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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 behaviour 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:

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

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.

2.6. Plasma cortisol analyses

Plasma cortisol concentrations were quantified by custom radioimmunoassay [14] using a commercially available antibody (Abcam) and ^{3}H-cortisol (PerkinElmer). The primary antibody shows a 100% specificity towards cortisol and a cross reactivity of 0.9% with 11-deoxycortisol, 0.6% with corticosterone, and $<$0.01% with 11-deoxycorticosterone, progesterone, 17-hydroxyprogesterone, testosterone and oestradiol. Inter- and intra-assay variations were 12.5 and 3.5%, respectively.

2.7. Analysis of behaviour

A characteristic fright reaction in salmon is an immediate dive to the bottom of the tank, that can easily be estimated by image analysis [15]. However, in the present study all groups in all trials descended to avoid the CS light flashes, although seemingly fastest in the early trials, and it was therefore difficult to use the diving response to estimate anticipation of chasing in this way. Instead, the synchronized positive rheotactic orientation of salmon maintaining a fixed position in the current was used as indication of a non-stressed behaviour in resting conditions. Thus, the number of fish turning and swimming with the current direction crossing a vertical line of reference during the CS provided a measure of the behavioural response in anticipation of chasing. As the group behaviour was especially chaotic when fish descended at the onset of the CS making analysis difficult, the first 10 s of the CS period were excluded. The 20 s immediately before the onset of the CS was used as baseline level.

2.8. Statistical analyses

All statistical analyses were done with R software system Version 2.12.0 (Copyright 2010, The R Foundation for Statistical Computing, Vienna, Austria). Effect of trial number on the oxygen hyperconsumption response was evaluated by fitting the data to a Non-linear Least Square model:

$$\text{Response} = a + b \cdot e^{(trial-1)c} \qquad (2)$$

using the “nls” method. Here, $a + b$ corresponds to the modelled initial response (trial 1), a is the modelled residual response at trial ∞, b is the portion of the initial response that eventually is lost, and c is the exponential decay rate (trial^{-1}), i.e. how steeply the line drops towards a. The relationship between the exponential decay rate c and percent change per trial is given by the formula: % per trial $=$ $100^*(1 - \exp.(c))$. The model was run on each triplicate group to obtain triplicate a, b and c values which were tested between procedure groups by Welch's two sample t-test. The effects of conditioning procedure (conditioned versus unconditioned), duration of chasing (15 s versus 5 min) and interaction effects of these variables on the hyperconsumption response to CS only (Day 9) were tested with linear model.

Effects of conditioning procedure, duration of chasing, and time of sampling (before versus after chasing), and interaction effects of these variables, on plasma cortisol concentration were tested by linear model. The data did not adhere to a Gaussian distribution and were therefore log transformed (Ln). Effects on conditioning procedure and duration of chasing on the cortisol response to CS only were tested similarly but without “time of sampling” as variable.

Differences in behaviour (number of fish crossing a vertical line swimming in the current direction) between procedure groups were tested with Mann–Whitney U-test with Bonferroni correction for multiple comparisons. No within-procedure differences between triplicates were found (generalized linear model for quasipoisson distributed data, all p-values $>$ 0.05), and triplicates were pooled before tests between procedures, with all analysed trials pooled for each procedure groups. The level of significance was set to 0.05.

3. Results

3.1. Oxygen consumption

Oxygen consumption increased markedly during chasing in all trials (Fig. 1). In the first trial, the oxygen consumption was back to baseline levels around 40 min after chasing in the groups chased for 15 s, but after 5 min of chasing consumption remained markedly elevated for more than 40 min (Fig. 1). The initial response was similar also in later trials, with a clear peak during chasing and immediately thereafter. However, oxygen consumption returned to baseline levels faster with increasing experience, and in trial 14 the consumption was back to

