Frustrative reward omission increases aggressive behaviour of inferior fighters

Marco A. Vindas¹,², Ida B. Johansen², Sergio Vela-Avitua¹, Karoline Sletbak Norstrud², Marion Aalgaard³, Bjarne O. Braastad¹, Erik Höglund⁴ and Øyvind Øverli¹

¹Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, PO Box 5003, Ås 1432, Norway
²Department of Biosciences, University of Oslo, PO Box 1041, Blindern, Oslo 0316, Norway
³Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Palackého tř. 1/3, Brno 61242, Czech Republic
⁴Department of Marine Ecology and Aquaculture, Danish Institute for Fisheries Research, North Sea Centre, PO Box 101, Hirtshals 9850, Denmark

Animals use aggressive behaviour to gain access to resources, and individuals adjust their behaviour relative to resource value and own resource holding potential (RHP). Normally, smaller individuals have inferior fighting abilities compared with larger conspecifics. Affective and cognitive processes can alter contest dynamics, but the interaction between such effects and that of differing RHPs has not been adjudged. We investigated effects of omission of expected reward (OER) on competing individuals with contrasting RHPs. Small and large rainbow trout (Oncorhynchus mykiss) were conditioned to associate a light with reward. Thereafter, the reward was omitted for half of the fish prior to a contest between individuals possessing a 36–40% difference in RHP. Small control individuals displayed submissive behaviour and virtually no aggression. By contrast, small OER individuals were more aggressive, and two out of 11 became socially dominant. Increased aggression in small OER individuals was accompanied by increased serotonin levels in the dorsomedial pallium (proposed amygdala homologue), but no changes in limbic dopamine neurochemistry were observed in OER-exposed individuals. The behavioural and physiological response to OER in fish indicates that frustration is an evolutionarily conserved affective state. Moreover, our results indicate that aggressive motivation to reward unpredictability affects low RHP individuals strongest.

1. Introduction

Agonistic behaviour is well conserved among animal populations and typically enhances an individual’s reproductive success and survival [1]. It is believed that animals closely monitor their environment and adjust their effort relative to the value of the contested resource as well as their energy levels and fighting ability [2–8]. This contest outcome may often be predicted when there is a divergence in resource holding potential (RHP, associated with overall size and energy reserves) and/or value expectation [2,3,6,7]. Aggression in mammals is associated with emotional responses, such as anger and frustration [9,10]. In psychological terms, emotions, such as frustration, anger and resentment may lead to a ‘loss of temper’ which in turn promotes aggressive behaviour [11]. These emotional responses are often linked with expectancy of value and therefore may increase the individual’s motivation to escalate levels of aggression [2,7,12,13]. Frustration, defined as ‘an aversive motivational state preceded by the omission of an expected reward (OER)’ [14], has been categorized as a potent trigger of intense aggression in mammals [15–17] and birds [18,19]. Even though there is evidence of cognitive abilities in fishes [20,21], it remains under debate whether these abilities renders them capable of complex emotions [22,23]. However, recent evidence suggests that fish species show both behavioural and neuroendocrine responses...
to potentially frustrating situations [24–27], suggesting that this affective state is well conserved and has evolutionary roots within the early vertebrate lineage [24].

Individuals with differing RHP may have different value expectancy based on their energy reserves, which may alter contest dynamics. This, in turn, may provoke inferior fighters (i.e. with lower RHP) to react with increased investment in aggressive contests [27,12,13]. Possible effects of unpredictable reward conditions in combination with unequal RHP during social competition have not been ascertained in fish species. Here, we address this question in juvenile rainbow trout (Oncorhynchus mykiss), which like many other salmonids, are territorial and establish and maintain social hierarchies through agonistic behaviour both in nature and in captivity [28–30]. Responses to omission of a palatable, but energetically insignificant food reward, during a social contest in pairs of size mismatched (i.e. differing RHP’s) rainbow trout are examined.

