The acute stress response of red porgy, Pagrus pagrus, kept on a red or white background

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Abstract

The skin colour of red porgy, Pagrus pagrus, can be modified by exposure to different background colours. Red and white background colours brighten the dark skin colour that develops under common culture conditions in red porgy. To assess whether skin colour is also modified by aquaculture related handling stress, we subjected red porgy to 5 min of netting stress combined with air exposure. Fish kept on a white background have: (1) a lighter skin colour, which is not influenced by an acute stressor, (2) a less saturated red colour, which significantly decreases 24 h post-handling, and (3) a similar hue as fish kept on a red background. The first plasma parameters to rise after application of the stressor are cortisol, lactate and Na⁺; then, glucose levels rose. Other plasma ions (Ca²⁺, Cl⁻, K⁺) were not affected up to 2 h post-stressor, but had decreased at 8 and 24 h after handling. Plasma pH decreased over the first 2 h post-handling, indicative of plasma acidosis upon air exposure. The acidosis then coincided with increases in plasma lactate levels. As αMSH levels were not significantly affected by the stressor while cortisol levels showed a five to tenfold increase, we suggest that following acute stress in red porgy, plasma cortisol release is controlled by ACTH, perhaps in combination with a sympathetic stimulation.

Keywords: Red porgy; Pagrus pagrus; αMSH; Acute stress responses; Teleosts; Background adaptation

1. Introduction

In fish, exposure to harmful or potentially harmful stimuli causes an integrated stress response similar to that in mammals. The chromaffin cells in the head kidney receive signals from the hypothalamus via the sympathetic nervous system to produce and release catecholamines. The hypothalamus further releases neuroendocrine factors that can induce the release of corticotrope signals from the pituitary gland that stimulate the interrenal steroid producing cells of the head kidney to produce and release cortisol into the bloodstream. These two pathways are called hypothalamic–sympathetic–chromaffin (HSC) cell axis and hypothalamic–pituitary–interrenal (HPI) axis, respectively (Wendelaar Bonga, 1997).

In aquaculture, fish are frequently exposed to stressful situations such as handling and confinement. For red porgy, Pagrus pagrus, a relatively new species in aquaculture belonging to the Sparidae (Kentouri et al., 1995; Pavlidis et al., 2000), it was demonstrated that stress evoked by crowding results in moderately elevated cortisol levels (Rottlant et al., 1997; Rottlant and Tort, 1997). During handling-induced stress these values doubled. In a study by Arends et al. (1999), the stress response of the gilthead seabream (Sparus auratus), another sparid fish, was tested by exposure of the fish to handling. The response of fish that
had previously been exposed to crowding stress was significantly lower than fish that had not been previously stressed. Such attenuation of the acute stress response during chronic stress has been described in detail (Wendelaar Bonga, 1997).

An important problem in commercial rearing of red porgy is a marked darkening of the body. Wild red porgy exhibit a pink, silvery body colouration, but in aquaculture the body colour darkens and the brightness decreases markedly. A hormone classically related to skin darkening is α-melanophore stimulating hormone (αMSH). This hormone can induce skin darkening by causing the stellar-shaped pigment cells (melanophores) to disperse their black pigment (melanin) granules within the cytoplasmic processes of the cell (Bagnara and Hadley, 1973; Baker et al., 1984). More recently, it has been shown that αMSH is also involved in the stress response in some fish species (Baker et al., 1984; Lamers et al., 1992; Wendelaar Bonga, 1997), and in one report αMSH was designated a corticotrope (Balm et al., 1995).

Adaptation of fish to a dark background can raise the blood levels of αMSH (Baker et al., 1984; Burton, 1993), although this phenomenon appears to be species-specific. In red porgy, previous research by our group (Salm et al., 2004b) and others (Rotllant et al., 2003) on background adaptation has shown that the skin colour lightens on a white background. Also under blue light illumination red porgy develop a lighter body colour (Szisch et al., 2004).

