GONAD MORPHOLOGY and HISTOLOGY OF THE A ENDEMIC HORMUZ CICHLID, *IRANOCICHLA HORMUZENSIS* COAD, 1982 FROM MEHRAN RIVER, SOUTHERN IRAN

Hamid Reza Esmaeili*, Zohreh Ganjali, and Maliheozaman Monsefi

Department of Biology, College of Sciences, Shiraz University, Shiraz, P.O. Box 71454, Iran

Abstract

The gGonad morphology and histology of the Hormuz cichlid *Iranocichla hormuzensis*, a maternal mouthbrooder endemic fish species from Mehran River, southern Iran was investigated. Based on the size, shape and weight of the gonads, degree of occupation of the body cavity, presence or absence of ripe oocytes, or milt, diameter of the oocytes in the ovary, and histological observations, six typical gonad maturation stages were described for both males and females using macroscopic and microscopic criteria. Based on the percentage of late gonad maturation stages (IV, V, VI) and high frequency of large oocytes it was concluded that the Iranian cichlid spawns at the end of winter and beginning of spring. These stages were correlated to the gonadosomatic index.
Key words: Iranian cichlid, *Iranocichla hormuzensis,* Reproduction, Gonad histology

***Corresponding author:** Hamid Reza Esmaeili (esmaeili@susc.ac.ir)

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Introduction

The family Cichlidae comprises about 150 genera and 1300 species, making it the second largest perciform family (Nelson 2006). Cichlids are found in fresh and brackish waters of Central and South America, Africa, Madagascar, the Levant, southern India, Sri Lanka and southern Iran. Cichlids are an important group of relatively large and often colorful aquarium fishes. They exhibit a broad range of morphological, ecological and behavioral variation. The Iranian cichlid or Hormuz cichlid, *Iranocichla hormuzensis* Coad, 1982 (Fig.1) is the only cichlid species in Iran (Coad 1982, Berra 2001). It belongs to Tillapiines (*Danakilia, Konia, Oreochromis, Tilapia, Sartherodon, Tristramell*a). *Iranocichla. hormuzensis* is endemic to two provinces of southern Iran (Fars and Hormuzgan) in waters draining into the Persian Gulf at the Strait of Hormuz. This species is found in waters of high temperature and mineral content. The Hormuz cichlid has been studied mainly on the basis of morphological, karyological and anatomical characters (Coad 1982, Abdoli 2000, Esmaeili et al. 2006). No study on the gonad histology of this cichlid fish has been published (Coad 2008). This study presents the first detaileds description of gonad histology of the Iranian cichlid, *Iranocichla hormuzensis*.

Materials and Methods

Study site and sampling

The fish specimens were collected from.the Mehran River at 27°04'889''N, 54°28'360''E, Alt. 282 m, Hormuzgan province. *I. hormuzensis* is locally called Mahi -e- Karoo and it is usually eaten by local people when it is available in large numbers during spring.

A total of 377 individuals specimens were collected monthly from December, 2002 to December, 2003 using a dip net. The specimens were preserved in 10% formalin until examination.

Total length (TL) and standard length (SL) of the preserved specimens were measured to the nearest 0.05 mm using vernier caliper. Preserved specimens were later blotted dry with a tissue paper and weighted to the nearest 0.001 g (total weight, W).

We dissected the fishes and examined the gonads to determine the stage of maturity. Sex and stage of maturity were ascertained macroscopically and microscopically and the weight of preserved gonads (GW) was taken to the nearest 0.001g. Stages of maturation were classified as follows: I, immature; II, developing; III, maturation IV, ripe; V, spawning (running); VI, spent (Babiker and Ibrahim 1979, Tacon *et al.* 1996).

The gonad samples were dehydrated in ascending grades (70%, 90% and 100%) of alcohol, embedded in paraffin, sectioned (5-7µm) and stained with haematoxylin and eosin (Bancroft and Stevens 1991). To examine the monthly changes in the gonads for estimating spawning season, the gonadosomatic index (I_G), was calculated by:

I_G= (weight of gonads/ weight of fish) × 100 (Nikolsky1963). ANOVA was used to test significant differences of the gonadosomatic index in different months. Pearson linear regression method was used to test the relationship between ovarian stages and I_G.