Although the list of signalling molecules involved in agonistic behaviour is expanding, serotonin (5-HT) and dopamine (DA) have been consistently associated with aggression [10,30,31]. Here, we measured 5-HT and DA levels and turnover in forebrain regions suggested to be homologous to mammalian areas associated with the control of emotional aggression, the dorso-medial (Dm; proposed amygdala homologue) pallium and the ventral (Vv) and lateral (Vl) parts of the ventral telencephalon (proposed homologue to the mammalian septal areas related to the lateral septum (LS)) [32,33]. In addition, we investigated 5-HT and DA neurochemistry in the dorsolateral pallium (Dl; proposed hippocampus homologue) owing to its proposed role in learning, memory and reward [32,33].

2. Material and methods

(a) Experimental environment and animals

The experiment was conducted at the fish facility at the Norwegian University of Life Sciences from April 2012 to August 2012. The experimental fish were reared at this fish facility in indoor experimental tanks (Ø = 3 m, volume = 7 m³) on a 24 h light regime, following established routines by the university. Two groups of 24 fish (total n = 48, 1-year-old rainbow trout, commercial strain, Aquagen AS) with a mean fork length of 25.1 ± 0.4 cm and weight of 189.4 ± 9.1 g (mean ± s.d.) were used. Experimental fish were initially anaesthetized in water containing 0.1 g l⁻¹ MS-222 (Finquel, Argent Chemical Laboratories, Redmond, WA, USA) in order to weigh and measure body length of each individual. The experiments were carried out in eight glass aquaria (100 × 50 × 50 cm, volume = 250 l) divided into four equally sized sections by plastic walls in order to house four fish individually in each aquarium (electronic supplementary material, figure A). The aquaria were kept under a 24 h light regime. This was achieved by the use of a fluorescent light tube (Sylvania, Standard, F36W/133-T8) positioned 20 cm above the water surface of each tank. A recirculating system supplied aerated water to the aquaria at a flow rate of 2 l min⁻¹. The overall oxygen saturation was 80 ± 0.07% and temperature was maintained at 14.3 ± 2.1°C. In order to create an asymmetric dyadic contest model, a big fish (average 246 ± 52 g) was placed next to a small fish (on average 131 ± 31 g). In this way pairs possessed a significant difference in RHP, which was calculated following the method described by Dugatkin & Biederman [2]:

\[
\frac{\text{weight of bigger fish} - \text{weight of smaller fish}}{\text{weight of bigger fish}} \times 100.
\]

Mean RHP difference among fish pairs was 38%. This model was chosen since a 30% or higher difference in RHP typically elicits stereotypic submissive and dominant behaviour in the smaller and bigger individual, respectively, with the smaller fish often showing a complete suppression of aggressive behaviour [2,4,7].

Experimental fish were thereafter acclimatized for a period of 10 days during which individuals were monitored for feeding behaviour, signs of sickness and stress. Following acclimatization, a conditioning regime was conducted for a period of 8 days and finally, an OER paradigm was carried out for a period of 2 days.

Throughout the experiment (from acclimatization to final OER day), fish were hand fed 3 mm dry pellets (Skretting, Norway), corresponding to an equivalent of 1% of their body weight, every morning between 9.00 and 11.00. Individuals that did not start eating within the first 10 days of the experiment were excluded from the study (a total of four fish).

(b) Trace conditioning regime

Following acclimatization, a trace conditioning regime was carried out over a period of 8 days (three trials per day) using a flashing light as the conditioned stimulus (CS) and one small portion (approx. 0.5 g wet weight) of boiled shrimp (Pandalus borealis) as the unconditioned stimulus (US). The flashing light (1 s on and 1 s off) was delivered via a waterproof lamp containing a 12 V, 20 W light bulb hanging 10 cm above the middle of two adjacent compartments. The trials were conducted at 13.00, 13.35 and 14.10 every day and consisted of presenting the CS for 15 s, followed by a non-stimulus period (i.e. trace interval) of 20 s before the US. The use of shrimp has been reported before as a functional motivator in conditioning studies [34]. Small crustaceans are a highly preferred prey for wild salmonids [35–38], and shrimp probably represent an attractive reward compared with pellets. Indeed, after conducting a small pilot study, it was determined that trout demonstrated a stronger reaction to being fed shrimp compared with dry pellets (data not shown). Moreover, the use of a small quantity but higher quality reward eliminates any possible confounding effects of hunger as a motivational factor following the deprivation of reward during the OER.

