



Journal of Fish Biology (2011) **79**, 575–586

doi:10.1111/j.1095-8649.2011.03036.x, available online at wileyonlinelibrary.com

Stressed mothers – troubled offspring: a study of behavioural maternal effects in farmed *Salmo salar*

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(Received 22 June 2010, Accepted 12 May 2011)

Mature female Atlantic salmon *Salmo salar* were given intraperitoneal cortisol implants 1 week prior to stripping to examine the influence of simulated maternal stress on offspring boldness and social dominance. Behavioural tests originally designed to investigate stress responsiveness and coping styles in salmonids (*i.e.* feeding in isolation, dominance tests and acute confinement) were carried out on the offspring 1.5 years after hatching. In the feeding test, there were no differences between the two treatment groups in total feeding score or number of pellets eaten, but offspring from the cortisol-implanted females made more unsuccessful feeding attempts than offspring from control females. In dominance tests, there was no difference between controls and cortisol-treated fish regarding propensity to become socially dominant. A higher proportion of individuals with bite marks, however, was observed in the cortisol group when compared to controls. Cortisol-treated offspring that gained dominant rank in the dominance tests performed more aggressive acts after stable dominance–subordinate relationships were established compared to control winners. During acute confinement stress, offspring from cortisol-implanted females showed a reduction in the proportion of time they were moving compared to the controls. These results indicate that the maternal endocrine state at spawning affects several aspects of progeny behaviour potentially related to subsequent success and survival in farmed *S. salar*. © 2011 The Authors

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Key words: aggression; boldness; cortisol; dominance; stress; welfare.

INTRODUCTION

Many studies on animals have established that a wide variety of stressors experienced by breeding females can exert profound impacts on progeny characteristics (Huizink *et al.*, 2004). In mammals and birds, prenatally stressed offspring typically suffer from increased mortality, lowered birth mass, congenital abnormalities and

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immunological problems (Braastad, 1998; Eriksen *et al.*, 2003; Hayward & Wingfield, 2004; Huizink *et al.*, 2004; Janczak *et al.*, 2006; Merlot *et al.*, 2008). Diverse functions and systems can thus be influenced, but the most prominent outcome of prenatal stress is its impact on behavioural variables. Behavioural responses exhibited by stressed offspring generally include reduced stress coping and increased fearfulness in novel and aversive situations, impaired competitive capability, cognitive deficits and modified sexual and maternal behaviour (Braastad, 1998; Huizink *et al.*, 2004). A large body of work has attempted to reveal the plausible proximate mechanisms by which prenatal stress can influence offspring traits, and increased exposure to maternally derived glucocorticoids during the prenatal period is suggested to be the main mediator (Rhees & Fleming, 1981; Williams *et al.*, 1995; Huizink *et al.*, 2004).

Prenatal stress is commonly proposed to convey disadvantageous impacts on the progeny phenotype. Severe stress that is beyond an individual's coping capacity can produce maladaptive, pathological impacts. From an evolutionary perspective, however, the transfer of information from the maternal environment to the embryo can benefit offspring by calibrating their developing biological systems to environmental conditions experienced by the mother (Bakken, 1995; Braastad, 1998). For instance, in environments with high predation pressure it might be favourable for offspring to possess increased vigilance. This has been illustrated in studies performed on the common lizard *Lacerta vivipara*, a species in which the progeny migrates from their natal territory primarily within the first 2 weeks of life. High maternal corticosterone levels in the blood were reflected in the eggs and it was shown that individuals originating from eggs with high corticosterone concentrations were more attracted to their mother, searched more for shelter when stressed and dispersed less far compared to controls (De Fraipont *et al.*, 2000; Meylan *et al.*, 2002). Thus, transfer of 'inside information' from the mother to her offspring about the prevalent conditions could induce adaptive behavioural modifications that may optimize survival in risky environments.

