Effects of post-ovulatory oocyte ageing and temperature on egg quality and on the occurrence of triploid fry in rainbow trout, *Oncorhynchus mykiss*

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Abstract

The present experiment was designed to evaluate the effect of spawning season rearing temperature and post-ovulatory oocyte ageing on egg developmental success in rainbow trout, *Oncorhynchus mykiss*, assessed as percent survival at the eyeing, hatch and swim-up stages, and as percent occurrence of malformations and ploidy anomalies at yolk sac resorption. Moreover, female weight, egg wet weight, oocyte ATP concentration and ovarian fluid pH, osmolality and protein concentration were measured to investigate possible links with post-ovulatory oocyte ageing and egg developmental success. Thirty three mature females were held at 12°C and 10 at 17°C. Eggs and ovarian fluid were sampled at the time of detected ovulation and 1, 2 and 3 weeks later. Eggs were inseminated with fresh semen pooled from five males and embryo development was monitored until yolk sac resorption. Generally, survival rate decreased and malformation rate increased with an increase in post-ovulatory oocyte ageing. Egg developmental success was positively correlated to female weight and to ovarian fluid pH and osmolality. Ovarian fluid protein concentration significantly increased during post-ovulatory ageing, whereas oocyte ATP concentration remained constant. A significant increase in morphological anomalies was observed as soon as 7 days post-ovulation (dpo), though this effect was not correlated with decreased survival. Also, an increase in spontaneous triploidy was associated with increased post-ovulatory oocyte ageing and high temperature, sometimes in dramatically high proportions (50%).

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Keywords: Egg quality; Triploidy; Ageing; Temperature; *Oncorhynchus mykiss*

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1. Introduction

Egg quality is highly variable in cultured fish as well as in wild stocks (Kjørsvik et al., 1990), and may be a limiting factor to the success of several hatchery techniques such as artificial fertilization or triploidization and to successful mass production of alevins. Good quality eggs may be defined as those that exhibit high survival through the stages of embryonic development, and would also be expected to produce the healthiest and fastest growing fry (Bromage et al., 1994).

Among salmonids, variability in egg quality is due in part to effects of post-ovulatory oocyte ageing. In these species, mature oocytes are expelled at ovulation into the coelomic cavity where they remain immersed in ovarian fluid until spawning is triggered by environmental and social stimuli. Under farming conditions, these stimuli are absent. Therefore, oocytes remain in the body cavity until they are manually stripped by fish farmers. If they are not collected, they degenerate and are progressively resorbed. After ovulation, the proportion of fertilizable oocytes progressively decreases, at a rate which depends on donor females and external factors such as temperature. This post-ovulatory ageing period can last from several days to a few weeks, during which modifications progressively occur affecting egg viability (Escaffre et al., 1976; Kjørsvik et al., 1990), egg shape (Sakai et al., 1975) and egg biochemical composition (Craik and Harvey, 1984; Lahnsteiner, 2000; Lahnsteiner et al., 1999, 2001). However, it is not practical to know the exact time of ovulation, for it would require handling the broodfish on a daily basis. Therefore, it would be of interest to know more precisely the limits of post-ovulatory ageing compatible with egg developmental success, and to have reliable criteria for estimating post-ovulatory ageing and the quality of each spawn.

The present experiment was designed to evaluate the effect of post-ovulatory oocyte ageing on egg developmental success in rainbow trout \textit{Oncorhynchus mykiss}, assessed not only through survival rates at eyeing, hatch and swim-up stages, but also through the occurrence of malformations and ploidy anomalies at yolk sac resorption. Furthermore, measures of fish weight, oocyte wet weight and ATP concentration, and ovarian fluid pH, osmolality and protein concentration were recorded to investigate the effect of post-ovulatory ageing on these parameters and/or possible links with egg developmental success. Egg weight and ovarian fluid pH, osmolality and protein concentration have been previously correlated to survival rates at eyeing stage in rainbow trout (Lahnsteiner, 2000). However, relations between these parameters and the survival rates at later stages or the occurrence of malformations at yolk sac resorption have not been studied. Moreover, post-ovulatory oocyte ageing in \textit{Cyprinus carpio} has been associated with a decrease in oocyte ATP concentration, leading to an irreversible shortage of available metabolic energy that could alter cytoskeletal organization and therefore chromosomal repartition and cell divisions (Boulekbache et al., 1989). In order to accelerate post-ovulatory oocyte ageing and possibly reveal and amplify links between female, egg and ovarian fluid parameters and egg developmental success, some of the females were maintained at an abnormally high temperature (17 °C) during late oogenesis, beginning prior to spontaneous maturation and ovulation.
2. Materials and methods