Results

Size range

During this investigation a total of 377 specimens of Hormuz cichlid ranging in total length from 33.30 mm to 129.50 mm (S.E. = 0.887), standard length 27.40 to 110.90 mm (S.E.= 0.719) and total weight from 0.8 g to 65.79 g (S.E.= 0.41) were caught.

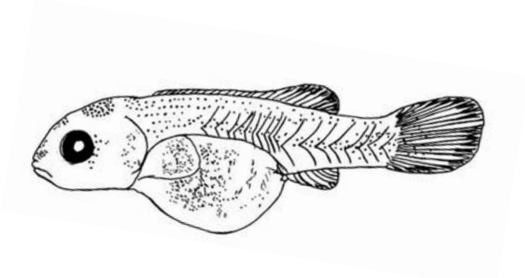
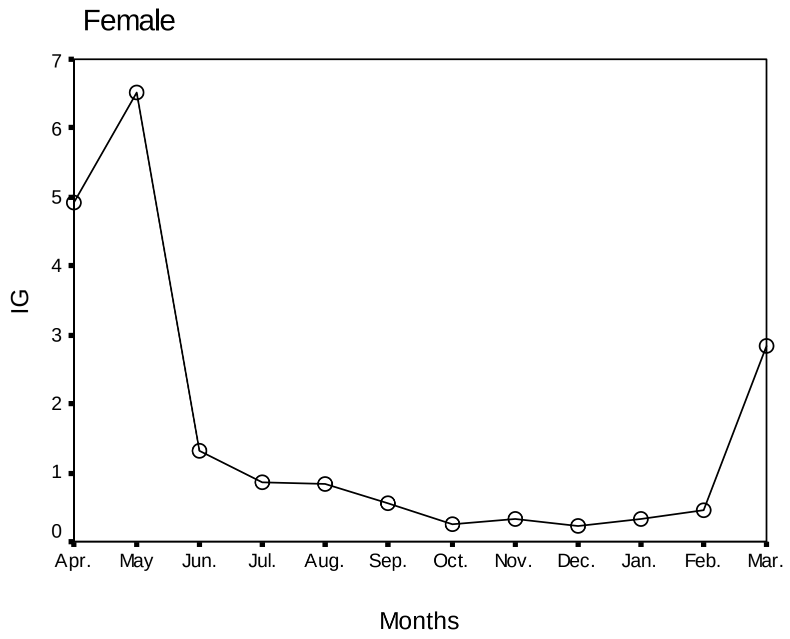


Figure 1: (a) Mouthbrooder Iranian cichlid with larvae collected from her mouth (L_T= 129 mm).
(b) Lateral view of larva (L_T=10 mm) collected from mouth of a female specimen

We observed significant differences of female and male I_G in different months (ANOVA, F=11.235, p<0.001). The female gonadosomatic index increased during October to April, peaked in May and decreased drastically in June showing the spawning season of this fish. It decreased slowly from end of June to reach the lowest value in October (Fig. 2). Almost the same pattern was observed in the I_G of the male (Fig. 2).

Maturity stages of gonads

Ovary: Based on the size and weight of the ovary, degree of occupation of the body cavity, presence or absence of ripe oocytes, diameter of the oocytes in the ovary, and histological observations, we described 6 maturation stages in the ovaries as follows:



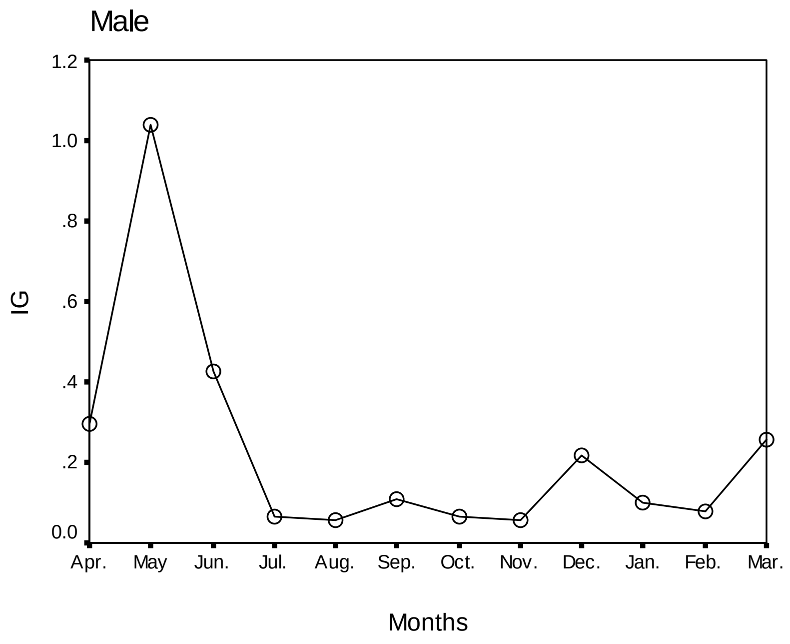


Figure 2: Monthly variation of mean I_G in female and male specimens of *I. Hormuzensis*

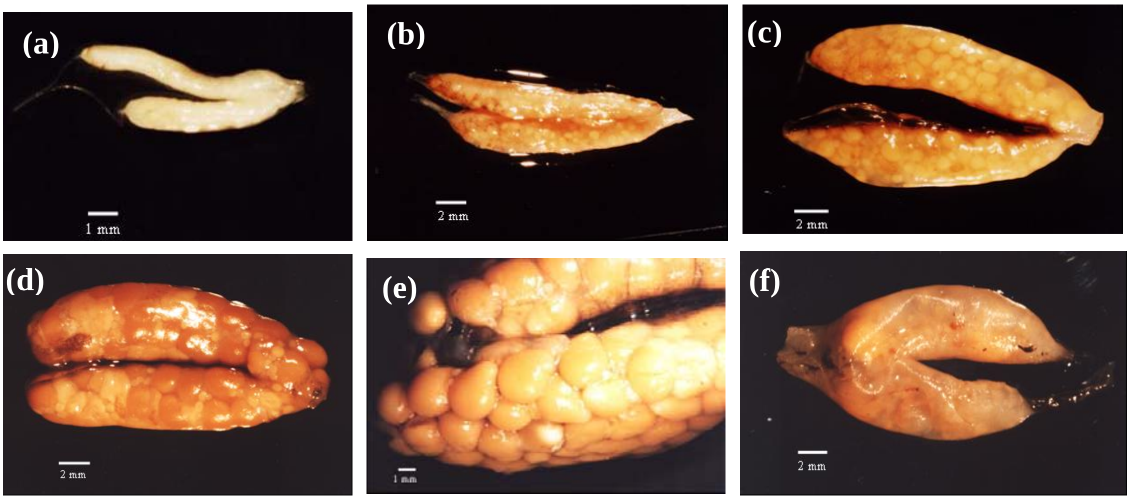


Figure 3: Morphological profiles of the six female maturity stages. (a) Immature stage, (b) Developing stage, (c) Maturation stage, (d) Ripe stage, (e) Spawning stage. (f) Spent stage.

Stage I (immature): Ovaries were small and transparent. They were thread-like and attached to the vertebral column. Oocytes were not visible to the naked eye. The mean diameter of oocytes was 0.121 mm (Fig. 3a). Small, round and transparent oocytes with a central nucleus were observed in histological sections of ovaries. Nucleoli were found in few oocytes. No lipid droplets were found. These cells had basophilic cytoplasm and an acidophilic nucleus. The ratio of nucleus to cytoplasm volume was high (Fig. 4a).

