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Effects of Post-Ovulatory Oocyte Ageing and Temperature on Egg Quality in Kutum *Rutilus frisii kutum*

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Abstract: Effects of post-ovulatory retention time in the ovarian cavity on egg quality were studied in kutum *Rutilus frisii kutum* at temperatures of 11 and 14°C. Partial volumes of eggs (20 g) were obtained from 10 individually identified females and stripped and fertilized at 24-hour intervals for 96 hours post-ovulation (HPO) (at 11°C) and at 12-hour intervals for 72 HPO (at 14°C). The results indicated that the highest eyeing and hatching rates (76% and 60% at 11°C; 81% and 71% at 14°C) and the lowest eyed-egg mortalities (20% at 11°C; 12% at 14°C) occurred in the eggs fertilized immediately (0-24 HPO at 11°C and 0-12 HPO at 14°C) after ovulation. Egg viability rates, was completely lost by 72-96 HPO at 11°C and 60-72 HPO at 14°C. Under both thermal regimes, eyed-egg mortalities increased with HPO ($P < 0.05$). These results demonstrate that egg stripping should take place within 168 degree (°C)-hours after ovulation and that complete loss of viability of the eggs occur by 672°C-hours after ovulation.

Key words: *Rutilus frisii kutum* · Ovulation · Over-ripening · Temperature · Egg quality

INTRODUCTION

Varying egg quality and loss of oocyte viability after ovulation are the limiting factors in reproduction and mass production of several fish species [1, 2]. In fact ovulated oocytes retained in the ovarian or body cavity undergo over-ripening due to gradual morphological and biochemical changes that negatively affect fertility and larval development [3-6]. So, over-ripening of the eggs has been identified as the most important factor influencing egg survival of many fish species [7, 8]. The time interval during which eggs remain viable differs from species to species and depends largely on temperature [9-14, 4, 2].

Kutum (*Rutilus frisii kutum*) is a cyprinid endemic to the Caspian Sea. It is a migratory anadromous fish with a reproductive period from early March to late April. There is great demand for kutum in Iran, so that the Iranian Fisheries Organization (Shilat) (www.shilat.com) currently runs a restocking program aimed at producing and releasing up to 200 million kutum fry annually in the Caspian Sea. Under the program, maturing fish are caught from the river inlets when they progress for spawning and are used for artificial breeding. However, in 30-40% of the adult females, eggs are not yet ripened completely when the fish are captured and therefore the fish must be kept until ovulation occurs. To date, the duration of egg viability and the optimum temperature for keeping eggs

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after ovulation have not been clarified for kutum, although such information is very important for effective mass production of this species.

The present study, therefore, was conducted to identify the retention time period during which unfertilized kutum eggs remain viable in the ovarian cavity after ovulation when cultured at different temperatures.

MATERIALS AND METHODS

Fish: Fish were captured in the Shazderud River, Babolsar, Iran, during their upstream migration and then transferred to Sadkin boxes set in the river. The fish were not fed during the experimental period. To confirm that ovulation had occurred and to collect gametes, fish were anaesthetized with 100 ppm tricaine methanesulfonate (methyl-aminobenzoate, MS222) to minimize stress and to make them easy to handle.

Egg Retention in the Ovarian Cavity: Fifty unovulated females that were expected to ovulate in the near future were captured from the upstream run. The state of ripeness was judged by gentle palpation of the abdomen. Fish for which the eggs could be removed by applying gentle pressure on the abdomen were considered to have already ovulated and were not used. The inspection was carried out within 24 hours after capture. Ten individuals weighing 1129 ± 50 g (mean ± Standard Error of Mean) were selected out of the sample of 50 for an experiment conducted at 11 ± 0.5°C. The temperature was set and adjusted by mixing waters obtained from the nearby river and urban water. The fish were transported to another holding box after colored tags were attached to the dorsal fin for individual identification. Before stripping the eggs, the body of each fish was wiped and cleaned with a towel. Next, a partial volume (20 g) of eggs (equivalent to ca. 1,630 eggs) was obtained individually from each female and the eggs were fertilized with mixed milt every 24 hours post ovulation (HPO) until 96 HPO. From each of 10 males weighing 892 ± 36 g, 2 ml of milt were collected at the same time that ovulated eggs were obtained for fertilization and the total volume of 20 ml was mixed gently; 0.5 ml of the mixed milt was used for each fertilization. Almost the same procedure was used for the experiment conducted at 14 ± 0.5°C as well. In both thermal regimes applied for the study, retention of fishes was performed in the same temperature when they caught. Ten females weighing 1,094 ± 42 g were used, but examination of ovulation and fertilization were performed every 12 hours until 72 HPO. Milt was obtained from another 10 males weighing 914 ± 62 g and used for each fertilization.