The darkening of the body colour of red porgy in aquaculture suggests that this fish experiences aquaculture conditions as a stressor. The purpose of this study was to investigate the stress response and the pigmentation response of red porgy to handling (netting combined with crowding) and light. The experiment was conducted in accordance with local regulatory requirements.

2. Materials and methods

2.1. Experimental setup

In the autumn of 2002, 100 fish weighing 371.7 ± 58.6 g were generously supplied by Interfish S.A., Greece. Upon arrival in the Institute of Aquaculture (Heraklion, Crete), fish were transferred to 5001 circular polyester tanks filled with natural sea water, continuously replaced with a mixture of fresh and recycled seawater. Salinity of the water was 40 psu and water renewal 100%/h. Fish were kept under normal day–night rhythm (16 L:8 D) and under a temperature of fresh and recycled seawater. Salinity of the water (these varied between 4.7 and 5.7 mg/l). Fish were fed with self-feeders containing INVE™ Pagrus feed (crude protein, 50%; crude fat after hydrolysis, 16%; crude fibre, 2%; crude ash, 10%; phosphorus, 1.4%; vitamin A, 12,500 IU; vitamin D3, 2500 IU; vitamin E, 300 mg; vitamin C, 2000 mg; copper sulfate + copper, 5 mg; ethoxyquinone; butylated hydroxytoluene).

Two weeks prior to the start of the experiment, fish were divided over 10 experimental tanks (10 fish per tank) and allowed to adapt to tanks with white (WBG) or red (RBG) coloured walls, mounted as described by Salm et al. (2004b). The experiment consisted of exposure of fish to 5 min of netting while being held above the water surface; in this way confinement and air exposure were simultaneously applied. Fish were sampled immediately after netting (time 0.05 h), or 2, 8 and 24 h after netting. Non-stressed fish, kept under similar background and illumination conditions, were sampled as controls one week prior to the netting experiment. The experiment was conducted in accordance with local regulatory requirements.

2.2. Sampling

Immediately following capture, some colour parameters of the skin of the fish were determined with a portable spectrophotometer (Hunter Lab Miniscan™XE) as described by Salm et al. (2004b). The parameters brightness (L*), observable colour or hue (h*) and colour intensity or chroma (C*) were calculated according to the CIELab system. Colour samples were taken from the control group, and from the experimental fish at the t = 0.05 h sampling and after 24 h. Next, the fish were euthanised in 0.2% phenoxethanol. At all sampling points, blood was drawn from the caudal vessels, with syringes containing 35 μl of 2% Na–EDTA to prevent clotting, and 50 μl (= 0.5 TIU) of aprotinin to prevent proteolysis of the peptide hormones. The blood was spun at 4°C for 5 min at 1000g, after which the supernatant plasma was stored in Eppendorf vials and quickly frozen. In general, both colour and blood sampling occurred within 5 min per fish.

2.3. Physiological parameters

The αMSH concentration in the plasma was determined as described by Arends et al. (1999). The antiserum used for the αMSH radio immunoassay cross-reacts for 100% with des-, mono- and di-acetyl αMSH (Vaudry et al., 1978), and was used in a final dilution of 1:60,000. Immuno complexes were precipitated by 7.5% (w/v) polyethylene glycol and 2.5% (w/v) bovine serum albumin. Cross-reactivity with ACTH is almost absent (<0.05%; Zoest et al., 1989). The detection limit was 25.2 pg/ml of sample. To validate the assay for red porgy plasma, serial dilutions (1:1) were made in assay buffer as described by Salm et al. (2004a). Regression analysis showed strict parallelism between the fits for the standard curve and the dilutions of red porgy plasma. To determine cortisol concentrations, a RIA was used as described in detail by
Arends et al. (1998). Radioactivity was quantified using a Cobra II \( \gamma \)-counter (Packard Instruments). Plasma glucose, lactate, ions (Na\(^+\), Cl\(^-\), K\(^+\) and Ca\(^{2+}\)) and pH were measured with a Stat Profile\textsuperscript{®} pHOx\textsuperscript{®} Plus L Analyser (Nova Biomedical).

