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Effects of maternal stress coping style on offspring characteristics in rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

Maternal size, age, and allostatic load influence offspring size, development, and survival. Some of these effects have been attributed to the release of glucocorticoids, and individual variation in these stress hormones is related to a number of traits. Correlated traits are often clustered and used to define the proactive and reactive stress coping styles. Although stress coping styles have been identified in a number of animal groups, little is known about the coupling between stress coping style and offspring characteristics. In the present study, plasma cortisol levels in ovulated mothers and cortisol levels in non-fertilized eggs from two rainbow trout (*Oncorhynchus mykiss*) strains selected for high (HR) and low (LR) post-stress plasma cortisol levels were compared. Offspring characteristics such as egg size, larval growth, and energy reserves also were compared between the two strains. Maternal plasma and egg cortisol levels were correlated, but no difference between the HR and LR strains was detected in either parameter. LR females produced larger eggs, and larvae with larger yolk sacs compared to HR females, however no differences in larval body size (excluding the yolk) was detected between strains. Considering that the HR and LR strains have a number of correlated behavioral and physiological traits that resemble the reactive and proactive stress coping styles, respectively, the results suggest that proactive mothers invest more energy into their offspring, producing larvae with larger energy reserves. It is possible that larger energy reserves in proactive larvae support the energy requirement for establishing and defending territory in salmonid fish. Furthermore, in the present study we found a positive relationship between mother plasma cortisol and egg cortisol; however neither mother plasma cortisol nor egg cortisol differed between strains. These results indicate that cortisol endowment from the mother to the offspring plays a minor role in the transfer of the behavioral and physiological traits which separates these strains.

Introduction

Intra female variation in propagule and offspring characteristics has been demonstrated in a wide range of plant and animal species (Bernardo, 1996a,b; McCormick, 1998; McEdward, 1996; Roach and Wulff, 1987; Strathmann, 1977, 1985). Many studies have investigated how parental characteristics are reflected in offspring size, development, and survival (Dejesus and Hirano, 1992; Dejesus et al., 1991; McCormick, 1998; Yamano et al., 1991). The general idea is that fecundity and offspring size are related to maternal age and body size (reviewed by Bernardo (1996a,b)).

Factors other than maternal size and age have also been shown to affect offspring fitness and survival. Animal studies have shown that suboptimal environmental conditions accompanied by an increased allostatic load, has a negative effect on an individual's reproductive success (Lordi et al., 2000; Moberg, 1987; Petitte and Etches, 1991; Pollard, 1984). In teleost fish, allostatic load has been shown to affect offspring size, and survival (Campbell et al., 1992, 1994). Some of these suppressive effects have been related to glucocorticosteroids (Wingfield and Sapolsky, 2003); which are stress hormones regulated by the hypothalamus pituitary adrenal/internal axis (HPA/HPI). For example, studies in rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) demonstrate that females exposed to confinement stress during oogenesis showed elevated plasma cortisol levels and produced smaller offspring with increased mortality rates (Campbell et al., 1992, 1994).

Individual variation in the HPA/HPI axis reactivity as a response to a stressor has been shown to be consistent over time (Schjolden et al., 2005), and to be a highly heritable trait (Pottinger and Carrick, 1999).

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Moreover, such variation has been demonstrated to correlate with other physiological and behavioral traits (Øverli et al., 2005; Ruiz-Gomez et al., 2008; Schjolden et al., 2005), and has been clustered together into two characteristic stress response patterns, the proactive and reactive stress coping styles (Koolhaas et al., 1999). A proactive stress coping style is characterized by a behavior that aims to counteract the challenge, such as aggression to obtain social domination. The physiological response is dominated by the sympathetic nervous system response and release of catecholamines (Koolhaas, 2008; Koolhaas et al., 1999; Korte et al., 2005). A reactive individual, on the other hand, responds to a challenge with immobility and shows a generally more flexible behavior, and the physiological stress response is dominated by the HPA/HPI axis and the release of glucocorticoids. Although stress coping styles have been identified in a variety of animal groups (Koolhaas et al., 1999), to our knowledge, the effects of the maternal stress coping style and concomitant HPA/HPI axis reactivity on offspring characteristics have not been investigated.

