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ORIGINAL PAPER

Fluctuating asymmetry in farmed Atlantic salmon (*Salmo salar*) juveniles: also a maternal matter?

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Abstract Developmental stability reflects the degree to which phenotypic expression is unaffected by random accidents or developmental noise. Developmental stability may be measured by phenodeviance or fluctuating asymmetry (FA), and estimation of developmental stability has attracted substantial interest because it appears to represent a relatively simple method to identify sub lethal stress exposure and to assess animal welfare. As a part of a long-term study, the work presented here primarily aimed to investigate impacts on developmental instability in farmed salmon offspring ten months post hatch attributable to maternal cortisol administration prior to spawning and mild hyperthermia exerted during incubation. Main results show that maternal cortisol enhancement increased the level of FA in pectoral and pelvic fins, but did not affect the frequency of malformations in offspring. Mild hyperthermia during incubation increased weight and fork length and also increased pelvic fin FA. Malformed fish were heavier and longer than the normal ones, and pelvic fin asymmetry was positively related to condition factor. These results illustrate plausible lasting impacts on offspring development due to the maternal endocrinological state at spawning and indicate that developmental instability in farmed salmon juveniles may mirror aspects of the broodstock's housing conditions.

Keywords Developmental stability · Prenatal stress · Cortisol · Salmon · Aquaculture · Welfare

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Introduction

Organisms demonstrate regularity of their phenotypes, and such regularity is claimed to endorse superior performance (Møller and Swaddle 1997). In reality however, we are all morphologically imperfect, but some are more so than others. Developmental stability refers to the competence of an organism to construct an intended phenotype under a given range of environmental and genetic conditions (Møller and Swaddle 1997). Failure to complete such stable phenotypic development can be assessed by the level of fluctuating asymmetry (FA), or the frequency of phenodevi-

ants. FAs denote diminutive, accidental departures from symmetry in otherwise bilateral symmetrical characters, whereas phenodeviance encloses relatively large and conspicuous morphological anomalies, such as having the heart on the right side of the body cavity, or the presence of an even number of fingers on a hand (Ludwig 1932; Van Valen 1962; Møller and Swaddle 1997). Development of the left and right sides of a paired trait is presumably controlled by an identical set of genetic instructions, and to produce the ideal phenotype individuals are recurrently buffered against developmental insults by employing homeostatic mechanisms (Møller and Swaddle 1997). However, morphological accuracy is in mammals, birds and fish shown to be disturbed by various biotic and abiotic stressors (Møller and Swaddle 1997). Under optimal conditions energy dissipation is at minimum, whereas during a stress response an organism has less energy accessible to distribute to the various life functions, hence compromising these momentarily or enduringly and diverting energy from less necessary control mechanisms (Campbell and Emlen 1996). It is thus suggested that flaws in symmetry may reflect perturbations experienced during ontogeny and thereby represent responsive, consistent indices of preceding environmental challenges (Møller and Swaddle 1997).

The appliance of developmental instability is diverse in life sciences, including human medicine and pollution monitoring. Recently it has also been assumed to denote an objective, non-invasive and integrated measure of animal welfare (Møller and Swaddle 1997; Møller 1998). Individuals exhibiting a high level of developmental stability are suggested to have a selective pro compared to those with lower developmental stability, as symmetrical morphology generally signals high phenotypic quality (Møller and Swaddle 1997). Indeed there is substantial evidence that asymmetry and phenodeviance are associated with reduced fitness; abnormal organisms have reduced survival, growth and fecundity, as well as lowered immune competence (Møller and Swaddle 1997). Additionally, anomalous organisms are reported to have reduced competitive ability and be more fearful and less sexually attractive than their normal counterparts (Thornhill and Gangestad 1993; Møller and Swaddle 1997). In humans, FA is related to increased incidence of behavioral anomalies like hyperactivity, mental retardation, learning difficulties and schizophrenia (Thornhill and Møller 1997). While the degree of FA and phenodeviance are associated with reduced fitness or performance, we have little specific knowledge regarding how the distinct anomalies reduce biological function.

While a significant number of studies have investigated the impact of diverse environmental stressors on developmental perturbations, few experiments have explored maternal effects. However, some have reported that maternal effects may affect the offspring's level of developmental stability (Livshits et al. 1988; Kieser et al. 1997; Polak 1997). For example, in humans advanced maternal age and cardiovascular diseases and sickness may increase FA in newborns (Livshits et al. 1988). Also, Kieser et al. (1997) found that a combination of maternal obesity and smoking was a predictor of fluctuating dental asymmetry in offspring. Conversely, Smith et al. (1982) failed to find an effect on dental asymmetry in children of mothers suffering from diabetes, hypothyroidism and hypertension. Although the findings on maternal effects on the developmental stability of progeny are relatively miscellaneous, Thornhill and Møller (1997) concluded in a review that poor maternal condition indeed can impair developmental precision of the offspring.

In a given group of cultured salmon one can record a relatively high percentage of fish inflicted with diverse malformations in skeletal and soft tissue. These abnormalities are found to arise due to a wide range of environmental factors and might signify suboptimal rearing conditions (Barahona-Fernandes 1982; Divanach et al. 1996; Koumoundouros et al. 2001). Malformations are economically costly, as they require manual sorting and also reduce the affected animal's performance, such as swimming stamina, feed conversion ratio, growth rate, survival and susceptibility to stress and pathogens (Barahona-Fernandes 1982; Andrades et al. 1996; Divanach et al. 1996; Koumoundouros et al. 2001; Claireaux et al. 2005). Previous research into the aetiology of malformations in farmed teleosts has largely neglected a possible

maternal influence. Prenatal factors should however not be excluded, as these have been found to affect offspring morphology in mammals and birds, possibly via the action of maternal steroids (Braastad 1998; Eriksen et al. 2003). In fish, maternal transfer of vitellogenin, lipids and other nutrients are essential during egg maturation, and maternal steroids may also impinge on offspring characters (Schreck et al. 2001; Eriksen et al. 2006a, b). However, whilst intermediate levels might be crucial for regulating foetal development, excess could be disadvantageous. Cortisol, for instance, is often secreted during stressful experiences, and is anti-developmental and suppresses growth (Schreck 1992). In intensive aquaculture, salmonid broodstock is commonly exposed to stressful procedures that may induce various physiological alterations in the fish, such as increments in circulating cortisol (Campbell et al. 1994; Schreck et al. 2001). Little information has so far been generated to determine whether these events cause measurable impacts on the developmental trajectories of the offspring.