2.1. Fish

Two-year-old rainbow trout weighing 1300 ± 300 g (mean ± S.D.) and belonging to a spring-spawning strain were obtained from INRA experimental fish farm in Brittany (Station Expérimentale du Drennec, F 29 Sizun). The fish were brought to the laboratory (INRA-SCRIPE in Rennes) 2 to 4 weeks before the expected time of spontaneous ovulation and spermiation, and held in recirculated water units. Water temperature in the fish farm was about 12 °C. In the laboratory, 12 males and 33 females were held at 12 °C, whereas 10 other females were submitted to a progressive 2-day rise in temperature to 17 °C, and kept at that temperature until the end of the experiment. For both temperatures, fish were maintained under natural photoperiod. Each fish was individually tagged with a numbered spaghetti tag inserted under the dorsal fin. All manipulations (tag insertion, ovulation check and gamete collection) were performed under anaesthetic in phenoxy-2-ethanol at 0.15 ml/l.

During the month preceeding experimentation, the broodstock were fed a commercial diet (Biomar™, Brande, Denmark) composed of 42% protein, 22% fat, 6.4% ash, 1.8% cellulose, and supplemented with 14,000 UI/kg of vitamin A, 3000 UI/kg vitamin D3 and 210 mg/kg vitamin E. Feeding rate was approximately 1% body weight per day.

2.2. Gamete collection

Twice a week, females were checked for ovulation by manual pressure on the abdomen. Approximately 40 ml of eggs and ovarian fluid were sampled when ovulation was first detected (0–3 days post-ovulation—dpo), and again 7, 14 and 21 days later. Three groups of five eggs were immediately taken from each sample, weighed, frozen in liquid nitrogen, and stored at −20 °C until ATP analysis. The remaining eggs and ovarian fluid were stored at 10 °C until further processing (1 h maximum).

On each day of egg collection, 12 mature males were anaesthetized. Urine was first evacuated from the urinary bladder by gentle localized pressure. Semen samples were then collected in polystyrene tubes by manual pressure on the abdomen and cooled at 4 °C until further processing (1 h maximum). The motility of spermatozoa from each sample was checked under a microscope as previously described (Labbe and Maisse, 2001), and samples exhibiting poor motility were discarded.

2.3. Fertilization assays and evaluation of egg developmental success

Immediately prior to fertilization, the ovarian fluid was drained from each individual egg sample and the sample was divided in two parts (approximately 250 to 400 eggs per group). Fifteen microliters of a pool of semen obtained from five males presenting the highest sperm motility were added to each group of eggs, and 15 ml of Actifish® solution (sperm motility activating saline solution, IMV, L’Aigle, France) adjusted to the fertilization temperature (10 °C) were pipetted onto the eggs. Five minutes later, the sperm motility activating solution was drained and egg batches were transferred into individual
incubators in recirculated water units at 10 °C. Water temperature and chemistry were routinely monitored and maintained relatively constant over the entire incubation period. Dead eggs and embryos were periodically removed and survival rates were estimated in relation to the initial egg number at the following developmental stages: eyeing, hatch and completion of yolk sac resorption. In addition, the percentage of morphological abnormalities (spinal cord torsion, head or caudal fin malformations, etc.) and ploidy anomalies (observed by flow cytometry) were assessed at yolk sac resorption.