Apart from having direct effects on fitness related traits in offspring, cortisol plays an important role in the regulation of gluconeogenesis and increases the metabolic rate in fish (Chan and Woo, 1978; Vijayan and Moon, 1994; Vijayan et al., 1991). Furthermore, several studies on larval fish showed that offspring depend on maternal energetic and hormonal endowment, which is available to them through the yolk-sac, for growth and development (Hwang et al., 1992; Schreck et al., 1991). This was also demonstrated in a study by Sloman (2010) where cortisol delivery by immersion to newly spawned eggs resulted in higher metabolic rate and ammonia excretion in brown trout larvae; compared to untreated larvae, further suggesting a stimulatory role of cortisol in larval development. However, a similar treatment affected developmental rhythms, but not developmental rate, in damsel fish (McCormick and Nechaev, 2002).

Maternal stress coping style and the associated HPA/HPI axis reactivity could also affect the behavior of the offspring. For example, studies performed on rats demonstrated that repeated stress and high levels of corticosteroids during pregnancy affected the HPA axis reactivity and induced anxiety like behavior in the offspring (Richardson et al., 2006). The coupling between stress in mother fish and the effects on behavior and HPA/HPI axis reactivity in the offspring are much less studied in fish. Recent studies on salmonid fish demonstrated that prefertilization injections of cortisol to the mother fish or immersion of unfertilized eggs in cortisol baths affected the behavior of the offspring (Espmark et al., 2008; Sloman, 2010). Even if the above mentioned studies suggest that variation in HPA/HPI axis reactivity in the mother could affect the behavior as well as development in the offspring, there has to our knowledge not been any investigations on the coupling between stress coping style, maternal cortisol endowment to the offspring, and offspring characteristics.

The aims of the present study were to investigate if differences in maternal stress coping style were reflected in offspring size and development. Furthermore we wanted to examine if the observed differences were related to differences in maternal cortisol endowment to the offspring in rainbow trout. To achieve this, we investigated cortisol level in nonfertilized eggs and plasma cortisol level in ovulated mothers, egg size, and larval development in two strains of rainbow trout selected for high (HR) and low (LR) post-stress plasma cortisol response. These strains were established in 1996 by Dr. Tom Pottinger (UK Natural Environment Research Council Center of Ecology and Hydrology, Windermere, UK), using a commercial strain of rainbow trout. Selection was based on the post-stress plasma cortisol response to a standardized stressor. These two strains have become a well established model for studying stress coping styles in fish (Höglund et al., 2008; Øverli et al., 2007; Ruiz-Gomez et al., 2008; Schjolden et al., 2005, 2006). Moreover the general pattern of behavioral and neuroendocrine responses demonstrated by LR and HR trout is consistent with the proactive and reactive stress coping styles found in mammals and birds (Øverli et al., 2004, 2005, 2007; Ruiz-Gomez et al., 2011; Schjolden et al., 2006).

Materials and methods

All animal procedures used in this study followed the policy and ethics as described by FELASA, Federation of European Laboratory Animal Science Associations, Denmark.

Experimental protocol

Eggs and larvae used in this study originated from the fifth generation of HR and LR rainbow trout selected on their cortisol response to a standardized stressor. Mother fish reared at the Technical University of Denmark, DTU Aqua, The North Sea Research Centre, Hirtshals, Denmark, were manually stripped on two separate occasions to produce batch 1 and batch 2. No significant differences in egg weight were found between batches (HR; $p = 0.14$, and LR; $p = 0.12$; *t*-test).