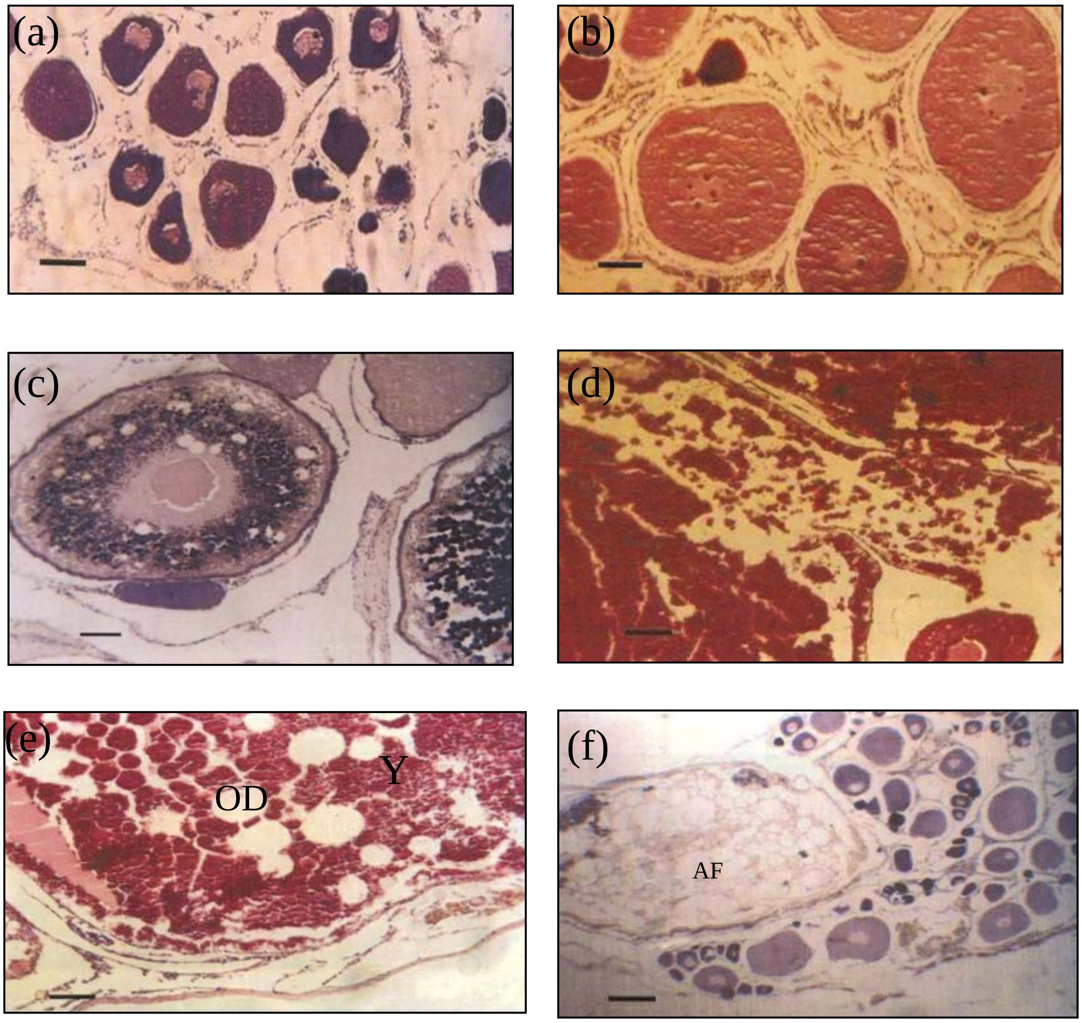


Figure 4: Histological profiles of the six female maturity stages. (a) Immature stage, scale bar = 70 µm. (b) Developing stage, scale bar = 70 µm. (c) Maturation stage, scale bar = 70 µm. (d) Ripe stage, scale bar=70 µm. (e) Spawning stage. Y, yolk; OD, oil droplet. scale bar = 70 µm. (f) Spent stage. AF, atretic follicle. scale bar = 200 µm.

Stage II (developing): Ovaries still were small and spindle shaped and restricted to the posterior part of the body cavity. Small yellow oocytes with an average diameter of 0.253 mm were visible to the naked eye (Fig. 3b). Histological sections showed that the developing oocytes exhibit a weak basophilic cytoplasm that was characterized by small lipid droplets. Yolk granules and a very thin follicular layer appeared gradually. Large peripheral nucleoli adjoining the nuclear envelope were distinguished. This stage is called a returning stage, because the ovaries after spawning will return to this stage to start oogenesis (Fig. 4b).

Stage III (maturation): Ovaries were yellow and large, positioned up to the middle part of body cavity. Yellow large oocytes with an average of 0.622 mm were clearly visible. They were densely packed (Fig. 3c). The growing follicles were characterized by spherical acidophilic granules (lipid droplets) and also a thin zona radiata surrounded by cubic follicular cells and theca layer. The size and number of yolk granules were also increased (Fig.4c).