(c) Omission of expected reward and dyadic pair contests

Following conditioning, an OER paradigm was conducted for a period of 2 days. In each aquarium, two fish experienced OER, while the remaining two served as controls. The paradigm consisted of the additional food reward (the extra ration of shrimp) being omitted after the trace interval. This was done randomly during two out of four trials on the first day of OER. On the second day of OER, only one trial of omitted reward was conducted before a 20 min dyadic contest was initiated 1 min immediately after the end of the trace interval by quickly removing the plastic wall separating mismatched contestants.

(d) Behavioural observations

Video recordings were used to study behavioural responses to the conditioning regime, and to monitor aggressive behaviour in each tank during dyadic contests. Recordings were made using waterproof cameras placed in front of each aquarium (Colour CCD cameras, IR–YC-25V, with a 3.6 mm lens) controlled by an MSH-video multcam surveillance system (M. Shafro & Co., Riga, Latvia). The system was set to record 1 min before initiation of the CS and stopped 2 min after US during the conditioning period. The recording stopped 5 min after the end of the trace interval on the first day of OER and 20 min after start of dyadic contest (i.e. before sampling) on the last day.

To determine the conditioning effect, each aquarium was divided into five horizontal sections (10 cm tall). Numbers
from 1 to 5 were assigned to the sections, from the top to the bottom. The conditioned response was determined by recording the fish’s position in the water column 10 s before the CS and at 18 s of the trace interval (i.e. before the US). The highest position the fish reached during the recorded time intervals was noted. Anticipatory behaviour was then assessed by subtracting the position before the US from the initial position (recorded immediately prior to the CS). More positive numbers indicate an elevated position in the water column in response to CS and thus, increased expectancy of reward.

The description of aggressive interactions between rainbow trout provided by Noakes & Leatherland [28] was used to quantify display time and total aggression during the dyadic contest. Total number of charges, nips and chases for a period of 20 min were recorded for each fish, along with the time until contests were resolved (i.e. one clearly dominant and one clearly subordinate were evident).

(e) Sampling protocol

On the last day of the experiment, OER and control fish were sampled immediately after the 20 min dyadic contest. Fish were netted and immediately euthanized with an overdose of Metacaine, at a concentration of 0.4 g l⁻¹ of water until there was no observable opercular movement. Following this, body length and body weight were measured and a blood sample was taken from the base of the caudal fin. Thereafter, fish were decapitated and the brains were quickly excised. Brains were placed in a container with Tissue-Tek O.C.T. compound and immediately frozen in melting isopentane. Frozen brains were then placed in individually labelled tubes and stored at −80 °C for later analysis. Following centrifugation for 5 min at 10 000 r.p.m. and 4 °C, blood plasma was stored at −80 °C for later analysis.

(f) Plasma cortisol analysis and brain neurochemistry

Plasma levels of cortisol were measured by specific radioimmunoassay (RIA) using the method described by Sørensen et al. [39]. Prior to RIA, steroids were extracted with ethyl acetate (Merck, 1:5 plasma, ethyl acetate). Following a brief 30 s vortex and 2 min centrifugation at 14 000 r.p.m., 10 μl of the supernatant was collected for analysis. Samples were assayed in duplicate, and 50 μl of hydrocortisone (1, 2, 6, 7-3H (N), Amersham Health) and a ratio of cortisol antibody (donkey anti cortisol, AbD Serotec) to assay buffer of 1:6.