For batch 1 we used eggs originating from 5 LR females and 5 HR females. The eggs from each strain were mixed and then fertilized by a mix of sperm originating from 3 LR and 3 HR males, respectively. Eggs were incubated at DTU Aqua until they were eyed, and they then were transported to the University of Copenhagen, Marine Biological Laboratory (MBL), Helsingør, Denmark. At MBL the HR and LR eggs were incubated, in batches of 100 eggs for each strain, in circular containers with netted bottoms, which allowed water exchange. The containers were partly submerged in 25 l aquariums, the water of which was exchanged every second day. Water was aerated and held at 10 °C ± 2 °C during the incubation period. The larvae were cultured in a 24-hour darkness regime throughout the entire testing period. Ten individuals from the HR and LR strains were randomly selected from the egg pool (female origin unknown) and isolated in small circular containers. Hatching time was monitored and after hatching was completed the larvae were then used to measure volume specific oxygen consumption (VO_2) and to calculate the volume of the larval yolk sac and the larval body; methods are described in the following sections.

Ten HR and ten LR mothers were used to produce the second batch of eggs. Mother fish were weighed and blood samples were taken post-stripping, to analyze maternal blood plasma cortisol content. Unfertilized eggs from each mother fish were sampled, and 15 ml (approximately 60 eggs) of eggs were frozen for cortisol analyses.

The mass (g) of 20 eggs from each female was measured directly after stripping.

Yolk-sac and larval body volumes

Photographs of the 20 individuals used to calculate VO_2 were taken at; 433, 483 and 523 DDF. To quantify larval body and yolk sac volumes, 2-D digital images of larvae were processed using image J (version 1.42, National Institute of Health, Bethesda, MD, USA). The yolk sac volume was calculated using the formula described by Bagarinao (1986) in which the yolk sac is considered to be a prolate spheroid (Eq. (1)), where *a* is the length of the yolk sac and *b* is the height of the yolk sac (Fig. 1). The larval body volume was calculated by adding the volume of the head (sphere) and the body (cylinder) using Eq. (2) where r_d is the radius of the body, *e* is the larval length and r_c is the radius of the larval head (Fig. 1).

$$\text{Yolk volume} = \frac{\pi * a * b}{6} \tag{1}$$

$$\text{Larval body volume} = \left(\pi * r_d^2 * e\right) + (4 * \pi) * \left(\frac{r_c^3}{3}\right) \tag{2}$$

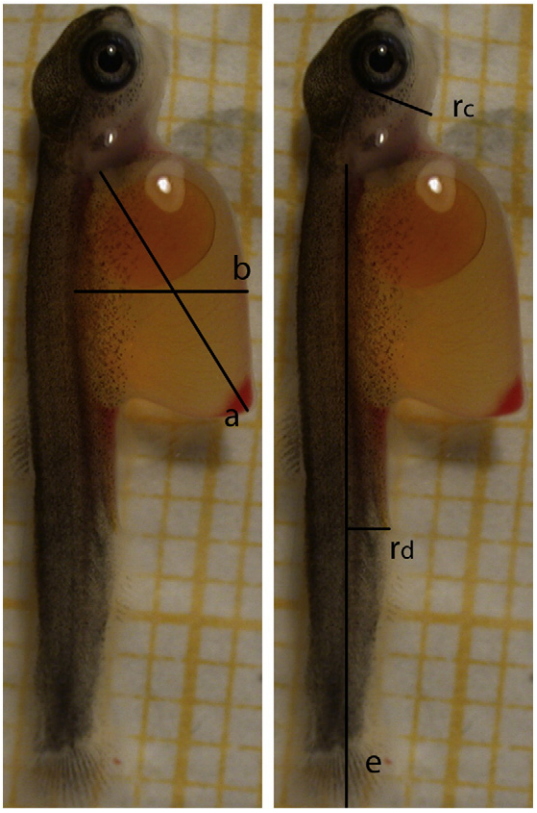

Fig. 1. The following parameters were used to calculate yolk sac and larval body volumes in rainbow trout larvae; *a*, yolk-sac length; *b*, yolk-sac height; r_c, head radius; r_d, larval body radius; *e*, larval body length.

Oxygen consumption

Oxygen consumption ($mgO_2/h/mm^3$) was measured repeatedly for all 20 of the isolated individuals on three different test occasions, 433, 483 and 523 day degrees post-fertilization (DDF). Larvae were removed from their holding containers and placed in the respiratory chambers for oxygen measurements, after which they were returned to the holding containers until the next test occasion. The timing of the test occasions in the present study was chosen so that the experimental animals could be tested at three times between hatching and time of emergence. The time of hatching, 418 DDF and the time of emergence 530 DDF were based on previous observations from our lab on these strains.

