25 was attached to the transparent cover and used for calibration between measurements. The time for the pigment measuring procedure, from netting to the completion of measuring, was approximately 40 s. Immediately after the second skin pigmentation measurement, the fish were anaesthetised (500 mg l^{-1} ethyl-*m*-aminobenzoate methanesulphonate) and blood (approximately 1 ml) was collected from the caudal vasculature, using a syringe pre-treated with 1.5 mg of EDTA. Blood samples were rapidly transferred to Eppendorf tubes containing aprotinin (Sigma, A1153, 1 mg ml^{-1} blood) and were centrifuged at 1500 ***g*** for 10 min at 4 °C. Following

centrifugation, the blood plasma was removed, divided into samples, frozen on dry ice and stored at –80 °C. Following blood sampling, the fish were killed by decapitation, and the brain was rapidly removed (within 2 min) and divided into telencephalon (excluding olfactory bulbs), hypothalamus (excluding the pituitary gland), optic tectum and brain stem (including the medulla and part of the spinal cord). Each brain division was wrapped in aluminium foil, frozen in liquid nitrogen and stored at –80 °C. Measurements of skin pigmentation and sampling of blood and brain tissue was always performed between 10:00 and 12:00 h.

Assays

The frozen brain samples were homogenised in 4 % (w/v) ice-cold perchloric acid containing 0.2 % EDTA and 40 ng ml^{-1} epinine (deoxyepinephrine, the internal standard), using a Potter–Elvehjem homogenizer (optic lobes, cerebellum and brain stem) or an MSE 100 W ultrasonic disintegrator (telencephalon and hypothalamus).

5-HT, 5-hydroxyindoleacetic acid (5-HIAA), dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC, a major dopamine metabolite), norepinephrine and 3-methoxy-4-hydroxyphenylglycol (MHPG, a major norepinephrine metabolite) levels were quantified using high-performance liquid chromatography (HPLC) with electrochemical detection, as described by Höglund et al. (2000). Samples were quantified by comparison with standard solutions of known concentrations and corrected for recovery of the internal standard using HPLC software (CSW, DataApex Ltd., the Czech Republic). The ratio of [metabolite]/[parent monoamine] was used as an index of brain monoaminergic activity. This is a more direct index of monoaminergic activity than brain levels of monoamine metabolites *per se*, since variance related to tissue sampling, and differences related to total levels of the parent monoamine and its metabolite, are reduced (Shannon et al., 1986).

Owing to the presence of interfering unidentified peaks in the chromatogram we were unable to quantify MHPG in the telencephalon and hypothalamus.

Blood samples were assayed for cortisol, ACTH and α-MSH levels. Cortisol analysis was performed directly on Arctic charr plasma without extraction, using a validated radioimmunoassay (RIA) modified from Olsen et al. (1992) as described by Winberg and Lepage (1998). Plasma concentrations of ACTH were determined by RIA as described previously (Balm and Pottinger, 1993; Balm et al., 1994) and plasma concentrations of α-MSH following Balm et al. (1995).

Statistical analyses

All data are presented as means ± S.E.M. and, since no differences were observed between the two experimental rounds, all values were pooled. The Mann–Whitney *U*-test was used to investigate differences in the number of aggressive acts performed on the black and white backgrounds. Data on plasma concentrations of α-MSH, ACTH and cortisol, on brain levels of monoamines and monoamine metabolites, and on ratios of monoamine metabolite to parent monoamine concentrations (i.e. [5-HIAA]/[5-HT], [DOPAC]/[dopamine] and [MHPG]/[norepinephrine]) were subjected to two-way multivariate analysis of variance (MANOVA), with social rank or control and background colour as dependent factors. In the case of skin colour data, a repeated-measures MANOVA was performed. If significant effects were indicated by variance analysis, the Sheffé test was used to investigate differences between fish of different social rank and differences between interacting fish and controls.

To investigate the relationships between [MHPG]/[norepinephrine] and plasma [ACTH], linear regression analysis was performed. In addition, a stepwise multiple regression analysis was performed to investigate correlations between plasma [ACTH], [α-MSH] and skin darkness.

To fulfil the assumption of normal distribution, data on plasma concentrations of ACTH and cortisol were log-transformed, whereas [MHPG]/[norepinephrine] ratios were subjected to arcsine transformation. All statistical analyses were performed using Statistica 5.1 (StatSoft Inc.) software.