Stage IV (ripe): Ovaries extended into the anterior portion of the body cavity and appeared dark yellowish. The weight of gonads increased but had not achieved their maximum amount. The mean of ova diameter was 1.45 mm (Fig. 3d).

Figure 5: Morphological profiles of the six male maturity stages. (a) Immature stage, (b) Developing stage, (c) Maturation stage, (d) Ripe stage, (e) Spawning stage. (f) Spent stage.

Histological sections showed that the relationship of nucleus to cytoplasm of oocytes was decreased progressively. In this stage a coalescence of lipids and yolk granules and the detachment of the follicular cell layer occurred. The nuclear envelope broke down and the thickness of the zona radiata was higher than previous stages (Fig. 4d).

Stage V (running): Ovaries were yellow and occupied most of the body cavity. They had achieved their maximum weight. Large, yellow and pear-like oocytes, full of yolk with an average diameter of 1.75mm were distinguishable (Fig. 3e). Oocytes are released by slight pressure. Oocytes were characterized by large mass of yolk and numerous large lipid droplets. The zona radiata was completely thick and had separated from the follicular layers (Fig. 4e). Blood vessels were clearly observed in the stroma of the ovary.

Stage VI (spent): This stage was typical of females after spawning. Ovaries were large and containinged few large oocytes that probably belonged to the same batch of ova that had just been spawned. They were likely to be rapidly eliminated. The mean ova diameter was drastically decreased, reaching 0.289 mm. Large atretic follicles were observed in the spent ovary (Figs. 3,4f).

The percentage of different stages in different months is presented in Fig. 6. Ovarian stages and I_G were correlated (r=0.558 p<0.001) (Fig. 7). A single female specimen of *Iranocichla hormuzensis* (TL= 129 mm; SL= 116.9 mm W= 38.2 g) having 153 larvae in her mouth was collected from Mehran River. Average length and weight of larvae were 10.2 mm and 0.021g respectively. They were in the yolk sac stage and the fins were developed (Fig.1).

Testes: Based on macroscopic and microscopic observations 6 stages of maturation were distinguished for males of *I. hormuzensis*, as below:

Stage I (immature): Testis was thread-like, thin and whitish gray (Fig. 5a). The mean I_G was 0.064. Spermatogonia and primary spermatocytes were the dominant cells of this stage. Spermatogonia had a light cytoplasm and a large nucleus. Some secondary spermatocytes having basophilic cytoplasm were also observed (Fig. 6a).