Incubation and Fertility Examination: All batches of fertilized eggs were transferred to the Shahid Rajayee Hatchery Center and placed in jar incubators with running water at 19°C until the eyeing and hatching stages were reached. The ratio of the number of embryos reaching the eyeing and hatching stages to the number of initially fertilized eggs (eyeing and hatching rates, respectively) were used as indices of egg viability [15-17]. Eyed-egg mortality, which is defined as the percentage of dead eyed-eggs in the total number of eggs that reached the eyed-egg stage, was also used as an index of viability. Eyeing was confirmed macroscopically 3-4 days after fertilization as presence/absence of eyes and hatching was examined by counting the number of hatched alevins 7-9 days after fertilization.

Statistical Analysis: The normality of the data was ascertained using the SPSS Software for Windows version 18. One-way ANOVA followed by Duncan's test were used to compare the viabilities (eyeing and hatching rates and eyed-egg mortality) of stored eggs with those of the control (first treatment). $P < 0.05$ was considered to be significant.

RESULTS

At 11°C, the eyeing and hatching rates for eggs fertilized immediately after ovulation (0-24 HPO) were 76.2 ± 1.7% and 60.7 ± 1.9% (mean ± SEM), respectively, which were significantly higher than those for eggs fertilized thereafter (Figure 1). The values then decreased linearly and reached 0% at 72-96 HPO for both eyeing and hatching rates. Statistically significant differences ($P < 0.05$) were detected among all HPOs for both eyeing and hatching rates. At 14°C, the eyeing and hatching rates were 81.5 ± 1% and 71.2 ± 1.7% at 0-12 HPO. Thereafter, they decreased over time linearly and finally dropped to 0% at 60-72 HPO (Figure 2). Significant differences were detected among all HPOs for both eyeing and hatching rates. The decreasing trends were similar to those seen at 11°C, but they were somewhat faster at 14°C. Under both thermal regimes, eyed-egg mortalities increased with HPO, but significant differences were not detected in some cases.

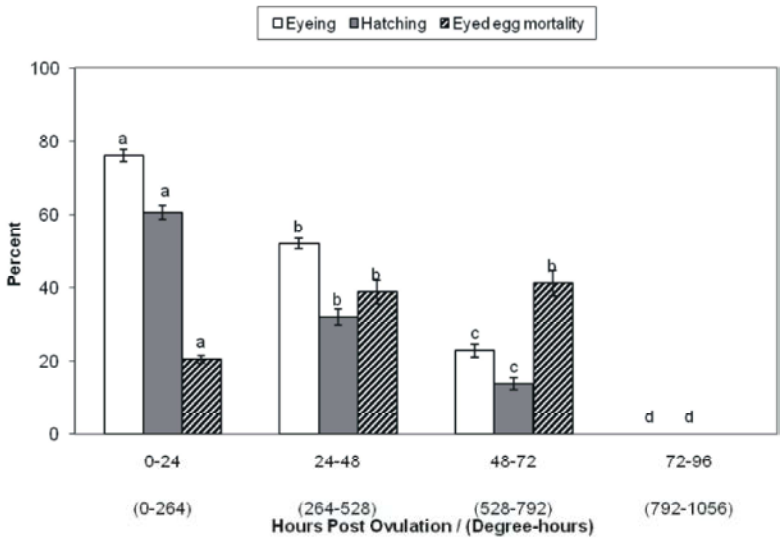

Fig. 1: Effects of the egg in vivo storage time at 11°C on eyeing, hatching and eyed egg mortality rates shown as mean ± SEM. Means sharing a common alphabetical symbol do not differ significantly. For each HPO, degree-hours were calculated by multiplying temperature to hours post ovulation