2.4. Statistics

Parameters were compared between groups using two-way analysis of variance (ANOVA), followed by Bonferroni or Dunnett C post-hoc tests to assess significance between mean values (all tests performed with SPSS 12.0 statistical software). Data on hue, an angular variable, were transformed and analysed according to circular statistical methods described by Zar (1999). Statistical differences were accepted at \( P<0.05 \). Values are shown as means ± standard deviation (SD).

3. Results

3.1. Colour parameters

The \( L^* \)-value was consistently higher in WBG fish compared to RBG fish (Fig. 1A). This difference was significant at all time points (\( P<0.05 \)). \( L^* \)-values were not influenced by the stressor, since there were no significant differences through time for fish kept on either background. The mean chroma (\( C^* \)) was higher in RBG fish, a difference that was significant before and 24 h after the start of the experiment (Fig. 1B). At 24.00 h post-stressor, \( C^* \) of WBG had decreased almost by 50\% (\( P<0.01 \)) compared to the \( C^* \) value immediately post-netting. \( C^* \) values of RBG fish were not influenced by the stressor. The hue of the fish was significantly elevated in RBG immediately after application of the stressor (0.05 h) compared to the control and 24.00 h RBG values (Fig. 1C; \( P<0.05 \)). Hues of fish kept on a white background were not significantly altered. On average, the hue was around 50° (red-orange) for fish kept on both backgrounds.

3.2. Physiological parameters

Na\(^+\) levels in the plasma showed an increase immediately after application of the stressor (Fig. 2A; \( P<0.05 \) for WBG fish). Levels increased up to 2.00 h post-netting (significantly higher than control values for both groups of fish) and had decreased to control values at 8.00 and 24.00 h.

The strongest rise immediately after application of the stressor was seen for plasma lactate and cortisol levels (Figs. 2 and 3). Plasma lactate levels were significantly elevated at this time point (0.05 h) in WBG fish (Fig. 2B; \( P<0.05 \)). At 2.00 h after netting, levels were significantly higher than control values in fish from both backgrounds (\( P<0.05 \)). At 8.00 and 24.00 h, levels had decreased below control levels in both groups. The pH of the plasma showed a significant decline at 2.00 h post-netting compared to control values in WBG fish (Fig. 2B). In RBG fish, plasma pH did not change significantly over time.

Plasma glucose did not increase until 2.00 h post-netting (Fig. 2C); this increase was significant for both groups of fish (\( P<0.05 \)). At 24.00 h, glucose levels had declined back to control levels.

Plasma cortisol values remained elevated compared to control values up to 8.00 and 24.00 h after netting (Fig. 3A). The increase was significant at 2.00 h post-netting (both backgrounds; \( P<0.05 \)), where levels had increased up to 300 ng/ml. There were no differences in the response to the stressor between RBG and WBG fish. Plasma αMSH levels showed a similar response pattern over time, but there were no significant differences between background colour treatments or through time (Fig. 3B).
Plasma Ca\(^{2+}\) levels tended to be elevated at 2.00 h post-netting in both groups, but these were not significantly higher than those in controls (Table 1). Ca\(^{2+}\) values decrease below control values at 8.00 and 24.00 h, a difference which was significant only in WBG. Plasma K\(^{+}\) and Cl\(^{-}\) levels remained similar to control values at 0.05 and 2.00 h post-netting, and showed a significant decrease at 8.00 and 24.00 h compared to the levels at 0.05 and 2.00 h post-netting (Table 1).

**Discussion**

Whereas background adaptation clearly modified the skin colour of red porgy, it did not alter the response to an acute stressor (netting the fish above the water surface). The physiological response to the stressor did not differ between RBG and WBG fish, and showed a strong increase in plasma Na\(^{+}\), lactate, glucose, and cortisol concentrations. αMSH levels were not correlated with skin colour in red porgy.