In fishes, maternally derived substances in the yolk may influence or modulate offspring development (Leatherland, 1999; Schreck *et al.*, 2001; Leatherland *et al.*, 2010). Intensive fish aquaculture operations often place broodstock in chronically or temporarily stressful situations. Stress experienced by a mature female might produce increased deposits of cortisol in the eggs, thus representing one pathway through which mature female fishes may affect the offspring phenotype. It is known that stress exerted upon maturing fishes can result in a general reproductive suppression, reflected by reduced levels of sex steroids and vitellogenin in plasma, decreased fecundity and oocyte size, as well as altered timing of ovulation (Carragher *et al.*, 1989; Campbell *et al.*, 1992, 1994; Schreck *et al.*, 2001). Furthermore, maternal stress may be detrimental to various physiological and morphological characters of the progeny, *e.g.* increasing offspring mortality, reducing embryo size at hatching and enhancing the incidence of morphological malformations (Campbell *et al.*, 1992, 1994; McCormick, 1998, 2009; Schreck *et al.*, 2001; McCormick & Nechaev, 2002; Eriksen *et al.*, 2006, 2007, 2008; Gagliano & McCormick, 2009).

Although former studies have demonstrated that exposing fish females to stress prior to spawning can be harmful to physiological and morphological development of the offspring, there is limited information on the more subtle effects this may have on behaviour. Considering the vast number of reports in mammals and birds on the

influence of the maternal environment on behavioural characters of the progeny, it is essential to investigate possible parallel mechanisms in fishes. Thus, the aim of the present study was to examine the impact of simulated maternal stress on offspring behaviour, in particular their fearfulness and competitive ability, in farmed Atlantic salmon *Salmo salar* L. 1758.

MATERIALS AND METHODS

MATERNAL CORTISOL ADMINISTRATION

Farmed *S. salar* broodstock from the SalmoBreed strain (mean \pm s.d. mass 8.12 ± 0.94 kg) were obtained from BOLAKS, Fusa, Norway. These fish were maintained in a group of 200 individuals in 25 m³ fibreglass tank supplied with aerated fresh water delivered at a rate of 500 l min⁻¹ at c. 7° C water temperature and reared under natural photoperiod for 60° N. Seven days prior to the expected spawning date, 31 mature females were selected, tagged (PAT tags, Os Husdyrmerkefabrikk; www.osid.no) and randomly divided into two groups, representing two implant treatments as described by Eriksen *et al.* (2006). Females in group 1 ($n = 15$) received sham injections containing only a vehicle, coconut oil (1 ml kg⁻¹), and served as a control group. In group 2 ($n = 16$), maternal stress was simulated by giving the females intraperitoneal implants (1 ml kg⁻¹) with coconut oil containing cortisol (Hydrocortisonum micronisatum; www.nmd.no). The injection solution was prepared by adding cortisol to liquid coconut oil at 40° C to yield a final cortisol concentration of 100 mg ml⁻¹ oil. Implanting glucocorticoids directly into the maternal organism may be advantageous as such a pharmacological approach should instigate less interindividual variation. In the peritoneal cavity, the implants solidify and release the hormone gradually over time. The dosage was selected on the basis of previous experiments where cortisol was titrated *in vivo* to give significant increments of cortisol, well within the physiological range of stressed fishes, *i.e.* <500 ng ml⁻¹ cortisol in blood serum (Specker *et al.*, 1994; Eriksen *et al.*, 2006). Prior to injections, the females were anaesthetized with metacaine (MS 222), weighed and then injected intraperitoneally with 1 ml of the emulsion kg⁻¹ body mass, using an 18 gauge needle connected to a plastic syringe. After implantation the females were transferred back to their original holding tank. At stripping 7 days later, the females were anaesthetized with metacaine and a sample of eggs (c. 0.2 l) was collected from each mature females in the two groups. One of the females in the cortisol group was immature and was thus discarded. The eggs from each treatment were pooled and fertilized by a mixture of milt obtained from two untreated males. Both males were used to fertilize all the eggs, hence avoiding male identification being confounded with treatment.

COLLECTION OF SERUM AND EGGS FOR HORMONE ANALYSIS

At stripping, samples of maternal blood and eggs were collected for blood analyses from control females, cortisol-implanted females and also from 10 untreated females. Blood was drawn from the caudal vein into siliconized evacuated blood collecting tubes (Venoject®; www.terumotmp.com) without additives, stored (at 4° C) overnight and centrifuged at 2000g for 20 min. Serum was separated and frozen at -20° C for later analysis. Unfertilized eggs were collected and frozen at -20° C. Cortisol was assayed by a radioimmunoassay (RIA) kit (Siemens Healthcare Diagnostics; www.medical.siemens.com). When assaying levels of cortisol in the eggs, two eggs per fish were first homogenized in 1 ml physiological saline in an Ultra-Turrax T25 (Janke & Kunkel GmBH, IKA Labortechnik; www.ika.com) followed by the addition of 5 ml diethyl ether. The ether phase was collected after freezing and evaporated under nitrogen. The residuum was dissolved in 1 ml phosphate buffer pH 7.4 containing 0.1 M NaCl, 0.05% (w/v) NaN₃ and 0.2% bovine serum albumin. Subsequent assays were as for serum samples.

