Description of the ovarian follicle maturation of the migratory adult female bulatmai barbel (*Luciobarbus capito,* Güldenstädt 1772) in captivity

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Abstract

The study aimed to investigate the maturation process of ovarian follicles and ovary structure of migratory form of female Bulatmai barbel (*Lucioarbus capito*). The histology of oogenesis coincided with that known from most teleosts. The ovarian structure was found to be cytovarian. The development of the oocytes is started from early May along with spawning and the degeneration of matured oocytes. The oocytes' development continues during summer and early fall and the ovary undergoes a dormant period in fall and winter. In the next spring, the next phase of oocyte development started along with the rising of water-temperature. During May and June the development of the oocytes is completed and the final maturation can occur if the environmental conditions are suitable. The Gonadosomatic Index values show a concordance with the oocyts maturation in the ovaries. The reproductive strategy, with long spawning periods during spawning season, found to be batch spawner with asynchronous oogenesis pattern. The results show that it can be matured while captivity.

Keywords: *Luciobarbus capito,* Reproduction, Ovarian follicle, Oocyte, Maturation

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Introduction

Bulatmai barbel (*Luciobarbus capito*), an anadromous cyprinid fish, can be found along the Caspian Sea's coastlines of Iran, Russia, Azerbaijan and inland waters of Georgia and Armenia. It migrates to most rivers of the Caspian Sea basin for spawning (Berg, 1964; Barimani, 1977; Kazancheev, 1981; Abdoli, 2000). Bulatmai barbels show a bimodal migratory behavior; some individuals migrate to the Caspian Sea, whereas others remain in the river of origin. In the former, winter migrants enter to rivers from January till March, whereas spring migrants migrate to rivers in June and July. The latter, i.e. the residents, permanently dwell on stony beds in the middle part of the rivers, which have an average annual temperature of 15-20 °C (Kazancheev, 1981; Abdoli, 2000). In wild, they accomplish spawning migration as a group and spawn, commonly one female with several males on gravels and stony beds in shallow current water. The female and male of Bulatmai barbel fulfill first-year spawning when they become five and four years old, respectively (Kazancheev, 1981; Abbasi et al., 1999; Abdoli, 2000). Shajiee et al. (2002) found a sex ratio of 3:1 for male:female fish in the western Caspian Sea (Guilan Province) and a life span of 8 years. The migratory form of this species, which is commercially important, has experienced a remarkable decline in the fishing yields of the Iranian Caspian Sea (Hossein, 1992; Coad, 2012) and is no longer seen in the annual fishing report of the bony fish production in the Iranian Caspian Sea (Abdolmaleki and Psuty, 2007; Coad, 2012). Kiabi et al. (1999) consider this species to be conservation dependent in the south Caspian Sea basin according to IUCN criteria.

The artificial propagation of several valuable fish species of the Caspian Sea, such as sturgeons and Kutum (*Rutilus frisii kutum*), in hatcheries and subsequent releasing their fingerlings into the Caspian Sea can be a pattern for recruiting the migratory form of Bulatmai barbel. Experiences with the artificial propagation of barbel (*Barbus barbus*) in the Czech Republic (Krupka, 1985; Kouril et al., 1988), a closely related species of Bulatmai barbel, show that artificial propagation and restocking levels can be applied as a proper strategy for recurring of Bulatmai barbel population in the short term (Cloud, 1995).

Following an unsuccessful effort for the artificial and semi artificial propagation of Bulatmai barbel using hormonal treatments such as HCG, P.G and LHR$_a$ (Iranian Fisheries Research Organization, 2002), this study was performed to elucidate potential causes or explanation for this failure. Hence, the present study was conducted to obtain information on (a) ovarian follicle maturation; and (b) ovary structure of the migratory female Bulatmai barbel. This information may be used to design effective techniques for artificial induction of gonad maturation and spawning, and the successful management of wild Bulatmai barbel stocks for a sustainable fishery.

Materials and methods

Fish material and Experimental desing

The migratory form of Bulatmai barbel can be caught in rivers during spawning migration. In total 250 Bulatmai barbels (male and female) were captured between May and July 2001 in the estuary of the Sefidrood and Polrood rivers (North Iran) by electroshocker and surrounding net. The fishes were transferred into an earthen pond (5000 m^2 - 7500 m^2) for further rearing. The reared specimens were sampled during one year from September 2001 till February 2002.