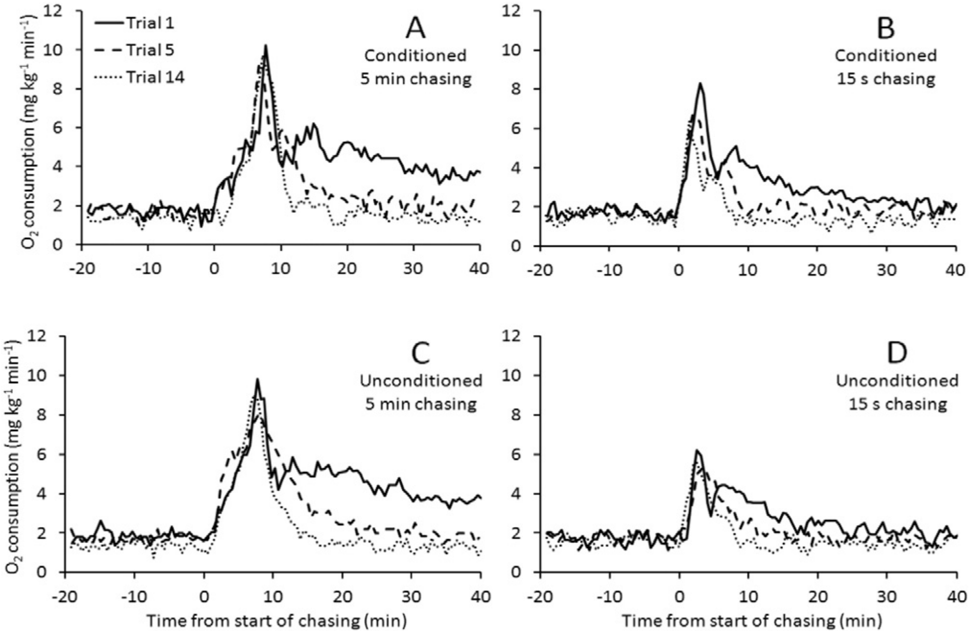

Fig. 1. Oxygen consumption of salmon parr before, during and after chasing for 5 min (A and C) or 15 s (B and D), with chasing either announced (A and B) or not announced (C and D) by a light signal. Trials 1, 5 and 14 were chosen as examples. The line represents mean consumption ($n = 3$).

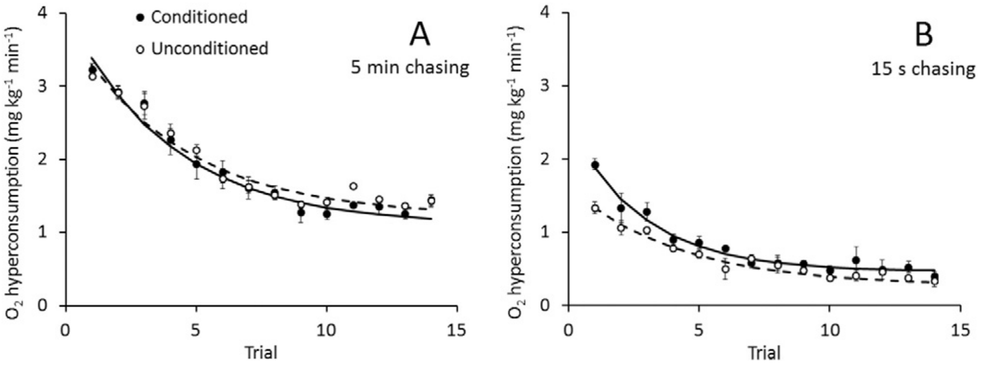

Fig. 2. Mean oxygen hyperconsumption rate (increase from baseline, mg O_2 kg^{-1} min^{-1}) during the 30 min following start of A) chasing for 5 min. The lines are given by the mean statistics from the Non-linear Least Square model (Eq. (2)); closed symbols and solid line: Conditioned 5 min, hyperconsumption $= 1.10 + 2.29 * \exp.((\text{trial} - 1) * -0.25)$; open symbols and dashed line: Unconditioned 5 min, hyperconsumption $= 1.22 + 2.08 * \exp.((\text{trial} - 1) * -0.23)$. B) chasing for 15 s. closed symbols and solid line: Conditioned 15 s, hyperconsumption $= 0.46 + 1.42 * \exp.((\text{trial} - 1) * -0.35)$; open symbols and dashed line: Unconditioned 15 s, hyperconsumption $= 0.27 + 1.06 * \exp.((\text{trial} - 1) * -0.24)$.

baseline level after around 8 min in the groups chased for 15 s and after around 15 min in the groups chased for 5 min (Fig. 1). No differences in oxygen consumption pattern were evident between conditioned and unconditioned fish, neither in peak consumption after chasing nor recovery rate (Fig. 1).