2.4. Collection and analysis of ovarian fluid

At the time of egg collection, an ovarian fluid sample was obtained from each egg sample using a thin glass pipette, and was centrifuged for 10 min at 4000 × g to discard any contaminating blood cells. One hour after sampling, pH of the supernatant was measured with a micro-electrode. Osmolality was determined by a Wescor 5500™ vapor pressure osmometer (Bioblock Scientific, Illkirch, France). Three measurements were made and mean value recorded. An ovarian fluid aliquot was stored at −20 °C for subsequent protein assay, performed according to the Bradford procedure (Sedmak and Grossberg, 1977) using a commercial kit (PIERCE, Rockford, IL, USA).

2.5. ATP extraction and assay

ATP extraction was performed as previously described for common carp (Boulekbache et al., 1989) with minor modifications. Five frozen oocytes (around 300 μl) were crushed in a 10-ml ice cold extraction medium (0.5 M perchloric acid, 4 mM EDTA). Each sample was vortexed then centrifuged for 10 min at 4000 × g and 4 °C. One milliliter of the intermediate phase was sampled and centrifuged for 12 min at 17,500 × g and 4 °C. The supernatant was neutralized by an excess of K2CO3 which was discarded following centrifugation for 5 min at 4000 × g and diluted to 10−3 with ATP Dilution Buffer, (Tris 40 mM, MgCl2·6H2O 20 mM, EDTA 2H2O 4 mM, pH 7.8). Luciferine–luciferase assay of ATP was performed using a Bioluminescence Assay Kit (Boehringer, Manheim, Germany). Bioluminescence was measured using a luminometer Top Count™ (Packard, Downers Grove, IL, USA).

2.6. Flow cytometry

At yolk sac resorption, alevins were over-anaesthetized, then frozen in liquid nitrogen and stored at −20 °C until ploidy analysis. Flow cytometry analysis was performed on 20 alevins per batch. Alevins were prepared as previously described (Lecommandeur et al., 1994). DAPI was used as the fluorochrome, and fluorescence was measured with a Partec CA II flow cytometer (Partec, Munster, Germany) equipped with UV illumination filters. The fluorescence intensity histogram obtained was evaluated by means of the DPAC software (Partec). For each individual alevin histogram, this software determines peak position and ploidy index. Ploidy index is calibrated at 2 with diploid fry. Triploid fry are characterized by one peak at 3, whereas mosaic fry show several peaks with various index values.
2.7. Statistical analyses

Statistical analyses were carried out using Statistica 5.0 software (Statsoft, Tulsa, OK, USA). Data expressed as proportions \((p)\) were transformed into arcsin \(\sqrt{p}\). Normality was assessed through Shapiro–Wilk tests and homogeneity of variances through Bartlett’s tests. The effects of post-ovulatory ageing and temperature were analyzed by multiway factorial analysis of variance. Paired Student \(t\)-test were performed to analyze differences between ageing times. Relations between measures were analyzed by linear regression. Random repartition of residues was checked a posteriori. For non-normal variables, non-parametric tests were performed: Kruskall–Wallis test and Wilcoxon \(t\) paired samples test. Variable correlations were studied by principal components analysis.

3. Results

3.1. Effects of post-ovulatory oocyte ageing and temperature on egg wet weight and ATP concentration, and ovarian fluid pH, osmolality and protein concentration

At 12 °C, egg wet weight and ovarian fluid concentration increased significantly during post-ovulatory oocyte ageing. Ovarian fluid pH and osmolality significantly decreased and oocyte ATP concentration remained constant. At 17 °C, the same trends were observed except for ovarian fluid protein concentration which remained constant, and for oocyte ATP concentration which significantly decreased after 14 dpo. At 0 and 7 dpo, no significant differences in these measures were observed between 12 and 17 °C. At 14 dpo, egg wet weight was higher and other measures were lower at 17 than at 12 °C (Table 1).