This pond was filled three weeks prior to the introduction of the fishes. The pond was fertilized to sustain primary production and benthos as natural food by adding two tons of cattle manure and 50 kg/ha urea . The fertilization continued during the rearing period using inorganic fertilizer (urea plus phosphate at 5 kg per ha/week) and cattle manure (5-20 kg per ha/week according to climatic conditions). In addition to the natural foods, the specimens were fed using of the commercial foods consist SFT (Trout broodstook food) and SFK (Kutum broodstook food) (from Chineh Company) at a rate of 1% body weight/day. DO and pH were recorded 6.2-8.1 and 7.2-8.4 during the whole rearing period, respectively. Other water quality parameters were between proper levels (based on Faridpak, 1984), for the warm water fish culture) during this period.

Determination of ovarian maturation

The ovarian follicles of four reared adult specimens were collected monthly from March till August 2002 (three specimens were sampled in March because of specimen shortage) and each one and half month from September 2001 till February 2002. The specimens' age were determined by counting annual circles on the scales according to Grant and Spain (1975) to select specimens of adult age.

The specimens' follicles were weighted using a digital balance with an accuracy of 0.01 g. and six follicle samples were taken from front, middle and end parts of each paired ovary (right and left). In total, 234 samples were gathered from the ovarian follicles of a total of 39 reared adult specimens during ten times sampling. These follicles were fixed into Bouin's fluid for 24 h, maintained in 70% alcohol for 1 week (Presnell and Schreibman, 1997). The preparatory process included 1-h submersion in three series of alcohol (80%, 96%, 100%) and two series of Xylene (Xylene I and II) and finally, embedding in paraffin. The ovaries were sectioned in 5 μm thickness in the transverse plan. They were stained with hematoxylin-eosine staining according to Pousti (1999). A total of 120 sections (20 sections per ovarian sample) were prepared for each ovary. The mounted slides of the ovaries and representative stages of oocytes development were observed and photographed using a Leica microscope equipped with a camera. The occupied areas of each developmental stage of oocyte on sections were measured using the pointing method with 66 randomly chosen plots per month (Fig. 1).
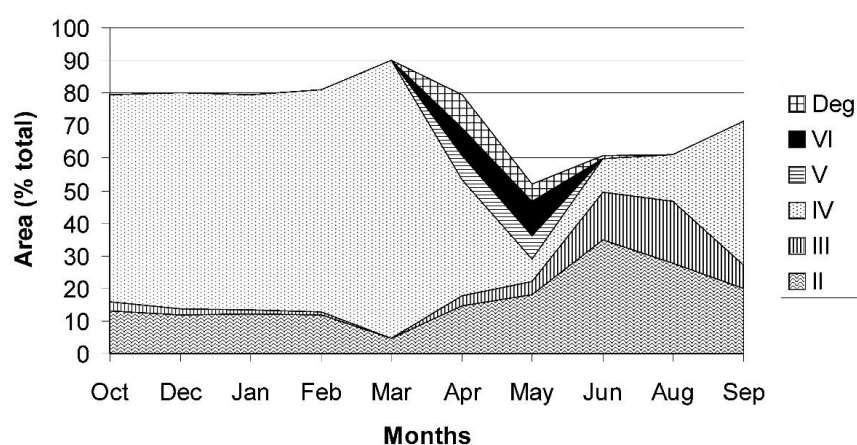
Figure 1: Monthly values of occupied areas for each developmental stages of oocyte (II= Stage II, III= Stage III, IV= Stage IV, V= Stage V, VI= Ovulation Stage VI, Deg= Degeneration stage). 10X magnification were used for each measured plot.

The oocyte maturity was classified into five follicle stages and two further stages; ovulation and degeneration stages according to the criteria of Wallace and Selman (1981) and Mojazi Amiri et al. (1996). The ovarian maturation was described based on the changes of the ovaries composition in terms of presence of different developmental stages of oocyte during one year. Oocyte development after spawning is not included in the stages of the reproductive cycle (Wallace and Selman, 1981). Also, the Gonado-somatic Index (GSI) was computed according to Nikolsky (1963).

Results

The ovary of Bulatmai barbel was found to be cytovarian, which implies that the ovules are released inside the ovary during ovulation and then they are conducted to the outside by a central canal and oviduct.

Morphology of oogenesis

The ovarian follicles present oocytes at five stages of maturation (I–V), followed with two further stages (i.e. ovulation and degeneration stages).

Chromatin nucleus stage (Stage I). The oocyte has the smallest size and an enlarged nucleus. Basophilic property or high ability to absorb haematoxylin staining (stains dark blue color in the sections) is another important attribute to detect them.