To test the trial-by-trial change in oxygen consumption statistically, the effect of trial number on the oxygen hyperconsumption during the 30-min period following start of chasing (i.e. the increase in mean consumption compared to the 30 min immediately before chasing) was tested. Oxygen hyperconsumption decreased with trial number in all groups, with the residual response a, portion of the initial response that was lost b and decay rate c different from 0 in all groups (all p-values < 0.05, $n = 3$ for each group, Fig. 2). When $a > 0$ the response is not fully ablated. No differences (all p-values > 0.05) in either a, b or c were found between conditioning procedures with the same chasing duration (5 min or 15 s). However, the initial response (trial 1) was somewhat higher in the conditioned fish chased for 15 s than in the unconditioned fish that were chased for 15 s (Fig. 2), with the proportion of the initial response lost b close to a significant difference ($p = 0.061$). The residual response a and portion of the initial response that was lost b did differ between conditioned fish chased for 5 min and 15 s (a: $p < 0.05$, b: $p < 0.01$), while the decay rate was not significantly different ($p > 0.1$).

The hyperconsumption response to CS only (Day 9) was negligible (mean for all groups: 0.047 mg kg^{-1} min^{-1}) with no effects ($p > 0.1$) of conditioning procedure or duration of chasing or interaction between these.

3.2. Plasma cortisol

After CS + chasing (Day 8), there was an effect of both chasing (before versus 1 h after, $p < 0.001$) and duration of chasing (15 s versus 5 min, $p < 0.001$) on plasma cortisol, with concentrations being higher 1 h after chasing than baseline and higher after 5 min chasing than after 15 s chasing (Fig. 3). Still, cortisol concentrations were relatively low in all groups, also 1 h after chasing. There was no effect of conditioning procedure ($p = 0.90$) and no interaction effects (procedure × duration of chasing: $p = 0.80$, procedure × chasing: $p = 0.99$). After CS only (Day 9), there was no effect of duration of chasing ($p = 0.32$) or conditioning procedure ($p = 0.94$), and no procedure × chasing interaction ($p = 0.17$) on plasma cortisol concentration 1 h after CS. The means concentrations of cortisol (ng ml^{-1}) ± S.E. in the treatment groups were: conditioned 15 s chased: 5.0 ± 0.8, unconditioned 15 s chased: 5.0 ± 0.6, conditioned 5 min chased: 4.8 ± 0.8, unconditioned 5 min chased: 4.2 ± 0.6.

3.3. Behavioural response to the CS

In all groups few fish were swimming with the current direction before the CS light flashes (Fig. 4), with no differences between conditioned and unconditioned groups, or between 5 min and 15 s chasing groups (all p-values > 0.1). However, during the CS, conditioned fish expressed a more chaotic swimming pattern with more fish swimming with the current direction than unconditioned fish (5 min chasing: $p < 0.001$; 15 s chasing: $p < 0.01$, Fig. 4). No differences were found between fish chased for 15 s and 5 min in neither the conditioned groups ($p > 0.1$) nor unconditioned groups ($p > 0.1$).