After three weeks of background adaptation, red porgy shows differences in colour pattern that are mainly attributable to the lightness of the skin. On a white background, fish generally have a lighter skin as indicated by a higher \(L^*\)-value. Chroma was higher in RBG fish than in WBG fish before and 24 h after the stressor, a difference that was absent immediately after application of the stressor. Upon stressing, the \(L^*\)-value did not change compared to control values. Chroma did not change after application of the...
stressor, yet was decreased in WBG fish after 24 h. Apparently, fish became paler (also indicated by increased L*-value through time in the WBG fish). Transient colour changes in fish are a well known phenomenon due to motile responses of the chromatophores (Burton, 2002; Fujii, 2000), but colour changes after stress are not well documented. Studies on social interactions in flounder indicate that social stress can result in colour pattern changes to signal submission or dominance (Höglund et al., 2000, 2002). Too little is known about social interactions in red porgy to translate these findings to a possible function of stress-induced paling. The hue of the body increased immediately after applying the stressor only in the RBG fish, although a similar trend of change was observed in WBG fish. This may indicate a transient shift of red porgy body colour from a red to a more yellow colour upon being stressed. However, since this shift comprises only a few degrees, the colour change seems minor and unlikely to be of physiological significance.

The lack of correlation between background colour and αMSH levels indicates that αMSH may not be involved in the skin pigmentation of red porgy. In previous studies on red porgy and related species such as the gilthead seabream, S. auratus, similar results were obtained. Szisch et al. (2004) and Salm et al. (2004b) found that adaptation to different backgrounds and illumination did not influence the plasma αMSH levels of red porgy. In amphibians the involvement of αMSH in background adaptation has been reported on numerous occasions (Fernandez and Bagnara, 1991; Högben and Slome, 1931; Roubos, 1997), but in fish the picture is much less clear. Eel, trout and catfish have been reported to show increased plasma levels of αMSH when kept on a black background (Baker et al., 1984), yet in flounder and sea bream such correlations are absent (Szisch et al., 2004). It seems that in the latter species, as in red porgy, other neurohormones such as catecholamines or melanin concentrating hormone (MCH) may have a more dominant role in colour change than αMSH.

The stress response of teleost fish comprises three subsequent stages: the primary stress response, characterised by increases in plasma catecholamines and cortisol; the secondary stress response wherein the former hormones influence energy mobilization and disturb hydromineral balance and the tertiary stress response which mainly involves long term inhibition of growth, immune functions and an inability to cope with additional stressors (Wendelaar Bonga, 1997). In this paper, we show that in red porgy, the primary stress response, after confinement and air exposure during netting above the water surface, is characterised by increased levels of cortisol, glucose, lactate and Na⁺ in the plasma. The very rapid increases in plasma glucose, lactate and Na⁺ levels suggest that these rises result from a catecholaminergic stimulation rather than from activation of the HPI-axis, and this is in line with the literature (Wendelaar Bonga, 1997). Air exposure leads to hypoxia and plasma acidosis (Arends et al., 1999; Vijayan et al., 1997) and indeed red porgy responds with a decrease in plasma pH at 5 min and 2 h after netting.

While Na⁺ levels show an increase immediately after application of netting stress, the levels of all other plasma ions measured are not significantly altered within 2 h after netting. This may indicate a selective gain of ions that enter the fish from the hyperionic environment. After 2 h however, all ion levels return to or drop below basal control levels, suggesting that the permeability of the gills has returned to normal and indicating that the hydromineral balance has actively and quickly been restored.

Upon exposure to an acute stressor such as netting above the water surface, cortisol levels in the plasma of red porgy increase around tenfold compared to basal cortisol values (0–32 ng/ml). Consensus exists that such swift increases are mediated by a release of corticotropin-releasing hormone (CRH) from the hypothalamus which stimulates the release of adreno-corticotropic hormone (ACTH) from the pituitary gland which, in turn, stimulates cortisol release by the interrenal tissue in the head kidneys (Wendelaar Bonga, 1997). A possible role for αMSH in the release of cortisol in tilapia has been described by Balm et al. (1995). Studies by Lamers et al. (1992) on this species indicate that the CRH–ACTH–cortisol axis predominates during acute stress situations while a TRH–αMSH–cortisol axis can be activated during chronic stress. In this study, the rise in plasma cortisol levels is not preceded by a rise in αMSH levels, indicating that in red porgy, an acute stressor activates the CRH–ACTH–cortisol axis. Arends et al. (1999) showed that 3 min of air exposure led to an increase of αMSH levels in the plasma of gilthead sea bream. These authors suggest that a stressor such as air exposure leads to acidosis in the plasma, resulting in increased release of αMSH. Due to high individual variation in plasma αMSH.