A few studies though have indicated that maternal stress might interrupt progeny morphogenesis in fish (Cerda et al. 1994; Morgan et al. 1999). Likewise, Eriksen et al. (2006a, b) recently showed that manipulating cortisol levels in mature farmed Atlantic salmon females affected various traits in their offspring, for instance survival rate and stress coping capability. Moreover, the frequency of morphological anomalies increased, thus suggesting a relationship between maternal endocrinology and the degree of developmental stability in the progeny. It was furthermore found that prenatal exposure to cortisol affected the growth pattern of the offspring in that elevated maternal cortisol increment caused growth retardation at early developmental stages, whereas this growth retardation was compensated later in life (Eriksen et al. 2006a, b).

In both fish and mammals, FA has been advocated as a retrospective, sensitive indicator of the capability of an organism to cope with various stressors faced during ontogeny (Valentine et al. 1973; Leary et al. 1991; Møller and Swaddle 1997; Øxnevad et al. 2002). However, the hypothesized relationship between sub-lethal stress and developmental instability has not always been supported (Palmer 1996; Björkstein et al. 2000). The frequency of gross abnormalities has been shown to be positively correlated with FA (Rasmuson 1960; Leary et al. 1984). It might therefore be expected that specimens with malformations also reveal minor FAs, whereas conversely, organisms that are asymmetric do not necessarily have to be inflicted with any malformations. Moreover, it is generally held that organisms are especially vulnerable to perturbations of morphological development during periods of rapid growth (Møller and Swaddle 1997). As a part of a long-term study, the present paper aims at investigating the impacts of maternal prespawning cortisol exposure and subsequent mild hyperthermia exerted during incubation of the eggs on size, condition factor and developmental instability in ten months old Atlantic salmon offspring. Measures of developmental instability will encompass both subtle FAs but also different obvious internal and external malformations. The possible correlation between FA and phenodeviance will be explored, and also the association between developmental instability and condition. Finally, it is discussed to what extent deviant morphology could represent a plausible indicator of a suboptimal maternal environment in intensive aquaculture.

Materials and methods

Fish, experimental set-up and procedures

This paper is part of a long-term experiment that was carried out during a period from December 2002 to December 2003. Farmed Atlantic salmon females (*Salmo salar*, of the Salmo Breed strain, mean weight 9.9 ± 1.3 kg) were obtained from Bolaks, Eikelandsosen (60°24'N). The fish were kept in a 25 m^3 fibreglass tank supplied with aerated fresh water delivered at a rate of 500 l min^{-1} at ~1°C under natural photoperiod. Six days prior to the expected spawning date, thirty mature females were selected, tagged (PAT tags, Os Husdyrmerkefabrikk) and randomly divided into three groups, representing three implant treatments. Table 1 outlines the experimental

Table 1 Experimental design

Offspring group	Maternal cortisol treatment mg cort/kg body weight	Temperature (°C) from fertilization to first feeding	Number of offspring to first feeding	Number of fish analyzed in each experimental group
1	0	8 ± 0.3	1775	44
2	50	8 ± 0.3	2084	47
3	100	8 ± 0.3	1848	51
4	0	10 ± 0.2	1576	44
5	50	10 ± 0.2	1284	43
6	100	10 ± 0.2	1162	45

set-up. In treatment 1, the females received sham injections containing only the vehicle and so served as controls. The fish in treatments 2 and 3 were injected intraperitoneally with coconut oil containing low and high levels of cortisol, respectively. The two injection solutions were prepared by adding cortisol (No. H–0888, Sigma Aldrich, Sigma Chemical Co., St. Louis, MO, USA) to liquid coconut oil at 40°C to yield a final concentration of 50 and 100 mg cortisol ml^{-1} oil. Prior to injections, the females were anaesthetized with metacaine (MS 222), weighed and then injected intraperitoneally with 1 ml of the emulsion per kg body weight. This resulted in an implant of 50 and 100 mg cortisol per kg for treatment 2 and 3, respectively (equivalent to 139 and 278 μmol kg^{-1}). Controls were injected with coconut oil, 1 ml kg^{-1}. After the implantation, all the females were returned to their original tank.

Six days later, the fish were stripped. The treated fish were again caught, anaesthetized with metacaine, and a sample of eggs (0.25 l) was collected from each of the mature females in the three groups: seven, eight and nine females in treatments 1, 2, and 3, respectively. Eggs within each treatment were pooled and fertilized by milt pooled from three untreated male salmon and further divided into two separate batches, which were then kept at two different temperatures from fertilization to first feeding. One served as a control (8 ± 0.3°C incubation temperature) and one batch was exposed to mild hyperthermia (10 ± 0.2°C incubation temperature). The two manipulations (cortisol administration and mild hyperthermia) generated six different offspring groups, apparently ranging from 'non-stressed' (group 1) to 'highly stressed' fish (group 6) (Table 1).

Collection of serum and eggs for cortisol analyses

Samples of maternal blood and eggs were collected for cortisol analyses from control females, the cortisol implanted females and also from ten undisturbed mature female salmon. Blood was drawn from the caudal vein into siliconized evacuated blood collecting tubes (Venoject®) without additives, stored at 4°C overnight and centrifuged at 3000 rpm (2000 *g*) for 10 min at 4°C. Serum was then separated and frozen (–20°C). Unfertilized eggs were collected and frozen at –20°C. Serum cortisol levels were measured in duplicates by a cortisol radioimmuno-assay (RIA, Hormone Laboratory, Aker University Hospital, Oslo, Norway) described by Aakvaag et al. (1978). Briefly, two 100 μl aliquots from each of the serum samples were added to 400 μl distilled water, and 5 ml diethyl ether for cortisol extraction. After ether evaporation the samples were resuspended into 200 μl of [^{3}H] cortisol and 200 μl antiserum, followed by incubation overnight at 4°C. Separation of antibody-bound and free radio-labeled cortisol was achieved by dextran-coated charcoal. The charcoal supernatant was counted in a liquid scintillation counter (Rackbeta, 1217, WallacOy, Finland). The RIA had a sensitivity range of 17-500 ng ml^{-1}, and the intra- and inter-assay coefficients of variation were 6 and 8%, respectively (Aakvaag et al. 1978). The values were not corrected for the extraction efficiency averaging 95%.

