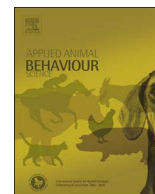




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## Oxygen consumption and swimming performance in Arctic charr with different pigmentation patterns

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### ABSTRACT

Pigmentation in animals often reflects behavioural and physiological traits, such as health status, stress responsiveness, and dominance. Individual variation in stress response and behaviour has earlier been proposed to be connected to differences in metabolism. The aim of this study was to investigate the connection between pigmentation, behaviour, and oxygen consumption in Arctic charr (*Salvelinus alpinus*). In this species, we have earlier found an association between number of carotenoid spots on the skin and physiological response to stress (stress coping style). Swimming endurance and respiration rates were estimated from video recorded behaviour and oxygen consumption in a swim tunnel. Flow velocity was kept as 1.6 BL (body lengths) s<sup>-2</sup> for 60 min, and oxygen consumption (mg min<sup>-1</sup> g<sup>-1</sup>) during that time was used as a proxy for metabolism. Oxygen consumption was negatively correlated with number of spots. Also behaviour scores from a principal component analysis varied with pigmentation, with a negative correlation between number of spots and behaviour scores connected with endurance. Fish with fewer spots rested more against the rear of the chamber, and fish with more spots were sooner, and more often, pressed to the rear grid by the water current. The variation in oxygen consumption in the swim tunnel indicates a relation between respiration rate, swimming endurance, and pigmentation in the Arctic charr. With earlier findings on covariations between spot numbers and stress coping in this species, there seems to be connections between stress coping style, behaviour, and metabolism. Thus, it would be possible to identify individual expression of these features, based on the pigmentation patterns of the fish.

### 1. Introduction

Pigmentation in animals often serve as a signal, indicating, for example, health status, immunity, dominance ranking, or sexual state (e.g. Kekalainen et al., 2010; Svensson and Wong, 2011; Vroonen et al., 2013; D'Alba et al., 2014; Johnson and Fuller, 2015). Thus, the intensity of coloration could vary over time according to the individual's condition, although pigmentation may also be heritable to some extent (e.g. Gorshkov, 2014; Nilsson et al., 2016; Rankin et al., 2016). Sometimes the covariations between pigmentation and behavioural and physiological traits are due to genetic pleiotropic effects (Ducrest et al., 2008). Individual variation in stress responsiveness is well-known in animals (Koolhaas et al., 2007), with animal populations ranging between proactive stress-coping style, with a low hypothalamic–pituitary–adrenal (HPA) axis reactivity, high aggression levels, and social dominance, and reactive stress-coping style, with a high HPA axis reactivity, low degree of aggression, and low social ranking (Koolhaas et al., 1999; Koolhaas et al., 2007). Both melanin- and carotenoid-based coloration have been connected to stress-coping in vertebrates. The

melanin production may in some cases be associated to glucocorticoid regulation, sexual activity, and aggressiveness (Roulin and Ducrest, 2011). Vertebrates with darker skin or feathers are generally more aggressive, sexually active, and resistant to stress compared to lighter individuals of the same species (Ducrest et al., 2008). Also carotenoid-based coloration could reveal an individual's ability to cope with stressors (Mougeot et al., 2010). In salmonid fish, the pigmentation in terms of melanin or carotenoid spots has been shown to be correlated with stress coping style (Kittilsen et al., 2009; Backström et al., 2014; Backström et al., 2015a). Furthermore, positive connections between carotenoid-based pigmentation and physical stamina or health have been found in several fish species (Nicoletto, 1991; Nicoletto and Kodric-Brown, 1999; Guderley and Couture, 2005; Johnson and Fuller, 2015).

It has been proposed that there are links between measures of performance in terms of physiology and behaviour and metabolism (Careau et al., 2008; Biro and Stamps, 2010; Martins et al., 2011; Binder et al., 2016; Metcalfe et al., 2016). Individuals vary in their acquisition and use of energy, and there may be a relationship between

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metabolic rate and behavioural syndrome/stress coping style (Huntingford et al., 2010; Killen et al., 2012; Holtmann et al., 2017). In general, animals with a higher metabolism are bolder and more aggressive (Metcalfe et al., 2016; Treberg et al., 2016). However, the connections may be dependent of context (Killen et al., 2011; Killen et al., 2012) or sex (Sichova et al., 2014).

The Arctic charr (*Salvelinus alpinus*) shows pronounced individual variation in skin coloration and density of light carotenoid-based spots. We have earlier found that there is a connection between pigmentation and stress coping style in a domestic strain of this species (Backström et al., 2014; Backström et al., 2015a). Arctic charr with few spots have lower plasma cortisol levels after a stressor (Backström et al., 2014), move more in a restraint test (Backström et al., 2016), and are more likely to become dominant (Backström et al., 2015a), compared to individuals with many spots. Thus, few spots indicate a proactive stress-coping style, while fish with many spots show a reactive stress-coping style (Koolhaas et al., 1999, 2007). We have also found that number of spots are heritable (Nilsson et al., 2016) and consistent over time (Brännäs et al., 2016). Thus, the density of light-coloured spots can be used as a non-invasive method in selective breeding to grade individuals into proactive or reactive individuals (Brännäs et al., 2016). In that context it is important to evaluate a possible relationship between spottiness and oxygen consumption. This may have an effect on the feed conversion factor which is an important economic as well as environmental factor in fish farming.

The aim of this study was to investigate a potential connection between pigmentation, metabolism and swimming endurance in the Arctic charr, estimated from behaviour and oxygen consumption in a swim tunnel. With earlier finding of connections between pigmentation and stress coping style, and between stress coping style and metabolism, we expected that fish with differences in spottiness also would differ in swimming performance and oxygen consumption.