REARING CONDITIONS

The eggs were incubated at the Aquaculture Research Station of Nofima Marin at Sundalsøra, Norway. Eggs from both groups were reared in five replicates to avoid confounding treatment effects with compartment. Fertilized eggs from each group were placed in separate compartments (10 cm × 15 cm), within trays in incubators supplied with a constant flow of fresh water. The temperature during incubation was $7.6 \pm 0.3^\circ \text{C}$ (mean \pm s.d.). From first feeding and throughout the experimental period all the fish were reared under the same kind of conditions to those normally applied in intensive aquaculture in Norway. The fish were kept under a continuous light regime and held in fibreglass tanks supplied with a constant flow of well-oxygenated fresh water at $8.6 \pm 1.7^\circ \text{C}$ (mean \pm s.d.). Commercial pelleted feed (Skretting; www.skretting.no) was delivered in excess to the fish every 30–60 min by automatic feeders. From first feeding onwards the offspring were held in groups consisting of *c.* 1000 individuals. After 4 months, 5000 individuals (2500 controls and 2500 from the cortisol group) were transported to NIVA's (Norwegian Institute of Water Research) research station at Solbergstrand. These fish were maintained in groups of 500 fish in 10 fibreglass cylindrical tanks (180 l) supplied with fresh water delivered at a rate of *c.* 6.1 min^{-1} at $8.6 \pm 0.8^\circ \text{C}$ (mean \pm s.d.) under natural photoperiod conditions for 60°N . Fish were hand-fed commercial pellets once a day, corresponding to 1–2 % of the biomass of the fish.

FEEDING IN ISOLATION AND SOCIAL DOMINANCE

One year after arrival in Solbergstrand, behavioural testing of the *S. salar* offspring was initiated. Prior to screening for boldness and social dominance, 40 fish (20 controls and 20 cortisol treated, four fish per holding tank, $17.2 \pm 3.1 \text{ g}$ mean mass \pm s.d., control fish $17.0 \pm 0.7 \text{ g}$, cortisol fish $17.3 \pm 0.7 \text{ g}$) were transferred from the rearing tanks to small glass aquaria (12 l) supplied with constantly flowing aerated fresh water at $9.1 \pm 0.3^\circ \text{C}$ (mean \pm s.d.), where each fish was kept in isolation. The observation aquaria were covered with opaque plastic on three sides and divided into two compartments by a detachable opaque PVC wall, each section contained one control fish or one cortisol-treated fish in mass-matched pairs (maximum mass difference 5%). Prior to transfer to social isolation the fish were anaesthetized and mass and fork length (L_F) recorded. While anaesthetized, all fish were fin clipped by a small cut in the upper or lower section of the caudal fin to distinguish between treatment groups. Fish were allowed to recover overnight before testing commenced the following day. For seven successive days feeding was performed by dropping feed pellets (Skretting) into the aquarium. Feeding was terminated either when the fish had refused three consecutive pellets or had consumed *c.* 2% of its body mass (eight pellets). Feeding behaviour was classified according to the criteria listed in Table I, in line with the methods applied by Øverli *et al.* (2006). Fish sometimes moved and attempted to ingest a food pellet, but failed to get it before it reached the bottom of the tank, and these were counted as missed feeding attempts.

After 7 days of feeding, the dividing screen in each aquarium was removed and fish were allowed to interact. Fish pairs were video recorded for 60 min. Videoing of fish behaviour was carried out on a single day, using one video camera, allowing filming of two test tanks (two couples, four fish) simultaneously. Previous experiments have shown that holding juvenile salmonids in isolation for *c.* 1 week with sufficient access to food is effective in inducing territoriality and motivation to express aggressive behaviour (Øverli *et al.*, 1999). In an

TABLE I. Point scores to grade feeding behaviour in *Salmo salar* after transfer to social isolation (after Øverli *et al.*, 2006)