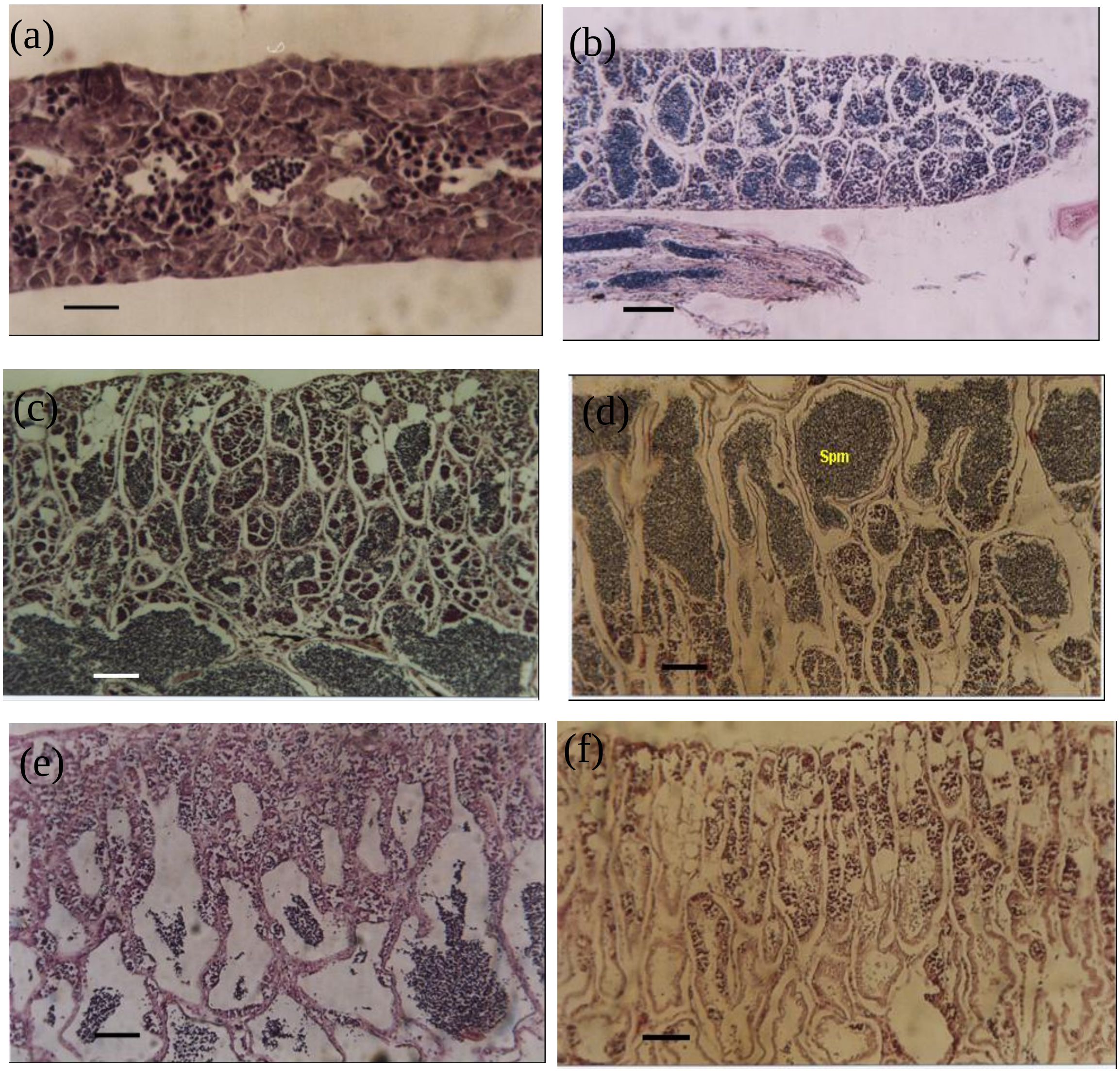


Figure 6: Histological profiles of the six male maturity stages. (a) Immature stage, scale bar = 20 µm. (b) Developing stage, scale bar = 70 µm. (c) Maturation stage, scale bar = 70 µm. (d) Ripe stage. Spm, sperms. scale bar=70 µm. (e) Spawning stage, scale bar = 70 µm. (f) Spent stage, scale bar = 200 µm.

Stage II (developing): Testes were opaque and gray with an average I_G of 0.091 (Fig. 5b). Primary and secondary spermatocytes, spermatid and sperm were observed in the tubules (Fig. 6b).

Stage III (maturation): The testes were yellowish gray and flat with an average I_G of 1.278 (Fig. 5c). In this stage, the number of large cells was decreased but the number of small cells (spermatids and sperms) was increased progressively (Fig. 6c).

Stage IV (ripe): The male gonads were flat (Fig. 5d) and mean I_G was increased (0.298). Tubules were characterized by having secondary spermatocytes, large numbers of spermatids and spermatozoa (Fig. 6d).

Stage V (running): Testis was a large flat structure occupying the whole body cavity. The anterior part of the testis was convoluted (Fig. 5e). The mean I_G value was 0.825. Milt was running by application of slight pressure on the abdominal region of the fish. The testes had well-defined lobules with a large number of spermatids and spermatozoa on their inner center. The predominant cells were spermatozoa with a dark blue stain related to their nucleus (Fig. 6e).

Stage VI (spent): The testes weight was decreased drastically (Fig. 5f) with a mean I_G of 0.087.The majority of tubules were empty. Few sperm were left in the tubules (Fig. 6f).

The fFrequency of different ovarian stages by months for *I. hormuzensis* and mean I_G in different gonad stages for male and female specimens of *I. hormuzensis* are given in Figs. 7 and 8.