Results

Effect of black and white background colour on agonistic behaviour

At the time of the first behavioural observation (30 min following pair formation) the dominant-subordinate relationship had already been established, and the dominant fish performed all the aggressive acts observed. Irrespective of background colour, the dominant fish usually moved directly above the substratum, close to the centre of the aquarium, frequently biting and nipping the subordinate fish. The subordinate fish in each pair stayed inactive close to the surface, often close to the walls or in a corner of the aquarium. A tendency towards declining levels of aggressive acts was observed on both backgrounds during the 5 days of social interaction (Fig. 1A). Dominant fish on the white background performed significantly higher numbers of aggressive acts during the 5 days of social interaction than dominant fish interacting on the black background (Mann–Whitney *U*-test, P=0.047, Fig. 1B).

Effects of social interaction and background colour on skin darkness

The skin darkness of isolated controls and of dominant and subordinate fish kept on a white or a black background are shown in Fig. 2. Prior to social interaction, there was no significant difference in skin darkness between fish subsequently becoming dominant and subordinate (P=0.99, on white background; P=0.96, on black background) or between fish that became dominant (P=0.74, on white background; P=0.99 on black background) or subordinate (P=0.38, on white background; P=0.43, on black background) and controls. However, background colour had a significant effect on the skin darkness of the fish ($F_{1,33}$=27.3, P=0.0009); fish kept on the black background were darker than those kept on the white

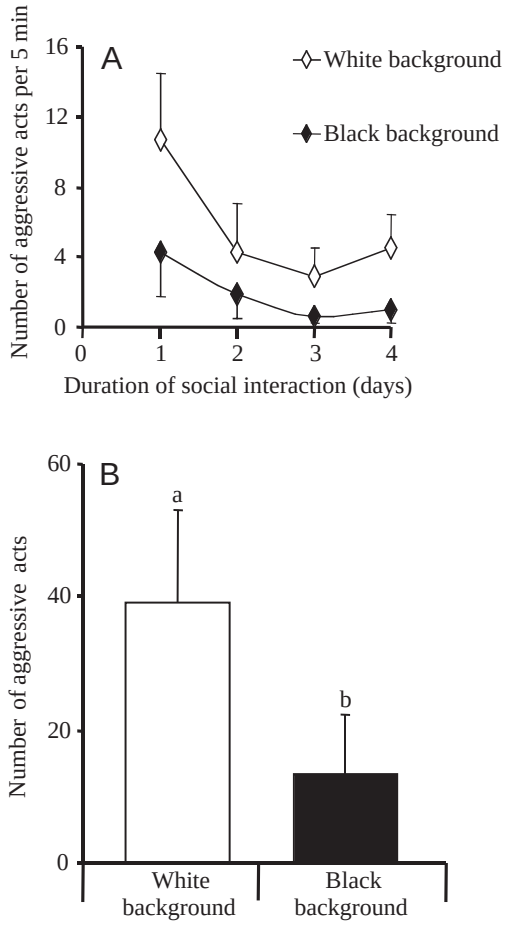

Fig. 1. The number of aggressive acts performed during each daily observation session (A) and the total number of aggressive acts (B) performed during four daily 5 min observation sessions by the socially dominant fish during social interaction in size-matched pairs of Arctic charr interacting on white or black backgrounds. Values are means ± S.E.M., N=7 pairs on a white and N=7 pairs on a black background. Different letters indicate a significant difference (P<0.05; Mann–Whitney U-test).

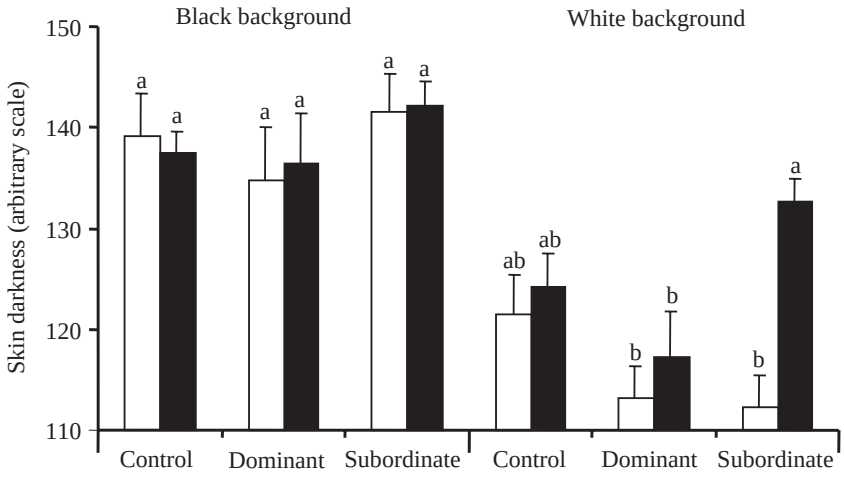

Fig. 2. Skin darkness of socially dominant and subordinate Arctic charr allowed to interact in pairs on a white (N=7) or black (N=7) background. Skin darkness was measured on a linear grey scale, where 0 is white and 255 is black, 24 h before (white bars) and after 5 days (black bars) of social interaction. Controls are fish that were visually isolated on the white (N=8) or black (N=8) background. Values are means + S.E.M. Means with no common superscript letters are significantly different (P<0.05; Sheffé *post hoc* test).