Perinucleolus stage (Stage II). The nucleoli are arranged along the internal side of the nucleus membrane and a follicle-encased oocyte can clearly be distinguished. In this stage, the intensity of the haematoxylin staining decreases in the oocyte cytoplasm (Fig. 2a).

Cortical alveoli stage (Stage III). The appearance of the cortical alveoli in the cytoplasm characterizes the initiation of this stage. Initially, they are seen in the cytoplasm, with their number and size increasing during further oocyte development. They occupy almost all of the perinuclear volume before the vitellogenetic stage starts, after which they

are restricted to a circular, thin layer under the cell membrane due to accumulation of the yolk proteins in the cytoplasm. They are also stained grey with haematoxylin-eosin staining (Fig. 2a).

Vitellogenic stage (Stage IV). This is the longest phase of oocyte development. The accumulation of the yolk corpuscles into the oocyte appear as a transparent layer around of the nucleus membrane. The yolk corpuscles gradually spread over the rest of the cytoplasm and fuse, thus forming the yolk disc. The follicular layer is also clearly distinguishable (Fig. 2a).

Migratory nucleus stage or mature follicle (Stage V). This stage is initiated by a movement of the elliptical nucleus towards the animal pole. Germinal vesicle break down (GVBD) also occurs after the migration of the nucleus. At this moment, the yolk corpuscles and lipid particles mix together within the oocyte, thus becoming an ovule (Fig. 2b).

Ovulation stage, i.e. the pulling out of the oocyte from the folliclar layer, occurs at the end of the GVBD.

Degeneration stage or absorption of the ovules is seen during the spawning season and in the case they were not able to find suitable conditions for spawning.

Maturation process of ovarian follicles: The composition of the ovaries in October, December, January and February were almost similar comprising the oocytes at stages II, III and IV (Table 1 and Fig. 1). This suggests a developmental dormancy of the ovary during the cold seasons (fall and winter). Most of the ovaries comprise oocytes at stage IV, with the majority being in the last phase of stage IV (Fig. 1a). In December, the

number of the oocytes at the third stage diminishes in contrast to the increase in the number of oocytes at stage IV. In January and February, oocytes at the third stage were rare.

In March, the surfaces of the ovarian stroma are reduced on the cross sections due to the expansion of the size of the oocytes at stage IV. In this month, the oocytes at stage IV are predominant and some oocytes at stage V and ovulation stage, along with a few oocytes at the degeneration stage, are present in the central part of the ovaries.

The oocytes at stage IV were also dominant during April. The oocytes at stages V and ovulated oocytes are observed sporadically among the oocytes at stage IV. The ovules were being absorbed while a large quantity of the oocytes at the third stage had entered into the beginning of stage IV, i.e. the previtellogenesis stage.

In May, an increase in the number of the ovulated eggs was accompanied by a decreasing in the number of ovules at the degeneration stage in the ovaries. The oocytes at second stage occupy most of the ovary. As a result of the degeneration of maturated ovules and spawning, the surfaces of the ovarian stroma occupy almost half of the sections' areas.

In June, the ovaries comprise the oocytes at stages II, III, IV, V and degeneration stage (Fig. 2b). The increase of the blood vessels in the ovaries, give them red color. The oocytes at the second stage are predominant and the number of the oocytes at the third stage was increasing due to the development of the oocytes at stage II. The oocytes at early

Ovulation stage and degeneration stage were rare. The ovarian stroma is diminished in comparison with that of the May (Fig. 2b).

In July, the ovaries are composed of the oocytes at stages II, III and IV. Most of the ovaries are occupied by oocytes at the second stage and a distinguishable quantity of the oocytes at stages III and IV are scattered among them (Fig. 2c).

The color of the ovaries had changed to grey in September and their compositions were similar to those of late October specimens, but with a higher ratio of oocytes at stages II and III. In addition, the quantity of oocytes at stage IV increased substantially in comparison to the July specimens, with most of them at an early phase of this stage (Fig. 2d).

Table 1: The composition changes of ovaries during one year sampling (Oocyte maturation stages (I–V), Ovulation stage (O), degeneration stage (D), present (+) and not present (-)).