Frozen brains were sliced with a SLEE Cryostat MNT machine (SLEE Mainz, Germany) at −19 °C in serial 30 μm sections, thaw mounted on glass slides and refrozen for microdissection. Neuroanatomy was confirmed using a forebrain rainbow trout thaw mounted on glass slides and refrozen for microdissection. (e) Transverse section of a rainbow trout telencephalon showing microdissected areas. (a) Diagram showing the dorsomedial pallium (Dm), the dorsolateral pallium (Dl) and the ventral (Vv) and lateral (Vl) parts of the ventral telencephalon. Adapted from [40]. (b) Microdissected areas on a stained (Cresyl violet Nissl method) brain, depicted with areas removed by a 300 μm punch needle on scale. (Online version in colour.)

Plasma cortisol analysis and brain neurochemistry (f) Plasma levels of cortisol were measured by specific radioimmunoassay (RIA) using the method described by Sørensen et al. [39]. Prior to RIA, steroids were extracted with ethyl acetate (Merck, 1:5 plasma, ethyl acetate). Following a brief 30 s vortex and 2 min centrifugation at 14 000 r.p.m., 10 μl of the supernatant was collected for analysis. Samples were assayed in duplicate, and 50 μl of hydrocortisone (1, 2, 6, 7-3H (N), Amersham Health) and a ratio of cortisol antibody (donkey anti cortisol, AbD Serotec) to assay buffer of 1:6.

Frozen brains were sliced with a SLEE Cryostat MNT machine (SLEE Mainz, Germany) at −19 °C in serial 30 μm sections, thaw mounted on glass slides and refrozen for microdissection. Neuroanatomy was confirmed using a forebrain rainbow trout atlas [40]. Microdissections were performed on the Dl as a whole (for the purposes of this study, we did not distinguish between Dl sub-regions), the Dm and the Vv/Vl as depicted in figure 1. Microdissections for the Vv/Vl area were collected until the appearance of the central part of the ventral telencephalon [40]. On average, between 25 and 35 punches were taken for the Dl, 15 and 20 for the Dm and 8 and 12 for the Vv/Vl area. All microdissections were done with a 300 μm (in diameter) needle on a BF-30 MP freezing stage (Physitemp Instruments, USA) at −14 °C. Punched tissue samples were immediately injected into 100 μl of sodium acetate buffer (containing 3 g of sodium acetate, 4.3 ml of 100% glacial acetic acid and 16 sodium hydroxide pellets in 1000 ml of Milli-Q water, the pH was corrected to 5.0 using phosphoric acid and 94.2 ng ml⁻¹ of 3,4-dihydroxybenzyl amine hydrobromide was added, to serve as an internal standard). Samples remained frozen until centrifuged at 17 000 r.p.m., for 5 min at 4 °C and the supernatant was decanted into 1.5 ml Eppendorf tubes for monoamine analysis with high-performance liquid chromatography (HPLC). Meanwhile, the pellets remaining at the bottom of the Eppendorf tube were frozen for later protein analysis using the Bradford protein assay. The HPLC system consists of a mobile phase containing 12.26 μM 1⁻¹ EDTA, 86.25 mM 1⁻¹ sodium phosphate and 1.4 mM 1⁻¹ sodium octyl sulfate in deionized water (resistance 18.2 MW), with 7% acetonitril brought to pH 3.1 with phosphoric acid. The system uses a solvent delivery system (Shimadzu, LC-10AD), an autoinjector (Famos, Spark), a reverse phase column (4.6 × 100 mm, Hichrom, C18, 3.5 mm) and an ESA Coulochem II detector (ESA, Bedford, MA, USA) with two electrodes at −40 and +320 mV. A conditioning electrode with a potential of +40 mV is used to oxidize possible contaminants before analysis. Brain [5-HT], [DA] and the concentration of their catabolites, 5-hydroxyindoleacetic acid (5-HIAA) and 3,4-dihydroxyphenylacetic acid (DOPAC), respectively, were quantified by comparing them with standard solutions of known concentrations and corrected for recovery of the internal standard using HPLC software (CSW, DataApex Ltd, the Czech Republic).