Each egg was homogenized in 1 ml physiological saline in an Ultra-Turrax T25 (Janke and Kunkel GmBH, IKA Labor Tecnik, D7818 Staufen i. Br., Germany) followed by the addition

of 5 ml diethyl ether. Subsequent extraction and assay were as for serum samples.

Incubation and rearing conditions

The fertilized eggs were transported to the research station of AKVAFORSK, Sunndalsøra, Norway. Eggs from each group were placed in separate compartments (10 × 15 cm), within trays in incubators supplied with a constant flow of fresh water. The temperature from fertilization to first feeding was held at 8 ± 0.3°C and 10 ± 0.2°C for group 1–3 and group 4–6, respectively, all in duplicate. From first feeding and throughout the experimental period, all the fish were reared under identical conditions, as normally applied in intensive aquaculture in Norway. The fish were kept under a regime of continuous light, and feed was delivered to the fish every 15 min by automatic feeders. From 810 d° (d°; day degrees~ water temperature each day multiplied with number of days) to 1530 d° the fish were held in cylindrical tanks (150 l) with a constant flow of fresh water delivered at a rate of 2 l min^{-1} at 12°C. From 1530 to 2490 d° the fish were maintained in groups with 900 individuals in cylindrical tanks (175 l) with fresh water supplied at 6 l min^{-1} at 8°C. Finally, from 2490 d° to the end of the experiment the fish were kept in groups with 750 individuals in cylindrical tanks (175 l) with fresh water, 15 l min^{-1} at 8°C. Each offspring group was kept in a separate tank.

Recorded parameters

Offspring mortality, weight, length, and frequency of malformations were recorded at hatch (510 d°), first feeding (810 d°), 1730 d°, and 2842 d° (Eriksen et al. 2006a, b). Ten months after hatching ~25 fish per group were euthanized with an overdose of MS–222, percussive stunned and immediately frozen at –20°C for later recordings of fork length, body weight, as well as FA and phenodeviance (internal and external malformations). Additionally, approximately 20 fish per group were X-rayed. Condition factor was calculated with the formula: [(weight(gm) × 100)/ $length^3$ (cm^3)].

When investigating impacts of stressors on FA in fish, bilateral asymmetry can be recorded of either metric or meristic characters. Asymmetry of meristic characters, e.g. number of fin rays, may be indicative of a suboptimal environment early in morphogenesis, due to the fact that most meristic characters develop at an early stage with minimal modification throughout lifetime. Contrarily, asymmetry of metric characters in organisms with continuous growth like fish, may originate due to stress experienced at any developmental stage, hence making it difficult to separate the impacts of stress exerted at different life stages. In the current study, the effects of maternal cortisol exposure and early hyperthermic exposure were to be examined. Therefore, FA was assessed by making right and left counts in situ on two bilateral meristic characters previously shown to exhibit increased FA in salmonids exposed to stress, i.e. pectoral fin rays and pelvic fin rays (Leary et al. 1983, 1984; Vøllestad and Hindar 1997). Phenodeviance was examined by dissecting the fish and observing external and internal anomalies. Malformations of bony structures were also investigated in X-rayed fish. Numerous morphological anomalies were surveyed, e.g. abnormalities of the head (malformed jaw and skull), vertebral column (fused vertebrae, lordosis, kyphosis), fins (missing, reduced size), operculum (shortened, folded), heart (missing septum transversum, situs inversus cordis), liver (dislocated) and spleen (dislocated, multiple).

Statistics

The statistical analyses were carried out by applying the SAS statistical package for personal computers (SAS 2000). Measurement error may be large in studies on bilateral asymmetry; hence the counts were repeated twice. No measurement errors were however detected, and the counts were pooled to generate one value per side per individual fish. Asymmetry may vary with trait size in various ways and it has been argued that the relation between FA and size should be inspected (Møller and Swaddle 1997). However, size might mirror particular aspects of condition, and thus adjusting for trait size may partially control for condition and create asymmetry

estimates that are independent of condition (Palmer 1994; Møller and Swaddle 1997). Nevertheless, length of the pectoral and pelvic fins was also measured in order to investigate whether there was an association between FA and fin size (Palmer 1994). No significant correlation between absolute FA and the size of pectoral and pelvic fins was detected; it was thus not needed to standardize FA values by correcting for size differences. For each character, asymmetry was first estimated as the signed difference between the right and left sides (FA = R–L). Several confounding factors can obscure estimates of FA (Palmer 1994; Møller and Swaddle 1997). FA is defined statistically in terms of its R–L distributions normally around zero. To certify that the asymmetry present is true FA, it is necessary to exclude the alternative possibilities of directional asymmetry (normal distribution with mean different from zero, i.e. skewed) and antisymmetry (typically a bimodal or platykurtic distribution with a mean of zero) (Palmer and Strobeck 1986; Palmer 1996). It was therefore tested for the presence of antisymmetry and directional symmetry by testing for mean = 0, by testing for skewness and kurtosis, and by visual inspection of the R–L distribution (Van Valen 1962; Palmer 1994). Subsequent to these initial steps to verify that the asymmetry present was true fluctuating asymmetry, FA was defined as the unsigned absolute value of the difference between the right and left sides (FA = |R–L|). As developmental stability is frequently not correlated between traits in the same individual, composite indices of FA may represent the general developmental stability more precisely than a measure of FA of each trait (Møller and Swaddle 1997). Therefore, in addition to single-character asymmetry, a composite asymmetry index for each individual was generated by summarizing the total number of asymmetrical fin rays ($|R–L|_{\text{pelvic fin rays}} + |R–L|_{\text{pectoral fin rays}}$). Using the GLM procedure (PROC GLM) it was then tested for impacts of cortisol treatment and mild hyperthermia and also possible interaction effects on fork lengths, body weights, condition factor and phenodeviance. The model contained maternal cortisol exposure and incubation temperature as independent variables, while fork length, body weight, condition factor and phenodeviance represented the dependent variables. Unsigned FA has a half-normal distribution, which violates the assumptions of most parametric statistics. Therefore, when testing for possible differences in FA between groups of offspring, a non-parametric Kruskal–Wallis test was applied. The Kruskal–Wallis test is identical to the parametric ANOVA performed on the ranks of the data. Finally, Pearson's correlation test was applied to test for correlations between size, condition factor, FA and phenodeviance.