Discussion

The gonad morphology and histology of the mouth brooding Iranian cichlid has not been already been described. This paper forms the first detailed attempt to investigate the gonad morphology and histology of this fish. The Iranian cichlid is a maternal mouth brooder in which the female churns the eggs in her mouth. Parental care occurs in 21% of families of teleost fishes (Gross and Sargent 1985). Most parental care (more than 95%) involves guarding of the eggs by the male (Specker and Kishida 2000). In the family Cichlidae, more than 1000 species exhibit parental care. The female *I. hormuzensis*, picks up the eggs in her mouth. The eggs hatch in her mouth and become hatched embryos (wrigglers). They remain in her mouth until they depart as free-swimming larvae (fry). As in other cichlid fishes, the release of young is followed by a care period or guarding, when the female takes the juveniles into her mouth when they are endangered. Our observations showed that a single female can keep as many as 153 juveniles of 10.2 mm total length and 0.021 g weight in her mouth.

Our findings demonstrate that tThe Iranian cichlid spawns at the end of winter and beginning of spring. This is evident from the I_G, percentage of late gonad maturation stages (IV, V, VI) and high frequency of large oocytes. The reproductive index for females was always higher than for males. ThisIt has been reported in some other fishes (Pajuelo and Lorenzo 2000, Esmaeili and Shiva 2006). Related to differences in the I_G, values between reproductively active males and females, in reviewing the reproductive biology of fishes it is noted that the I_G, values of males are commonly lower than those of females (Pajuelo and Lorenzo 2000). The cost of producing sperm is less than that for producing eggs (Buxton 1990). Although the number of gametes produced does not necessarily need to be a function of the size of the gonad, the difference in male and female gonadosomatic index suggests that the energy invested in gamete production by males is probably less than that invested by females (Buxton 1990, Pajuelo and Lorenzo 2000).

Sexual dimorphism has been reported for this fish. According to Coad (1982) head length is greater in females while pelvic fin length is smaller in females compared to males. Interorbital width is greater in males. Dorsal and anal fins are larger in males when expressed in terms of longest ray length in head length. The present study indicated that sexual dimorphism involves body proportions, fin lengths and pigmentation. Based on our observations on the fishes kept in an aquarium, sexual dimorphism was observed in the color of fish during the breeding season. The female has an overall silvery color with up to 9 faint to moderate flank bars. The dorsal fin has a black tilapia-mark on the posterior dorsal fin. The male is brick-red on the lower sides and underside of the head with black on the dorsal head surface in agreement with Schulz (2004).

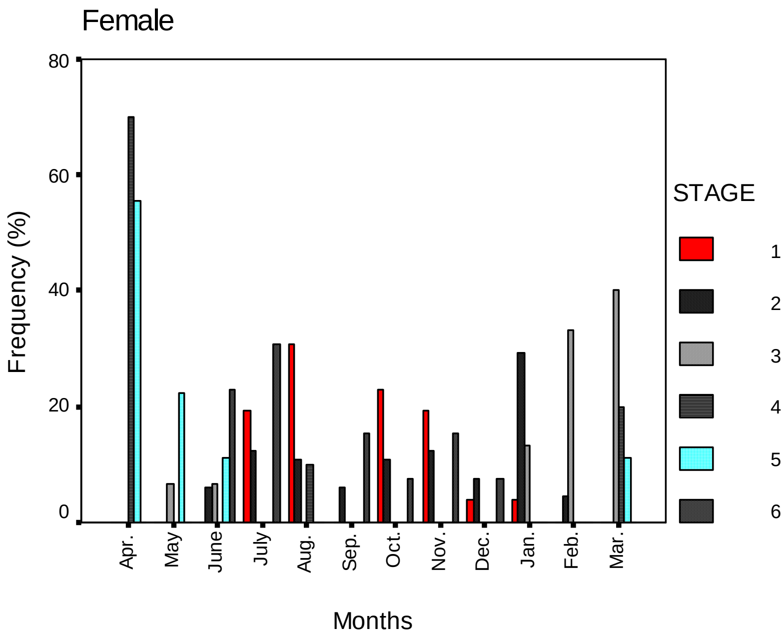


Figure 7: Frequency of different ovarian stages by months for *I. Hormuzensis*

Ovarian oocytes exhibited a broad variability in size distribution profiles, depending mostly on the maturity stages and also on individual fish variations. Egg size in *Iranocichla hormuzensis* was generally larger in larger fish.