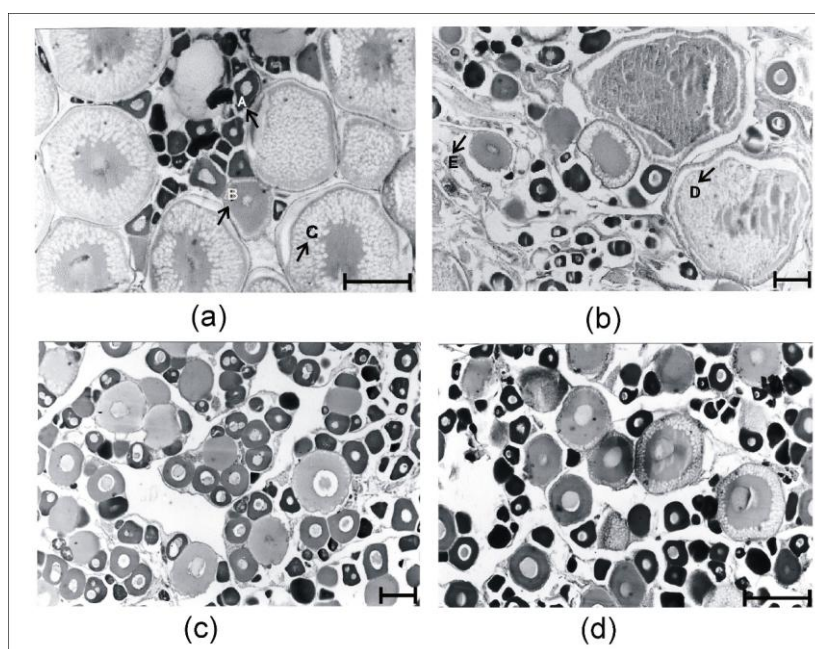| Sampling Time	II	III	IV	V	O	D
Oct. 2001	+	+	+	-	-	-
Dec.2001	+	+	+	-	-	-
Jan. 2001	+	+	+	-	-	-
Feb. 2001	+	+	+	-	-	-
Mar. 2001	+	-	+	-	-	-
Apr. 2002	+	+	+	+	+	+
May. 2002	+	+	+	+	+	+
Jun. 2002	+	+	+	-	-	+
Jul. 2002	+	+	+	-	-	-
Sep. 2002	+	+	+	-	-	-

Figure 2: Histological sections of the ovary of *Luciobarbus capito* at different stages of seasonal maturation in captivity: (a) Ovary in October (H&E) A= Oocyte at stage II, B= Oocyte at stage III, C= Oocyte at stage IV. (b) Ovary in June (H&E) D= Oocyte at stage V, E= the follicle remains. (c) Ovary in July (H&E). (d) Ovary in September (H&E). Scale Bar = 1mm

The Gonado-somatic Index (GSI)

The GSI of the reared female Bulatmai barbels was under 2% from June till September. An increase in GSI was observed to start from October, with a slow increase untill February, but followed by a rapid increase in April. During this period, GSI had increased from 2.14% in October to 4.48% in April. The lowest GSI was recorded in August (1.12%), which corresponded with a dominance of oocytes at stages II and III. The highest GSI was observed in April, when the oocytes at stages II, IV and V were predominant (Fig. 3).

Temperature

The fluctuation of water temperature during the sampling period shows a lowest value in February 2001 and maximal temperature in September 2002 (Fig. 4).
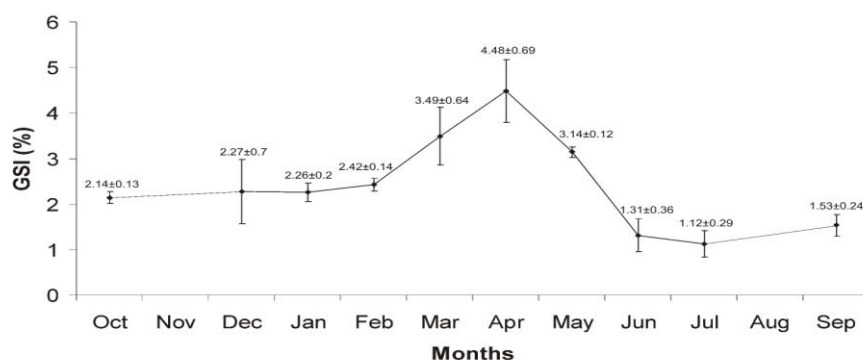

