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Effect of predictability on the stress response to chasing in Atlantic salmon (*Salmo salar* L.) parr

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HIGHLIGHTS

- Signalled predictability did not reduce stress level after chasing in salmon parr.
- Behavioural but not physiological responses to the predicting signal were found.
- With two daily chasing events salmon habituated within a week
- The rapid habituation may be due to other sources of predictability than the signal.
- Benefits of predictability may be limited if avoidance of stressors is not possible.

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ABSTRACT

The possibility to prepare for and respond to challenges in a proper manner is essential to cope with a changing environment, and learning allows fish to up or downregulate the stress response based on experience. The regulation of the response to predicted needs should be easier in more predictable environments. We exposed salmon parr to chasing of either 15 s (weak stressor) or 5 min (strong stressor) twice daily for a 7-day learning period, with chasing either announced by a 30 s light signal (conditioned) or not announced (unconditioned). The behavioural response to the light signal was different between the conditioned and unconditioned groups, demonstrating that conditioned groups associated the signal with chasing. We could, however, not demonstrate any effect on the stress response of anticipation. The fish habituated to repeated stress exposures with a similar decrease in oxygen hyperconsumption in all groups. Due to habituation, possible effects of predictable announcement of a stressor on the physiological stress response may not have been expressed in this study. Plasma cortisol concentrations 1 h after light signal and chasing the day after the training period was moderate in all groups although higher after 5 min chasing (13 ng ml^{-1}) than 15 s chasing (7 ng ml^{-1}). There was no physiological stress response after exposure to the light signal only after the learning period. We argue that the benefit of predictability of stressors is limited when the fish have no way to avoid the stressor.

1. Introduction

It is essential for animals to prepare for forthcoming events in order to respond in a proper manner and intensity. Predictability allows for learning when certain events are likely to occur [1] and decreases the degree of discrepancy between internal prediction (set values) and the reality (actual value) [2]. There are different kinds of predictability [3]; temporal predictability is when events occur at even intervals or at fixed times of day, whereas 'signalled' predictability is when events are announced by a cue. Pavlovian conditioning [4] is an example of signalled predictability. The constancy of the quality of the event, for instance the duration, represents a third type of predictability.

In accordance with the allostasis concept, fish facing a challenge (e.g. stress) should adjust their psychological and physiological responses according to actual demand and not spend more resources than necessary, and thus maintain stability through change [5]. Fish in predictable environments should be better to up- or down regulate their responses according to the expected stress intensity compared to fish in unpredictable environments [6]. Previous findings suggest that rats, birds and fish, when given a choice between predictable and unpredictable electric shocks generally prefer a

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shock with a highly signalled predictability, regardless of whether they actually are able to escape from it or not [7]. Although predictable negative events often reduce the cortisol response [6], results from studies relating predictability to physiological measures of stress are less consistent [3]. To what extent anticipation will allow fish to avoid the forthcoming stressor could influence the stress response [8]. In aquaculture conditions fish are confined in a limited water volume and have generally few possibilities to avoid the stressor.

Fish can learn to anticipate a stressor by conditioning to stimuli presented before the event [9]. However, during repeated exposure to stressful events the response may decrease over time by the process of habituation [10] regardless of whether fish go through a conditioning regime or not. For example, the oxygen hyperconsumption of salmon parr exposed to daily, sudden transitions from darkness to bright light is habituated at a rate of 24% per day [11]. In this way, over time, fish could cope with stressors that are not perceived as too aversive.

In the present study we investigate the effect of signalled predictability in groups of salmon parr that were repeatedly exposed to chasing events that are either announced by a signal or not. In order to disentangle the effects of habituation and anticipation we exposed the fish to two different durations of chasing. The effect of anticipation and the strength of the stress response were evaluated using behavioural (swimming pattern) and physiological (cortisol, oxygen hyperconsumption) analyses.

2. Materials and methods

2.1. Experimental fish and set-up

Atlantic salmon parr eggs were obtained from a commercial farm (Aqua Gen AS, Trondheim, Norway), and hatched at the Institute of Marine Research (IMR), Matre, Norway. After hatching, salmon fry were kept in an indoor circular tank (3 m diameter, 10,000 L). When the fry had reached the parr stage and a mean size of 48 g (360 fish bulk weight) they were transferred to the experimental tanks (squared 1.5 m tanks filled with 53 cm freshwater of 9.4 °C (min: 8.7 °C and max: 10.2 °C), volume 1200 L) and randomly divided into groups of around 400 individuals (18 kg m^{-3}) in each of 12 tanks. Each tank was covered with a lid furnished with two neon tubes and a small window through which the fish were sampled or stressed. The light regime was 24:0 L:D. Fish were fed *ad libitum* with feed (Nutra Olympic 2 mm, Skretting, Norway) delivered continuously throughout the 24-h cycle by automatic feeders (Arvo-Tec T drum 2000, www.arvotec.fi). The fish were allowed to acclimate for 20 days before the start of the experiment.