background. Social interaction on the white background had a significant effect on skin colour ($F_{3,33}$=6.23, P=0.025) and resulted in a significant skin darkening in subordinate fish (P=0.0015). Moreover, following social interaction on the white background, subordinate fish were significantly darker than dominant fish (P=0.031). Dominant fish on the white background were brighter than dominant fish kept on the black background (P=0.0033). There were no significant differences in skin darkness between controls and dominant fish (P=0.99) or subordinate and dominant fish (P=0.35) on the black background following social interaction (Fig. 2).

Effects of social interaction and background colour on plasma levels of cortisol, ACTH, α-MSH

Plasma [cortisol] ($F_{2,37}$=7.60, P=0.0017) and [ACTH] ($F_{2,37}$=12.90, P<0.0001) were significantly affected by social interaction, with subordinate fish tending to show the highest levels (Fig. 3A,C). Plasma [α-MSH] was not significantly affected by social interaction ($F_{2,35}$=1.35, P=0.270) (Fig. 3B). Moreover, there were no significant effects of background colour on plasma [cortisol] ($F_{1,37}$=0.19, P=0.66) or [ACTH] ($F_{1,37}$=0.69, P=0.410) (Fig. 3A,C). Plasma [α-MSH] showed a non-significant trend ($F_{1,35}$=3.20, P=0.083) towards elevated levels in fish kept on the black background (Fig. 3B). There was no significant effect of social interaction and background colour combined on either plasma [cortisol] ($F_{2,37}$=2.01, P=0.15) or [α-MSH] ($F_{2,35}$=1.35, P=0.78) (Fig. 3B,C). However, there was a trend towards elevated plasma [ACTH] ($F_{2,37}$=2.67, P=0.082) in subordinates on the white background as compared to subordinates on the black background (Fig. 3A), but this trend did not reach the level of statistical significance.

Even though neither background colour nor social rank had significant effects on plasma [α-MSH], a multiple regression analysis indicated that plasma [α-MSH] had a significant effect on skin darkness, explaining 23 % of the variance ($F_{2,33}$=6.24, P=0.002), whereas [ACTH] showed no significant correlation with skin darkness.

Effects of social rank and background colour on brain monoaminergic activity

There were no significant effects of

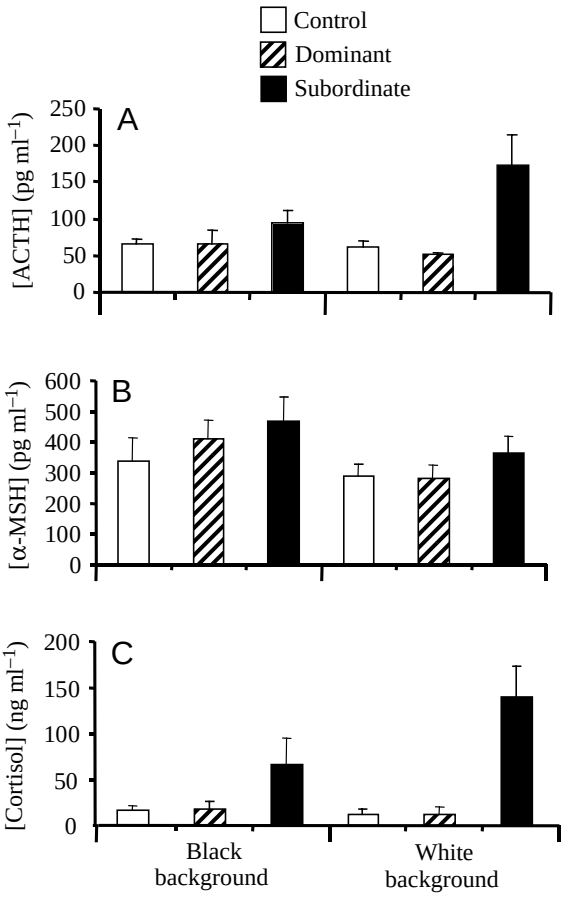

Fig. 3. Plasma concentrations of adrenocorticotropic hormone (ACTH) (A), α-melanocyte-stimulating hormone (α-MSH) (B) and cortisol (C) in socially dominant (N=7 for both black and white background) and subordinate (N=7 for both black and white background) Arctic charr after 5 days of social interaction in pairs on white or black background colour. Controls are fish that were visually isolated on a white (N=8) or black (N=8) background. Values are means + S.E.M. There were no significant differences between individual means (P<0.05; MANOVA followed by the Sheffé *post hoc* test on the combined effect of social position and background colour). For MANOVA statistics see Table 1.