Table 1
Measures of rainbow trout egg and ovarian fluid characteristics (± S.D.) during post-ovulatory ageing determined for 33 females at 12 °C and 10 females at 17 °C

<table>
<thead>
<tr>
<th>Egg and ovarian fluid characteristics</th>
<th>Temperature (°C)</th>
<th>Time of post-ovulatory ageing (days post-ovulation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Egg wet weight (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>55 ± 10 (a)</td>
<td>59 ± 9 (b)</td>
</tr>
<tr>
<td>17</td>
<td>62 ± 8 (a)</td>
<td>62 ± 10 (a*)</td>
</tr>
<tr>
<td>Egg ATP concentration (nmol/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>77 ± 20 (a)</td>
<td>80 ± 20 (a)</td>
</tr>
<tr>
<td>17</td>
<td>73 ± 25 (a)</td>
<td>68 ± 24 (a)</td>
</tr>
<tr>
<td>Ovarian fluid protein concentration (mg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1.8 ± 0.9 (a)</td>
<td>2.0 ± 1 (a)</td>
</tr>
<tr>
<td>17</td>
<td>1.4 ± 0.5 (a)</td>
<td>2.2 ± 1.3 (a)</td>
</tr>
<tr>
<td>Ovarian fluid pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>8.2 ± 0.2 (a)</td>
<td>8.1 ± 0.1 (b)</td>
</tr>
<tr>
<td>17</td>
<td>8.2 ± 0.1 (a)</td>
<td>8.1 ± 0.1 (b)</td>
</tr>
<tr>
<td>Ovarian fluid osmolality (mosM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>291 ± 12 (a)</td>
<td>285 ± 11 (b)</td>
</tr>
<tr>
<td>17</td>
<td>293 ± 9 (a)</td>
<td>280 ± 14 (b)</td>
</tr>
</tbody>
</table>

For each egg or ovarian fluid parameter: dissimilar letters represent significant differences \((P<0.05)\) between times of post-ovulatory ageing for a given temperature; an asterisk (*) represents a significant difference \((P<0.05)\) between 12 and 17 °C for a given time of post-ovulatory ageing.
3.2. Effects of post-ovulatory oocyte ageing and temperature on egg developmental success

When females were held at 12 °C, survival rates at eyeing, hatch and swim-up stages remained constant in eggs sampled at 0 and 7 dpo, and significantly decreased thereafter. When females were held at 17 °C, embryo survival rates decreased significantly between samples taken at 0 and 7 dpo, and embryos were no longer viable at 14 dpo (Fig. 1A–C).

Fig. 1. (A to D) Effect of post-ovulatory oocyte ageing (days post-ovulation—dpo) in females held at 12 or 17 °C on egg developmental success. The number of females for which data was measured is under parentheses. For each temperature, dissimilar letters represent significant differences (\(P<0.05\)) between times of post-ovulatory ageing. An asterisk represents a significant difference (\(P<0.05\)) between 12 and 17 °C for a given post-ovulatory ageing time. Error bars represent standard deviations.
Survival at 0 and 7 dpo for eggs held at 17 °C was lower than for those at 12 °C at each developmental stage, with the exception of survival at eyeing at 0 dpo, where the difference was not statistically significant.

The percentage of malformed alevins increased significantly after only 1 week of post-ovulatory oocyte ageing at 12 °C (Fig. 1D). It remained constant between 7 and 14 dpo, and decreased significantly thereafter. At 17 °C, it decreased significantly between 0 and 7 dpo down to a lower value than at 12 °C.

3.3. Effect of post-ovulatory oocyte ageing on egg quality inter-female variability

Coefficients of variation for egg developmental success were similar for data at 0 and 7 dpo, and showed relatively high variability among females. At 7 dpo, coefficients of variation were 38% for eyeing rate, 42% for hatching and swim-up rates, and 133% for malformation rate. These percentages increased further at 14 and 21 dpo (Fig. 2).