The Bradford protein analysis was conducted in order to relate monoamine concentrations to the protein level of each sample. In brief, pellets were re-suspended in 40 μl solution of 0.4 M sodium hydroxide solution. Samples were left at room temperature on a shaker for 7 days in order to totally dissolve all tissue. A Bradford reagent (BioRad protein assay concentrate) was diluted in Milli-Q water at a ratio of 1:5. Following re-suspension, 5 μl of each sample and standards in duplicates were placed in wells and 200 μl of reagent added. Plates were then analysed for a wavelength absorbance of 595 γ using a Wallac Victor 2 multilabel counter (Perkin Elmer Life Sciences, USA). All samples were compared to a standard curve consisting of five standards containing 0.05, 0.1, 0.2, 0.4 and 0.6 mg ml⁻¹ of bovine serum albumin.

(g) Statistical analysis

Regarding the behavioural response to the conditioning regime (i.e. vertical positioning in the water column), data obtained from large and small fish were pooled after confirming that there was no significant effect of size on this behaviour. Change in vertical position in response to the CS was then analysed by means of linear regression (on average daily values) in order to statistically
3. Results

(a) Conditioned response
The fish moved higher in the water column after presentation of the CS and this tendency increased with time during the conditioning regime (figure 2). A positive slope in the linear regression analysis, along with high $r^2$ indicates a steadily increasing tendency to move towards the surface in response to the CS during the 8 days of trace conditioning ($r^2 = 0.96, p < 0.001$).

(b) Dyadic contests under omission of expected reward
Time to first attack ($p = 0.02$), total display time ($p < 0.001$) and total time to achieve contest resolution ($p < 0.001$) were significantly increased in OER compared with control pairs (figure 3). Total aggressive acts were significantly different ($p < 0.001$) between groups (small and large control and OER). Furthermore, a Wilcoxon post hoc test revealed that aggression was elevated by OER in small ($p = 0.018$; figure 4), but not in large fish ($p = 0.15$). Curiously, in two out of 11 OER pairs, the smaller fish even achieved dominance. Contrary to the small OER fish, small control fish reacted with almost immediate submissive behaviour upon encountering the larger fish. More precisely, all but one small control fish never attacked its larger opponent, instead, they immediately avoided confrontation and attempted to escape from aggressive acts.

(c) Cortisol
There was no significant effect of OER treatment, size or interaction on plasma cortisol levels (effect of treatment: $F_{3,37} < 0.01, p = 0.99$, effect of size: $F_{size,3,37} = 3.37, p = 0.08$, interaction effect: $F_{3,37} = 0.7, p = 0.41$). Mean cortisol levels (± s.e.m.) were: 53.7 ± 14.86 ng ml$^{-1}$ for big and 150.4 ± 41.7 ng ml$^{-1}$ for small control fish, and 93.7 ± 33.7 ng ml$^{-1}$ for big and 86.4 ± 20.6 ng ml$^{-1}$ for small OER fish.

(d) Brain monoamine neurochemistry
All statistics for monoamine neurochemistry are summarized in the electronic supplementary material, table S1. There was a significant effect of size ($p = 0.006$) and an interaction effect
between size and treatment on 5-HT concentrations in the Dm of OER pairs, with significantly higher 5-HT levels in small OER fish compared with small control and all big fish ($p = 0.003$; figure 5). This was, however, not the case for the 5-HT catabolite 5-HIAA or the 5-HIAA/5-HT ratio, in the Dm or any other analysed brain area. Concentrations of 5-HT (figure 5) and 5-HIAA in the Vv/Vl areas were elevated in small compared with big fish, but no effect of treatment was observed here. A significant correlation was found between total amount of aggressive acts before contest resolution and 5-HT levels in the Dm ($r = 0.86$, $p = 0.01$; figure 6). No significant effects on 5-HT neurochemistry of either treatment or size were seen in the DI.