background colour on [5-HIAA]/[5-HT] ratios in any brain part (Tables 1, 2). However, background colour had a significant effect on telencephalic [DOPAC/[dopamine] ratios ($F_{1,37}$=6.17, P=0.017), fish kept on the black background showing higher values than fish on the white background (Tables 1, 2). In the optic tectum there was a significant effect of background colour on [MHPG]/[norepinephrine] ratios ($F_{1,32}$=7.10, P=0.012), fish on the white background showing a tendency towards elevated values (Tables 1, 2). However, there was no significant effect of background colour on [MHPG]/[norepinephrine] ratios in the brain stem (Table 2).

Social interaction had significant effects on brain [5-HIAA]/[5-HT] ratios (Tables 1, 2). Specifically, significant effects of social interaction were observed on [5-HIAA]/[5-HT] ratios in the brain stem ($F_{2,33}$=3.46, P=0.043), hypothalamus ($F_{2,28}$=4.18, P=0.026) and optic tectum ($F_{2,32}$=4.9, P=0.014), subordinate fish tending to display elevated [5-HIAA]/[5-HT] ratios.

The [MHPG]/[norepinephrine] ratios in the brain stem ($F_{2,33}$=7.88, P=0.0016) and optic tectum ($F_{2,32}$=7.3, P=0.0025), were also significantly affected by social interaction, subordinate fish showing elevated [MHPG]/[norepinephrine] ratios (Tables 1, 2). There were no significant effects of social interaction on brain [DOPAC]/[dopamine] ratio (Tables 1, 2).

The brain stem [MHPG]/[norepinephrine] ratio was also affected by background colour and social status combined ($F_{2,33}$=4.83, P=0.014), with elevated values in subordinate fish kept on the white background as compared to dominant fish (white P=0.038, black P=0.012) and controls (white P=0.0064, black P=0.038) on either the black or white background (Tables 1, 2). Moreover, in all fish taken together, brain stem [MHPG]/[norepinephrine] showed a significant correlation with plasma [ACTH] (r=0.52, P=0.0008).

Effects of social interaction and background colour, as well as the combined effects of these dependent variables, on brain ratios of [monoamine metabolite] to [parent monoamine] (i.e. [5-HIAA]/[5-HT], [DOPAC]/[dopamine] and [MHPG]/[norepinephrine]), were all reflected in similar changes in the concentration of the metabolites. Brain levels of monoamine neurotransmitters, in contrast, in most cases remained relatively constant. However, in the optic tectum, [dopamine] was significantly affected by background colour and social interaction, but not by background colour and social interaction combined (Table 2). Similarly, background colour and social interaction had significant effects on telencephalic [5-HT] and [norepinephrine], respectively (Table 2).

Discussion

Background matching is well known in fish, and differential environmental background colours were used in the present study in an attempt to manipulate the body colour of the fish. If dark body coloration signals social subordination, a darker fish may represent less of a threat and elicit less aggression than a conspecific displaying paler body coloration. The results from the present study seem to support this hypothesis. In pairs of fish interacting on a white background, both fish were initially pale in coloration and showed a high level of aggressive behaviour. However, the frequency of aggressive interactions declined over time, suggesting that this decline in aggressive behaviour was related to the fact that the fish becoming subordinate took on a darker body coloration. In pairs of fish interacting on a dark background, both fish were dark in coloration from the start, and the frequency of aggressive interactions was lower. Moreover, in pairs interacting on a dark background, the subordinate fish did not show any additional darkening of its body colour. In these pairs, the decline in aggressive interaction over time was less obvious than in pairs interacting on the white background.