Results

Effects of cortisol implants

The concentrations of cortisol in serum were 192.9 ± 39.1 ng ml^{-1}, 238.3 ± 37.1 ng ml^{-1}, 221.4 ± 37.1 ng ml^{-1} and 373.0 ± 37.1 ng ml^{-1} (means ± SE) in the untreated females, control females and the females receiving low and high doses of cortisol, respectively. As for cortisol in eggs, the concentrations were 51.3 ± 25.4 ng ml^{-1}, 37.6 ± 28.7 ng ml^{-1}, 123.1 ± 26.9 ng ml^{-1} and 123.7 ± 25.4 ng ml^{-1} (means ± SE), respectively. Intraperitoneal cortisol implants six days prior to stripping thus caused significant elevations in cortisol concentrations in maternal serum and egg. For specific details as regards efficacy of the implantation procedures, see Eriksen et al. (2006a).

In this study, no significant differences were found for any of the recorded parameters between offspring originating from females receiving low and high dosages of cortisol. In the statistical analyses, the offspring groups that originated from females implanted with 50 and 100 mg cort/kg were therefore pooled. This generated four offspring groups; 8°C—0 mg cort/kg, 8°C—50/100 mg cort/kg, 10°C—0 mg cort/kg and 10°C—50/100 mg cort/kg.

Size and condition factor

Mild hyperthermia exerted during incubation increased body length in the offspring ($F = 20.29$, df = 1, $P < 0.0001$; Table 2). Prenatal cortisol

Table 2 Fork length, body weight and condition factor in salmon offspring ten months post hatch (means ± SE)

Offspring group	Fork length (cm)	Body weight (gm)	Condition factor
8°C—0 cort	13.34 ± 0.39 a	34.93 ± 3.29 a	1.36 ± 0.02 ac
8°C—50/100 cort	14.12 ± 0.28 a	40.10 ± 2.38 a	1.35 ± 0.02 ab
10°C—0 cort	15.06 ± 0.40 b	50.59 ± 3.44 b	1.42 ± 0.02 cd
10°C—50/100 cort	15.46 ± 0.28 b	56.12 ± 2.35 b	1.47 ± 0.02 de

Dissimilar letters indicate statistical differences ($P < 0.05$)

exposure of the eggs also tended to enhance offspring fork length ($F = 2.99$, df = 1, $P = 0.08$). Likewise, mild temperature stress during incubation led to heavier offspring at ten months of age ($F = 29.67$, df = 1, $P < 0.0001$; Table 2) whereas maternal cortisol implants only tended to increase offspring body weight ($F = 3.39$, df = 1, $P = 0.07$; Table 2). The highest condition factor was observed in fish exposed to hyperthermia during incubation ($F = 19.33$, df = 1, $P < 0.0001$; Table 2), whereas prenatal cortisol challenge did not significantly affect condition factor at this stage. Intriguingly, the offspring that had been exposed to both prenatal cortisol increments and early temperature stress were longest, heaviest and had the highest condition factor (Table 2).

Fluctuating asymmetry

The means of none of the asymmetry distributions differed significantly from zero; hence the two characters pelvic fin rays and pectoral fin rays did not show directional asymmetry. Testing for kurtosis is essential when investigating the potential existence of antisymmetry. A kurtosis value greater than zero would indicate leptokurtosis, whereas a kurtosis value less than zero could indicate platykurtosis and possibly antisymmetry. Accordingly, whenever individual differences in developmental imprecision exist, signed FA should be leptokurtically distributed (Leung and Forbes 1997; Møller and Swaddle 1997). Kurtosis for pelvic and pectoral fin ray asymmetry was 2.39 and –0.28, respectively. Pectoral fin ray asymmetry was thus slightly platykurtic. However, visual inspection showed that the distribution was not antisymmetric. Platykurtic distributions might simply reflect that selection has already discarded the most asymmetrical specimens rather than the trait not exhibiting the features of FA (Møller and Swaddle 1997). Therefore, as indicated by tests and visual inspection, it was concluded that the distributions were neither directionally distributed nor antisymmetric, and that the asymmetry present was true FA.

The level of asymmetry for the two meristic characters was calculated as the absolute value of the R–L difference of each character. Maternal cortisol increment enhanced FA of both pectoral fin rays ($\chi^2 = 16.45$, df = 1, $P < 0.0001$; Fig. 1) and pelvic fin rays ($\chi^2 = 9.28$, df = 1, $P < 0.05$; Fig. 1). Hyperthermia during incubation increased FA of pelvic fins ($\chi^2 = 12.16$, df = 1, $P < 0.01$, Fig. 1), whereas asymmetry of pectoral fins was unaffected by early temperature stress (Fig. 1). The highest level of FA in pelvic fins was seen in offspring that had been challenged with both prenatal cortisol and hyperthermia. In all groups, the level of FA was generally higher in the pectoral fins than in the pelvic fins (Fig. 1). When examining the composite FA index, it was found that prenatal cortisol exposure increased this index ($\chi^2 = 21.93$, df = 1, $P < 0.0001$, Fig. 1). Hyperthermia during incubation did not significantly affect the composite FA index. The largest composite FA could be observed in offspring that originated from cortisol implanted females and had also been exposed to temperature stress during incubation.

Phenodeviance

Figure 2 shows fraction of fish with malformations ten months after hatch. Prenatal cortisol exposure did not significantly affect the frequency of malformations at this stage. However, there was a tendency of temperature stress to increase the occurrence of malformations ($F = 3.17$, df = 1, $P = 0.08$; Fig. 2). The highest number of morphological anomalies could be observed in offspring