Oxygen consumption of one HR and one LR individual was quantified simultaneously in two respirometers. To compensate for chamber differences, individual measurements were repeated twice in one of the two chambers on test day 1 and in the opposite chamber on test day 2. DDF and oxygen consumption are in this paper presented as an average for test days 1 and 2. Because the metabolic activity is presumed to occur in the body (excluding the yolk) (Kamler, 2008), individual metabolic rate was expressed as VO_2 of the larval volume excluding the yolk, also allowing us to repeatedly measure oxygen consumption in the same test subjects over time. Photographs were taken of each individual larva on both test days, and were later used to calculate the volumes of the larval body which then were used to calculate VO_2.

Oxygen consumption ($mgO_2/h/mm^3$) was quantified using the closed respirometry method, described by Steffensen et al. (1984) and Steffensen (1989), which we modified to accommodate the small size of the test animals used in this study. The yolk sac larvae were kept in two respiratory chambers constructed from 6.8 ml vials with rubber corks sealing off each chamber. Fiber optic cables, connected to Presesns Fibox 3 oxygen monitors, were inserted through the corks. Water was aerated and kept at 10 °C, using air stones and water coolers in the water supplying the respirometers. All larvae were kept in darkness during oxygen consumption measurements.

Each respiratory chamber was divided into two compartments, which were separated by a plastic mesh that was mounted with silicone. The test subject was placed in the upper compartment and a magnetic spinner, which prevented the development of oxygen layers, was placed in the lower compartment.

A pilot experiment was performed prior to the oxygen consumption quantification tests, to evaluate the acclimatization period in the chamber. We observed occasional fluctuations in oxygen consumption during the first 5 min. The system then remained stable for up to 3 h after inserting larvae into the chamber.

HR and LR larvae were left undisturbed in the respiratory chamber for 30 min, after which the chambers were sealed and oxygen consumption measurements initiated, and then for another 30 min during which measurement was taken. The first 10 min of the oxygen measurement period were excluded from the measurement period to eliminate disturbances associated with closing the respirometer, yielding a 40 minute acclimatization period and a 20 minute measurement period from which oxygen consumption could be calculated. Respirometry data was logged on a PC equipped with Labtech Notebook (Omega Engineering Inc, USA).

In the present study VO_2 was quantified by closed respirometry with a rather short acclimation period compared to that normally used in larger fish. This allowed us to measure oxygen consumption in 20 individuals (10 from each strain) during a short period of time.

Cortisol analysis

Egg cortisol content was measured using a method described by Szecsi et al. (2006). Cortisol extractions were taken from homogenized eggs, the solute was then purified and the sample was run through an HPLC to enhance the specificity of the ELISA quantification method.

From each female, 1.5 g of eggs were homogenized in 1.5 ml of PBS and resuspended in 15 ml of ethyl acetate for extraction. Ethyl acetate was separated from the tissue by centrifugation and evaporated under vacuum centrifugation at 29 °C heating. Dry residue was then resuspended twice (2×3 ml) in 30% v/v methanol–milli-Q water, filtered on a 0.2 μm filter, and loaded on a 500 mg Amprep C_{18} microcolumn. Impurities were washed out with 10 ml of milli-Q water and cortisol was eluted in 2.5 ml of 90% v/v methanol–milli-Q water. The subsamples were dried for by vacuum centrifugation, resuspended in 400 μl of HPLC buffer (0.01 M sodium dihydrogen phosphate), filtered through a 0.2 μm filter, and stored at −80 °C for further analysis.