Table 1. *Effects of social rank and background colour on the concentrations of monoamines and monoamine metabolites, and the ratio of concentrations of monoamine metabolite to parent monoamine neurotransmitter in Arctic charr*

	Social status and background colour					
	White			Black		
	Control	Dominant	Subordinate	Control	Dominant	Subordinate
Brain stem						
[5-HIAA]	170±23[a]	150.2±11[a]	210±18[a]	200±27[a]	150±15[a]	170±14[a]
[5-HT]	620±25[a]	640±63[a]	660±66[a]	810±88[a]	680±35[a]	810±88[a]
[DOPAC]	7.1±1.2[a]	5.9±2.3[a]	9.11±1.5[a]	9.6±1.6[a]	5.9±2.3[a]	8.6±1.2[a]
[DA]	290±20[a]	340±53[a]	320±31[a]	410±20[a]	320±36[a]	410±20[a]
[MHPG]	5.5±0.8[a]	6.0±1.3[a,b]	13±2.8[b]	8.3±0.8[a,b]	6.2±1.1[a,b]	8.2±1.3[a,b]
[NE]	820±23[a]	912±70[a]	856±47[a]	968±40[a]	886±65[a]	846±55[a]
[5-HIAA]/[5-HT]×10^{-3}	270±3.5[a]	240±8.0[a]	340±28[a]	240±22[a]	240±21[a]	260±12[a]
[MHPG]/[NE]×10^{-3}	7.0±1.1[b]	7.0±1.0[b]	17±3.2[a]	9.0±1.1[b]	7.0±1.2[b]	9.0±1.1[a,b]
[DOPAC]/[DA]×10^{-3}	36±13[a]	23±12[a]	31±11[a]	24±4.2[a]	36±11[a]	27±3.1[a]
Hypothalamus						
[5-HIAA]	105±20[a]	195±15[a]	192±39[a]	172±31[a]	146±23[a]	225±47[a]
[5-HT]	1200±270[a]	2100±125[a]	1800±350[a]	2400±245[a]	2100±125[a]	1800±350[a]
[DOPAC]	46±19[a]	19±5.0[a]	27±7.0[a]	32±6.7[a]	24±6.9[a]	34±4.2[a]
[DA]	1600±230[a]	1900±145[a]	1600±190[a]	2000±143[a]	1600±271[a]	1600±190[a]
[NE]	1300±182[a]	1400±130[a]	1100±220[a]	1400±170[a]	1400±130[a]	1400±180[a]
[5-HIAA]/[5-HT]×10^{-3}	66±8.0[a]	92±5.6[a]	130±19[a]	87±13[a]	99±35[a]	130±21[a]
[DOPAC]/[DA]×10^{-3}	29±9.3[a]	11±3.1[a]	20±7.0[a]	16±3.1[a]	40±32[a]	20±2.1[a]
Telencephalon						
[5-HIAA]	202±24[a]	241±16[a]	303±43[a]	154±30[a]	205±28[a]	302±42[a]
[5-HT]	950±254[a]	1100±110[a]	1210±310[a]	670±120[a]	890±±180[a]	1200±310[a]
[DOPAC]	82±7.3[a]	45±22[a]	40±14[a]	67±14[a]	45±22[a]	40±14[a]
[DA]	850±380[a]	380±87[a]	550±280[a]	320±54[a]	380±87[a]	330±75[a]
[NE]	1800±230[a]	2300±280[a]	2500±230[a]	1600±345[a]	1950±270[a]	1600±340[a]
[5-HIAA]/[5-HT]×10^{-3}	230±31[a]	220±12[a]	310±61[a]	240±25[a]	270±40[a]	320±60[a]
[DOPAC]/[DA]×10^{-3}	160±26[a]	90±27[a]	120±35[a]	210±23[a]	140±34[a]	26±63[a]
Optic tectum						
[5-HIAA]	80±12[a]	86±6.8[a]	120±16[a]	100±20[a]	94±9.5[a]	100±14[a]
[5-HT]	280±48[a]	310±24[a]	350±36[a]	280±48[a]	310±24[a]	350±36[a]
[DOPAC]	14±2.57[a]	18±1.36[a]	18±2.3[a]	15±1.6[a]	18±1.4[a]	18±2.3[a]
[DA]	370±30[a]	370±15[a]	320±12[a]	330±20[a]	320±18[a]	270±20[a]
[MHPG]	1.3±0.26[a]	2.5±0.89[a]	6.2±2.0[a]	1.0±0.28[a]	2.3±0.78[a]	2.4±0.47[a]
[NE]	730±56[a]	820±45[a]	890±84[a]	710±43[a]	900±160[a]	850±39[a]
[5-HIAA]/[5-HT]×10^{-3}	320±62[a]	260±36[a]	330±20[a]	320±40[a]	310±20[a]	390±36[a]
[MHPG]/[NE]×10^{-3}	1.9±0.49[a]	2.9±0.70[a]	7.0±2.20[a]	1.4±0.50[a]	2.7±0.31[a]	2.8±0.56[a]
[DOPAC]/[DA]×10^{-3}	41±0.0[a]	47±2.0[a]	49±9.0[a]	46±6.0[a]	49±6.0[a]	51±6.0[a]

Concentrations of metabolites are ng g^{-1} brain tissue.