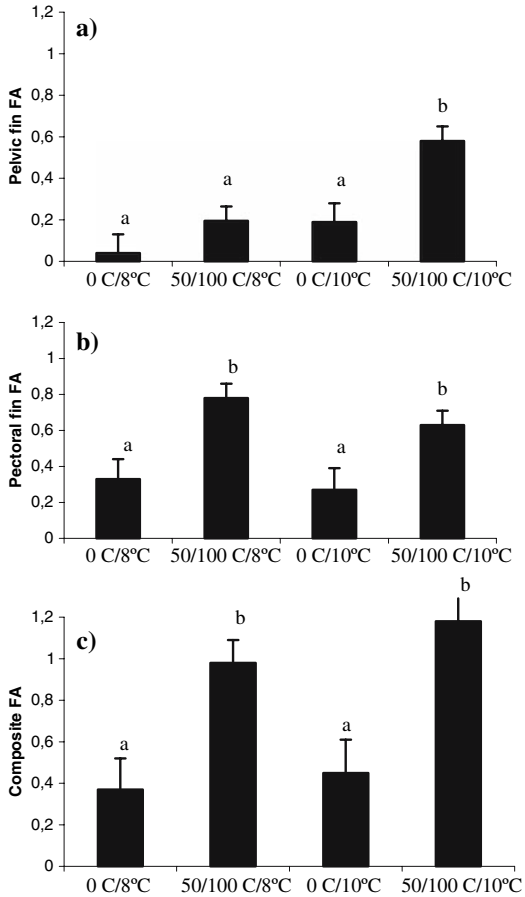

Fig. 1 Fluctuating asymmetry (mean ± SE) for the meristic characters pelvic fin rays (**a**), pectoral fin rays (**b**), and the composite asymmetry index (**c**). No significant differences were found between offspring originating from females receiving low and high dosages of cortisol. Thus, in the statistical analyses, the offspring groups that originated from females implanted with 50 and 100 mg cort/kg were pooled. This generated four offspring groups; 8°C—0 mg cort/kg, 8°C—50/100 mg cort/kg, 10°C—0 mg cort/kg and 10°C—50/100 mg cort/kg. Indications of maternal cortisol treatment and incubation temperature are given below bars (e.g. 0 C/8°C refers to 0 mg/kg maternal cortisol implantation and subsequent incubation temperature at 8°C). Different letters above bars indicate statistical differences ($P < 0.05$)

from cortisol implanted females that had also been incubated at 10°C. Various morphological malformations were observed, including anomalies of the vertebral column, fins, operculum, heart, liver, and spleen. The most common ones were splenic ($N = 23$) and cardiac anomalies ($N = 12$), where the fish typically displayed multiple

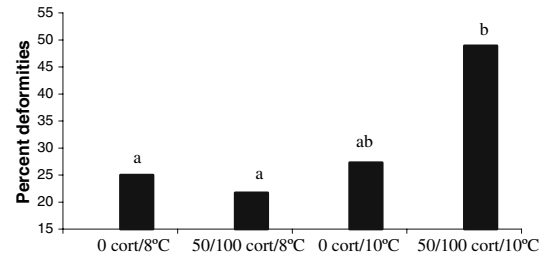

Fig. 2 Percentage of salmon offspring inflicted with malformations (vertebral column, fins, operculum, heart, liver, spleen), based on in situ observations. Indications of maternal cortisol treatment and incubation temperature are given below bars (e.g. 0 C/8°C refers to 0 mg/kg maternal cortisol implantation and subsequent incubation temperature at 8°C). Different letters above bars indicate statistical differences ($P < 0.05$)

spleens, a relocated spleen, situs inversus of the heart and absence of the septum transversum. Displacement of the liver ($N = 6$) and malformed fins ($N = 5$) were also found. There were no apparent disparities regarding different types and occurrence of these anomalies in the offspring groups, i.e., the treatments did not seem to impinge on the particular type of morphological abnormality.

Figure 3 shows the percentage of abnormalities in X-rayed fish. The highest frequency (25%) of vertebral malformations was seen in the offspring exposed to both prenatal cortisol and hyperthermia. However, no obvious impacts on vertebral malformations could be observed as a consequence of maternal cortisol exposure and temperature stress during incubation (Fig. 3).

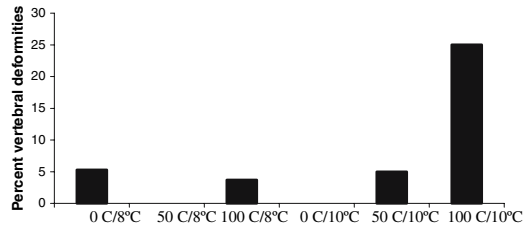

Fig. 3 Percent salmon offspring displaying anomalies in the vertebral column, identified by X-ray. Indications of maternal cortisol treatment and incubation temperature are given below bars (e.g. 0 C/8°C refers to 0 mg/kg maternal cortisol implantation and subsequent incubation temperature at 8°C)

Table 3 Pearson correlation coefficients between developmental instability, size and condition factor

	Length	Weight	Condition factor	Composite FA	Pelvic fin (FA)	Pectoral fin (FA)
Weight	0.95*					
Condition	0.16	0.38*				
Composite FA	–0.04	–0.06	0.09			
Pelvic fin FA	0.02	0.05	0.19*	0.71*		
Pectoral fin FA	–0.03	–0.05	–0.01	0.78*	0.12	
Phenodeviance	0.16*	0.18*	0.12	–0.03	–0.03	0.00

(*$P < 0.05$)

Relationship between developmental instability, size and condition factor

Correlations between developmental instability, size, and condition factor are shown in Table 3. It was found that pelvic fin asymmetry was positively correlated to condition factor ($P < 0.05$). Moreover, phenodeviance was correlated with fork length ($P < 0.05$) and weight ($P < 0.05$); the specimens inflicted with malformations were longer and heavier than the normal ones. Logically, positive correlations between the composite FA index and pelvic ($P < 0.0001$) and pectoral fin asymmetry ($P < 0.0001$), between length and weight ($P < 0.0001$), and also between condition factor and weight ($P < 0.0001$) were found. No correlation was however seen between asymmetry in pelvic and pectoral fins. Likewise, FA and phenodeviance were not related to each other. It should be mentioned that given the relatively large number of correlations tested, the statistical evidence for the correlations is somewhat weak.

Discussion

Developmental stability, including measures of FA and phenodeviance, signifies deviations from the ideal morphological state. Bilaterally symmetrical traits are somewhat universal in nature and estimation of developmental stability has thus attracted substantial interest because it appears to represent a relatively simple method to identify sub lethal stress exposure and assess animal welfare (Møller and Swaddle 1997). This study was designed primarily to investigate whether maternal cortisol implantation 6 days before stripping and an additional stressor, i.e. mild hyperthermia exerted during incubation affected developmental stability in farmed Atlantic salmon juveniles. It was found that maternal cortisol administration increased the level of FA in pectoral and pelvic fins in the offspring, but did not affect the frequency of malformations. Furthermore, mild hyperthermia exerted during incubation increased weight and fork length and also increased pelvic fin FA. Finally, it was demonstrated that malformed fish were heavier and longer than the normal ones, and that pelvic fin asymmetry was positively related to condition factor.