The resuspended samples were injected into a 250×4.6 mm column packed with C_{18}-silica gel (5 μm particle size), and the chromatogram was developed using a four-step gradient eluent (1: 45% v/v methanol in 0.01 M sodium dihydrogen phosphate buffer (pH=5.3) between 0 and 25 min; 2: 51% v/v between 26 and 65 min; 3: 64% v/v between 66 and 80 min; and 4: 45% v/v between 80 and 90 min). Chromatography was performed at 4 °C with 1 ml/min flow rate. The elution of the steroid was monitored by U.V. absorbance at 239 nm (Furuta et al., 2004). Cortisol standards demonstrated that the retention time for cortisol was very stable using this method, and the cortisol was sampled between 40 and 45 min. One milliliter from the HPLC elute was dried under vacuum centrifugation, and resuspended in the same volume of ELISA buffer. Egg and maternal blood plasma cortisol levels were quantified with ELISA kits (Neogen, #402710). Cortisol was extracted from the blood plasma by ethyl ether which then was evaporated in a vacuum centrifuge at 29 °C.

Procedural losses of egg cortisol were estimated by spiking subsamples of eggs (1.5 g) from each female with a known amount of

cortisol (450 ng of hydrocortisone, HO888 — 1G Sigma, approximately 10 times higher than the endogenous cortisol). The difference in cortisol content between the spiked and unspiked samples was used to calculate the average recovery rate, which then was used to determine the amount of cortisol in the egg samples.

Statistics

All values are presented as means ± standard error of the mean. T-tests were used to detect differences in maternal weight, hatching time, maternal plasma cortisol, and egg cortisol values between the HR and LR strains. To investigate if strain origin affected egg weight an analysis of covariance, ANCOVA was performed using strain origin as the independent variable, egg weight as the dependent variable, and maternal post stripping weight as a covariate. A Spearman rank correlation test was used to examine the relationships among maternal plasma cortisol level, egg cortisol level and egg size. To detect statistical differences in larval body volume, yolk sac volume, and oxygen consumption between strains over time, we used a repeated measurement ANOVA, with sampling time as the within-subject factor and strain origin as the between-subjects factor. A Tukey HSD post-hoc test was used to further analyze these differences.

Statistical analyses were conducted in statistica (version 9.0, StatSoft, Inc, Tulsa, USA).

Results

Mother fish weight (post-stripping) and egg weight

There was a significant difference ($F_{1,17} = 43.14$, $p < 0.001$) in egg mass between HR and LR eggs, however, this difference was not an effect of differences in maternal weight (post stripping) ($F_{1,17} = 3.94$; $p = 0.065$), Fig. 2.

Hatching time, yolk sac volume and larval body volume

No significant differences in hatching time were observed between isolated individuals from each strain ($t = 0.00$, $p = 1.00$, $df = 9$). Hatching for all individuals, from both strains, occurred during DDF 418.

The repeated measurements ANOVA indicated a significant strain by DDF interaction for yolk volume after hatching ($F_{2,34} = 7.30$, $p = 0.032$). Independent of strain, for both HR and LR larvae, the yolk volume decreased significantly with increasing DDF ($F_{2,34} = 280$, $p < 0.001$). For HR larvae yolk absorption resulted in a significant decrease in yolk volume from 433 to 483 DDF ($p < 0.001$) and from 483 to 523 DDF ($p < 0.001$). The same general pattern was found in the LR strain. Yolk volume was significantly smaller at 483 compared to 433 DDF ($p < 0.001$) and at 523 compared to 483 DDF ($p < 0.001$). Independent of DDF, yolk volumes differed between strains, with HR larvae generally having smaller yolk volumes for all experimental DDFs ($F_{1,17} = 41$, $p < 0.001$; 433 DDF, $p < 0.001$; 483 DDF, $p < 0.001$; 523 DDF, $p < 0.001$) (Fig. 3).

The repeated measurement ANOVA did not show a significant strain by DDF interaction for the larval body volume ($F_{2,34} = 0.59$, $p = 0.55$). Independent of strain effect ($F_{2,36} = 200$, $p < 0.001$), the larval body volume changed over the experimental period. This resulted in a significant increase in larval body volume over DDF, with larger body volumes at DDF 483 compared to 433 DDF (HR, $p < 0.001$; LR, $p < 0.001$) and at 523 DDF compared to 483 DDF (HR, $p < 0.001$; LR, $p < 0.001$). Independent of DDF, no significant differences in larval body volume between strains were observed ($F_{1,18} = 0.01$, $p = 0.92$) (Fig. 3).