3.4. Effects of post-ovulatory oocyte ageing and temperature on the occurrence of triploid and mosaic fry

When oocytes were sampled at 0 dpo from females held at 12 °C, no triploid offspring were observed. However, triploid fry were observed among the progeny of some females at 7 dpo (50% for one female at 7 dpo), and the average proportion of triploids increased significantly at 14 and 21 dpo (over 50% for four females). For females held at 17 °C, the average incidence of triploidy was significantly higher than at 12 °C, reaching 17% as early as 0 dpo (Fig. 3A).

Mosaicism was also observed among the offspring, including from females stripped as early as 0 dpo and held at 12 °C. Although the proportion remained low, it did

![Fig. 2. Change in the coefficients of variation for eyeing, hatching, swim-up and malformations rates, as a function of egg post-ovulatory ageing at 0, 7, 14 and 21 days post-ovulation (dpo), at 12 °C. Data come from separate incubations of the eggs from 33 females.](image-url)
significantly increase during post-ovulatory oocyte ageing at 12 °C, from 0.5% at 0 dpo to 2.5% at 21 dpo (Fig. 3B).

3.5. Relations between female, egg and ovarian fluid characteristics, and egg developmental success

Linear regression analyses indicated that female weight and ovarian fluid osmolality were positively correlated to survival at eyeing, hatch and swim-up stages (Table 2). A high rank correlation was observed between ovarian fluid pH and survival rates at eyeing stage (Spearman’s $r = 0.36$, $P < 0.001$), although linear regression did not show a significant correlation. Such apparent discrepancy is due to a strongly discontinuous

<table>
<thead>
<tr>
<th>Female or ovarian fluid characteristic</th>
<th>Developmental stage</th>
<th>Pearson correlation coefficient</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female weight</td>
<td>Eyeing</td>
<td>0.22</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td>Hatching</td>
<td>0.21</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>Yolk sac resorption</td>
<td>0.21</td>
<td>0.024</td>
</tr>
<tr>
<td>Ovarian fluid osmolality</td>
<td>Eyeing</td>
<td>0.25</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Hatching</td>
<td>0.26</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Yolk sac resorption</td>
<td>0.26</td>
<td>0.003</td>
</tr>
</tbody>
</table>
distribution of survival rates, most pH values lower than 8 being associated with null or very low rates.

Ovarian fluid protein concentration, egg wet weight and ATP concentration were not significantly correlated to egg developmental success.

3.6. Correlations between variables

Principal Component Analysis demonstrated that eyeing, hatching and swim-up rates were strongly positively correlated with each other, whereas they were poorly correlated with the percentage of malformations at yolk sac resorption. Furthermore, the percentage of triploid fry was not correlated to the percentage of malformations at yolk sac resorption. Finally, post-ovulatory ageing was negatively correlated with survival rates and positively correlated with the percentage of malformations and triploids at yolk sac resorption (Table 3).

4. Discussion

To better understand the factors which affect egg quality in fish and which measures correlate well with high developmental success, many criteria have been investigated (Kjörsvik et al., 1990), but none is able to fully account for observed variability in egg survival.

The present experiment was designed to amplify egg quality variation, in order to detect possible links with characteristics which could be used as egg quality predictors. Two factors were controlled for this purpose: post-ovulatory oocyte ageing and temperature. Stress due to successive fish handling is another factor which could be involved, although it was shown to be of minor importance for progeny survival in rainbow trout when suffered during final maturation (Contreras-Sanchez et al., 1998). Several characteristics that have already been shown to be related to egg quality in

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**Table 3**

<table>
<thead>
<tr>
<th>Survival at eyeing</th>
<th>Survival at hatching</th>
<th>Survival at yolk sac resorption</th>
<th>Malformed alevins</th>
<th>Post-ovulatory ageing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival at eyeing</td>
<td>0.97</td>
<td>0.96</td>
<td>0.67</td>
<td>−0.53</td>
</tr>
<tr>
<td></td>
<td><em>P</em> = 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival at hatching</td>
<td>0.99</td>
<td>0.59</td>
<td>−0.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>P</em> = 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival at yolk sac resorption</td>
<td>0.58</td>
<td>−0.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>P</em> = 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malformed alevins</td>
<td>0.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of triploid alevins</td>
<td>−0.31</td>
<td>−0.36</td>
<td>−0.22</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td><em>P</em> = 0.014</td>
<td><em>P</em> = 0.004</td>
<td><em>P</em> = 0.002</td>
<td><em>P</em> = 0.02</td>
</tr>
</tbody>
</table>