In the present study gonad developments was arbitrarily classified into six stages. These stages were based on the size and weight of the ovary, degree of occupation of the body cavity, presence or absence of ripe oocytes, diameter of the oocytes in the ovary, oocyte shape, vitellogenesis, size of oil droplets and yolk vesicles and presence of atretic oocytes. They were strongly correlated to the I_G and correspond to the chronological succession. Hence the spawning season could be determined. Ovarian development has been described in some other cichlid fishes like *Oreochromis niloticus* (Babiker and Ibrahim 1979). In this species, 5 or 6 development stages have been defined based on histological criteria. They described the change in the ovary from immature gonad (stage I) to ovulation (stage V or VI). Tacon *et al.* (1996) also defined six stages according to follicle size of *O. niloticus*. Among cichlid fish belonging to the group "tilapia" (genus *Tilapia*, *Oreochromis* and *Sarotherodon*), the rhythm of ovarian development has been shown to depend on the influence of various external factors such as temperature (Bogomolnaya and Yaron 1984), photoperiodic rhythm (Hyder 1972) and social and behavioral factors such as visual, olfactory and sound stimuli from conspecifics (Silvermann 1978).

We did not study the reproductive behavior of the Iranian cichlid in the field but Schulz (2004) observed fish in the field (Hormuzgan province) and found each male occupying a territory defending a nest about 1 m from each neighbouring nest. The nests were made on light grey, fine sand and consisted of a pit approximately 15 cm in diameter. The pit was black because of anoxic conditions below the sand surface. The actual nest was about the same as the body length of the fish (8-10 cm) and lay at the centre of the pit. The pit was surrounded by a rim about 1.5 cm high with an internally indented margin. Simpler pits are built where building materials are unavailable. Females were present in schools in deeper water in the river centre. Individual females swam purposefully to the nest defended by the male. The male directed the female to the nest centre with folded up fins while the female spread her fins and showed radiating colour changes. Spawning occurred immediately and neighboring males intervened continuously at a speed that did not allow full analysis of the movements. A defending male would chase away an intruding male allowing another male into the unprotected nest to mate with the female. A clutch of eggs were always inseminated by a whole group of males. Hence it seems that Iranian cichlid is a polygamous mouthbrooder. Polygamy predominates among mouthbrooders (Barlow 1991). In this mating system, males are usually, but not always larger and more colourful than females (Barlow 1991).

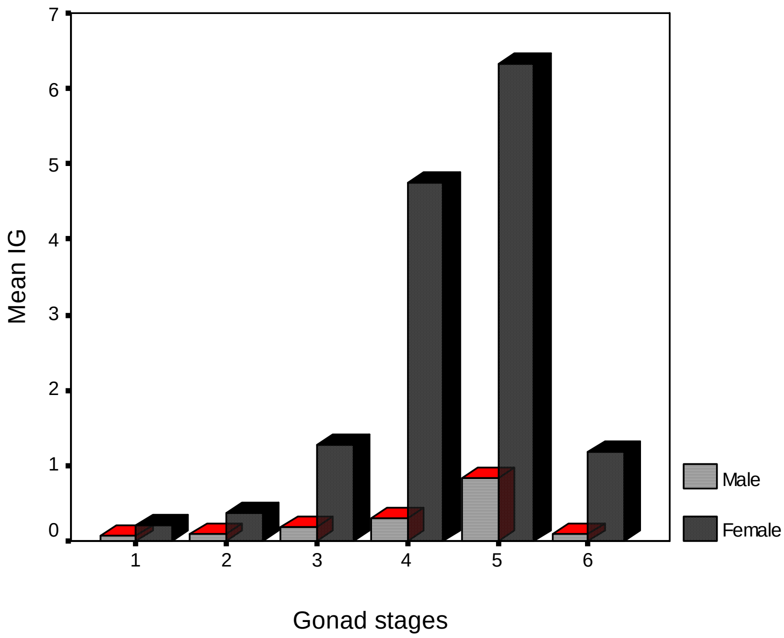


Figure 8: Mean I_G in different gonad stages for male and female specimens of *I. hormuzensis*

It seems that evolution of parental care in this fish like many other teleosts is associated with an increase in egg size and a corresponding reduction in the number of eggs. Parental behavior can be considered as a significant investment by one of the parents, which is rewarded by increased survival of the offspring (Sargent et al. 1987). As such, this investment should represent an energy cost detrimental to the subsequent reproductive cycle (Williams1966).

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