Fish were allowed to interact in pairs for 5 days on a white or black background. Controls were fish that were visually isolated on a white or black background.

Values are means ± S.E.M. from 5–8 individuals.

Different letters indicate significant differences (P<0.05) (MANOVA followed by the Sheffé *post hoc* test on the combined effect of social position and background colour; for MANOVA statistics, see Table 2).

MHPG was not detected in the hypothalamus and telencephalon (see Materials and methods).

5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; DOPAC, 3,4-dihydroxyphenylacetic acid; DA, dopamine; MHPG, 3-methoxy-4-hydroxyphenylglycol; NE, norepinephrine.

O'Connor et al. (1999) showed that Atlantic salmon (*Salmo salar*) parr display darkening of the body and sclera in response to social subordination when interacting in pairs on a light-coloured substratum. In their experiment, they observed a sudden change to a darker colour, occurring at the moment a fish stops being aggressive and becomes subordinate. In response to this darkening of the subordinate fish, the behaviour of its dominant opponent immediately changed and, as a result, the number of attacks on the subordinate rapidly declined. Thus, darkening of the body colour appears to act as

Table 2. *Effects of social status, background colour and social status and background colour combined on the concentrations of monoamines and monoamine metabolites, and the ratio of concentrations of monoamine metabolite to parent monoamine neurotransmitter in Arctic charr*

	ANOVA		
	Social status	Background colour	Social status and background colour
Brain stem			
[5-HIAA}	$F_{2,33}$=1.8; P=0.18	$F_{1,33}$=0.087; P=0.77	$F_{2,33}$=1.9; P=0.17
[5-HT]	$F_{2,33}$=0.60; P=0.55	$F_{1,33}$=2.3; P=0.14	$F_{2,33}$=1.1; P=0.34
[DOPAC]	$F_{2,33}$=0.049; P=0.95	$F_{1,33}$=2.5; P=0.12	$F_{2,33}$=1.1; P=0.33
[DA]	$F_{2,33}$=0.46; P=0.63	$F_{1,33}$=2.2; P=0.15	$F_{2,33}$=3.0; P=0.062
[MHPG]	$F_{2,33}$=5.0; P=0.012	$F_{1,33}$=0.28; P=0.60	$F_{2,33}$=3.8; P=0.032
[NE]	$F_{2,33}$=0.087; P=0.92	$F_{1,33}$=0.73; P=0.40	$F_{2,33}$=2.3; P=0.11
[5-HIAA]/[5-HT]	$F_{2,33}$=3.5; P=0.043	$F_{1,33}$=4.1; P=0.053	$F_{2,33}$=1.1; P=0.34
[MHPG]/[NE]	$F_{2,33}$=7.9; P=0.0016	$F_{1,33}$=1.8; P=0.19	$F_{2,33}$=4.8; P=0.014
[DOPAC]/[DA]	$F_{2,33}$=0.19; P=0.83	$F_{1,33}$=0.20; P=0.65	$F_{2,33}$=0.77; P=0.47
Hypothalamus			
[5-HIAA]	$F_{2,35}$=2.9; P=0.068	$F_{1,35}$=0.24; P=0.63	$F_{2,35}$=1.6; P=0.21
[5-HT]	$F_{2,28}$=0.80; P=0.92	$F_{1,28}$=0.20; P=0.59	$F_{2,28}$=2.2; P=0.12
[DOPAC]	$F_{2,35}$=1.1; P=0.32	$F_{1,35}$=0.032; P=0.86	$F_{2,35}$=0.468; P=0.63
[DA]	$F_{2,35}$=0.21; P=0.81	$F_{1,35}$=0.088; P=0.77	$F_{2,35}$=1.8; P=0.18
[NE]	$F_{2,35}$=0.37; P=0.37	$F_{1,35}$=0.63; P=0.43	$F_{2,35}$=0.34; P=0.70
[5-HIAA]/[5-HT]	$F_{2,28}$=4.18; P=0.026	$F_{1,28}$=0.29; P=0.59	$F_{2,28}$=0.23; P=0.80
[DOPAC]/[DA]	$F_{2,35}$=0.0054; P=0.95	$F_{1,35}$=0.25; P=0.62	$F_{2,35}$=1.6; P=0.29
Telencephalon			
[5-HIAA]	$F_{2,37}$=3.9; P=0.029	$F_{1,37}$=8.1; P=0.0068	$F_{2,37}$=0.70; P=0.50
[5-HT]	$F_{2,37}$=0.38; P=0.68	$F_{1,37}$=4.7; P=0.036	$F_{2,37}$=0.26; P=0.76
[DOPAC]	$F_{2,37}$=2.5; P=0.097	$F_{1,37}$=0.55; P=0.46	$F_{2,37}$=1.5; P=0.23
[DA]	$F_{2,37}$=0.26; P=0.77	$F_{1,37}$=0.98; P=0.33	$F_{2,37}$=1.0; P=0.37
[NE]	$F_{2,37}$=3.7; P=0.034	$F_{1,37}$=1.9; P=0.17	$F_{2,37}$=0.12; P=0.88
[5-HIAA]/[5-HT]	$F_{2,37}$=1.7; P=0.19	$F_{1,37}$=0.27; P=0.60	$F_{2,37}$=0.24; P=0.78
[DOPAC]/[DA]	$F_{2,37}$=2.6; P=0.086	$F_{1,37}$=6.2; P=0.017	$F_{2,37}$=0.82; P=0.44
Optic tectum			
[5-HIAA]	$F_{2,32}$=4.4; P=0.020	$F_{1,32}$=0.027; P=0.87	$F_{2,32}$=0.47; P=0.63
[5-HT]	$F_{2,32}$=0.33; P=0.71	$F_{1,32}$=0.30; P=0.59	$F_{2,32}$=1.52; P=0.23
[DOPAC]	$F_{2,32}$=0.46; P=0.64	$F_{1,32}$=1.7; P=0.20	$F_{2,32}$=0.81; P=0.45
[DA]	$F_{2,32}$=5.0; P=0.012	$F_{1,32}$=5.9; P=0.021	$F_{2,32}$=0.024; P=0.98
[MHPG]	$F_{2,32}$=5.3; P=0.010	$F_{1,32}$=4.4; P=0.043	$F_{2,32}$=2.26; P=0.089
[NE]	$F_{2,32}$=2.4; P=0.11	$F_{1,32}$=0.013; P=0.90	$F_{2,32}$=0.38; P=0.68
[5-HIAA]/[5-HT]	$F_{2,32}$=4.9; P=0.014	$F_{1,32}$=0.71; P=0.40	$F_{2,32}$=1.2; P=0.31
[MHPG]/[NE]	$F_{2,32}$=7.3; P=0.0025	$F_{1,32}$=7.1; P=0.012	$F_{2,32}$=2.1; P=0.13
[DOPAC]/[DA]	$F_{2,32}$=1.4; P=0.26	$F_{1,32}$=0.23; P=0.88	$F_{2,32}$=0.47; P=0.62