Correlations were regarded as significant when *P* < 0.05.
various fish species were measured, including female weight, oocyte wet weight and ATP concentration, and ovarian fluid pH, osmolality and protein concentration (Boulekbach et al., 1989; Lahnsteiner, 2000). Moreover, in addition to classical criteria used to assess egg quality, i.e. simple survival rate at various developmental stages, the proportions of fry with malformations or ploidy anomalies at yolk sac resorption were also determined.

Ovarian fluid pH and osmolality or female weight were the only factors which were significantly correlated to egg developmental success. The correlation with ovarian fluid pH appears not to be the result of a linear relationship, but of a threshold effect—most of poor quality eggs being associated with an ovarian fluid pH below 8. In Cyprinidae, ovarian fluid is produced by the epithelium of the ovarian cavity which shows a high secretory activity (Lahnsteiner et al., 1997). There is no ovarian cavity sensu stricto in salmonids, and the ovarian fluid is likely produced by the post-ovulatory ovary, which is known to undergo rapid morphological and probably secretory changes. The relationship between ovarian fluid pH and egg quality could thus result from the coincident changes in characteristics of the ovarian secretions and loss of developmental potential due to egg ageing. This hypothesis concords with the observation of a significant decrease in ovarian fluid pH from 8.1 at 0 dpo to 7.9 at 21 dpo in rainbow trout (Lahnsteiner, 2000). However, caution is required when comparing ovarian fluid pH values reported by different laboratories. Measures of pH will increase more or less slowly depending on the time between ovarian fluid collection and pH measurement, and on storage temperature during this period. Such progressive change in the acido–basic balance of ovarian fluid is expected when it is removed from the body cavity and placed in contact with air. In the present study, variations of more than 0.5 pH units were observed on the same samples when storage temperature was changed and measurement time delayed. Therefore, it is important to standardize the protocol for pH measurement of ovarian fluid, and to be cautious in the use of pH as an egg quality indicator.

Regarding ovarian fluid osmolality, low values might result either from water inflow into the coelomic cavity due to the loosening of genital papilla after successive forced egg release, or to changes in the ovarian secretions. In both cases, lowered ovarian fluid osmolality could promote premature egg hydration and lower egg fertilizability. Low ovarian fluid osmolality could therefore be a good indicator of poor egg quality. The observed absence of correlation of egg wet weight with survival rates corresponds to the point of view expressed by Bromage et al. (1992) that no reliable data have been able to confirm such correlation in rainbow trout. However, the present observation of a positive correlation between donor female weight and fry developmental success appears original and suggests that faster growing fish could give better quality eggs relative to smaller fish reared in the same conditions, and ovulating at the same age and almost at the same time.

Finally, as previously reported (Lahnsteiner, 2000; Lahnsteiner et al., 1999), a significant increase in ovarian fluid protein concentration was observed during post-ovulatory ageing. Changes in ovarian fluid of rainbow trout stripped only once were similar to those of fish submitted to sequential stripping (Lahnsteiner, 2000). This increase is therefore more likely due to changes in secretory activity of the post-
ovulatory ovary rather than to egg damage after sequential stripping and handling of fish. In the present experiment, protein concentration was correlated with post-ovulatory oocyte ageing, but not with egg quality in contrast to previous observations (Lahnsteiner, 2000).