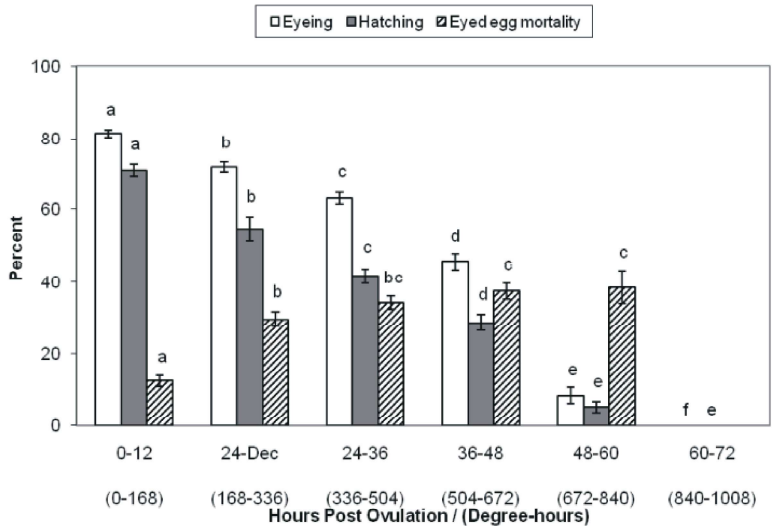

Fig. 2: Effects of the egg in vivo storage time at 14°C on eyeing, hatching and eyed egg mortality rates shown as mean ± SEM. Means sharing a common alphabetical symbol do not differ significantly. For each HPO, degree-hours were calculated by multiplying temperature to hours post ovulation

DISCUSSION

The results revealed that the highest eyeing and hatching rates and the lowest eyed-egg mortalities occurred when eggs were fertilized just after ovulation, irrespective of temperature; this finding is similar to that reported for several fish species [18, 9, 10, 2]. Complete loss of egg viability was observed 72-96 HPO at 11°C and at 60-72 HPO at 14°C. Trends showing simple decrease in the eyeing and hatching rates with elapsed time after ovulation, however, are not always general *in vivo* storage of eggs depending on species. An initial increase in these trends has been documented in salmonids and closely related species [13]. The lack of an initial increasing trend in the present study might be due to the relatively longer time intervals between successive strippings used in this study (i.e., 24 and 12 hours for the 11 and 14°C experiments). Experiments in which eggs are

stripped at shorter time intervals immediately after ovulation are needed to determine whether an initial increase in eyeing and hatching rates occurs in kutum.

The time period during which eggs remain viable after ovulation, which guarantees egg fertility, has been reported for a variety of species: 30 minutes for striped bass (*Morone saxatilis*) [14]; 1.5 hours for tilapia (*Sarotherodon mossambicus*) at 18-20°C [11]; 2 hours for Asian catfish (*Pangasius hypophthalmus*) at 28-29°C [12] and for the neotropical teleost fish *Prochilodus marggravii* at 18 and 26°C [2]; 9 hours at 20°C and 5 hours at 24°C for the South American catfish (*Rhamdia sapo*) [10]; 12 hours for gold fish (*Carassius auratus*) [4]; 5 to 15 days for rainbow trout (*Oncorhynchus mykiss*) at 8-17°C [19, 18, 15, 20, 5, 13, 21, 22]; and 30-40 days for Caspian brown trout (*Salmo trutta caspius*) at 7°C [9]. As shown in these studies, the acceptable post-ovulatory stripping time differs from species to species and also depends on storage temperature. Comparison of the present results to the above-mentioned reports reveals that kutum eggs have a relatively short time period after ovulation during which successful fertilization can occur. Nearly short time interval between ovulation and over-ripening of the eggs in kutum renders the timing of manual stripping a critical step in hatchery management. The over-ripening of eggs has been reported to be as an important factor affecting mate choice and thereby the integrity of the population genetic structure [23]. Therefore the short time needed for over-ripening of kutum eggs also emphasizes removing environmental changes caused by humans like dam building which can delay migration and spawning of the fish resulting poor fertilization because of rapid loss of egg quality in this species.

Increasing eyed-egg mortalities with HPO, detected in both thermal regimes of this study, is in accordance with the previous study [24] which reported that mortality of eyed eggs may be considered as a sign of progress of over-ripening caused by time of storage.

Since the rate of most physiological processes is greatly affected by temperature, it is more appropriate to express the optimal post-ovulatory stripping time and the time needed for complete over-ripening of the eggs in terms of degree (°C)-hours rather than simply hours as a practical unit in hatcheries. Summarizing the present study, the best stripping time was estimated to lie before 168 degree-hours after ovulation and completely over-ripening of the eggs occurred from 672 degree-hours after ovulation. Using noted, each hatchery can perform according to the daily water temperature to obtain best quality eggs. In addition, the data demonstrated that retention of kutum eggs inside the ovarian cavity is an effective technique for restocking programs of this species.

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