Fish were allowed to interact in pairs for 5 days on a white or black background. Controls were fish that were visually isolated on a white or black background.

MHPG was not detected in the hypothalamus and telencephalon (see Materials and methods).

5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; DOPAC, 3,4-dihydroxyphenylacetic acid; DA, dopamine; MHPG, 3-methoxy-4-hydroxyphenylglycol; NE, norepinephrine.

a social signal announcing defeat and/or subordinate social status in both Atlantic salmon (O'Connor et al., 1999) and Arctic charr. However, the higher number of aggressive acts observed in dominant fish on the white background in the present study could also be related to the fact that subordinate fish are more visible against a pale background. This explanation is less likely since the frequency of aggressive acts displayed by the dominant fish declined over time as the subordinate darkened and thus became even more visible against the white background.

In Arctic charr, skin darkening of subordinates appears to be related to the chronic social stress experienced by subordinate individuals and is possibly mediated by a stress-induced elevation in plasma levels of α-MSH (Höglund et al., 2000). In the present study, there was a significant correlation between plasma levels of α-MSH and skin darkness, even though the

effects of social rank or background colour on plasma levels of α-MSH did not reach the level of statistical significance. A visual social signal reflecting the activation of the physiological stress response has also been reported in the lizard *Anolis carolinensis* (Summers and Greenberg, 1994; Korzan et al., 2000). However, in *A. carolinensis*, the signal (darkening of the eyespots) indicates social dominance and reflects an acute elevation of plasma catecholamine levels. In Arctic charr, darkening of the body colour seems to reflect chronic HPI axis activation, lasting as long as the stressor, i.e. the dominant fish, is present (Höglund et al., 2000). The darkening of the body coloration and sclera of subordinate Atlantic salmon may be a less persistent but more rapidly activated response (O'Connor et al., 1999). Rapid changes in body coloration, and the visual pattern of the body, signalling intent or motivational state during agonistic interactions, are often mediated by neural mechanisms (e.g. Demski, 1992) or by rapid changes in circulating plasma catecholamine levels (Bentley, 1998). The technique used to quantify skin darkness in the present study, involving netting and immobilisation of the fish, did not allow us to study rapid stress-related changes in body coloration or visual pattern of the body mediated by neural mechanisms or circulating plasma catecholamines.