4. Discussion

Conditioned Atlantic salmon parr associated a light signal (CS) with an upcoming chasing event and responded to the CS by changing their swimming pattern, demonstrating that the fish anticipated the stressor. Yet, neither cortisol nor oxygen consumption responses were altered. The physiological stress response was more evident after the stronger stressor (longer chasing time), but conditioning did not reduce the response regardless of the intensity of the stressor. Presentation of the CS only did not result in elevated cortisol levels neither in conditioned nor unconditioned parr, although the CS induced a behavioural response in the conditioned groups. Physiological responses are not always in accordance with behavioural responses (see [16] for a review). Folkedal et al. [17,18] found that in salmon, behavioural measures such as reduced anticipatory behaviour and feed intake were

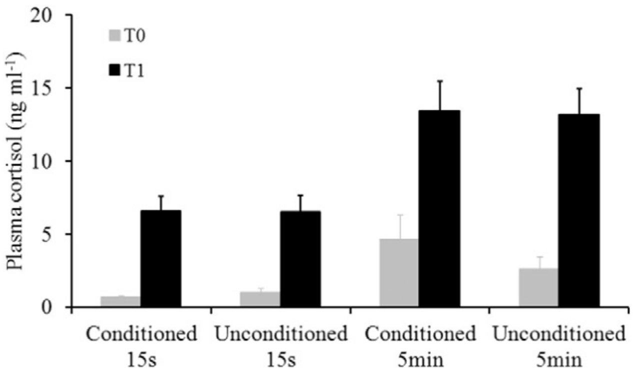

Fig. 3. Plasma cortisol concentrations (mean + S.E.) before (T0) and 1 h after (T1) CS. Fish were subjected to chasing for 15 s or 5 min after 7 days with 2 daily chasing trials with chasing either announced (Conditioned) or not announced (Unconditioned) by a CS.

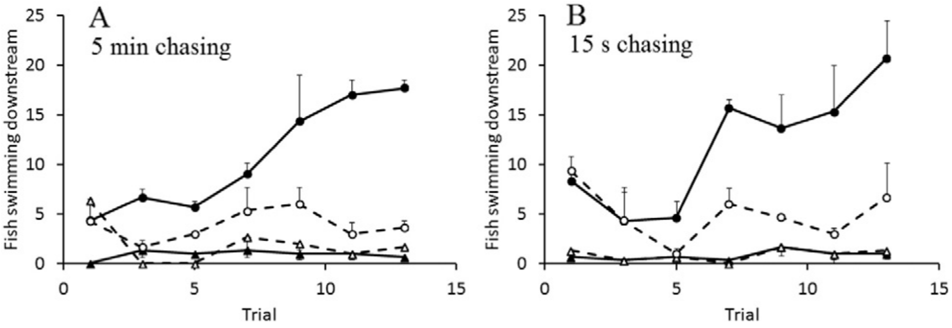

Fig. 4. Mean number of fish observed crossing a vertical line swimming with the water current direction 20 s before (triangles) and 20 s after (circles) the CS light signal (with 10 unobserved seconds immediately after the start of the signal). In conditioned groups (filled symbols) the light signal announcing chasing, while in unconditioned groups (open symbols) the fish were chased 2 h after the CS. A) chasing duration of 5 min, B) chasing duration 15 s. Error bars indicate S.E. of 3 tank groups.

affected longer after stress than physiological measures such as cortisol and oxygen consumption.

There might be several reasons why predictability of the stressor did not reduce the measured stress response. The biological relevance of light flashes and chasing and the natural link between these stimuli could be questioned as they do not occur in the wild. However, sudden changes in light intensity (shadows) are naturally linked with danger (predators). The behavioural response to the light-CS shows that the CS was associated with chasing, and a lack of a natural link between stimuli is not the most likely explanation of the low effect of predictability. As chasing occurred in the same manner every time with no severe consequences, the fish could habituate to the procedure, and habituation to the stressor resulted over time in a low physiological stress response in all treatment groups. Habituation may in this way have masked possible effects of the announcement of chasing. The observed mean maximum cortisol response of up to 13 ng per ml is moderate compared to values of approximately 200 ng per ml found in earlier stress studies [19,20]. This could mean that the "strong" stressor was not strong enough to disentangle the effect of habituation and conditioning. Furthermore, high swimming activity connected to chasing in itself could increase cortisol levels. The stronger increase in cortisol concentrations after 5 min than 15 s of chasing supports that the energy demand required for forced swimming plays a role [21,22] even if chasing was not longer perceived as aversive. In addition, unconditioned fish might have anticipated what was about to happen already when the window of the tank was removed [23], and the regular times of chasing could permit unconditioned fish to anticipate the stress by temporal predictability [3,24].