Table 1
Plasma ion levels in red porgy adapted to a red and white background after 5 min of netting and air exposure at time 0

<table>
<thead>
<tr>
<th></th>
<th>K (mEq/l)</th>
<th>Cl (mEq/l)</th>
<th>Ca (mEq/l)</th>
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<tbody>
<tr>
<td></td>
<td>Red</td>
<td>White</td>
<td>Red</td>
</tr>
<tr>
<td></td>
<td>5.53 ± 0.18</td>
<td>5.53 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>180.7 ± 2.6</td>
</tr>
<tr>
<td>0.05 h</td>
<td>6.02 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.62 ± 0.37</td>
<td>185.4 ± 2.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.00 h</td>
<td>6.28 ± 0.74</td>
<td>5.62 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>179.4 ± 4.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>8.00 h</td>
<td>3.86 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.06 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>165.3 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>24.00 h</td>
<td>3.86 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.93 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>162.5 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Significance between values is denoted by: a vs. b = P < 0.05. Values sharing the same letter are not significantly different; nsd = no significant differences.
levels in red porgy we cannot confirm this hypothesis, although there appears to be a trend on both backgrounds for αMSH levels to rise within the first 2 h after application of the stressor. The release of cortisol was not accompanied by an increase of ACTH levels in the plasma, which led these authors to conclude that perhaps the increase of cortisol resulted from sympathetic activity: acetylcholine, the neurotransmitter of parasympathetic fibres stimulates interrenal cells directly to release cortisol (Arends et al., 1999). With respect to an involvement of αMSH in cortisol release, it is noteworthy to mention that recent research in carp has shown a direct projection of CRH-containing neurons on the pars intermedia of the pituitary gland (Huising et al., 2004) and thus hypothalamic CRH-neuron activity would result in αMSH release. However, as yet only an MC2 receptor has been found in the head kidney of fish, which is selective for ACTH. An MC5 receptor could not be demonstrated and this would exclude αMSH as a corticotropic (Klovins et al., 2004; Metz et al., 2005). On the other hand, the pleiotropic nature of this hormone, combined with numerous observations on plasma increases during acute stress indicates that αMSH may have an as yet unknown function in the stress response (e.g., food intake or immunological responses; Cerda-Reverter et al., 2003; Luger et al., 2003).

The results of this study suggest that for red porgy, handling induced stress can evoke swift and strong responses, particularly in plasma cortisol, lactate and Na⁺ levels. Since handling is unavoidable in aquaculture, cultured red porgy will experience stress frequently. However, the observed darkening in red porgy under these conditions can not be attributed to the elevated αMSH levels. Skin colour was not correlated with plasma αMSH levels. Adaptation to a white background could lighten the skin, yet without any involvement of αMSH. Background colour did not alter the stress response to netting; for fish from either a red or white background the response followed a similar pattern: immediate increases in cortisol, lactate and Na⁺, followed by increased glucose levels and a drop in plasma pH. From 8 h onwards, most parameters were restored.

Our results emphasize the need to evaluate neural and endocrine involvement in skin colour regulation. The current trend to diversify fish aquaculture has brought several species into the attention of researchers. Amongst other factors, this has markedly increased the number of fish species currently studied in the field of body colour regulation, and it is becoming clear that enormous differences occur in the main pathways to regulate the skin colour. For the red porgy, it seems that not αMSH but catecholaminergic pathways are more important in control of skin colour during aquaculture related stress.

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References