Oxygen consumption

The repeated measurement ANOVA did not indicate any significance for the strain by DDF interaction in VO_2 ($F_{2,36} = 0.02$, $p = 0.97$). Independent of strain effect, both the HR and LR larvae were significantly affected by DDF ($F_{2,36} = 7.60$, $p < 0.001$). VO_2 decreased over the experimental period, with higher VO_2 at 433 DDF compared to 483 DDF (HR, $p < 0.001$; LR, $p < 0.001$), and 583 DDF compared to 523 DDF (HR, $p = 0.007$; LR, $p < 0.001$) for both strains. Independent of DDF, the ANOVA did not indicate a significant effect of VO_2 between strains ($F_{1,18} = 0.46$, $p = 0.50$) (Fig. 4).

Mother fish blood plasma cortisol and egg cortisol levels

There was no significant difference between the two strains in maternal plasma cortisol level ($t = 0.46$; $p = 0.65$; $df = 18$). HR females had a blood plasma cortisol level of 198.65 ± 22.48 ng/ml, and LR individuals had a cortisol level of 227.29 ± 37.16 ng/ml. In addition, no significant difference in egg cortisol level was detected between strains ($t = 1.08$; $p = 0.30$; $df = 18$). The cortisol content in the eggs originating from the HR females was 23.09 ± 4.71 ng/g, compared to eggs originating from LR females was 16.67 ± 3.13 ng/g. A Spearman rank correlation test showed no correlation ($r_{sp} = -0.00$, $p = 0.99$, $df = 18$) between egg weight and egg cortisol content, but a correlation ($r_{sp} = 0.55$, $p < 0.01$, $df = 18$) between maternal fish plasma cortisol level and egg cortisol level was observed (Fig. 5).

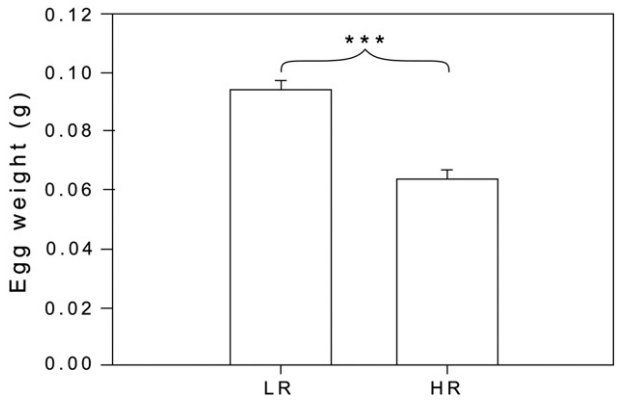

Fig. 2. Adjusted means of egg weight in HR and LR eggs by maternal weight (post-stripping). Eggs were sampled post-fertilization; n = 10 individuals per strain (mean per female consisted of n = 20 from each female, 10 HR and 10 LR). *** indicates a significant difference at $p < 0.001$.

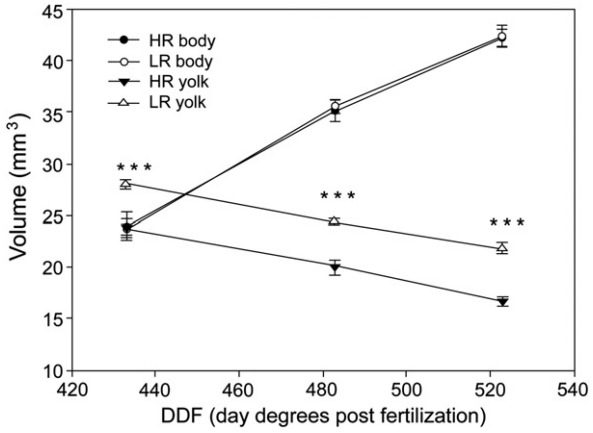

Fig. 3. Repeated measures of yolk sac and larval body volumes in HR and LR larvae sampled at 433, 483 and 523 day degrees post-fertilization. n = 10 individuals per strain. *** indicates $p < 0.001$ between HR and LR strains at the same test time.