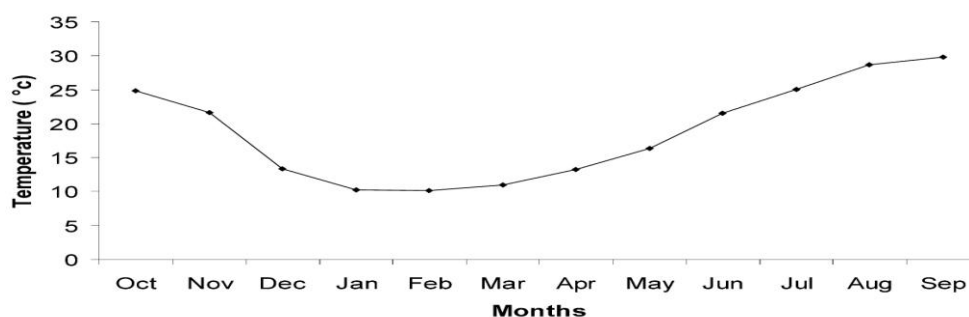**Figure 3: Monthly values of females Gonado-Somatic Index of the reared bulatmai barbel**

Figure 4: Monthly values of water-temperature in the rearing pond

Discussion

The oocyte development pattern observed in this species is similar to that of most other teleosts (Wallace and Selman, 1981; Begovec and Wallace, 1988; Selman and Wallace, 1989; Tyler and Sumpter, 1996; Kaymaram et al., 2011). Adults of Bulatmai barbel show a new ovarian development phase along with spawning or degeneration of ovules, of which the vitellogenesis had been completed the year before, in May. In this phase, a large number of oocytes develop from a perinucleolus stage to a cortical alveoli stage and then vitellogenesis gradually completes in these oocytes during summer and fall. These oocytes experience a dormant period in winter, a quietness period also seen in other cyprinids such as common carp (*Cyprinus carpio*) (Bieniarz et al., 1979). A next phase of the ovarian development is associated with a rising of water temperature during April, with a peak reached in GSI, in April. After this peak, ovulation gradually occurs during about one month, with a subsequent onset of a new phase of ovarian development. The composition of ovaries at different developmental stages can indicate differences in the reproductive strategies of fish. For example, the presence of a similar group of maturated ovules or substantial difference in the ovarian composition during spawning season, respectively, indicates short term and long term spawning strategies (Takashima and Hibiya, 1995). The ovarian development strategy in the Bulatmai barbel should be considered to correspond to batch spawner with an asynchronous oogenesis, because it shows the presence of oocytes in different developmental stages, from the second stage to the degeneration stage, at the same time during the spawning season. Oocytes are followed by a gradual and sporadic ovulation during the spawning season (from late April till mid June). A batch spawner with asynchronous oogenesis can spawn a few eggs every day throughout the whole spawning season, with the ovaries containing oocytes at the various maturation stages and thus allowing spawning to occur repeatedly over an extended period of time (Wallace and Selman, 1981). So, the Bulatmai barbel may spawn over an extended period depending on the availability of stony river beds, mature males and favorable

temperatures. Otherwise, oocytes are gradually absorbed. It does seem that the conspicuity of the ovules at the degeneration stage can suggest a lack of proper conditions to spawn in the rearing pond. This process continues until ovulation or degeneration of all oocytes that had completed their vitellogenesis during the year before.

In some *Barbus* species, multiple spawning during a single spawning season has been reported. Poncin (1988) reported eight spawning events in *Barbus barbus* in the Czech Republic during one spawning season. Hosseini (1992) also suggested multiple spawning in the Caspian Sea barbel (*Barbus brachycephalus)* during a single spawning season, relying on oocytes obtained from stripping.

The results demonstrate the feasibility to rear female Bulatmai barbel in captivity up to maturation of the follicles. So, it will be possible to rear broodstock in earth pond, thus both female (this study) and male Bulatmai barbel (Eagderi et al., 2006) for artificial propagation.

As for preliminary unsuccessful efforts to artificially propagate of Bulatmai barbel using hormonal treatments, it does seem advisable to apply techniques which expose female to hormones during the presence of a maximal number of oocytes sensitive to hormonal induction. Oocytes have been shown to be most susceptible to hormonal induction at stage V when germinal vesicle migrates and breaks down (Goetz 1983; Patino and Thomas 1990; Kagawa et al., 1994; Kraak et al., 1998; Bahre Kazemi et al., 2010). Presence of oocytes at stage V in Bulatmai barbel were observed from early May on, but the conversion of all oocytes

to stage V lasts more than one month. So, inducing Bulatmai barbel only once, like in synchronous spawners, would not be useful because only a small fraction of the oocytes would react to hormonal induction and the rest of them may degenerate due to handling or stress. Hence, applying a slow release implant for induced spawning can be suggested (Fornies et al., 2001). Also, a further investigation on fluctuations of sex hormones during spawning period is recommended.

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