There were no significant effects of size nor treatment on DA neurochemistry in the DI and Dm. However, both DA ($p = 0.03$), DOPAC ($p = 0.005$) and DOPAC/DA ($p = 0.008$) in the Vv/Vl area were significantly and positively affected by size. There was neither a significant treatment effect, nor an interaction effect between size and treatment on DA neurochemistry in this area (Vv/vl).

4. Discussion

Our results demonstrate that experience of omission of expected reward (OER) is associated with increased aggressive behaviour during establishment of a subordinate–dominant relationship in juvenile rainbow trout. Moreover, the magnitude and nature of aggressive behaviour appears to be determined by the individual’s RHP. More precisely, small individuals facing a larger opponent after OER reacted with increased contest investment as indicated by increased levels of aggression. In consequence, the duration of fights for dominance was longer after OER, and so was the duration of aggressive displays prior to actual physical contact between opponents. We also note that in two out of 11 contests, small OER fish became socially dominant despite their considerable RHP disadvantage. Further, we report here, to our knowledge for the first time, a link between OER-induced-increased aggressive behaviour and elevated serotonin (5-HT) concentrations in the dorsomedial pallium (Dm).

The results from this experiment corroborate previous observations that fishes are capable of trace conditioning [34,41–43]. To our knowledge, this is the first report showing that rainbow trout are capable of maintaining such an association for 20 s, which is considerably longer than what has been reported previously (3.4 s; [43]). This ability to predict a US temporally from a CS is critical, as it indicates that the fish are capable of acknowledging that there is indeed a reward omission. Interestingly, this study suggests that it is the quality rather than the quantity of the food reward which is most important for increased aggressive behaviour. In our study, the fish were successfully trace conditioned to react to a small piece of shrimp, which represents an energetically insignificant but highly palatable reward. Thereafter, OER resulted in a strong behavioural response despite the fact that the fish had already been fed a ration of food pellets prior to the OER treatment.

OER pairs showed longer latencies to attack and prolonged display times and small OER fish performed more aggressive acts than small controls. It is believed that individuals facing and engaging in aggressive contests readily monitor and assess their ability to win in order not to take unnecessary risks and squander resources. In this context, small individuals (i.e. low RHP) competing against much larger ones (i.e. high RHP) should respond with almost immediate submission...
Correlation between the concentration of 5-HT in the dorsomedial pallium (Dm) and total aggressive acts performed by small fish under OER. Note that the 5-HT value used for fish that did not perform any aggressive acts represents an average (circle). Statistics: Pearson’s correlation analysis.

![Graph showing correlation](image)

Figure 6. Correlation between the concentration of 5-HT in the dorsomedial pallium (Dm) and total aggressive acts performed by small fish under OER. Note that the 5-HT value used for fish that did not perform any aggressive acts represents an average (circle). Statistics: Pearson’s correlation analysis.

Contrary to this, in accordance with our results, Dugatkin & Ohlsen [7] found that smaller pumpkinseed fish (Lepomis gibbosus) expecting a larger reward than their bigger opponents, initiated fights despite their size disadvantage. Indeed, there is evidence suggesting that smaller/subordinate individuals may invest more in aggressive contests when value expectancy is higher [12,44]. It is thus possible, although speculative, that small OER individuals escalated aggression owing to higher food motivation in a hope of achieving access to the expected resource. Disregarding the specific selective mechanism, our results conclusively demonstrate how fish react to discrepancies between reality and expectancy, a response that appears to be conserved in vertebrates [12,44].

Further, the elevated aggression observed in small OER-exposed fish was accompanied by higher 5-HT levels in the Dm. Contradictory to this finding, several studies on fish [45–47] and other animals [17,48–52] suggest that 5-HT is the primary inhibitory regulator of aggression. It has been shown, however, that short-term increases in serotoninergic activity do not inhibit agonistic activity during combative social interactions. Instead, inhibition of aggression by 5-HT appears to occur during long-term exposure [53]. Moreover, brain region-specific 5-HT activity has been reported in microdialysis studies in mice which showed that 5-HT release was lower in the prefrontal cortex but not in the nucleus accumbens during dyadic contests [54]. In our study, there also appears to be a localized effect on 5-HT which is associated with increased aggression. 5-HT levels only increased in the Dm and only in small OER-exposed individuals. The observed link between 5-HT and aggression adds to previous studies showing a context- and region-dependent regulatory action of this monoamine on aggressive behaviour [53].