In the present setting we could thus not demonstrate that the ability to anticipate a stressor reduces the stress response. This could be because of methodological limitations, or because predictability has limited effect on the stress response of salmon, or a combination of these. Earlier studies on the effects of predictability of aversive stimuli on physiological stress responses have in fact given variable results [3]. It has been suggested earlier that when aversive stimuli are predictable they result in a lower stress response in animals because they provide feedback on safe periods, when aversive stimuli are absent [25,26]. However, no difference was found in baseline cortisol levels between the treatments in periods between stress exposures suggesting that conditioned fish were not less stressed than unconditioned fish. It is possible that the already low baseline levels in the unconditioned groups may not have allowed a further decrease in the conditioned groups.

Anticipation of the stressor may not help the parr to cope with the situation when nothing can be done to avoid/escape the stressor. Active responses may allow an animal to avoid aversive stimuli, and the control of the stressor could reduce the physiological stress response [27]. Carpenter and Summers [8] found that rainbow trout that learned to escape an aggressor did not show an increased cortisol response in contrast to fish that had not learned to escape. In larger holding units or in their natural environment, fish can cope by swimming away from danger, and anticipation of a stressor may then reduce the stress response, but this behaviour is not possible in the restricted space within the present experimental design and in intensive aquaculture in general.

An interesting methodological finding of our study is that whereas the initial oxygen hyperconsumption response to the stressor is not changed by repeated exposures, the recovery rate in consumption after chasing is. First time exposure to 5 min chasing resulted in a long-lasting (>40 min) stress response, but when fish had experienced chasing a number of times, they still initially reacted with the same intensity but recovered more rapidly. The fish could have learned that when the stress ends they could anticipate a long period without disturbance. Therefore, while the peak of the response could be the required physiological support for the change in physical activity, the enhanced speed of recovery may be interpreted as increased predictability and controllability of the stressor [28]. Hence, when studying habituation to repeated stimuli in animals, the temporal dynamics of the stress response requires particular attention with regard to when the response is recorded: in studies where only one single instantaneous response is measured we risk to not get the full picture (see also [29]).

We could not demonstrate that conditioned salmon parr that were able to anticipate a stressor became less stressed than unconditioned parr. Exposing fish to stronger stressors may allow us to disentangle the effects of habituation and conditioning. Although the lacking effect of conditioning could have several reasons, our findings suggest that knowledge of what will take place in the near future does not always make fish cope with stress in a different way. The change in behaviour in conditioned fish already during the conditioning signal before a stressful event could in fact increase the time the fish experience stress. Anticipation of stress may thus not always have a positive effect and it may be critical if the fish have the option to avoid the stressor by an active response, and this option is generally not present under farming conditions. Unpredictability related to husbandry routines may increase stress levels [30]. In future studies it would be interesting to test if conditioning connected to a harmless procedure that is initially identical to a stressful procedure [31] could enable the fish to distinguish between the two procedures and thereby reduce unnecessary stress.

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References

[1] P. Sterling, Principles of allostasis: Optimal design, predictive regulation, pathophysiology and rational therapeutics, in: J. Schulkin (Ed.), Allostasis, homeostasis and the costs of physiological adaptation, Cambridge University Press, Cambridge, UK 2004, pp. 17–64.

[2] H. Ursin, H.R. Eriksen, The cognitive activation theory of stress, Psychoneuroendocrinology 29 (2004) 567–592, http://dx.doi.org/10.1016/S0306-4530(03)00091-X.

[3] L. Bassett, H.M. Buchanan-Smith, Effects of predictability on the welfare of captive animals, Appl. Anim. Behav. Sci. 102 (2007) 223–245, http://dx.doi.org/10.1016/j.applanim.2006.05.029.

[4] I.P. Pavlov, *Conditioned Reflexes*. An investigation of the physiological activity of the cerebral cortex, Oxford Univ. Press, Oxford, UK, 1927.