Results of this study confirm previous reports on the deleterious effect of post-ovulatory oocyte ageing (Sakai et al., 1975; Springate et al., 1984) and of high prespawning temperature (Billard and Gillet, 1981) on fish embryo survival. Moreover, they show that the percentages of morphological and ploidy anomalies significantly increased as early as 7 dpo and that they are poorly correlated with survival rates. Therefore, to fully estimate egg quality, it is essential to consider the alevin malformation rate and ploidy level in addition to survival rates at eyeing, hatch and swim-up stages. Finally, the present results show that the percentage of morphological anomalies was not correlated with the percentage of triploid alevins at yolk sac resorption, in contrast with previous report on Silurus glanis (Varkonyi et al., 1998). However, our comparison between morphological abnormality and triploidy rates does not take into account the number of deformed fry which died prior to yolk sac resorption, but only those which survived.

An increasing occurrence of triploidy among alevins as a function of post-ovulatory ageing has been observed previously in S. glanis (Varkonyi et al., 1998) and Oncorhynchus masou masou (Yamazaki et al., 1989). In the present study, triploidy was observed at 12 °C as early as 7 dpo, sometimes in high proportions (50%), and the mean proportion increased significantly during oocyte post-ovulatory ageing. Moreover, at high temperature, triploid fry occurred earlier and more frequently. Thus, although the occurrence of spontaneous triploids was reported as an uncommon phenomenon in rainbow trout under hatchery conditions (Cuellar and Uyeno, 1972; Thorgaard et al., 1982; Miller et al., 1994), the present results suggest that triploid fry could frequently and spontaneously occur when broodfish are checked for ovulation at low frequency and/or are held at high temperature. Although conditions prior to and during spawning, such as stress or sub-optimal water quality, could also play a role, post-ovulatory oocyte ageing seems to be a key factor in the occurrence of this phenomenon.

It can be hypothesized that post-ovulatory ageing alters fertilization mechanisms. The failure of one of the meiotic divisions followed by fertilization of the unreduced oocyte is the most likely mechanism by which triploidy is achieved (Mong et al., 1974; Chourrout, 1980). Post-ovulatory oocyte ageing cannot disturb the first meiotic division which is completed before ovulation as in most vertebrates. Instead, triploidy is probably associated with disturbance of the process of second polar body extrusion. The cause of this disturbance, which was likely the cause behind occurrence of mosaicism, could be related to changes in oocyte cytoskeletal organization during post-ovulatory ageing, such as has been described in mouse eggs (Webb et al., 1986).

Finally, the disruption of cytoskeletal organization could be linked to a reduction of oocyte energy reserves during post-ovulatory ageing. In common carp, a depletion of oocyte ATP levels was observed during post-ovulatory oocyte ageing (Boulekbache et al., 1989). In contrast, no change in the oocyte ATP concentration between ovulation and 14 dpo was observed in the present study. The ATP concentration was decreased only at 21 dpo and seemed then to characterize nonviable eggs. The stability of rainbow
trout egg ATP concentration during post-ovulatory ageing thus appears to be in agreement with the previous observations in chinook salmon (*Oncorhynchus tschawytscha*) showing that ATP concentration remained constant over a 5-day period while stored in various media (Wending et al., 2000). ATP concentration in rainbow trout eggs was similar to that observed in chinook salmon eggs (2.61 nmol/egg versus 2.5 nmol), and was 100 times higher than in carp eggs (Boulekbache et al., 1989). Such high concentrations might explain that ATP is not a limiting factor during post-ovulatory ageing in salmonids.

In conclusion, post-ovulatory oocyte ageing has important deleterious effects on progeny survival and is also associated with an increased incidence of triploidy. Although egg quality and embryo survival can be influenced by many factors, post-ovulatory oocyte ageing appears as a key factor which should be rigorously controlled before analyzing the contribution of other genetic or environmental factors. Moreover, the sensitivity to post-ovulatory ageing is variable among females. The coefficients of variation for developmental criteria are high and increase during post-ovulatory ageing. Such variability in the individual response to egg ageing suggests an involvement of genetic factors, in which case selection for broodstock with decreased sensitivity could be envisioned.

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References