## 2. Methods

### 2.1. Study animals

The study was carried out 24–26 May, 2016, at the Aquaculture Centre North in Kälärne, Sweden (62°58'N, 16°50'E). We used one-year old Arctic charr from the 7th generation of the Swedish Arctic charr breeding program (Nilsson et al., 2010). The fish were bred and kept in the stocking facilities in tanks (10 m<sup>3</sup>) supplied with running water from the nearby lake Ansjön at natural temperatures, with a photoperiod set to 12 h dark/12 h light. The fish were fed continuously by automatic feeders with commercial pellets (Biomar, 4 mm, [www.biomar.com](http://www.biomar.com)) at 1.0–1.5% of body mass per day depending on temperature. The Arctic charr used in this study had a body length (mean ± SD) of 25.4 ± 1.8 cm, and a body mass of 220.5 ± 55.3 g (n = 18).

### 2.2. Experimental set-up

The fish were tested in a 30 L swim tunnel (SW10150, Loligo® systems, Tjele, Denmark) (Fig. 1) with a chamber size of 55 × 14 × 14 cm. A speed control and an external motor were used to adjust the water current in the test chamber. Flow calibrations were made with a flow velocity meter (Swoffer Instruments Inc®, MODEL 3000, Seattle USA), to determine the correlation between RPM measures on the motor controller and flow velocity (cm s<sup>-1</sup>). A pilot study was performed to determine optimal test flow to 1.6 body lengths per second (BL s<sup>-1</sup>). With this speed the fish had to swim intensively but was not completely exhausted after the 60 min of high flow exposure.

Oxygen (% saturation) and temperature in the chamber were measured continuously, using a fibre optic oxygen probe and a temperature probe, registering via Witrox 1 (Loligo® Systems) to a computer. A handheld oxygen and temperature instrument (YSI model 55, YSI Inc., Yellow Springs, USA) was used to validate the measurements. The temperature in the swim tunnel during the experimental period was 9–10 °C.

The day before the start of the experiment, ca 20 Arctic charr were taken randomly from the stocking facilities and placed in a holding tank (1 m<sup>3</sup>) near the stream tank. After each test the individual fish was placed in another similar tank, to make sure that each fish was used only once. The fish were not fed before the trial, but in the second tank they were fed manually after the trial. When the trials were finished, all fish were returned to their original location.

Before each swimming trial, a fish was taken from the holding tank, anaesthetized with Tremaine methane sulfonate (MS-222, 0.15 g L<sup>-1</sup>), and length and body mass were measured. The fish was then placed into the fish test chamber to acclimate for 1 h. During this time the water was aerated with an air stone, and water current kept at a minimal speed to distribute the oxygen evenly in the water. Following the 1 h recovery period, the aeration was stopped and the swim tunnel sealed, to prevent any addition of oxygen from the outside. The speed of the water was increased, first to 0.6 BL (body lengths) s<sup>-1</sup> and then by 0.1 BL s<sup>-1</sup> per minute during a 10 min period, up to 1.6 BL s<sup>-1</sup>, according to the ramp velocity test described in (Jain et al., 1997). The test fish was filmed from the side to analyse the swimming behaviour during 1 h with a flow velocity of 1.6 BL s<sup>-1</sup>. After this, the fish was removed from the chamber, anaesthetized, photographed on the left side using a Canon EOS 500D digital camera and a box with a transparent lid (see also Backström et al., 2015a). Immediately after photographing, the fish was sampled for blood via the caudal vein, and after recovering from the anaesthesia it was placed in a holding tank. The blood was subsequently centrifuged at 10,000g for 5 min; the plasma was collected and stored at -20 °C until further analysis. These samples were made to investigate whether oxygen consumption and behaviour were connected to stress responsiveness.

Plasma was analysed for cortisol using a commercial enzyme linked immunosorbent assay (ELISA) kit (product # 402710, Neogene Corporation, Lexington, USA). Each sample was run in duplicate during a single assay with an intra-assay coefficient of variation of 1.0%.

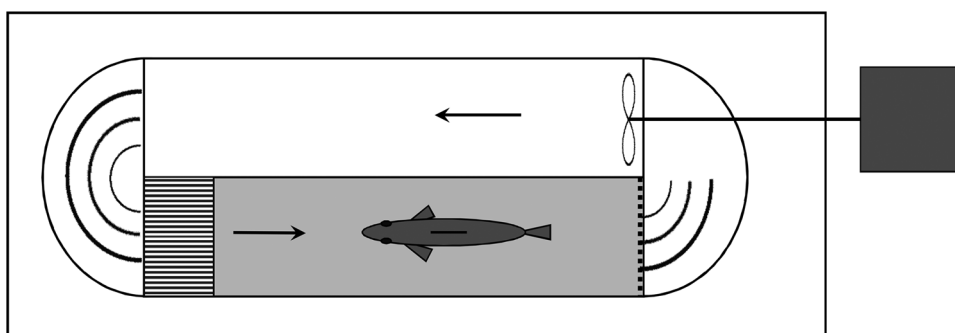


Fig. 1. Swim tunnel (30 L) used for trials on swimming performance and oxygen consumption in Arctic charr. Shaded area shows the swim chamber. Flow velocity is created by a propeller, driven by an electric motor (black square), with speed control. The water is entering the chamber through a honeycomb structure for laminar flow (striped area). At the rear end of the chamber is a metal grid (broken line). Arrows indicate the direction of the water.

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