Mild hyperthermia exerted during incubation significantly increased fork length and weight in the progeny. Maternal cortisol exposure, however, did not significantly affect fork length and weight. Eriksen et al. (2006a, b) previously demonstrated that both enhanced cortisol levels in mature females and increased incubation temperature reduced growth in salmon offspring at early developmental stages, whereas this growth restriction was recompensed later in life. Thus, although the offspring had impaired growth early in life, the subsequent growth compensation may reflect adaptive mechanisms if maternal stress indicates environmental conditions where rapid growth in offspring is advantageous to better acquire resources, defend territories, avoid predators and finding mates. The observed growth pattern compares well with findings reported from studies where females have been exposed to challenges during sexual maturation (fish) or pregnancy (mammals) (Braastad 1998; Contreras-Sànchez et al. 1998; Schreck et al. 2001). The impacts of prenatal stress on the growth trajectories of the progeny have been discussed previously (Eriksen et al. 2006a, b). When examining the condition factor, it was found that exposing embryos to mild hyperthermia during incubation

increased this index in ten months old offspring, whereas prenatal cortisol exposure did not significantly affect it. The highest condition factor was observed in the offspring that had been exposed to both prenatal cortisol increment and also subsequent hyperthermia. Condition factor and other length–weight relations are frequently applied as non-invasive indicators of vigour. Declines in condition factor may reflect alterations in energy storage, metabolism, and feeding behavior due to environmental challenges, but might also happen for other reasons than stress, for instance seasonal and life stage changes. A high condition factor is normally regarded as positive, reflecting healthy, vital animals. In intensive aquaculture, slim fish have thus traditionally been discarded, preferring those with high condition factor. However, Gjerde et al. (2005) recently showed that malformed Atlantic salmon were shorter, lighter and had higher condition factor than normal fish. Likewise, Claireaux et al. (2005) demonstrated that condition factor was positively related to anomalous cardiac shape and reduced swimming performance in rainbow trout. High condition factor may hence not necessarily imply healthy, vital specimens; it is more likely that an 'optimal' condition factor would be somewhere between starvation and obesity (Claireaux et al. 2005).

The current experiment further revealed that prenatal cortisol exposure increased the level of FA in pectoral and pelvic fins. In addition, hyperthermia exerted during incubation enhanced pelvic fin asymmetry and tended to increase the frequency of phenodeviants. Both pelvic fin FA and the composite FA index were highest in progeny whose mothers had been implanted with cortisol and who were later exposed to temperature stress. In teleosts, the extent of FA and phenodeviance have been measured in numerous experiments to assess the degree of exposure to various environmental challenges, e.g. suboptimal incubation temperature, low pH, high densities, elevated levels of mercury, insecticides, or other industrial pollutants and starvation (Valentine and Soulè 1973; Laale and Lerner 1981; Leary et al. 1992; Møller and Swaddle, 1997; Grønkjær and Sand 2003). Indeed, several studies reveal increased level of asymmetry as a consequence of environmental challenges (Valentine and Soulè 1973; Leary et al. 1992; Østbye et al. 1997; Campbell et al. 1998; Mazzi and Bakker 2001; Øxnevad et al. 2002). By contrast, others have provided limited support to the view that stress challenges homeostatic mechanisms, with subsequent increased incidence of asymmetrical or deviant structures (Jagoe and Haines 1985; Vøllestad et al. 1998; Panfili et al. 2005). Scarce knowledge, however, exists regarding the impacts on morphological characteristics of the progeny that are due to stress experienced by the broodstock. Yet, a few studies in fish have reported enhanced frequency of malformed offspring if the broodstock are stressed during sexual maturation (Cerda et al. 1994; Morgan et al. 1999; Eriksen et al. 2006a, b).

The evolution of 'adaptive' growth rate and its impact on other life history dispositions is claimed to represent an ignored topic in biology (Robinson and Wardrop 2002). As for developmental stability, it is generally held that asymmetrical organisms have lower growth rates than symmetrical ones (Møller and Swaddle 1997). However, organisms may be more vulnerable to perturbations in morphological development during periods of intensive growth. In farm animals, individuals with rapid growth more often show morphological anomalies, signifying that high growth rates should be recognized as a risk factor per se (Olsson 1978). Robinson and Wardrop (2002) found evidence in three-spined sticklebacks, Gasterosteus aculeatus, that FA was increased in fast growing individuals. Likewise, Elliott et al. (1995) demonstrated that Chinook salmon, Oncorhynchus tshawytscha, with high condition factor also more frequently had various abnormalities. In the current study, positive correlations between size, condition factor, and developmental instability were found. The deviant fish were longer and heavier than the normal ones and pelvic fin asymmetry was also positively related to condition factor. This could imply that high growth rates might have instigated developmental disturbances. However, this finding probably reflects two separate outcomes of prenatal cortisol challenge rather than causal mechanisms linking rapid growth and anomalous morphology. Obvious malformations in internal organs and fin

ray asymmetry are rather unlikely to develop post hatch (Gorodilov 1996; Geir Totland, personal communication). The notion that increased cortisol levels in mature females inhibited initial growth (Eriksen et al. 2006a) indeed supports the suggestion that prenatal cortisol exposure may induce both growth compensation with subsequent high condition factor, and also increased FA and phenodeviance, but that these outcomes are independent of each other, i.e. the deviant morphology seen in cortisol exposed offspring are not caused by the growth compensation.

It has been claimed that some of the published studies have been somewhat too optimistic regarding the universality of correlation between the impact of a preceding environmental challenge and developmental instability. Recently, a more careful and circumspect approach has emerged to the ubiquitous applicability of developmental instability as a simple, attractive method to discover stress in animals and plants (Merila and Bjørklund 1995; Palmer 1996; Bjørkstein et al. 2000). As for phenodeviance, it is normally quite unusual within populations, appearing in less than 4% of the population (Jones 1988). Development has to be extensively perturbed before these radical phenotypes appear, and such anomalies thus represent rather insensitive measures of developmental stability. The use of gross abnormalities will only be feasible and valuable indicators of developmental stability if the population is severely stressed or if a large number of traits are measured (Møller and Swaddle, 1997). Given that approximately one third of all studies fail to support the idea that developmental stability is decreased due to environmental and genetic stresses, the method is thus far from universal (Bjørkstein et al. 2000). Besides, non-appearance of developmental instability does not necessarily imply that the organism has been reared without any exposure to stress (Anne et al. 1998). If FA is a good indicator of overall developmental stability, and if anomalous morphology was to be a reliable indicator of the optimality of the broodstock's environment during sexual maturation, one could expect that phenodeviants also might display increased asymmetry. Indeed, phenodeviants are often reported to also exhibit increased asymmetry (Rasmuson 1960, Adams and Niswander 1967; Barden 1980; Malina and Buschang 1984). Leary et al. (1984) found a positive relationship between FA and phenodeviance in salmonids. Such a correlation was however not found in the present study, and the specific reason for this is unknown. FA was assessed by examination of pectoral and pelvic fin rays, whereas phenodeviance was mainly assessed by recording anomalies in internal organs, e.g. liver, spleen, and heart. In salmon, internal organs start to develop before the fins (Gorodilov 1996). It might be that stress susceptibility varies between traits, and also between different developmental stages during embryogenesis. Also, it could be that the causal mechanisms generating subtle asymmetries and gross phenodeviance are distinct, with subsequent lack of correlation between FA and malformations.