Points	Behaviour
0	Fish does not respond to food
1	Fish eats only pellets that fall directly in front, does not move to take food
2	Fish moves to take food, but returns to original position
3	Fish moves freely between food items and consumes all food that is presented

experimental environment with no possibility to escape or withdraw, the situation described above is highly stressful for the subordinate individual. After initial fights, subordinate fish typically show little or no aggression towards the dominant individual and commonly move to and remain in a corner of the aquarium while dominant fish often move around the whole territory often chasing the subordinate fish (Øverli *et al.*, 1999). In all pairs the outcome of the fights was recorded from video recordings, *i.e.* subordinate and dominant fish were identified based on the aforementioned criteria. Additionally, 5 min after the establishment of observable dominance–subordinate relationships, the number of aggressive acts performed by dominant fish was counted for a period of 10 min. Aggressive acts included nips (a bite given towards a closely located individual without any prior approach), attacks (a rapid approach towards an individual, often finished with a bite) and chases (a succession of two or more attacks towards the same fleeing individual). Subsequent to the test, fish were netted and euthanized with an overdose of metacaine. Body mass, L_F , fish identity and presence of bite marks were recorded.

FEEDING IN ISOLATION AND ACUTE CONFINEMENT

Forty (20 controls and 20 cortisol-treated, four fish per holding tank) mass-matched test fish (19.3 ± 3.3 g, mean mass \pm s.d., maximum mass difference 5%, control fish 19.3 ± 0.8 g, cortisol fish 19.3 ± 0.7 g) were selected and isolated in the observation aquaria as described above. Prior to the transfer, the fish were anaesthetized and mass and L_F recorded. Fish were allowed to recover overnight before testing started the next day. For 1 week fish were hand-fed to satiety or to a maximum of 2% body mass per day and daily foraging behaviour was recorded. After 7 days of feeding in isolation, one-half of the experimental fish (10 controls and nine cortisol fish) were subjected to a confinement test (30 min in a perforated transparent 1.5 l plastic box, submerged in their observation aquaria). The fish were video filmed and time spent moving was later recorded for a period of 30 min following transfer to the confinement box. This was done by triggering a stopwatch when the fish was moving and stopping it when the fish was stationary. The classification of ‘moving’ was restricted to active locomotion actually transporting the fish or active swimming towards the walls of the confinement box. Directly after the confinement period, fish were euthanized with an overdose of metacaine.

STATISTICAL METHODS

The statistical analyses were performed with JMP, version 6 (SAS Institute Inc.; www.sas.com). One-factor ANOVA was used to test the effects of maternal cortisol administration on cortisol levels in maternal blood and eggs, as well as behaviour in the feeding test, number of aggressive acts in the dominance test and activity level during acute confinement. Assumptions of normality were examined with a goodness of fit test. A likelihood ratio χ^2 test was applied when investigating categorical data, *i.e.* potential group differences in social rank and percent of fish with bite marks. Mean values are given with s.e. unless stated otherwise.

RESULTS

EFFECTS OF CORTISOL IMPLANTS ON MATERNAL SERUM AND EGGS

Intraperitoneal cortisol implantation resulted in a significant increase in maternal serum cortisol ($F_{1,29} = 24.05$, $P < 0.001$). At stripping, the concentrations of cortisol in maternal serum were 356.9 ± 19.7 and 491.5 ± 19.1 ng ml⁻¹ in the control group and cortisol group, respectively. Similarly, cortisol-implanted females had significantly more cortisol in their eggs when compared to controls (71.1 ± 5.7 and 2.7 ± 4.8 ng egg⁻¹; $F_{1,24} = 61.44$, $P < 0.001$). The cortisol concentration in eggs

from untreated females ($1.36 \pm 9.1 \text{ ng egg}^{-1}$) was significantly lower than eggs from cortisol-implanted females ($F_{1,23} = 4.95$, $P < 0.05$).

FEEDING IN ISOLATION AND SOCIAL DOMINANCE

There were no significant differences between the two treatment groups regarding total feeding score ($F_{1,38} = 0.0$, $P = 1.00$) or total number of pellets eaten ($F_{1,38} = 1.04$, $P = 0.32$). When compared to the controls, however, offspring from the cortisol-implanted females showed an increased percentage of unsuccessful feeding attempts during 7 days of feeding (10.09 ± 2.09 v. 3.21 ± 2.21 ; $F_{1,34} = 5.08$, $P = 0.03$).