[5] P. Sterling, J. Eyer, Allostasis: a new paradigm to explain arousal pathology, in: S. Fisher, J. Reason (Eds.), Handbook of Life Stress, Cognition and Health, Wiley, New York 1988, pp. 629–649.

[6] L. Galhardo, J. Vital, R.F. Oliveira, The role of predictability in the stress response of a cichlid fish, Physiol. Behav. 102 (2011) 367–372, http://dx.doi.org/10.1016/j.physbeh.2010.11.035.

[7] P. Badia, J. Harsh, C.C. Coker, B. Abbot, Choice and the dependability of stimuli that predict shock and safety, J. Exp. Anal. Behav. 26 (1976) 95–111, http://dx.doi.org/10.1901/jeab.1976.26-95.

[8] R. Carpenter, C. Summers, Learning strategies during fear conditioning, Neurobiol. Learn. Mem. 4 (2009) 415–423, http://dx.doi.org/10.1016/j.nlm.2009.01.009.

[9] J.B. Overmier, K.L. Hollis, Fish in the think tank: learning, memory and integrated behavior, in: R.P. Kesner, D.S. Olton (Eds.), Neurobiology of Comparative Cognition, Lawrence Erlbaum Associates, Hillsdale, USA 1990, pp. 204–236.

[10] D.A. Lieberman, Learning: Behaviour and Cognition, Wadsworth, Belmont, CA, 2000.

[11] O. Folkedal, T. Torgersen, J. Nilsson, F. Oppedal, Habituation rate and capacity of Atlantic salmon (*Salmo salar*) parr to sudden transitions from darkness to light, Aquaculture 307 (2010) 170–172, http://dx.doi.org/10.1016/j.aquaculture.2010.06.001.

[12] J. Nilsson, T.S. Kristiansen, J.E. Fosseidengen, A. Fernö, R. Van den Bos, Learning in cod (*Gadus morhua*): long trace interval retention, Anim. Cogn. 11 (2008) 215–222, http://dx.doi.org/10.1007/s10071-007-0103-6.

[13] J. Nilsson, T.S. Kristiansen, J.E. Fosseidengen, L.H. Stien, A. Fernö, R. van den Bos, Learning and anticipatory behaviour in a “sit-and-wait” predator: the Atlantic halibut, Behav. Process. 83 (2010) 257–266, http://dx.doi.org/10.1016/j.beproc.2009.12.008.

[14] M. Gorissen, N.J. Bernier, R. Manuel, S. de Gelder, J.R. Metz, M.O. Huising, G. Flik, Recombinant human leptin attenuates stress axis activity in common carp (*Cyprinus carpio* L.), Gen. Comp. Endocrinol. 178 (2012) 75–81, http://dx.doi.org/10.1016/j.ygcen.2012.04.004.

[15] L.H. Stien, S. Bratland, I. Austevoll, F. Oppedal, T.S. Kristiansen, A video analysis procedure for assessing vertical fish distribution in aquaculture tanks, Aquac. Eng. 37 (2007) 115–124, http://dx.doi.org/10.1016/j.aquaeng.2007.03.002.

[16] P. Jensen, F.M. Toates, Stress as a state of motivational systems, Appl. Anim. Behav. Sci. 53 (1977) 145–153, http://dx.doi.org/10.1016/S0168-1591(96)01156-2.

[17] O. Folkedal, L.H. Stien, T. Torgersen, F. Oppedal, R.E. Olsen, J.E. Fosseidengen, V.A. Braithwaite, T.S. Kristiansen, Food anticipatory behaviour as an indicator of stress response and recovery in Atlantic salmon post-smolt after exposure to acute temperature fluctuation, Physiol. Behav. 105 (2012) 350–356, http://dx.doi.org/10.1016/j.physbeh.2011.08.008.

[18] O. Folkedal, T. Torgersen, R.E. Olsen, A. Fernö, J. Nilsson, F. Oppedal, L.H. Stien, T.S. Kristiansen, Duration of effects of acute environmental changes on food anticipatory behaviour feed intake, oxygen consumption, and cortisol release in Atlantic salmon parr, Physiol. Behav. 105 (2012) 283–291, http://dx.doi.org/10.1016/j.physbeh.2011.07.015.