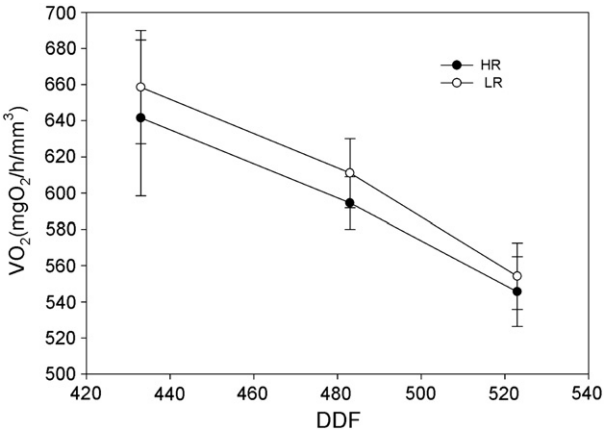

Fig. 4. Repeated measures of individual volume specific oxygen consumption (VO_2) in eggs and larvae originating from HR and LR rainbow trout; n = 10 individuals/strain.

Discussion

Several studies have reported that exposure to stress during oocyte development influences egg size in fish. Contreras-Sanchez et al. (1998) showed that rainbow trout that had been exposed to stress during early vitellogenesis produced larger eggs compared to fish stressed during late vitellogenesis. Campbell et al. (1992) showed that fish with a high stress level prior to spawning produced smaller eggs compared to fish with low stress levels. In the present study, females originating from the HR strain produced smaller eggs compared to females from the LR strain. In a previous study on these two strains Pottinger and Carrick (2000), reported similar differences, however they could not exclude the possibility that the observed egg size difference was an effect of differences in the size of the mother. In the present study, the differences in egg size between the HR and LR strain was present in similar sized females, indicating that mother fish size is not the predominant factor influencing egg size in these strains. That smaller eggs and larvae (total larval volume including yolk sac) were observed in the strain selected for high post stress blood plasma cortisol response (HR) in the present study agrees with results from other studies demonstrating that exposure to prolonged stress or high levels of plasma cortisol in the mother fish results in smaller offspring (Campbell et al., 1992, 1994). However, it is important to note that the two strains do not differ in basal cortisol levels (Pottinger and Carrick, 1999) which indicate that the differences in egg size between the two strains is a result of higher HPI-axis reactivity and concomitant elevated stress induced plasma levels of cortisol in the HR strain.

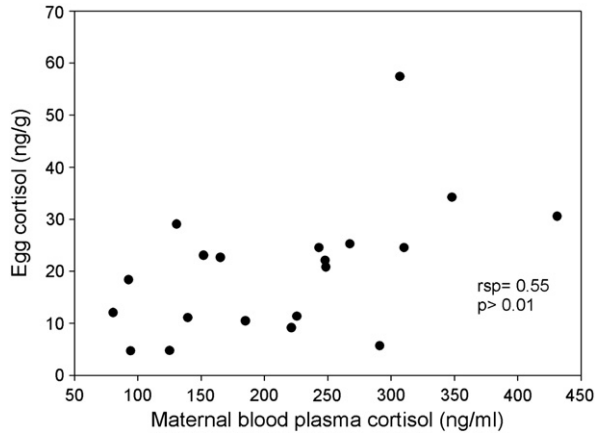

Fig. 5. Relationship between post-stripped maternal blood plasma cortisol level and post-fertilized egg cortisol level in HR and LR trout.

An increasing body of evidence has demonstrated that the HR and LR strains resemble the reactive and proactive stress coping styles, respectively (Overli et al., 2005; Ruiz-Gomez et al., 2011; Schjolden et al., 2005). In salmonid fish, individual variation in developmental rate seems to be related to trait variation typical of the two stress coping styles (Vaz-Serrano et al., 2011); larvae with an early time to emergence from spawning nests show proactive characteristics such as being bolder and more aggressive, and having a higher metabolic rate than late emergence larvae (Metcalfe and Thorpe, 1992; Metcalfe et al., 1995). In the present study, we did not detect differences in development between the HR and LR strains; the two strains did not differ in hatching time, metabolic rate, larval growth rate, or yolk consumption. However, a clear difference in yolk sac size was observed between strains; the proactive LR larvae had larger energy reserves compared to the reactive HR larvae throughout the experimental period. Furthermore it is important to keep in mind that in the present study, observations occurred on a daily basis and it is possible that differences in hatching times could have become apparent if the observation time had been shortened.