In the pair-wise dominance tests there was no significant difference between controls and cortisol-treated fish regarding propensity to become socially dominant ($\chi^2 = 2.66$, d.f. = 1, $P = 0.1$; Table II). In 19 of the 20 pairs one dominant and one subordinate individual could clearly be identified after initial fights. Controls became dominant in 12 of the 20 pairs, whereas offspring from cortisol-treated females gained a dominant rank in seven pairs. In the last pair of fish it was not possible to conclude which fish was dominant or subordinate.

For a total period of 10 min subsequent to establishment of dominance–subordinate relationships it was observed that cortisol-treated offspring that obtained a dominant rank displayed a higher number of aggressive acts than control offspring that became dominant ($F = 4.23$, d.f. = 1, 17, $P = 0.04$; Table II). During this period, no aggressive acts were performed by subordinate fish.

A higher proportion of individuals with bite marks was observed in the cortisol group when compared to controls ($\chi^2 = 5.96$, d.f. = 1, $P = 0.01$; Table II).

FEEDING IN ISOLATION AND ACUTE CONFINEMENT

During the 7 days of feeding in isolation, no significant differences between the two treatment groups were found regarding total feeding score ($F_{1,36} = 0.06$, $P = 0.81$) or the total number of pellets eaten ($F_{1,36} = 0.66$, $P = 0.42$).

TABLE II. Dominance, aggressive acts, occurrence of bite wounds and time spent active during confinement in *Salmo salar* offspring originating from cortisol-implanted mothers and controls (values are means \pm S.E.)

Group	Per cent dominant fish†	Aggressive acts‡	Per cent bite wounds§	Time spent active
Control	60.0	14.1 \pm 5.6	7.9	33.6 \pm 4.8
Cortisol	35.0	34.3 \pm 7.4*	26.3*	7.2 \pm 5.0*

* $P < 0.05$.

†Percent of offspring becoming dominant in size-matched pairs.

‡Number of aggressive acts performed by dominant fish for a period of 10 min after hierarchy establishment in pairs.

§Percent of individuals with bite wounds.

||Time spent moving (per cent of 30 min observation time) during acute confinement.

†§Likelihood ratio χ^2 test.

‡||One-factor ANOVA.

Offspring from cortisol-implanted females showed reduced percentage of time spent moving under acute confinement when compared to controls ($F_{1,17} = 5.65$, $P = 0.03$; Table II).

DISCUSSION

The influence of the prenatal environment on developing organisms has been the subject of substantial research (Braastad, 1998; Huizink *et al.*, 2004). Although broods from stressed and unstressed females may diverge in some respects under normal living conditions, the majority of the differences are manifested when the offspring are exposed to challenging or stressful situations (Braastad, 1998; Huizink *et al.*, 2004). Data presented in the current study indicate that the maternal endocrine state at spawning affects several aspects of progeny behaviour in farmed *S. salar*. These stress-induced impacts may have ramifications for the offspring's success and survival in culture or in natural conditions.

In most species of fishes, rapid growth promoted by a high food intake is highly advantageous, especially in early life stages (Beitinger, 1990). Disruption of normal feeding behaviour is believed to signal stress in fishes, and elevated levels of cortisol is one mechanism underlying feeding suppression during periods of stress (Beitinger, 1990). In the current study, feeding in isolation did not differ between the two offspring groups regarding total feeding score or number of pellets eaten. The scale in the feeding test applied in the present experiment is based on prolonged studies of identifiable patterns in salmonid feeding behaviour, and fishes are typically reported to advance through every step before becoming completely confident in the new environment, consuming food *ad libitum* and utilizing all available space when feeding (Øverli *et al.*, 2006). It remains unclear whether a high feeding score would indicate boldness (because the anxiogenic effect of novelty wears off quickly) or simply increased hunger (because the anorectic impact of stress diminishes quickly); it is, however, suggested that the total feeding score may reflect how quickly fishes resume normal feeding in a novel environment, thus indicating risk-taking propensity or boldness in salmonids (Øverli *et al.*, 2006; Ruiz-Gomez *et al.*, 2008). In the current study most fish seemed to adapt quite well to the novel environment in terms of high feed intake soon after transfer (the majority of the fish started to feed on day 2), leading to the speculation that transfer from group rearing to being isolated in new aquaria was not particularly stressful. Recently, the feeding test has been found to be highly affected by previous growth history of the fish (Ruiz-Gomez *et al.*, 2008; Kittilsen *et al.*, 2009a). Salmonids are reported to demonstrate substantial plasticity in foraging behaviour, risk estimation and growth patterns (Damsgård & Dill, 1998; Ali *et al.*, 2003), and individuals may become bolder and more aggressive when competing for resources if their need for these resources is high (Damsgård & Dill, 1998; Morrell *et al.*, 2005). Prior to the behavioural tests in the present study the fish demonstrated relatively poor growth, and this may explain why a large proportion of fish showed rapid reversal of stress-induced anorexia when fed in isolation. In conclusion, the feeding test as an indicator of stress sensitivity may have limited value if earlier growth history and feeding motivation of the fishes are not also taken into account.