[19] I.E. Einarsdóttir, K.J. Nilssen, Stress responses of Atlantic salmon (*Salmo salar* L.) elicited by water level reduction in rearing tanks, Fish Physiol. Biochem. 15 (1996) 395–400.

[20] A. Madaro, R.E. Olsen, T.S. Kristiansen, L.O.E. Ebbesson, T.O. Nilsen, G. Flik, M. Gorissen, Stress in Atlantic salmon: response to unpredictable chronic stress, J. Exp. Biol. 218 (2015) 2538–2550, http://dx.doi.org/10.1242/jeb.120535.

[21] M.M. Vijayan, C. Pereira, T.W. Moon, Hormonal stimulation of the hepatocyte metabolism in rainbow trout following an acute handling stress, Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 108 (1994) 312–329, http://dx.doi.org/10.1016/0742-8413(94)00024-5.

[22] J.S. Christiansen, E.H. Jørgensen, M. Jobling, Oxygen consumption in relation to sustained exercise and social stress in Arctic charr (*Salvelinus alpinus* L.), J. Exp. Zool. 260 (1991) 149–156, http://dx.doi.org/10.1002/jez.1402600203.

[23] J.P. Topál, V. Csányi, Interactive learning in the paradise fish (*Macropodus opercularis*): an ethological interpretation of the second-order conditioning paradigm, Anim. Cogn. 2 (1999) 197–206.

[24] A. Fernö, I. Huse, J.E. Juell, Å. Bjordal, Vertical distribution of Atlantic salmon (*Salmo salar* L.) in net pens: trade-off between surface light avoidance and food attraction, Aquaculture 132 (1995) 285–396, http://dx.doi.org/10.1016/0044-8486(94)00384-Z.

[25] J.S. Lockard, Choice of a warning signal or no warning signal in an unavoidable shock situation, J. Comp. Physiol. Psychol. 56 (1963) 526–530, http://dx.doi.org/10.1037/h0041552.

[26] M.E. Seligman, Chronic fear produced by unpredictable electric shock, J. Comp. Physiol. Psychol. 66 (1968) 402–411, http://dx.doi.org/10.1037/h0026355.

[27] J. Weinberg, S. Levine, Psychobiology of coping in animals: the effects of predictability, in: S. Levin, H. Ursin (Eds.), Coping and Health, Plenum Press, New York, USA 1980, pp. 39–59.

[28] J.M. Koolhaas, A. Bartolomucci, B. Buwalda, S.F. de Boer, G. Flügge, S.M. Korte, P. Meerlo, R. Murison, B. Olivier, P. Palanza, G. Richter-Levin, A. Sgoifo, T. Steimer, O. Stiedl, G.v. Dijk, M. Wöhr, E. Fuchs, Stress revisited: a critical evaluation of the stress concept, Neurosci. Biobehav. Rev. 35 (2011) 1291–1301, http://dx.doi.org/10.1016/j.neubiorev.2011.02.003.

[29] L. Bejder, A. Samuels, H. Whitehead, H. Finn, S. Allen, Impact assessment research: use and misuse of habituation, sensitisation and tolerance in describing wildlife responses to anthropogenic stimuli, Mar. Ecol. Prog. Ser. 395 (2009) 177–185, http://dx.doi.org/10.3354/meps07979.

[30] A. Fernö, G. Huse, P.J. Jakobsen, T.S. Kristiansen, J. Nilsson, Fish Behaviour, Learning, Aquaculture and Fisheries, in: C. Brown, K.N. Laland, J. Krause (Eds.), Fish Cognition and Behavior, Wiley-Blackwell, Oxford, UK 2011, pp. 359–404.

[31] M. Bakken, R. Moe, A. Smith, Radio telemetry: a method of evaluation stress and learning ability in the silver fox (*Vulpes vulpes*), Proceedings of the International Society of Applied Ethology, 3rd Joint Meeting 1993, pp. 591–594.