Meanwhile, 5-HT and 5-HIAA levels in the Vv and VI were elevated in small individuals of both treatment groups, indicating that 5-HT signalling in these brain regions is associated with social submission. Indeed, 5-HT has a well-characterized role in coordinating the neurochemical stress response in fishes, where 5-HT activity increases in response to stress [30,55,56]. This is supported by previous observations in rainbow trout showing that 5-HT activity increases in stressed subordinate compared with dominant individuals [56,57]. In mammals, the LS is associated with promoting the expression of behaviour under aversive and stressful conditions [58] and has been suggested to have a role in contextual information processing and regulation of motivation together with the hippocampus [59]. Even though the homology of fish’s Vv/Vl areas to the mammalian LS is still debated [60], these areas may still operate some of the same regulatory functions [33]. Hence, an effect of social subordination on Vv/Vl 5-HT signalling and a possible role for this area in emotional stress reactivity in fishes is suggested by our results. Conversely, 5-HT neurochemistry in the DI was not affected by either OER or size. This suggests that 5-HT neurochemistry in this brain region is not involved in regulating short-term responses to dyadic contests or OER. Regarding the Dm response discussed above, we do not interpret the increase in 5-HT in the Dm of small OER-exposed fish as a response to social subordination, as it only occurred after OER and not in control fish.

Numerous studies have suggested that increased DA in the brain is associated with higher aggression and social dominance in rodents [61,62], reptiles [63] and fishes [64–66]. In our study, neither treatment nor size appeared to be associated with changes in DA neurochemistry in the DI or Dm. In the Vv/Vl area however, DA neurochemistry was significantly higher in all large individuals, which could reflect their dominant social status, because the majority of these individuals became socially dominant following dyadic contests. This conclusion however needs to be verified by studying a higher number of large subordinate individuals. Further studies looking at possible functions regulated by limbic areas in fishes are needed in order to elucidate their role in aggression and stress reactivity.

Plasma cortisol levels reported in this study indicate a considerable stress reaction to the dyadic contest in both subordinate and dominant individuals. Subordinate/small controls displayed particularly high cortisol levels (on average 150 ng ml$^{-1}$) as compared with big/dominant controls (on average 54 ng ml$^{-1}$). This corresponds to plasma cortisol patterns previously seen in rainbow trout directly after fights for social dominance [67]. Meanwhile, in OER pairs, the discrepancy between small and big contestants was less pronounced (on average 94 ng ml$^{-1}$ in small fish). We speculate that cortisol levels may remain high on both contestants owing to an extended fight time.

In summary, our results demonstrate that experience of reward unpredictability is associated with increased aggressive behaviour and that the magnitude of this response is determined by RHP. The behavioural and physiological response to OER in fish indicates that frustration is an evolutionarily conserved affective state. Emotional responses (e.g. frustration) are thought to represent adaptive evolutionary strategies contributing to an animal’s fitness in a changing world [68]. For example, it could be speculated that inferior fighters with increased value expectancy may in some way gain from a bigger investment in agonistic behaviour against superior fighters [2,7,11–13]. The adaptive value of such intensified agonistic behaviour, however, remains to be resolved. The possibility also emerges that inferior competitors are more sensitive to reward unpredictability or environmental unpredictability in general. In future studies, it would be of interest to investigate whether other types of reward unpredictability also provoke a specific aggressive response in inferior fighters.

This work was conducted in accordance with the laws and regulations controlling experiments and procedures on live animals in Norway and was approved by the Norwegian Animal Research
References

22. Jacobsen LA, Hansen LP. 2001 Feeding habits of wild and escaped farmed Atlantic salmon, Salmo