A light bulb (12 V, 21 W) positioned immediately below the surface at the tank wall was used to deliver the conditioned stimulus (CS, see below). A camera (Seavision Subsea Light, Scan Secure, Norway) positioned midwater in the centre of the tank pointing towards the wall was used to record fish behaviour and distribution. Oxygen saturation (% of air saturation) in the water was recorded every 30 s throughout the experiment using a probe (Oxyguard Commander, Oxyguard International, Denmark, www.oxyguard.dk) positioned 20 cm above the bottom near the tank wall.

All experiments were approved by the Norwegian Experimental Animal Committee (Forsøksdyrutvalget, 2012/236291–1).

2.2. Procedure

The fish were chased manually with a brush twice daily with 6-hour interval (morning and afternoon) for 15 s (weaker stressor) or 5 min (stronger stressor) for a 7-day period, in total 14 trials. In the conditioned groups a conditioning stimulus (CS) that consisted of 30 s of light flashes (1 s interval) from the light bulb was given right before stress exposure and thus announcing chasing. The CS light was switched off outside the CS period. Due to practical limitations there was a delay of on average 13 ± 7 s from the moment the CS ended to the opening of the window. The opening of the window defined the start of chasing. In the unconditioned control groups the CS was given 2 h after chasing. The combinations of chasing duration and conditioning procedure thus resulted in four treatments groups (in triplicates): conditioned 15 s: light flashes + chasing for 15 s; conditioned 5 min: light flashes + chasing for 5 min; unconditioned 15 s: chasing for 15 s + light flashes 2 h later; unconditioned 5 min: chasing for 5 min + light flashes 2 h later. Video recordings of the CS period, starting 20 s before the onset of the CS, were analysed with respect to the behavioural response.

2.3. Cortisol response to chasing

After the 7-day learning period (Day 8), plasma from 10 fish in each tank was sampled for cortisol analysis before chasing (after presentation of the CS and opening of the window) as well as 1 h after chasing. To avoid that the conditioned groups received a stronger stimulation than the unconditioned groups, the CS was presented to all groups, but 3 min before chasing in the unconditioned groups to avoid that the CS became associated with chasing. In reward conditioning of cod [12] and halibut [13] conditioned response was weak or absent after >60 trials when the time gap between a light-CS and a food-US was 2 min, and we therefore assumed that a 3-min gap was long enough to prevent association here. The sample fish were rapidly netted and anaesthetised with an overdose of metacain (>150 mg l^{-1}, FINQUEL vet., ScanAqua AS, Årnes, Norway) buffered 1:1 with sodium bicarbonate, and blood was drawn from the caudal veins using 1-ml heparinised syringes fitted with 20G needles. Blood samples were centrifuged at 13,000 rpm for 3 min, and the plasma stored at −80 °C for later analyses.

2.4. Cortisol and oxygen response to CS only

The subsequent day (Day 9) all groups were presented with the CS only in order to test if the light signal had become associated with chasing and induced stress responses of different intensities in fish exposed to 15 s and 5 min chasing. Ten fish per tank were sampled 1 h after the CS and blood samples for cortisol analyses were collected as described above. The oxygen consumption in response to the CS only was also calculated.

2.5. Analysis of oxygen consumption

Oxygen consumption rate (VO_2, mg O_2 kg^{-1} min^{-1}) for each replicate group was measured by using the experimental tanks as open respirometers. Experiments in similar tanks and similar oxygen consumption rates suggest that the diffusion of air oxygen into the system is negligible compared to consumption of the fish (T. Torgersen, Institute of Marine Research, personal communication). The oxygen consumption rate per tank was calculated by measuring the difference in oxygen content between influent and effluent water. The following equation was used:

$$VO_{2_t} = Vol \cdot Sol \cdot \frac{Sat_t - Sat_{t-1}}{100\delta_t} + Flow \cdot Sol \cdot \frac{Sat_{in} - Sat_t}{100}, \quad (1)$$

where *Vol* is the tank volume, *Sol* is the solubility of oxygen at prevailing temperature and conductivity conditions, Sat_t is the oxygen saturation at time t, $\delta_t = 30$ s, *and* Sat_{in} is the oxygen saturation in the influent water (in this case 100.8 ± 0.42%, mean ± S.D.). Oxygen hyperconsumption for each trial was calculated by subtracting the average consumption rate for the 30-min period immediately before chasing (baseline consumption) from the average rate during the 30-min period following after start of chasing.