An aggressive and active life style, as is characteristic of the proactive stress coping style may be coupled with high energy utilization and metabolic rate. The reactive stress coping strategy, on the other hand, is characterized by an energy conserving strategy (Korte et al., 2005). For example, in common carp (*Cyprinus carpio*), proactive individuals show higher routine metabolic rates (Huntingford et al., 2010). In salmonids the timing of emergence from spawning nests and the establishment of a territory have been shown to be of great selective importance to juvenile fish (Brannas, 1987; Einum and Fleming, 2000; Elliott, 1986). Initial establishment of a profitable territory can result in a bimodal distribution of individual growth potential, suggesting selection for two energetically different strategies after emergence from the spawning nests (Titus and Mosegaard, 1991). If the variation in yolk sac size observed in the present study is continuous during the time of emergence, the energy reserves in the proactive (LR) strain could supply the energetic resources needed for the demanding process of establishing profitable territories. In the HR fish, on the other hand lesser yolk reserves may reflect more energy conserving behavior after emergence from the spawning nests, which is characteristic of the reactive stress coping style. However, if such differences in reproductive strategies are a general feature separating stress coping styles in a wider group of animals needs to be further investigated.

Several studies demonstrate that plasma corticosteroids, mainly cortisol, elevates in sexually mature fish. Generally, this elevation declines during spawning but in some cases a second elevation may occur in spawning and post-spawning individuals (Phillips et al., 1959; Schmidt and Idler, 1962). Previous studies performed on the HR and LR strains demonstrated great variability in the magnitude of the difference in post-stress cortisol response in these strains, and it has been suggested that these divergent cortisol results could be related to the reproductive status of the fish (reviewed by Øverli et al. (2005)). The lack of apparent differences in plasma cortisol level in the present study could be due to the experimental conditions used (e.g., mother fish not being exposed to stress under standardized circumstances, and were subjected to handling stress prior to stripping such as netting, anesthetizing, and stripping).

Sloman (2010) reported that cortisol exposure to eggs, prior to fertilization, was reflected in the behavior and physiology of brown trout. This suggests that the cortisol available to the offspring in the ovarian fluid could be important for the behavior and physiology in the offspring. In the present study we employed the same procedures during stripping and fertilization of the LR and HR females, including netting, anesthetizing, as has been used for earlier generations for these strains. However these procedures did not induce detectable differences in plasma and/or egg cortisol levels between strains. Moreover, the present study demonstrates a positive relationship

between egg and mother plasma cortisol. The results presented here suggests that maternal cortisol endowment to the offspring, through the ovarian fluid, does not play a key role in the transfer of the behavioral and physiological differences which exist between the HR and LR strains (Øverli et al., 2005; Ruiz-Gomez et al., 2011; Schjolden et al., 2005). However it is important to keep in mind that in the present study we had a low sample number, 10 females from each strain, and it cannot be excluded that a larger sample size could have revealed differences between the strains. This needs to be further investigated.

Conclusion

We propose that the observed differences in egg size and larval yolk sac size between the HR and LR strains reflect alternative reproductive strategies for reactive and proactive stress coping styles. A proactive female invests more energy into her offspring, and produces larger eggs, and larvae with large energy reserves (yolk reserves); in contrast, a reactive female produces small eggs and larvae with small energy reserves. Furthermore, we could not detect any significant difference in mother plasma cortisol and/or egg cortisol between the HR and LR strains, however a positive relationship between mother plasma and egg cortisol was detected. These results suggest that cortisol endowment from the mother to the offspring is not the key factor in the transfer of the behavioral and physiological differences that separates the HR and LR rainbow trout strains.

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