Offspring from cortisol-treated females also demonstrated a higher frequency of unsuccessful feeding attempts when fish moved to take food items, but did not

succeed in capturing them. Direct exposure to stressors has previously been reported to impair feeding capability in fishes, *e.g.* reflected by reduced ability to capture and ingest prey (Beitinger, 1990). As far as is known, this is the first time prenatal stress has been found to have an effect on offspring feeding efficiency for any animal. In both wild and cultured fishes, competent feeding strategies are vital to optimize growth, and the possible impact of prenatal stress on foraging behaviour should be investigated further.

In fishes, much research has focused on the causes and consequences of social subordination (Winberg & Lepage, 1998; Øverli *et al.*, 1999, 2002; Sloman *et al.*, 2001; Gilmour *et al.*, 2005). There are many potential detriments related to being subordinate, such as enhanced levels of glucocorticoids, increased susceptibility to pathogens and impaired growth rate (Abbott & Dill, 1989; Pottinger & Pickering, 1992; Sloman *et al.*, 2001). The possible impact of the prenatal environment on offspring competitive ability has to some extent been investigated in birds and mammals. Eriksen (2001) and Janczak *et al.* (2006, 2007) exposed chick *Gallus gallus domesticus* embryos to corticosterone and documented that this treatment made them less competitive later in life. Similarly, stressing farmed fox vixens *Vulpes vulpes* was found to produce cubs with diminished competitive capacity (Bakken, 1995). In contrast, Kemme *et al.* (2008) reported in guinea pigs *Cavia aperea f. porcellus* that prenatal stress did not affect social rank. In fishes, however, little is known about the potential effects of maternal stress on offspring dominance. In the present study, controls became dominant in 12 of the 20 pairs, whereas progeny from cortisol-treated females gained a dominant rank in seven pairs. This difference was, however, not significant. Thus, there is a need for further experiments on this topic.

Social dominance, on the other hand, may also involve stress, especially in unstable social environments when dominant individuals may frequently be involved in aggressive acts (Sapolsky, 1992). In the current study, number of nips, attacks and chases performed by the dominant fish was quantified after dominance relationships were established. During this period, dominant cortisol fish demonstrated a higher frequency of aggressive acts compared to control winners. Furthermore, an enhanced proportion of individuals with bite wounds was observed in the cortisol group in comparison with the control fish, with cortisol-treated offspring showing the highest number of wounds. These results could imply that dominant cortisol fish applied a more offensive strategy to achieve and maintain dominant status. Similarly, Sloman (2010) recently showed in brown trout *Salmo trutta* L. 1758 that exposure of eggs to cortisol produced more aggressive offspring. As prenatal stress is commonly associated with enhanced reactivity in challenging situations (Braastad, 1998; Huizink *et al.*, 2004), it might be speculated that the cortisol winners in the current study were more stressed in the pair tests in comparison with the control winners. Øverli *et al.* (2004) studied the phenomenon of displaced aggression in rainbow trout *Oncorhynchus mykiss* Walbaum (1792) and argued that subordinate individuals may represent stress-reducing agents, in that aggressive acts directed towards these subordinates could be a behavioural stress-coping strategy for the dominant fish. In rodents, biting another individual has been reported to serve as an outlet for stress (Levine *et al.*, 1989). In primates, individuals that lose fights often attack a subordinate spectator that was not participating in the original conflict (Virgin & Sapolsky, 1997). Clearly, more work is required to determine the behavioural mechanisms underlying the enhanced damage sustained by juveniles

from stressed mothers if these are to be managed and to reduce these impacts on cultured populations.