Finally, it should be mentioned that, even if the groups were reared in duplicate from fertilization to first feeding, offspring from different experimental groups were kept in disparate containers from first feeding until the end of the experiment, with one tank per treatment. This is of course a predicament with the experimental design, as it implies that the study is basically not replicated. However, there are several arguments that tank effects may be relatively unlikely in this study. From first feeding and throughout the experimental period, all the tanks shared the same water source and thus had water with equal physical and chemical properties, including temperature. The six tanks were positioned in close proximity to each other, and had identical illumination and water current conditions. The feeding regimes and all the daily routines performed by the caretakers were similar in the six tanks. In addition, due to the growth of the offspring, the fish were moved to new tanks three times during the experimental period, thus eradicating potential tank effects. Nevertheless, future experiments should include replication to allow for possible tank effects.

In his review Bernardo (1996) emphasized the ubiquitous nature of maternal influences on progeny characteristics and the potential of these influences to affect adult life stages. Likewise, non-genetic maternal contributions have recently been demonstrated to fortify the 'silver spoon effect' in various taxa, where the early life history

characteristics of an individual excessively affects its later success (Hofer and East 2003). Findings in the present study indicate that developmental trajectories of progeny may be significantly altered if salmon females have elevated levels of glucocorticoids during the final periods of gametogenesis, thus accentuating the importance of including maternal effects when investigating the origin of the diverse morphological anomalies frequently seen in intensive aquaculture. This may be interesting both from a fundamental point of view, but also due to its significance for cultured species because it discloses mechanisms that can link maternal factors to offspring viability and productivity.

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References

Aakvaag A, Bentdal Ø, Quigstad K, Walstad P, Rønningen H, Fonnum F (1978) Testosterone and testosterone binding globulin (TeBG) in young men during prolonged stress. Int J Androl 1:22–31

Adams MS, Niswander JD (1967) Developmental noise and congenital malformation. Gen Res 10:313–317

Andrades JA, Becerra J, Fernandez-Llebrez P (1996) Skeletal deformities in larval, juvenile and adult stages of cultured gilthead sea bream (Sparus aurata L.). Aquaculture 141:1–11

Anne P, Mawri F, Gladstone S, Freeman CD (1998) Is fluctuating asymmetry a reliable biomonitor of stress? A test using life history parameters in the soybean. Int J Plant Sci 159:559–565

Barahona-Fernandes MH (1982) Body deformation in hatchery reared European sea bass Dicentraechus labrax (L): types, prevalence and effect on fish survival. J Fish Biol 21:239–249

Barden HS (1980) Fluctuating dental asymmetry: a measure of developmental instability in Down's syndrome. Am J Phys Anthropol 52:169–174

Bernardo J (1996) Maternal effects in animal ecology. Amer Zool 36:83–105

Björkstein TA, Fowler K, Pomiankowski A (2000) What does sexual trait FA tell us about stress? Trends Ecol Evol 15:163–166

Braastad BO (1998) Effects of prenatal stress on behaviour of offspring of laboratory and farmed mammals. Appl Anim Behav Sci 61:159–180

Campbell PM, Pottinger TG, Sumpter JP (1994) Preliminary evidence that chronic confinement stress reduces the quality of gametes produced by brown and rainbow trout. Aquaculture 120:151–169

Campbell WB, Emlen JM (1996) Developmental instability analysis of BKD-infected spring Chinook salmon, Oncorhyncus tshawytscha, prior to seawater exposure. Oikos 77:540–548

Campbell WB, Emlen JM, Hershberger WK (1998) Thermally induced chronic developmental stress in coho salmon: integrating measures of mortality, early growth and developmental instability. Oikos 81:398–410

Cerda J, Carrillo M, Zanuy S, Ramos J, de la Higuera M (1994) Influence of nutritional composition of diet on sea bass, Dicentrarchus labrax L., reproductive performance and egg and larval quality. Aquaculture 128:345–361

Claireaux G, McKenzie DJ, Genge AG, Chatelier A, Aubin J, Farrell AP (2005) Linking swimming performance, cardiac pumping ability and cardiac anatomy in rainbow trout. J Exp Biol 208:1775–1784

Divanach P, Boglione C, Menu B, Koumoundouros G, Kentouri M, Cataudella S (1996) Abnormalities in finfish mariculture: an overview of the problem, causes and solutions. In: Chatain B, Saroglia M, Sweetman J, Lavens P (eds) Seabass and seabream culture: problems and prospects. Int. Workshop, Verona, Italy, October 16–18, 1996. Eur Aquacult Soc, Oostende, Belgium, pp 45–66

Elliott DG, Pascho RJ, Palmisano AN (1995) Broodstock segregation for the control of bacterial kidney disease can affect mortality of progeny Chinook salmon (Oncorhynchus tshawytscha) in seawater. Aquaculture 132:133–144

Eriksen MS, Haug A, Torjesen PA, Bakken M (2003) Prenatal exposure to corticosterone impairs embryonic development and increases fluctuating asymmetry in chickens (Gallus gallus domesticus). Brit Poult Sci 44:690–697

Eriksen MS, Bakken M, Espmark Å, Braastad BO, Salte R (2006a) Pre-spawning stress in farmed Atlantic salmon Salmo salar: maternal cortisol exposure and hyperthermia during embryonic development affect offspring survival, growth and incidence of malformations. J Fish Biol 69:114–129

Eriksen MS, Espmark Å, Braastad BO, Salte R, Bakken M (2006b) Long term effects of maternal cortisol exposure and mild hyperthermia during embryogeny on survival, growth and morphological anomalies in farmed Atlantic salmon Salmo salar offspring. J Fish Biol (in press)