Social subordination is stressful and is known to affect brain monoaminergic activity (Winberg and Nilsson, 1993). In the present study, we observed an elevation of brain [5-HIAA]/[5-HT] ratios in subordinate fish, which agrees well with earlier studies (Winberg and Nilsson, 1993). Together with an activation of the brain 5-HT system, subordinate fish also showed an activation of the HPI axis, as indicated by elevation of plasma levels of ACTH and cortisol. The central 5-HT system is believed to stimulate HPI axis activity, and it has been suggested that brain 5-HT plays a key role in the control and integration of behavioural and neuroendocrine stress responses in both teleosts (Winberg and Nilsson, 1993; Winberg et al., 1997; Winberg and Lepage, 1998) and mammals (Chaouloff, 1993; Dinan, 1996).

Brain [MHPG]/[norepinephrine] ratios in subordinate fish on the white background followed the same pattern as [5-HIAA]/[5-HT] ratios, and there was also a significant positive correlation between [MHPG]/[norepinephrine] ratios in the optic tectum and plasma [ACTH]. In mammals, stress is known to activate the brain norepinephrine system (reviewed by Stanford, 1993), and Øverli et al. (1999) reported elevated brain [MHPG]/[norepinephrine] ratios and a positive correlation between brain [MHPG]/[norepinephrine] ratios and plasma cortisol levels in subordinate rainbow trout. Furthermore, in agreement with the results of the present study, Höglund et al. (2000) observed a strong relationship between the [MHPG]/[norepinephrine] ratios in the optic tectum and plasma [ACTH] in Arctic charr following 5 days of social interaction on a pale background. In the present study, we observed a significant elevation of brain stem [MHPG]/[norepinephrine] ratios in subordinate fish kept on white background, and the observation that plasma [ACTH] and [cortisol] followed the same general pattern as brain [MHPG]/[norepinephrine] ratios supports the suggestion that the central norepinephrine system plays a role in the regulation of the teleost HPI axis.

Pale background colour, resulting in an elevation of plasma concentrations of melanin-concentrating hormone (MCH) and a decrease in plasma levels of α-MSH, has been reported to have a suppressive effect on the stress-induced elevation of circulating plasma concentrations of cortisol and ACTH in rainbow trout (*Oncorhynchus mykiss*) (Baker and Rance, 1981; Gilham and Baker, 1985; Baker et al., 1985). However, in the present study, subordinate fish kept on the white background showed a tendency to higher plasma [ACTH] and [cortisol] than subordinates on black background. Moreover, the effect on brain stem [MHPG]/[norepinephrine] ratios was only observed in subordinates on the white background. Thus, fish on the white background seemed to display a more persistent stress response, which is probably explained by the fact that subordinates on the white background were exposed to a more intense social stress, as a result of the greater number of aggressive acts performed by the dominant fish. Unfortunately, in the present study, the amount of plasma available did not allow us to quantify plasma levels of MCH.

In the present study, social subordination resulted in skin darkening, but only in fish interacting on a white background. The lack of skin darkening in subordinates on a black background could be due to the lower levels of aggression and thus reduced levels of social stress experienced by subordinates on the black background, compared with those on the white background. Another explanation for the lack of skin darkening in subordinates interacting on a black background could be that fish acclimated to a black background had reached a maximum level of skin darkness and could not become darker in response to social subordination. The control of α-MSH release from the pituitary pars intermedia is multifactorial and is not fully understood, but there is evidence to suggest that pituitary α-MSH is under inhibitory control by dopamine and norepinephrine, by nerves projecting down from hypothalamus to the pars intermedia of the pituitary (Bentley, 1998). In the present study, we observed a tendency to higher plasma levels of α-MSH in fish kept on a black background, but there was no indication of any decrease in hypothalamic dopamine or norepinephrine activity in these fish.

In conclusion, the results of the present study further support the hypothesis that skin darkening serves as a social signal in Arctic charr, acting to reduce unnecessary fights and energy loss in an established dominance hierarchy (O'Connor et al., 1999; Höglund et al., 2000). Fish on a white background showed a brighter skin colour and in these pairs the dominant fish performed more aggressive acts than did dominant fish on a black background. This observation may be explained by a dark fish representing less of a threat, and thus eliciting less aggression, than a pale conspecific. Higher levels of aggression resulted in a more intense social stress, as indicated by elevated brain norepinephrine activity, in subordinate fish kept on a white background. The more intense stress experienced by

subordinates kept on the white background may explain why socially induced skin darkening in subordinate fish was observed only on this background. However, another explanation could be that fish acclimated to a black background colour had already reached a maximum level of skin darkening.

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