Offspring from cortisol-implanted *S. salar* mothers were found to spend less time active in the acute confinement test compared to controls. Espmark *et al.* (2008) similarly reported that *S. salar* offspring from cortisol-implanted females spent more time frozen on the bottom in a novel environment, and argued that this could be attributed to enhanced levels of stress. Studies of rodents and primates have shown that prenatal stress can affect the behavioural responses of offspring when exposed to novel or aversive environments, typically reflected by a reduction in activity (Braastad, 1998; Huizink *et al.*, 2004). Many inconsistencies have been found (Thomson, 1957; Braastad, 1998; Roussel *et al.*, 2004) and in fishes, increased amount of locomotor activity during acute confinement is commonly associated with elevated concentrations of cortisol in individual fish, thus indicating high levels of stress (Øverli *et al.*, 2006; Kittilsen *et al.*, 2009a, b). Hence care must be taken when interpreting activity levels in acute stress tests as various motivational systems may underlie this behaviour, for instance general exploration, specific appetitive behaviour and fear-induced flight or escape. In conclusion, it remains difficult to determine whether reduced activity demonstrated by cortisol offspring in the confinement test is in fact due to increased levels of stress in these specimens or simply represents an enhanced prevalence of stationary resting.

In the current study, maternal stress was simulated by giving mature females intraperitoneal cortisol implants 1 week prior to stripping. The experimental procedure was successful in significantly increasing cortisol content in cortisol-implanted females above that found in control females. The implanted cortisol dose was based on former experiments where cortisol was titrated *in vivo* to give significant increments still within the physiological range of stressed fish (Specker *et al.*, 1994; Eriksen *et al.*, 2006). Unfortunately, there are few data on the cortisol concentration in eggs from stressed salmonids. Stratholt *et al.* (1997) used a 1 min chase twice daily for 2 weeks prior to ovulation and reported a concomitant increase in stressed coho salmon *Oncorhynchus kisutch* (Walbaum 1792) eggs (25.3 ng g⁻¹) as compared to the controls (9.9 ng g⁻¹). In the laboratory, cortisol levels varying from 2.2 to 58.2 ng egg⁻¹ have been found in eggs from untreated salmon females at stripping (unpubl. data). While it seems that the cortisol content of the eggs used in the current study was rather high, the fact that eggs from non-stressed females may range up to 58.2 ng egg⁻¹ suggests it may not have been abnormally elevated. In the study by Eriksen *et al.* (2006), mature *S. salar* females were provided with cortisol implants during a period with especially low water temperature (*c.* 1° C). In the present experiment, cortisol was implanted when the water temperature was rather high (*c.* 7° C), probably making the cortisol implant more potent, as cortisol is temperature sensitive.

While previous research suggests that the impacts of maternal stress on progeny of fishes may be marked and detrimental, the specifics are unclear, particularly when placed in an aquaculture context. The present research suggests that offspring from stressed mothers may be more easily affected by environmental challenges that often occur during intensive culture. Such challenges may generate stress and fear in an endocrine system primed for stress, with chronic activation of the HPI axis and its detrimental outcomes. Farm production is dependent on the rapid growth of fishes to market size, with minimal damage. Farmed fishes, however, are reared

at high stocking densities, and the distances between the individuals are low, predisposing fishes to a high number of behavioural interactions and fighting over food and space. If maternal stress affects offspring behavioural characteristics, this may adversely influence aspects such as food conversion efficiencies through altering social dynamics. These behavioural changes may in the end have repercussions for growth patterns, injury rates and biomass production in intensive *S. salar* farming. Taken together, the present findings demonstrate that the maternal hormonal regime at stripping may affect various aspects of progeny behaviour in farmed *S. salar*. This underscores the necessity of considering maternal effects when investigating rearing conditions, social dynamics and animal welfare in farmed fishes.

The procedures in the experiment have been approved by the local responsible Laboratory Animal Science specialist under the surveillance of the Norwegian Animal Research Authority (NARA) and registered by the Authority. This study has thus been conducted in accordance with the laws and regulations controlling experiments on live animals in Norway. The authors greatly appreciate the valuable assistance of the technical staff at Bolaks (Eikelandsofen), Nofima Marin (Sunnalsøra) and NIVA (Solbergstrand). M.S.E.'s contribution was financially supported by the Norwegian Research Council, project no. 173261/I10.

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