Gjerde B, Pante JR, Bæverfjord G (2005) Genetic variation for a vertebral deformity in Atlantic salmon (Salmo salar). Aquaculture 244:77–87

Gorodilov YN (1996) Description of the early ontogeny of the Atlantic salmon, Salmo salar, with a novel system

of interval (state) identification. Env Biol Fish 47:109–127
Grønkjær P, Sand MK (2003) Fluctuating asymmetry and nutritional condition of Baltic cod (Gadus morhua) larvae. Mar Biol 143:191–197
Hofer H, East ML (2003) Behavioral processes and costs of co-existence in female spotted hyenas: a life history perspective. Evol Ecol 17:315–331
Jagoe CH, Haines TA (1985) Fluctuating asymmetry in fishes inhabiting acidified and unacidified lakes. Can J Zool 63:130–138
Jones KL (1988) Smith's recognizable patterns of human malformation, 4th edn. Saunders, Philadelphia
Kieser JA, Groeneveld HT, daSilva PCF (1997) Dental asymmetry, maternal obesity and smoking. Am J Phys Anthropol 102:133–139
Koumoundouros G, Divanach P, Kentouri M (2001) The effect of rearing conditions on development of saddleback syndrome and caudal fin malformations in *Dentex dentex* (*L.*). Aquaculture 200:285–304
Leary RF, Allendorf FW, Knudsen KL (1983) Developmental stability and enzyme heterozygosity in rainbow trout. Nature 301:71–72
Leary RF, Allendorf FW, Knudsen KL (1984) Superior developmental stability of heterozygotes at enzyme loci in salmonid fishes. Am Nat 124:540–551
Leary RF, Allendorf FW, Knudsen KL (1991) Effects of rearing density on meristics and developmental stability of rainbow trout. Copeia 1:44–49
Leary RF, Allendorf FW, Knudsen KL (1992) Genetic, environmental and developmental causes of meristic variation in rainbow trout. Acta Zool Fennica 191:79–95
Leung B, Forbes MR (1997) Modeling fluctuating asymmetry in relation to stress and fitness. Oikos 78:397–405
Livshits G, Davidi L, Kobyliansky E, Ben-Amital D, Levi Y, Merlob P (1988) Decreased developmental stability as assessed by fluctuating asymmetry of morphometric traits in preterm infants. Am J Med Gen 29:793–805
Ludwig W (1932) Das Rechts-Links problem im Tierreich und beim Menschen. Springer, Berlin
Laale HW, Lerner W (1981) Teratology and early fish development. Am Zool 21:517–533
Malina RM, Buschang PH (1984) Anthropometric asymmetry in normal and mentally retarded males. Ann Hum Biol 11:515–531
Mazzi D, Bakker TCM (2001) Acid stress increases pelvic spine asymmetry in juvenile three-spined sticklebacks. J Fish Biol 59:582–592
Merila J, Bjørklund M (1995) Fluctuating asymmetry and measurement error. Syst Biol 44:97–101
Morgan MJ, Wilson CE, Crim LW (1999) The effects of stress on reproduction in Atlantic cod. J Fish Biol 54:477–488
Møller AP (1998) Developmental instability as a general measure of stress. Adv Stud Behav 27:181–213
Møller AP, Swaddle JP (1997) Asymmetry, developmental stability and evolution. Oxford University Press, Oxford, UK
Olsson SE (1978) Osteochondrosis in domestic animals—introduction. Acta Rad Diag 9–14(Suppl): 358
Palmer AR (1994) Fluctuating asymmetry: a primer. In: Markow TA (ed) Developmental instability: its origin and evolutionary implications. Kluwer Academic, Dordrecht, pp 335–364
Palmer AR (1996) Waltzing with asymmetry. Is fluctuating asymmetry a powerful new tool for biologists or just an alluring new dance step? BioScience 46:518–532
Palmer AR, Strobeck C (1986) Fluctuating asymmetry: measurement, analysis, patterns. Ann Rev Ecol Syst 17:391–421
Panfili J, Durand JD, Diop K, Gourene B, Simier M (2005) Fluctuating asymmetry in fish otoliths and heterozygosity in stressful estuarine environments (West Africa). Mar Freshwat Res 56:505–516
Polak M (1997) Ectoparasitism in mothers causes higher positional fluctuating asymmetry in their sons: implications for sexual selection. Am Nat 149:955–974
Rasmuson M (1960) Frequency of morphological deviations as a criterion of developmental stability. Hereditas 46:511–536
Robinson BW, Wardrop SL (2002) Experimentally manipulated growth rate in threespine sticklebacks: Assessing trade offs with developmental stability. Env Biol Fish 63:67–78
Schreck CB, Contreras-Sanchez W, Fitzpatrick MS (2001) Effects of stress on fish reproduction, gamete quality and progeny. Aquaculture 197:3–24
Schreck CB (1992) Glucocorticoids: metabolism, growth and development. In: Schreibman MP, Scanes CG, Pang PKT (eds) The endocrinology of growth, development and metabolism in vertebrates. Academic Press, New York, pp 367–392
Smith BH, Garn SM, Cole PE (1982) Problems of sampling and inference in the study of fluctuating dental asymmetry. Am J Phys Anthropol 58:281–289
Thornhill R, Gangestad SW (1993) Human facial beauty: averageness, symmetry and parasite resistance. Hum Nat 4:237–269
Thornhill R, Møller AP (1997) Developmental stability, disease and medicine. Biol Rev 72:497–548
Valentine DW, Soulè ME, Samollow P (1973) Asymmetry analysis in fishes: a possible statistical indicator of environmental stress. Fish Bull 71:357–370
Van Valen L (1962) A study of fluctuating asymmetry. Evolution 16:125–142
Vøllestad LA, Hindar K (1997) Developmental stability and environmental stress in Salmo salar (Atlantic salmon). Heredity 78:215–222
Vøllestad LA, Fjeld E, Haugen T, Øxnevad SA (1998) Developmental instability in grayling (Thymallus thymallus) exposed to methylmercury during embryogenesis. Env Poll 101:349–354
Østbye K, Øxnevad SA, Vøllestad LA (1997) Developmental stability in perch (Perca fluviatilis) in acidic aluminium-rich lakes. Can J Zool 75:919–928
Øxnevad SA, Heibo E, Vøllestad LA (2002) Is there a relationship between fluctuating asymmetry and reproductive investment in perch (Perca fluviatilis)? Can J Zool 80:120–125